On the Origin of Self-Incompatibility Haplotypes: Transition Through Self-Compatible Intermediates

Marcy K. Uyenoyama,* Yu Zhang* and Ed Newbigin

*Department of Biology, Duke University, Durham, North Carolina 27708-0338 and
†School of Botany, University of Melbourne, Victoria 3010, Australia

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ABSTRACT

Self-incompatibility (SI) in flowering plants entails the inhibition of fertilization by pollen that express specificities in common with the pistil. In species of the Solanaceae, Rosaceae, and Scrophulariaceae, the inhibiting factor is an extracellular ribonuclease (S-RNase) secreted by stylar tissue. A distinct but as yet unknown gene (provisionally called pollen-S) appears to determine the specific S-RNase from which a pollen tube accepts inhibition. The S-RNase gene and pollen-S segregate with the classically defined S locus. The origin of a new specificity appears to require, at minimum, mutations in both genes. We explore the conditions under which new specificities may arise from an intermediate state of loss of self-recognition. Our evolutionary analysis of mutations that affect either pistil or pollen specificity indicates that natural selection favors mutations in pollen-S that reduce the set of pistils from which the pollen accepts inhibition and disfavors mutations in the S- RNase gene that cause the nonreciprocal acceptance of pollen specificities. We describe the range of parameters (rate of receipt of self-pollen and relative viability of inbred offspring) that permits the generation of a succession of new specificities. This evolutionary pathway begins with the partial breakdown of SI upon the appearance of a mutation in pollen-S that frees pollen from inhibition by any S- RNase presently in the population and ends with the restoration of SI by a mutation in the S- RNase gene that enables pistils to reject the new pollen type.

In the form of GSI expressed in the Solanaceae (and also the Rosaceae and Scrophulariaceae; Broothaerts et al. 1995; Xue et al. 1996), an extracellular ribonuclease (S- RNase) inhibits the growth of incompatible pollen tubes in the style (McClure et al. 1990; Lush and Clarke 1997). In poppy, which lacks a style, GSI rejection entails the arrest of pollen tube growth at the stigma. The S protein encoded by the poppy S locus appears to act as a stigmatic signal molecule that, upon binding to surface receptors, induces an increase in calcium ion concentration in incompatible pollen tubes (Franklin et al. 1995). The striking variety of genetic and physiological mechanisms employed by these systems constitutes compelling evidence for multiple independent evolutionary origins of SI.

Descended from diverse phylogenetic origins, these systems of SI exhibit a remarkable evolutionary convergence to single-factor regulation at the level of classical genetics. Another shared feature is the very large number of distinct specificities estimated to segregate at the S locus, ranging from 12 to nearly 200 (Lawrence 2000). Classical questions include the nature of the selective forces and ecological contexts that permit the maintenance of this extraordinary diversity (Wright 1939; Fisher 1958, Chap. 4).

In this article, we explore the origin of new GSI specificities through the analysis of population genetic models. We address the evolutionary dynamics of mutations that alter SI recognition between pollen and pistil,
which induces a transient or permanent loss of self-incompatibility.

**Bipartite structure: SSI in Brassica:** In Brassica, the S-locus genotype of the pollen parent determines the specificities expressed by pollen. Proteins borne in the pollen coating determine pollen specificity (Stephen-son et al. 1997), with a membrane-bound receptor kinase mediating recognition by the stigma (Nasrallah et al. 1994). The S-locus resides in a structurally complex and gene-dense region of the genome that appears to segregate as a single unit (Boyes et al. 1997; Suzuki et al. 1999). Shaplotypes include SCR (S-locus cysteine-rich protein; originally named SP11; Suzuki et al. 1999), which encodes the specificity-determining proteins in the pollen coat (Schopper et al. 1999; Takayama et al. 2000), and SRK (S-locus receptor kinase), which controls recognition of pollen specificity by the stigmatic epidermal cells (Takasaki et al. 2000).

**GSI in the Solanaceae:** Early irradiation studies of GSI systems established the bipartite structure of the S-locus by generating mutations that separately disrupted SI expression in pollen and style (Lewis 1954; see review by Golz et al. 2000). Pollen-part mutations disrupt expression of pollen specificity while preserving stigmatic rejection, and style-part mutations disrupt stigmatic rejection while preserving pollen specificity. Transformation of solanaceous plants with an SRNase construct conferred the ability to reject pollen expressing the new specificity, without affecting the specificity expressed by the pollen of the transgenic plants (Lee et al. 1994; Murfett et al. 1994). A gene distinct from the gene that encodes SRNase controls the specificity expressed by pollen (Dodd et al. 1999). This as yet unidentified gene is generally called pollen-S.

Experiments designed to detect recombination between the loci that control the specificities rejected by the style and expressed by pollen have yielded negative results in all SI systems studied to date (see Lewis 1949, for example). Indeed, such recombination would presumably impair SI by permitting the expression of different specificities in pollen and style. In the solanaceous system, the S-locus resides in an extensive genomic region (comprising perhaps 1 Mb of DNA; McCubbin and Kao 1999) over which recombination is suppressed (or generates only unbalanced chromosomes that are immediately eliminated). These findings support the hypothesis that the origin of a new specificity entails a series of coevolved point mutations at two or more genes within the S-locus.

**Stylar specificity:** Sequence comparisons of SRNases that determine different stylar phenotypes may afford insight into the determination of stylar specificity. SRNases segregating within species show extraordinarily high divergence, with amino acid sequence similarity ranging from 40 to 80\% (Ioerger et al. 1990). In a comparative study of SRNases derived from Nicotiana alata, Kheyr-Pour et al. (1990) recognized five hyperva-
Charlesworth and Charlesworth (1979) also studied the fate of mutations that impair expression in pollen only, pistil only, or both. An unusual feature of their models is that pollen produced by the same plant (self-pollen) and pollen produced by a different plant (non-self-pollen) do not appear to compete with one another for fertilization within pistils. In virtually all other models of SI, the fraction of ovules fertilized by a given compatible pollen type corresponds to the quantity of that type of pollen received normalized by the total compatible pollen received (see, for example, Wright 1939). This construction entails that all plants set the same number of seeds, reflecting the definition of gametophytic self-incompatibility as a prezygotic process. The Charlesworth and Charlesworth (1979) models do not incorporate a normalization of this kind; further, the fraction of seeds set by self-pollen in plants that carry mutations with impaired SI function corresponds to a parameter rather than to a function of genotypic frequencies.

