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

Manuscript received August 7, 2000
Accepted for publication January 10, 2001

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.

SELF-incompatibility (SI) systems prevent fertilization of a flowering plant by its own pollen (see de Nettancourt 1977). Under heteromorphic SI, plants of different mating types exhibit different floral morphologies, which promote between-type and reduce within-type fertilization. Under homomorphic SI, different mating types have similar floral morphologies, with fertilization inhibited upon the expression of the same specificity in pollen and pistil. In gametophytic self-incompatibility (GSI) systems, a given pollen grain or tube expresses the specificities encoded in its own haploid genome, and in sporophytic self-incompatibility (SSI) systems one or more of the specificities of the plant that produced it.

Model systems for which the characterization of the molecular basis of SI is most advanced include Brassica (Nasrallah *et al.* 1985), the Solanaceae (Anderson *et al.* 1986), and the field poppy *Papaver rhoeas* (Franklin *et al.* 1995). In the form of SSI expressed in Brassica, recognition of pollen coat proteins by a receptor kinase that spans the membranes of stigmatic epidermal cells induces withholding of hydrating factors required for pollen germination (Stephenson *et al.* 1997; Schopfer *et al.* 1999; Takasaki *et al.* 2000; Takayama *et al.* 2000).

Corresponding author: M. K. Uyenoyama, Department of Biology, Box 90338, Duke University, Durham, NC 27708-0338.
E-mail: marcy@duke.edu

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 (Stephenson *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). *S*-haplotypes include *SCR* (*S*-locus cysteine-rich protein; originally named *SP11*; Suzuki *et al.* 1999), which encodes the specificity-determining proteins in the pollen coat (Schopfer *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 stylar rejection, and style-part mutations disrupt stylar rejection while preserving pollen specificity. Transformation of solanaceous plants with an *S*-RNase 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 *S*-RNase controls the specificity expressed by pollen (Dodds *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 *S*-specificity entails a series of coevolved point mutations at two or more genes within the *S*-locus.

Stylar specificity: Sequence comparisons of *S*-RNases that determine different stylar phenotypes may afford insight into the determination of stylar specificity. *S*-RNases 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 *S*-RNases derived from *Nicotiana alata*, Kheyr-Pour *et al.* (1990) recognized five hypervariable regions. Upon expansion of the taxonomic sampling to include sequences from *Petunia inflata* and *Solanum chacoense*, subsequent analyses confirmed the hypervariability of two regions identified by the previous study, designating them HVa and HVb (Ioerger *et al.* 1991; Tsai *et al.* 1992). In their study of variation among *S*-RNases from all three families known to exhibit this form of GSI, Ishimizu *et al.* (1998) detected a significant excess of nonsynonymous over synonymous substitution in four regions: two corresponded to HVa and HVb, with each remaining region situated close to another hypervariable region proposed by Kheyr-Pour *et al.* (1990). Comparison to homologous fungal RNases for which the crystal structure has been solved indicated that the hypervariable regions of *S*-RNases likely correspond to surface loop structures (Ishimizu *et al.* 1998; Parry *et al.* 1998).

While many haplotypes show a great number of differences, some naturally occurring *S*-RNases that function as distinct SI specificities differ by relatively few nonsynonymous substitutions (Saba-El-Leil *et al.* 1994). Such observations suggest that the minimum number of substitutions required to generate a new specificity may be relatively low. The large sequence differences observed among currently segregating *S*-haplotypes may in large part reflect the antiquity of their most recent common ancestor, estimated at >27 million years (my) in the Solanaceae (Ioerger *et al.* 1990) and 40 to 50 my in Brassica (Uyenoyama 1995).

One-mutation models: Fisher (1961) explored recombination between the genes that control the pistil and pollen components of SI as a mechanism for the generation of new *S*-specificities. On the basis of the understanding of the day of the vertebrate immune system, his model addressed the origin of new antigens expressed by pollen, with recognition by specific antibodies in the pistil assumed to arise simultaneously. Fisher proposed that a pistil that rejects two pollen specificities would also reject pollen that expresses a recombinant of those specificities. This scheme preserves self-incompatibility throughout the rise of the recombinant haplotype. Under Fisher's model, recombinant pollen can fertilize all pistils except those that bear the recombinant or both parental haplotypes, and expression of the recombinant in the pistil directs rejection against recombinant pollen alone. Fisher showed that the reduced rate at which recombinant pollen initially encounters incompatible pistils permits the invasion of the recombinant haplotype into the population. All specificities segregate at the equilibrium state, though in unequal frequencies: the equilibrium frequency of the recombinant haplotype lies below that of each parental haplotype, which in turn is less common than each nonparental haplotype. Fisher suggested that an *S*-locus region comprising several polymorphic antigen genes could generate a very large number of specificities (for 10 biallelic genes, $2^{10} = 1024$ specificities).