Two-mutation models: Matton et al. (1999) constructed a “dual-specificity” SRNase that rejected two pollen specificities in S. chacoense. Naturally occurring SRNases $S_1$ and $S_2$ differ at only 10 amino acids, including 3 in HVa and 1 in HVb (Saba-El-Leil et al. 1994). Transformation of plants with an $S_1$ sequence in which the 4 HVa and HVb residues had been substituted to match the $S_3$ sequence conferred the ability to reject $S_3$ pollen (Matton et al. 1997). Transformants bearing an $S_3$ sequence in which the 3 HVa sites but not the HVb site had been substituted rejected neither $S_1$ nor $S_2$ pollen (Matton et al. 2000). Substitution of 2 of the 3 HVa sites and the HVb site produced the remarkable dual-specificity construct, which caused the rejection of both $S_1$ and $S_3$ pollen (Matton et al. 1999). Matton et al. (1999) proposed that new $S$-specificities may arise through a pathway that begins with a mutation that confers recognition by the pistil of both an existing pollen specificity and a pollen specificity not yet present in the population, continues with the appearance of the new pollen specificity, and terminates with a stylar mutation that restricts rejection to the new pollen specificity alone. Charlesworth (2000) questioned whether the multiplicity of mutations required in the same haplotype lineage is too large to explain the generation of the many specificities known to exist.

Uenoyama and Newbigin (2000) argued that whether a given pathway can in fact generate new specificities depends on the evolutionary dynamics among the ancestral haplotype, the derived haplotype, and their intermediates. Their simple analysis of mutations that modify elements of the SI reaction in either pollen or pistil while preserving rejection of self-pollen revealed an evolutionary advantage associated with mutations that restrict the set of SRNases from which pollen accept disablement and an evolutionary disadvantage associated with mutations that cause pistils to accept pollen specificities nonreciprocally. They noted that in the pathway proposed by Matton et al. (1999) the mutation that generates the new single-specificity haplotype appears to cause the pistil to accept pollen bearing the dual-specificity ancestral haplotype in a nonreciprocal manner. The ancestral haplotype would drive such a mutation to extinction. In contrast, a mutation in pollen-S that distinguishes among formerly neutral variations among SRNases expressing a given specificity would in fact succeed in invading the population and replacing the ancestral haplotype.

In this article, we continue the exploration of the origin of new specificities by expanding consideration to evolutionary intermediates rendered self-compatible by mutations that affect either pollen or pistil SI function. All mutations considered here are subject to selection as a consequence of their impairment of SI. We show that the selective pressures generated by reduction in pollen susceptibility and nonreciprocal pollination dominate the evolutionary process, even though absolute linkage between the regulators of pollen and pistil function consigns them to a common evolutionary fate.

**MODEL STRUCTURE**

Shaplotypes comprise an $A$ component (analogous to the SRNase gene), which controls the pollen specificity rejected by the pistil, and a $B$ component (analogous to pollen-S), which controls the pistil specificity from which pollen accepts inhibition. Fully functional haplotype $S$, corresponds to $AB$, in which the common subscript signifies the mutual recognition of the $A$ and $B$ components.

We denote the initial population, comprising $n$ functional haplotypes, by $\{S_i\}$, for $i$ assuming values from 1 to $n-1$ and $S$, the haplotype in which the new mutation will occur. Mutations within haplotype $S$, may alter only the specificity rejected by the pistil (generating haplotype $S_i$) or only the specificity expressed in pollen ($S_j$). Figure 1 summarizes the SI phenotypes associated with the haplotypes. Pollen tubes bearing haplotype $S_i(A_{s+1}B_j)$ express specificity $B_j$, which accepts disablement by $A_s$ SRNase, while pistils bearing $S_i$ produce a new SRNase directed against a pollen specificity not yet present in the population. Haplotype $S_i(A_{s+1}B_{s+1})$ encodes the $A$, SRNase and a new pollen specificity that accepts disablement by an SRNase not yet present in the population.

Upon the successful invasion of the initial mutant haplotype ($S_i$ or $S_j$), the population converges to a new equilibrium state (for example, $\{S_i S_j S_k\}$), comprising the single mutant together with all, some, or none of the original haplotypes. We then consider the fate of the double mutant haplotype $S_{s+1}(A_{s+1}B_{s+1})$, which corresponds to a new, full-function specificity.

Table 1 summarizes the variables representing genotypic and allelic frequencies. To maintain tractability
while permitting arbitrary numbers of Shaplotypes, we assume that all genotypes of a given class (for example, $S_iS_i$ and $S_jS_j$) occur in equal frequency. In a study of the evolution dynamics of sporophytic SI (UYENOYAMA 2000), explicit stochastic numerical simulation of the full system exhibited negligible departures from the reduced, fully symmetric system, which assumed equal frequency among genotypes within class. Variable $a_i$ denotes the frequency of a genotype carrying $S_i$ together with any of the other nonmutant haplotypes ($SS, 1 \leq i < n$); and $G$ denotes two distinct nonmutant haplotypes other than $S_i$ ($SS, 1 \leq ij < n, i \neq j$). Frequencies of carriers of the single mutant include $a_i$, corresponding to the heterozygote with any of the nonancestral full-function haplotypes (for example, $S_jS_j, 1 \leq i < n$); $a_i$, the heterozygote with the ancestral nonmutant haplotype (for example, $S_iS_i$); and $a_i$, the single mutant homozygote (for example, $S_iS_i$). Frequencies of carriers of the double mutant include $a_i$, corresponding to the heterozygote with any of the nonancestral full-function haplotypes ($SS, 1 \leq i < n$); $a_i$ the heterozygote with the ancestral haplotype ($SS, 1 \leq i < n$); and $a_i$, the heterozygote with the single mutant haplotype (for example, $S_iS_j$).

These variables account for the frequencies of all genotypes in the population:

$$(n-1)(a_1+a_2)+a_1+a_2+a_1+a_2+G\left(\frac{n-1}{2}\right)=1.$$

(1)

Self-pollen comprises a proportion $s$ ($0 \leq s \leq 1$) of the pollen received by any individual plant. Compatible pollen tubes, irrespective of origin, compete on an equal basis for fertilization. All plants set the same number of seeds. Inbred offspring (derived from self-pollen) survive to reproduction at rate $s$ ($0 \leq s \leq 1$) relative to outbred offspring (derived from non-self-pollen).