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" *S*-RNase that rejected two pollen specificities in *S. chacoense*. Naturally occurring *S*-RNases S_{11} and S_{13} 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_{11} sequence in which the 4 HVa and HVb residues had been substituted to match the S_{13} sequence conferred the ability to reject S_{13} pollen (Matton *et al.* 1997). Transformants bearing an S_{11} sequence in which the 3 HVa sites but not the HVb site had been substituted rejected neither S_{11} nor S_{13} 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_{11} and S_{13} 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.

Uyenoyama 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 *S*-RNases 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 *S*-RNases 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

S-haplotypes comprise an *A* component (analogous to the *S*-RNase 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_i corresponds to A_iB_i, 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 $\{S_i, S_n\}$, for i assuming values from 1 to $n - 1$ and S_n the haplotype in which the new mutation will occur. Mutations within haplotype S_n may alter only the specificity rejected by the pistil (generating haplotype S_a) or only the specificity expressed in pollen (S_b). Figure 1 summarizes the SI phenotypes associated with the haplotypes. Pollen tubes bearing haplotype $S_a(A_{n+1}B_n)$ express specificity B_n, which accepts disablement by A_n *S*-RNase, while pistils bearing S_a produce a new *S*-RNase directed against a pollen specificity not yet present in the population. Haplotype $S_b(A_nB_{n+1})$ encodes the A_n *S*-RNase and a new pollen specificity that accepts disablement by an *S*-RNase not yet present in the population.

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

Table 1 summarizes the variables representing genotypic and allelic frequencies. To maintain tractability

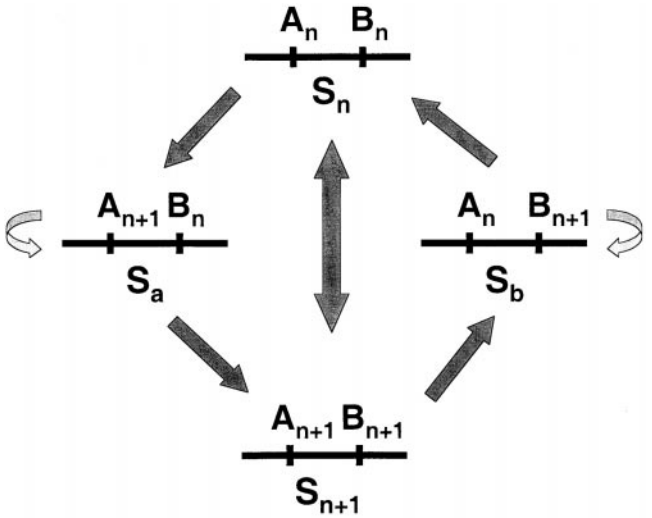

FIGURE 1.—Self-incompatibility phenotypes of *S*-haplotypes. Arrows indicate the direction of transmission through pollen. Double-headed arrows join mutually compatible haplotypes. Single-headed arrows indicate that pollen bearing the haplotype at the base of the arrow fail to accept inhibition by the *S*-RNase encoded by the haplotype at the head, while the reciprocal pollination is incompatible.

while permitting arbitrary numbers of *S*-haplotypes, we assume that all genotypes of a given class (for example, S_1S_n and S_2S_n) occur in equal frequency. In a study of the evolutionary 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 c_0 denotes the frequency of a genotype carrying S_n together with any of the other nonmutant haplotypes (S_iS_n, $1 \leq i < n$); and G denotes two distinct nonmutant haplotypes other than S_n (S_iS_j, $1 \leq i,j < n$, $i \neq j$). Frequencies of carriers of the single mutant include c_1, corresponding to the heterozygote with any of the nonancestral full-function haplotypes (for example, S_aS_i, $1 \leq i < n$); c_2, the heterozygote with the ancestral nonmutant haplotype (for example, S_aS_n); and c_3, the single mutant homozygote (for example, S_aS_a). Frequencies of carriers of the double mutant include c_4, corresponding to the heterozygote with any of the nonancestral full-function haplotypes (S_iS_{n+1}, $1 \leq i < n$); c_5, the heterozygote with the ancestral haplotype (S_nS_{n+1}); and c_6, the heterozygote with the single mutant haplotype (for example, S_aS_{n+1}). These variables account for the frequencies of all genotypes in the population:

$$(n-1)(c_0 + c_1 + c_4) + c_2 + c_3 + c_5 + c_6 + G\binom{n-1}{2} = 1. \quad (1)$$

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 σ ($0 \leq \sigma \leq 1$) 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_n , S_b, and S_{n+1} occur in the pollen pool and in the population in frequencies p, p_n, p_b, and p_{n+1}, respectively;

$$p = [c_0 + c_1 + c_4 + G(n-2)]/2 \quad (2)$$

$$p_n = [c_0(n-1) + c_2 + c_5]/2 \quad (3)$$

$$p_b = c_1(n-1)/2 + c_2/2 + c_3 + c_6/2 \quad (4)$$

$$p_{n+1} = (c_4 + c_5 + c_6)/2, \quad (5)$$

for which

$$p(n-1) + p_n + p_b + p_{n+1} = p'(n-1) + p'_n + p'_b + p'_{n+1} = 1. \quad (6)$$