**Pollen-part mutation**: A full dynamical description of the genotypic frequencies, with the subsequent generation denoted by primes, appears in the APPENDIX. Haplotypes $S_{i}$ ($1 \leq i < n$), $S_{i}$, $S_{j}$, and $S_{i+1}$ occur in the pollen pool and in the population in frequencies $p_{i}$, $p_{i+1}$, and $p_{k}$, respectively:

$$p = \frac{[a_0 + a_1 + a_2 + G(n-2)]}{2}$$

(2)

$$p_{i} = \frac{[a_0(n-1) + a_1 + a_2]}{2}$$

(3)

$$p_{i+1} = \frac{(a_1 + a_2 + a_i)}{2}$$

(4)

for which

$$p(n-1) + p_i + p_{i+1} = p \cdot (n-1) + p_i + p_i + p_{i+1} = 1.$$  

T, the average viability among offspring, corresponds to

<table>
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<th>Variable</th>
<th>No.</th>
<th>$A$ locus mutation</th>
<th>$B$ locus mutation</th>
</tr>
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<td>$a_0$</td>
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<td>$SS$</td>
<td>$SS$</td>
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<tr>
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<td>$SS$</td>
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<td>$SS$</td>
<td>$SS$</td>
</tr>
<tr>
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<td>$n-1$</td>
<td>$SS$</td>
<td>$SS$</td>
</tr>
<tr>
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<td>$SS$</td>
<td>$SS$</td>
</tr>
<tr>
<td>$a_6$</td>
<td>1</td>
<td>$SS$</td>
<td>$SS$</td>
</tr>
<tr>
<td>$G$</td>
<td>$(n-1)\choose2$</td>
<td>$SS$</td>
<td>$SS$</td>
</tr>
</tbody>
</table>

**TABLE 1**

Self-incompatibility genotypes and phenotypes
\[ T = 1 - \left( \frac{c_1(n-1)}{2N_1} + \frac{c_2}{2N_2} + \frac{c_3}{N_3} \right) s(1 - \sigma); \] (7)

and the \( N_i \) represent the fractions of the total pollen received by pistils of genotype class \( i \) that are compatible:

\[
\begin{align*}
N_0 &= (1 - s)(1 - p - p_s) \\
N_1 &= s/2 + (1 - s)(1 - p - p_s) \\
N_2 &= s/2 + (1 - s)(1 - p) \\
N_3 &= s + (1 - s)(1 - p) \\
N_4 &= (1 - s)(1 - p - p_s) \\
N_5 &= (1 - s)(1 - p - p_s - p_{s+1}) \\
N_6 &= (1 - s)(1 - p - p_s - p_{s+1}) \\
N_7 &= (1 - s)(1 - 2p).
\end{align*}
\] (8)

Manipulation of the genotypic recursions provides expressions for the haplotype frequencies in the next generation:

\[
T'p' = \frac{p}{2} - \frac{c_1(1 - \sigma)}{8N_1} \\
\quad + \left[(n - 2)\left(\frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} \right)\right] \times p(1 - s)/2 \quad (9)
\]

\[
T'_p = \frac{p}{2} - \frac{c_2(1 - \sigma)}{8N_1} \quad + \left[\frac{c_1(n-1)}{N_1} + \frac{c_2\left(\frac{n-1}{2}\right)}{N_2}\right] \times p(1 - s)/2 \quad (10)
\]

\[
T'_s = \frac{p}{2} - \left[\frac{c_1(n-1)(1 - 3\sigma)}{4N_1} + \frac{c_1(1 - 3\sigma)}{4N_2} + \frac{c_1(1 - 2\sigma)}{N_1}\right] s/2 \\
\quad + \left[(n - 1)\left(\frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} \right)\right] \times p(1 - s)/2 \quad (11)
\]

\[
T'_{s+1} = \frac{p_{s+1}}{2} \\
\quad + \left[(n - 1)\left(\frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} \right)\right] \times p_{s+1}(1 - s)/2. (12)
\]

(These expressions reflect transmission of haplotypes through pollen and egg cells. Pollen expressing haplotype \( S_k \) is compatible in pistils of all genotypes except those bearing \( S_{k+1} \). The rate of transmission of \( S_k \) through outcross- and self-pollen is

\[ P = \left[(n - 1)\left(\frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} + \frac{c_1}{N_1} \right)\right] p(1 - s) \]

and the \( N_i \) represent the fractions of the total pollen received by pistils of genotype class \( i \) that are compatible:

\[
\begin{align*}
N_0 &= (1 - s)(1 - p - p_s) \\
N_1 &= s/2 + (1 - s)(1 - p - p_s) \\
N_2 &= s/2 + (1 - s)(1 - p) \\
N_3 &= s + (1 - s)(1 - p) \\
N_4 &= (1 - s)(1 - p - p_s) \\
N_5 &= (1 - s)(1 - p - p_s - p_{s+1}) \\
N_6 &= (1 - s)(1 - p - p_s - p_{s+1}) \\
N_7 &= (1 - s)(1 - 2p).
\end{align*}
\]

This last expression indicates that the number of \( S_k \) haplotypes transmitted through egg declines as the rate of receipt of self-pollen \( (s) \) increases and the viability of inbred offspring \( (\sigma) \) decreases. Because one-half the genes held by offspring derives from egg cells and one-half from pollen cells, the total transmitted frequency of \( S_k \) is

\[ T'p' = (E + P)/2, \] (15)

which reduces to (11) upon rearrangement. The new frequencies of the functional haplotypes similarly reflect transmission through egg and pollen.

**Style-part mutation:** Genotypic frequencies in the next generation appear in the appendix. In the pollen pool, haplotype frequencies \( p, p_s, \) and \( p_{s+1} \) are defined in (2), (3), and (5), and \( p_s \) (frequency of \( S_k \)) corresponds to the right side of (4). \( T \), the average viability among offspring, is

\[ T = 1 - \left[\frac{c_1(n-1)}{2N_1} + \frac{c_2}{2N_2} + \frac{c_3}{N_3}\right] s(1 - \sigma); \] (16)

and the \( N_i \) give the fraction of compatible pollen received:

\[
\begin{align*}
N_0 &= (1 - s)(1 - p - p_s - p_s) \\
N_1 &= s/2 + (1 - s)(1 - p - p_{s+1}) \\
N_2 &= (1 - s)(1 - p - p_s - p_{s+1}) \\
N_3 &= s + (1 - s)(1 - p_{s+1}) \\
N_4 &= (1 - s)(1 - p - p_{s+1}) \\
N_5 &= (1 - s)(1 - p - p_s - p_{s+1}) \\
N_6 &= s/2 + (1 - s)(1 - p_{s+1}) \\
N_7 &= (1 - s)(1 - 2p).
\end{align*}
\] (17)

Among offspring, the frequency \( T'p' \) of a functional haplotype other than \( S_k \) corresponds to (9); the remaining haplotype frequencies are
For larger \( n \), \( S_b \) increases only if \( \sigma \) exceeds the single threshold value determined by (A1) in the appendix. Implicit differentiation indicates that this threshold value increases with the fraction of self-pollen received \( (s) \) and the number of functional haplotypes \( (n) \). Higher rates of receipt of self-pollen and greater numbers of functional haplotypes tend to oppose the rise of the single mutant, necessitating higher viability of inbred offspring \( (\sigma) \) to ensure the invasion of \( S_b \). In the limit as \( n \) becomes very large, \( S_b \) increases when rare in \( \{S_n, S_s\} \) only if
\[
\sigma^2 s - 3 \sigma + 1 < 0. \tag{24}
\]
Near the state of fixation of \( S_b \), the mean viability of offspring \( (T) \) lies close to its minimum value:
\[
1 - s + \sigma s. \tag{25}
\]
This state resists the invasion of all original full-function haplotypes if
\[
\sigma > \frac{1 - s}{3 - 2s}. \tag{26}
\]
Unlike the condition for the initial increase of \( S_b \) (A1), (26) is independent of \( n \).

In a two-dimensional plot of \( \sigma \) (ordinate) against \( s \) (abscissa), condition (A1) demarcates the region above a monotonically decreasing curve (Figure 2) and (26) the region above a monotonically decreasing curve (Figure 2). Together, the conditions determine four parameter regions, corresponding to qualitatively different evolutionary outcomes: decrease of \( S_b \) when rare and when near fixation (region E), increase when rare and when near fixation (region S), decrease when rare and increase near fixation (region D), and increase when rare and decrease near fixation (region P).

Figure 3 depicts the dependence of condition (A1) on the number of functional haplotypes. For small \( n \), the curve specified by (A1) lies near the right boundary, minimizing regions D and E. For very large \( n \), the intercept of the curve with the abscissa approaches zero, minimizing region P.

\[
\begin{align*}
Tp'_{b} &= p_b/(n - 1) \left[ \frac{\sigma/(N_1 + \sigma)}{N_0} + \frac{G/(n - 1)}{N_0} \right] \\
&\times p_b(1 - s)/2
\end{align*}
\tag{18}
\]

\[
\begin{align*}
Tp'_{c} &= p_c/(n - 1) \left[ \frac{\sigma/(N_1 + \sigma)}{N_0} + \frac{G/(n - 1)}{N_0} \right] \\
&\times p_c(1 - s)/2 \\
&- \left[ \frac{s(n - 1)(1 - 3\sigma)}{4N_1} + \frac{s(1 - 2\sigma)}{N_0} + \frac{s(1 - 3\sigma)}{4N_0} \right]/2
\end{align*}
\tag{19}
\]

\[
T_{p_{c+1}} = p_{c+1}/2 \left[ \frac{\sigma(n - 1)}{N_0} + \frac{G/(n - 1) + \sigma^2/(n - 1)}{N_0} \right] \\
\times p_{c+1}(1 - s)/2 - \frac{s\sigma(1 - \sigma)}{4N_0}, \tag{20}
\]

for which
\[
p(n - 1) + p_a + p_c + p_{c+1} = p'(n - 1) + p'_a + p'_c + p'_{c+1} = 1. \tag{21}
\]

\section*{RESULTS}

**Pollen-part mutation: Introduction of the single mutant:** All individuals in the initial population are outbred \( (T = 1) \) and carry two distinct functional haplotypes, with each genotype occurring in equal frequency \( (G = \sigma = 1/(\zeta)) \). Equations 9–11 indicate that if the viability of offspring derived by selfing is at least one-half that of outbred offspring \( (\sigma > \zeta/2) \), the per-gene rate of increase of the nonfunctional haplotype \( S_b \) uniformly exceeds that of any of the existing functional haplotypes,
\[
T p'_{b}/p_b > T p'/p_a, \tag{22}
\]
signifying that \( S_b \) increases to fixation upon its appearance in \( \{S_n, S_s\} \) in any frequency.

Introduced in low frequency, haplotype \( S_b \) invades \( \{S_n, S_s\} \) for \( n < 5 \) and for \( n \) sufficiently small to satisfy
\[
\begin{align*}
&\quad sn^2(n - 4) - (1 - s)8(n - 1)(n - 2) \\
&+ (1 - s)(n^3 - 2n^2 - 9n + 16) < 0. \tag{23}
\end{align*}
\]

Figure 2.—Four evolutionary outcomes of the introduction of haplotype \( S_b \) into populations with \( n = 10 \) functional haplotypes. Values of the relative viability of inbred offspring \( (\sigma) \) and self-pollen fraction \( (s) \) corresponding to region \( S \) permit \( S_b \) to increase both near extinction and near fixation. \( S_b \) increases when rare but not near fixation in region \( P \), decreases near extinction but increases near fixation in region \( D \), and decreases in both ranges in region \( E \).

\[\text{Relative viability of inbred offspring (} \sigma \text{)}\]

\[\text{Self-pollen fraction (} s \text{)}\]

\[\text{P \quad E \quad D}\]
Polymorphic states: Parameter values corresponding to regions P and S ensure the increase of $S_i$ upon its introduction in low frequency into $\{S_i, S_n\}$. Because the fixation of $S_i$ is unstable in region P, the invasion of $S_i$ implies convergence to a polymorphic state at which all full-function haplotypes ($\{S_i, S_n, S_j\}$) or all but the parental haplotype $S_i$ ($\{S_i, S_n\}$) segregate. In region S, the fixation of $S_i$ is locally stable, suggesting that the invasion of $S_i$ can result in complete self-compatibility upon the extinction of all full-function haplotypes.

Deterministic iteration of the recursion system indicates that under almost all parameter combinations in region S, the introduction of $S_i$ ends in its fixation, corresponding to a total loss of SI. Under parameter combinations in a neighborhood close to the intersection of the increasing curve (invasion of $S_i$) and decreasing curve (fixation of $S_i$; see Figure 2), the population converges to a state of partial self-compatibility, reflecting the maintenance of full-function haplotypes together with $S_i$.

Our numerical explorations indicate that with the exception of parameter combinations very close to the threshold (A1) that determines whether $S_i$ increases when rare in $\{S_i, S_n\}$, the presence of $S_i$ causes its parental haplotype $S_n$ to decline to extinction. In the exceptional cases, $S_i$ may segregate in stable polymorphism with $S_n$ and the other full-function haplotypes.

We examine the conditions that permit $S_i$ to invade $\{S_i\}$. In the absence of both $S_i$ and $S_{i+1}$, $S_i$ causes the rejection of no pollen and $S_i$ pollen encounters rejection in no pistil. Only three genotypic classes exist: $SS_i$ (frequency $G_i$), $S_Si(e_i)$, and $S_Si(e_i)$. In such populations, $S_i$ increases when rare if

$$\sigma^2 s^2 (n - 1)(n - 3) - \sigma s (n - 3)(3n - 5 + 2s) + n^2 s - 2n(2 + s) + 8 - 3s < 0. \quad (27)$$

This condition holds uniformly for $n < 6$; for larger $n$, $S_i$ invades for relative viabilities of inbred offspring ($\sigma$) greater than the single root in $(0, 1)$ of the quadratic on the left side of (27). Implicit differentiation confirms that this threshold value of $\sigma$ also increases with $s$ and $n$. The minimum value of $\sigma$ that permits the invasion of $S_i$ in the presence of $S_n$ [from (A1)] only slightly exceeds that in its absence [from (27)]; consequently, the simpler condition (27) provides a close approximation to (A1), especially for large $n$ or $s$.

In the narrow parameter region lying between these two thresholds, $S_i$ invades in the absence of $S_n$ but not in its presence. Under some conditions, $S_i$ may initially decline in frequency upon its introduction into $\{S_i, S_{i+1}\}$, but then increase as its own presence causes the exclusion of $S_n$. In such cases, the introduction of $S_i$ can result in its maintenance even in regions E and D.

Introduction of the double mutant: Region P comprises parameter values that permit the initial invasion of $S_i$ but not its fixation, implying that the population converges to a polymorphic state ($\{S_i, S_n\}$ or $\{S_i, S_n, S_j\}$), in which $S_i$ segregates together with functional haplotypes. Expressions (11) and (12) indicate that the per-gene rate of increase of $S_{i+1}$ exceeds that of $S_i$ ($T_{ip+1}^*/p_{i+1}$ if the first bracketed term of (11) is positive, which is clearly true if the viability of outbred offspring exceeds that of inbred offspring by more than threefold ($\sigma < \frac{3}{2}$). Because the instability of the fixation of $S_i$ [violation of (26)] ensures $\sigma < \frac{1}{2}$, $S_{i+1}$ invades all stable polymorphisms arising under region P, causing the extinction of $S_i$ and restoring the population to full self-incompatibility.

Within regions S and D [satisfying (26)], the fully self-compatible state of fixation of $S_i$ resists the invasion of any of the original full-function haplotypes. Expression (12) indicates that $S_{i+1}$ increases when rare near such states $[e_i = 1, T$ given by (25)] only if the viability of outbred offspring exceeds that of inbred offspring by more than twofold ($\sigma < \frac{1}{2}$). In the absence of any other full-function haplotypes, carriers of $S_{i+1}$ express incompatibility against all pollen and the entire population derives from seeds set by $S_iS_i$ individuals alone.

Region S also admits stable polymorphisms comprising $S_i$ together with full-function haplotypes for values of $\sigma$ close to the minimum (A1) required for the invasion of $\{S_i, S_n\}$ by $S_i$ (see preceding section). Parameter combinations under which such polymorphisms arise appear to lie in the neighborhood of the intersection of the increasing and decreasing curves of the kind depicted in Figure 2. This neighborhood becomes vanishingly small as $n$ increases. The relative viability of inbred offspring at the intersection itself is always less than one-third ($\sigma < \frac{1}{3}$) because the declining curve (26) never exceeds this value. For $n > 21$, the increasing
curve (A1) extends above one-third, but in this range the subregion that admits stable polymorphisms is very small or nonexistent. These findings suggest that the appearance of the double mutant $S_{e+1}$ near any polymorphism that arises in region S will result in the exclusion of the single mutant $S_e$ and restoration of the population to full self-incompatibility.

Numerical analysis of such polymorphic states in region S indicates that if the initial polymorphism includes $S_e, S_s$ persists in the population while $S_s$ declines to extinction upon the invasion of $S_{e+1}$. Through this pathway, the number of full-function Shaplotypes can increase from $n$ to $n + 1$. Because $S_s$ declines to extinction upon the invasion of $S_s$ under all but a small set of parameter combinations (see preceding section), we conclude that the number of full-function Shaplotypes can increase, but only under restrictive conditions.

Within regions D and E, $S_s$ declines in frequency upon its appearance as a rare mutant in {$S_s, S_s$}. However, $S_s$ in fact succeed in invading after an initial decline if its introduction causes the extinction of $S_s$. As discussed in the preceding section, the parameter region that permits the invasion of $S_s$ in the absence of $S_s$, but not in its presence (A1) is quite small. For $n < 21$, this region entails $\sigma < \frac{1}{2}$, which would ensure that the appearance of the double mutant $S_{e+1}$ into polymorphic, partially self-compatible, states would restore full SI by excluding $S_s$. For larger values of $n$, $\sigma > \frac{1}{2}$ in this intervening region, although the region is very small for large $n$. Although we have not thoroughly explored the evolutionary dynamics in cases in which $\sigma > \frac{1}{2}$, our preliminary results indicate that $S_{e+1}$ excludes $S_s$ in this situation as well.

Results of our analytical and numerical studies of the fate of $S_s$, bearing a pollen-part mutation, indicate that with the delimited exceptions noted, emergence of a new Shaptype and restoration of full SI occur primarily in region P (Figure 2). In this region, the appearance of the single mutant ($S_s$) results in convergence to a polymorphic state of partial self-compatibility, with the parental haplotype ($S_s$) almost always excluded. Upon the subsequent appearance of the double mutant ($S_{e+1}$), the single mutant declines to extinction, resulting in the generation of a new Shaptype and the return of the population to full SI, generally without an increase in the number of $S$ specificities.

**Style-part mutation:** Introduction of the single mutant: If the viability of outbred offspring exceeds that of inbred offspring by at least threefold, the per-gene rate of increase of haplotype $S_s$ uniformly exceeds that of $S_s$, $T_p'/p_s > T_p/p_s$, for $\sigma > \frac{1}{2}$, from (18) and (19)], which ensures the extinction of $S_s$. Alternatively, $S_s$ drives $S_s$ to extinction if inbred offspring have at least one-half the viability of outbred offspring ($T_p'/p_s > T_p/p_s$ for $\sigma > \frac{1}{2}$). Comparison of (9) and (19) after the extinction of $S_s$ and before the entrance of $S_{e+1}$ ($q_5 = c_2 = c_1 = c_5 = q_5 = 0$) indicates that $\sigma > \frac{1}{2}$ in fact ensures the extinction of all functional haplotypes ($T_p'/p_s > T_p/p_s$).

In the remaining parameter range ($\frac{1}{2} < \sigma < \frac{1}{2}$), $S_s$ increases when rare in {$S_s, S_s$} only under (24), the limiting condition for the invasion of the pollen-part mutant $S_s$ for arbitrarily large $n$. Consequently, the invasion of $S_s$ requires more stringent conditions (higher viability of inbred offspring) than the invasion of $S_s$ when $S_s$ is independent of $n$, unlike $S_s$.

These results indicate that the system exhibits three qualitative behaviors: substitution of $S_s$ for $\sigma > \frac{1}{2}$ (region S in Figure 4), stable polymorphism for $\sigma < \frac{1}{2}$ but satisfying (24) (region P), and extinction (region E). In the pistil, $S_s$ directs a rejection response against no pollen currently in the population, although it continues to express the $S_s$ specificity in pollen. In the absence of $S_s$, $S_s$ is indistinguishable from a haplotype that lacks all self-incompatibility function. Comparison of Figures 2 and 4 illustrates that the rejection of $S_s$ pollen by pistils carrying $S_s$ opposes the introduction of the single mutant $S_s$ and transforms its evolutionary fate.

**Polymorphic states:** Numerical iteration of the system of recursions in the absence of $S_{e+1}$ indicates that for values of $\sigma < \frac{1}{2}$ but sufficiently large to ensure the initial invasion of $S_s$ into {$S_s, S_s$} (24), the population converges to a stable polymorphism comprising all haplotypes ($S_s, S_s, S_s$). Numerical analysis of the expressions for the equilibrium frequencies of the genotypes (see Appendix) confirms the existence of a single polymorphic state for this range of $\sigma$ values.
Introduction of the double mutant: Numerical iteration of the full recursion system indicates that the invasion of haplotype $S_{n+1}$ near the polymorphic state $\{S, S_n, S\}$ always fails.

DISCUSSION

Genetic costs of outcrossing: “Cost of meiosis”: Genetic modifiers that enhance the rate of self-fertilization without affecting contributions through outcrossing increase in frequency provided that the fitness of outbred offspring exceeds that of inbred offspring by less than twofold (Kimura 1959; Maynard Smith 1971). This twofold cost of meiosis (or, more specifically, cost of outcrossing) reflects that a given gene may be transmitted through both male and female gametes to an offspring derived by selfing, but through only the female gamete to an offspring derived by outcrossing. This cost reflects independence between the rate of self-fertilization and the rate of fertilization of the outbred offspring of other individuals. Alternatively, the enhancement of self-fertilization may directly affect production of outcrossed offspring: for example, production of a male gamete and a female gamete may require comparable investment (isogamy; Maynard Smith 1978) or greater self-fertilization may entail reduced pollen export (pollen discounting; Holtsinger et al. 1984).

Expression of self-incompatibility affects genetic transmission to offspring derived by outcrossing as well as by self-fertilization. Rejection by the pistil of pollen bearing a similar haplotype ensures that compatible pollen is more dissimilar than randomly sampled pollen. This self-targeted rejection tends to inflate the cost of outcrossing beyond twofold. The greater than twofold cost that opposes the invasion of functional haplotypes into fully self-compatible populations (see, for example, Charlesworth and Charlesworth 1979; Uyenoyama 1988) also arises in systems in which individual plants partition pollen production into pollen for deposit on their own stigmas and pollen for export to other plants (Steinbach and Holtsinger 1999).

Invasion of haplotypes with impaired function: We describe the transmission through both egg and pollen cells of haplotypes that express incomplete SI function. Our results indicate that the conditions for the invasion of such haplotypes become more stringent (require higher minimum viability of inbred offspring) as the rate of receipt of self-pollen $(s)$ and number of functional haplotypes $(n)$ increase.

We first consider the rate of transmission through seeds set by a focal individual. Haplotype $S_i$ bears a mutation in the pollen component that accepts disablement only from a novel $SR$-RNase that is not yet present in the population. When rare, $S_i$ occurs both in heterozygotes $(frequency \, e_i$) and in heterozygotes, which also bear $S_j$ $(\epsilon_2)$ or any of the other full-function haplotypes [total frequency $(n - 1) \epsilon_2$]. Homozygotes may transmit a copy of a particular $S_i$ haplotype through both egg and pollen to their self-fertilized seeds, but through only egg to seeds set by pollen received from other plants. The factor $(1 - 2\epsilon)$ against the $\epsilon_2$ term in the expression for the transmitted frequency of $S_i$ (11) reflects the classical twofold cost of outcrossing: relative to outbred offspring, inbred offspring survive at a lower rate $(\sigma)$ but have a twofold higher probability of carrying the haplotype. In heterozygotes, $S_i$ occurs in one-half of the transmitted egg cells, but in all compatible self-pollen. The factor $(1 - 3\epsilon)$ against the $\epsilon_1$ and $\epsilon_2$ terms in (11) reflects that heterozygotes transmit threefold more copies of $S_i$ to inbred offspring than outbred offspring. Haplotype $S_j$ bears a mutation in the pistil component that directs rejection against only a novel pollen specificity not yet present in the population. Rates of transmission of $S_i$ also exceed those of fully functional haplotypes by a factor of two or three [see (19)]. Our finding that higher rates of receipt of self-pollen $(s)$ tend to discourage the invasion of haplotypes with impaired function appears to reflect that as $s$ increases, rare haplotypes occur more often in homozygous form, in which they benefit from a twofold rather than threefold advantage in transmission.

We now consider transmission through pollen exported to other plants. Pollen bearing any of the $n$ fully functional haplotypes is incompatible with $n - 1$ of the $\frac{n}{2}$ common genotypes in the population. Because $S_i$ encodes the pollen specificity of $S_i$, exported pollen bearing $S_i$ encounters incompatible pistils at the same rate as pollen bearing a full-function haplotype. Exported pollen bearing $S_a$ which accepts disablement in no pistils, has higher fertilization success than pollen bearing any full-function haplotype. This advantage intensifies as outcrossing rates increase, and becomes negligible as the number of functional haplotypes becomes very large, under which the rate of encounter with incompatible pistils becomes vanishingly small even for full-function haplotypes. Accordingly, the condition for the invasion of $S_i$ (A1) converges to that for $S_i$ (24) as $n$ approaches arbitrarily large values.

Extinction of functional haplotypes: Condition (26) ensures that full-function haplotypes decline to extinction in populations rendered self-compatible by the near-fixation of $S_i$ in agreement with earlier results [see (9a) of Uyenoyama 1988]. In contrast with our findings for the invasion of rare haplotypes with impaired function, lower rates of receipt of self-pollen $(s)$ promote the maintenance of full-function haplotypes.

We compare the transmission of a rare full-function haplotype $S_i$ in $SS_i$ individuals $(frequency \, e_i)$ to that of one of the $S_j$ haplotypes in $SS_j$ $(frequency \, e_j)$. Because pollen bearing rare haplotypes encounter incompatible pistils with negligible frequency, full-function and impaired-function haplotypes have equal rates of transmission through exported pollen. $SS_i$ individuals may transmit the focal $S_i$ haplotype through both pollen and egg...
cells to seeds set by self-pollen, but through only egg cells to seeds set by pollen from other plants. The total rate of transmission of the focal $S_1$ haplotype is

$$s\sigma + (1 - s)/2.$$  \hspace{1cm} (28)

In contrast, the expression of SI in $SS_b$ pistils excludes $S_1$ from competition for fertilization among the self-pollens. As a result, the expected numbers of $S_1$ haplotypes transmitted to seeds set by self- and non-self-pollen are identical. Haplotype $S_1$ is transmitted to the offspring generation at a higher rate than is $S_1$ only if

$$s\sigma + (1 - s)/2 > \left[ \frac{s\sigma^2 + (1 - s)}{s/2 + (1 - s)} \right]/2,$$  \hspace{1cm} (29)

which reduces to (26).

**Prospects for the origin of new specificities:** Mutations that maintain SI: Uyenoyama and Newbigin (2000) discussed the evolutionary fate of mutations that modify the pollen or pistil components of SI without permitting self-compatibility. Functional interactions between the $S$-RNase gene ($A$) and pollen-$S$ ($B$) of a given haplotype might be preserved even in the presence of nonsynonymous substitutions in certain regions of the proteins. For example, Kakeda et al. (1998) showed that substitution of a number of residues in the hydrophilic surface loops of the stigmatic $S$ protein of *P. rhoeas* had no detectable effect on the activity or specificity of the rejection response as assessed by an *in vitro* assay. Substitution of even a strictly conserved residue in the region (hydrophilic loop 6) shown to contribute to pollen recognition had little effect on SI expression if replaced by a comparably acidic residue (Asp $\rightarrow$ Glu), although a more basic residue (Asp $\rightarrow$ His) eliminated activity. We distinguish mutations that alter functional interactions between the A and B components from those that preserve them. Mutations of the former kind may include, for example, substitutions in the $B$ locus that expand or shift the recognition region in such a way as to permit discrimination among formerly neutral variants at the $A$ locus. By affecting SI recognition, such mutations expose themselves to selection, and also endow the formerly neutral variation at the interacting gene with new functional and selective significance.

Pathway I of Uyenoyama and Newbigin was intended to depict the generation of a new specificity through the segregation of neutral variation in $B$ followed by a mutation in $A$ that affects function ($A_1B_1 \rightarrow A_1^*B_1^* \rightarrow A_1^*B_1^*$), in which the asterisk indicates a variant that is neutral at the time of its appearance). While the mutation that changes $B_1$ to $B_1^*$ initially does not affect recognition, it becomes functionally significant upon the appearance of $A_1^*$, which enables pistils to discriminate between pollen that express $B_1$ and $B_1^*$. Pathway II involves neutral variation first in $A$, followed by a functional mutation in $B$ ($A_1B_1 \rightarrow A_1^*B_1 \rightarrow A_1^*B_2$). Unlike $B_1$, $B_2$ distinguishes between $A_1$ and $A_1^*$.

The analysis presented by Uyenoyama and Newbigin (2000) demonstrated that nonreciprocal transmission through pollen engenders a key selective pressure. A clear selective advantage accrues to mutations that cause pollen to accept disablement from a smaller set of pistils. Nonreciprocal pollen transmission disrupts the symmetric selection pressures that maintain multiple $S$-haplotypes in equal frequencies. Natural selection disfavors mutations that cause pistils to accept pollen from genotypes that reject their pollen. The mutation that generates $A_2$ from $A_1$ in pathway I is of the latter kind: while $A_1$ rejects both $B_1$ and $B_1^*$, $A_2$ accepts $B_1$ pollen. In contrast, the mutation that generates $B_2$ from $B_1$ in pathway II is of the former kind: $B_2$ accepts disabling from $A_1$ while $B_1$ accepts disabling from $A_1^*$. Consequently, it is the ancestral form ($A_1B_1$) that replaces the derived form ($A_1^*B_1^*$) in pathway I and the derived form ($A_1^*B_2$), that replaces the ancestral form in pathway II. Nonreciprocal transmission through pollen can exclude modifications of existing specificities (pathway I) or drive the origin of new specificities (pathway II).

**Mutations that permit self-compatibility:** Major features that distinguish the evolutionary scenarios explored here from those considered by Matton et al. (1999) and Uyenoyama and Newbigin (2000) include the partial breakdown of SI and the consequent expression of inbreeding depression. Only extremely intense inbreeding depression, corresponding to regions $E$ and $D$ in Figure 2 (A1) or to region $E$ in Figure 4 (24), ensures the unconditional exclusion of mutations that impair SI.

Rick’s (1986) survey of natural populations of *Lycopersicon peruvianum* in Peru included only one self-compatible accession. Most (49 of 53) of the plants tested from that population were self-compatible, in sharp contrast with all other populations, including a neighboring self-incompatible population that was very similar in morphology and habitat. Genetic crosses between the two populations showed that self-compatible individuals produce a glycoprotein ($S_r$) that shares several biochemical properties with $S$-RNases, with the significant exception of ribonuclease activity (Bernatzky and Miller 1994; Kowyma et al. 1994). Isolation and characterization of the gene that encodes this glycoprotein (Royo et al. 1994) revealed that $S_r$ differs from all functional $S$-RNases by the substitution of a histidine residue known to be essential for RNase activity in homologous fungal RNases (Kawata et al. 1990). Because RNase activity is essential to the stylar SI rejection reaction (Huang et al. 1994), $S_r$ likely represents a loss-of-function mutant and not a change-of-specificity intermediate from which a new full-function haplotype may eventually arise. Nonetheless, $S_r$ serves as an example of a mutant with impaired SI function that appears to have established itself in stable polymorphism with functional Salleles.

Our present analysis of the evolutionary dynamics of mutations that permit self-compatibility supports the view (Uyenoyama and Newbigin 2000) that regulators
of pollen specificity and the stylar rejection reaction evolve under different selective pressures, even though absolute linkage commits them to a common evolutionary fate. Selection strongly favors mutations that cause pollen to restrict the set of pistils from which they accept pollen. In the event of a new mutation, selection favors subsequent mutations in the mutant haplotype that restore SI by enabling pistils to recognize and reject the new pollen specificity.

We have described the set of parameter combinations under which a succession of new specificities can arise through this evolutionary pathway. Mutations of the first kind, generating pollen that express a novel specificity rejected by no pistil presently in the population, increase in the population for parameter values corresponding to regions P and S in Figure 2. In region S, such self-compatible, single-mutant haplotypes almost always converge to fixation, rendering the population fully self-compatible. Such states represent the permanent loss of SI, resisting the exclusion of the self-compatible mutant by haplotypes bearing compensating mutations that would permit rejection of the novel pollen specificity. In region P, the invasion of the single-mutant haplotype always results in stable polymorphism with full-function haplotypes. In such partially self-compatible populations, selection strongly favors subsequent mutations that permit pistils to recognize and reject pollen that express the mutant specificity. Such full-function double-mutant haplotypes uniformly invade the population and exclude their single-mutant progenitors, thereby restoring full-self-incompatibility.

Coevolutionary changes in various components of reproduction may affect prospects for the restoration of SI. For example, the predominantly self-compatible natural population described by Rick (1986) showed reduced flower size relative to a neighboring self-incompatible population that was similar in several other respects. Further, Lewis and Crowe (1957) argued that during a period of self-compatibility, the absence of selection favoring the preservation of SI may permit the increase of mutations in the pistil determinant that disable the rejection mechanism altogether. The consequences of adaptation to self-compatibility for the evolutionary fate of compensating mutations in the S locus that would restore SI remain unexplored.

**Divergence of lineages:** Restoration of SI through the evolutionary pathway we have explored reflects the generation of a new full-function Shaplotype, generally without an increase in the number of Shaplotypes. The full-function double mutant ($S_{n+1}$) causes the extinction of the self-compatible intermediate ($S_i$) from which it descends. With the exception of cases in which the invasion of $S_i$ fails to exclude its ancestral Shaplotype ($S_0$), the generation of the new haplotype represents a specificity shift within an Shaplotype lineage (extinction of $S_i$ before invasion of $S_{n+1}$), but not a bifurcation of that lineage (coexistence of $S_i$ and $S_{n+1}$). We conjecture that the rate of branching of Shaplotype lineages may depend critically on population structure. For example, distinct Shaplotypes independently derived in different subpopulations from a common ancestral form may coexist upon their subsequent introduction into the same subpopulation. We are continuing our exploration of these evolutionary processes.

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**LITERATURE CITED**


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**APPENDIX**

**Pollen-part mutation: Genotypic frequencies in the next generation are denoted by primes**

\[ T_0' = \left[ \frac{c_1}{N_s} + G(n - 2) \right] (1 - \sigma) p_n/2 + \frac{c_1(n - 2) + c_2}{N_s} (1 - \sigma) p_n/2 \]

\[ T_0' = \frac{c_1(n - 1)}{2N_e} + \frac{c_2}{2N_e} (1 - \sigma) (p_n + p_{n-2}) \]

\[ T_1' = \frac{c_1(n - 1)}{2N_e} + \frac{c_2}{2N_e} (1 - \sigma) (p_n + p_{n-2}) \]

\[ T_1' = \frac{c_1(n - 1)}{N_s} + \frac{c_2}{N_s} (1 - \sigma) p_n/2 \]

\[ T_1' = \frac{c_1(n - 1)}{N_s} + \frac{c_2}{N_s} (1 - \sigma) p_n/2 \]

\[ T_1' = \frac{c_1(n - 1)}{N_s} + \frac{c_2}{N_s} (1 - \sigma) p_n/2 \]

\[ T_1' = \frac{c_1(n - 1)}{N_s} + \frac{c_2}{N_s} (1 - \sigma) p_n/2 \]
Genotypic frequencies in the next generation are denoted by primes

\[ T'_{c} = \left[ \frac{c(n-1)}{N_{c}} + \frac{c_{1}}{N_{c}} \right] \frac{(1-s)p_{c+1}}{2} \]

\[ T'_{g} = \left[ \frac{c_{2}+c_{1}+c_{1}}{2N_{c}} \right] \frac{(1-s)p_{g}}{2} + \left[ \frac{c_{1}}{N_{c}} + \frac{c_{1}+c_{1}+G(n-3)}{N_{c}} \right] \frac{(1-s)p_{g}}{2} \]

in which \( p_{g} \) and \( p_{g+1} \) are defined in (2), (3), and (5); \( p_{g} \) corresponds to the right side of (4); \( T \) is given in (16); and the \( N_{c} \) are given in (17).

High viability of inbred offspring (\( \sigma > \frac{1}{2} \)) ensures that \( S_{c} \) introduced in any frequency into \( S_{c}, S_{g} \) excludes the functional haplotypes, while low viability (\( \sigma < \frac{1}{2} \)) ensures the exclusion of \( S_{c} \) (see results). For values of \( \sigma \) in the range (\( \frac{1}{2} > \sigma > \frac{1}{2} \)) that permit the invasion of \( S_{c} \) into \( S_{c}, S_{g} \) (24), the population converges to the polymorphic state \( S_{g}, S_{g}, S_{c} \) described by

\[ c_{1} = \frac{p_{g}(1+s-2s \sigma) - (1-s)}{T(1-\sigma)(n-1)} \]

\[ c_{2} = \frac{4(1-T)(1-\sigma)[s/2 + (1-s)(1-\sigma)]}{(n-1)(1-\sigma)^{2}} \]

\[ c_{3} = \frac{p_{g}(1+s-2s \sigma)}{T(1-s) \sigma} \]

\[ c_{4} = \frac{(1-T)(3s-1)}{s(1-\sigma)^{2}} \]

\[ G = \frac{2(1-2\sigma)[s(1-\sigma)^{2} - (1-T)(1-\sigma)(3s-\sigma) + 2s(1-\sigma)^{2}]}{(n-1)(n-2)(1-\sigma)^{2}} \]

\[ p_{g} = \frac{T(3s-1) - sa^{2}}{(1-s)(1-\sigma)} \]

\[ p = \frac{s(1-s) + 2s \sigma^{2} + T(3-8s-s+2s \sigma)}{2(n-1)[s(1-\sigma) - (1-T)(1+s-2s \sigma)]} \]

in which \( p(n-1) + p_{g} + p_{g} = 1 \) and \( T \) is a root of

\[ p_{g}(1-s)(1-T)Q_{3} - (1-T)Q_{2} = 0, \quad (A2) \]

in which

\[ Q_{2} = T^{2}((1-s) + 2s(3s-3)(1-3s-2s \sigma)) \]

\[ - (1-s)^{2}(1-2s \sigma) \]

\[ - 2T(1-s + 2s(1-\sigma) \sigma^{2} - s(1-\sigma)(1-\sigma)^{2} + s(1-\sigma)^{2})(1-2s) \]

\[ Q_{3} = 2T^{2}((1-s) + 2s(3s-3)(1-3s-2s \sigma)) \]

\[ - T(1-s - 3s + s \sigma^{2}) + (1-2s)(1-s - 3s) \]

\[ - 2s \sigma^{2}(4-3s-4s \sigma^{2}) + s(5-5s-2s \sigma^{2} - 4s \sigma^{2}) \]

\[ - s(1-s)(2(1-\sigma)(1-s + s \sigma^{2}) - s(3s-1)(1-2s)) \]

Valid equilibria correspond to roots of the cubic (A2) that lie in the range

\[ \frac{(1-s)(1-\sigma) + sa^{2}}{3s-1} > T > \frac{sa^{2}}{3s-1} \]

\[ \frac{s(1-\sigma)^{2} + (1-s)(3-5s) + 2s(1-\sigma)^{2}}{2s(1-\sigma)^{2} + (1-s)(3-5s)} \quad (A3) \]