T, the average viability among offspring, corresponds to

TABLE 1

Self-incompatibility genotypes and phenotypes

		A locus mutation		*B* locus mutation	
Variable	No.	Genotype	Rejects	Genotype	Rejects
c_0	$n-1$	S_iS_n	S_i, S_n, S_a	S_iS_n	S_i, S_n
c_1	$n-1$	S_iS_a	S_i, S_{n+1}	S_iS_b	S_i, S_n
c_2	1	S_nS_a	S_n, S_a, S_{n+1}	S_nS_b	S_n
c_3	1	S_aS_a	S_{n+1}	S_bS_b	S_n
c_4	$n-1$	S_iS_{n+1}	S_i, S_{n+1}	S_iS_{n+1}	S_i, S_b, S_{n+1}
c_5	1	S_nS_{n+1}	S_n, S_a, S_{n+1}	S_nS_{n+1}	S_n, S_b, S_{n+1}
c_6	1	S_aS_{n+1}	S_{n+1}	S_bS_{n+1}	S_n, S_b, S_{n+1}
G	$\binom{n-1}{2}$	S_iS_j	S_i, S_j	S_iS_j	S_i, S_j

$$T = 1 - \left[\frac{c_1(n-1)}{2N_1} + \frac{c_2}{2N_2} + \frac{c_3}{N_3}\right]s(1-\sigma); \quad (7)$$

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

$$\begin{aligned}
N_0 &= (1-s)(1-p-p_n)\\
N_1 &= s/2 + (1-s)(1-p-p_n)\\
N_2 &= s/2 + (1-s)(1-p_n)\\
N_3 &= s + (1-s)(1-p_n)\\
N_4 &= (1-s)(1-p-p_b-p_{n+1})\\
N_5 &= (1-s)(1-p_n-p_b-p_{n+1})\\
N_6 &= (1-s)(1-p_n-p_b-p_{n+1})\\
N_G &= (1-s)(1-2p). \quad (8)
\end{aligned}$$

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

$$\begin{aligned}
Tp' &= p/2 - \frac{sc_1(1-\sigma)}{8N_1}\\
&\quad + \left[(n-2)\left(\frac{c_0}{N_0}+\frac{c_1}{N_1}+\frac{c_4}{N_4}\right) + \frac{c_2}{N_2}+\frac{c_3}{N_3}+\frac{c_5}{N_5}+\frac{c_6}{N_6}\frac{G\left(\frac{n-2}{2}\right)}{N_G}\right]\\
&\quad \times p(1-s)/2 \quad (9)\\
Tp_n' &= p_n/2 - \frac{sc_2(1-\sigma)}{8N_2} + \left[\frac{c_4(n-1)}{N_4} + \frac{G\left(\frac{n-1}{2}\right)}{N_G}\right]\\
&\quad \times p_n(1-s)/2 \quad (10)\\
Tp_b' &= p_b/2 - \left[\frac{c_1(n-1)(1-3\sigma)}{4N_1} + \frac{c_2(1-3\sigma)}{4N_2} + \frac{c_3(1-2\sigma)}{N_3}\right]s/2\\
&\quad + \left[(n-1)\left(\frac{c_0}{N_0}+\frac{c_1}{N_1}\right) + \frac{c_2}{N_2}+\frac{c_3}{N_3}+\frac{G\left(\frac{n-1}{2}\right)}{N_G}\right]\\
&\quad \times p_b(1-s)/2 \quad (11)\\
Tp_{n+1}' &= p_{n+1}/2\\
&\quad + \left[(n-1)\left(\frac{c_0}{N_0}+\frac{c_1}{N_1}\right) + \frac{c_2}{N_2}+\frac{c_3}{N_3}+\frac{G\left(\frac{n-1}{2}\right)}{N_G}\right]\\
&\quad \times p_{n+1}(1-s)/2. \quad (12)
\end{aligned}$$

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

$$P = \left[(n-1)\left(\frac{c_0}{N_0}+\frac{c_1}{N_1}\right) + \frac{c_2}{N_2}+\frac{c_3}{N_3}+\frac{G\left(\frac{n-1}{2}\right)}{N_G}\right]p_b(1-s)$$
$$+ \left[\frac{c_1(n-1)}{2N_1} + \frac{c_2}{2N_2} + \frac{c_3}{N_3}\right]s\sigma \quad (13)$$

and the rate through egg cells is

$$\begin{aligned}
E &= \frac{c_1(n-1)[s\sigma/2 + (1-s)(1-p-p_n)]}{2N_1}\\
&\quad + \frac{c_2[s\sigma/2 + (1-s)(1-p_n)]}{2N_2}\\
&\quad + \frac{c_3[s\sigma + (1-s)(1-p_n)]}{N_3}\\
&\quad + \frac{c_6(1-s)(1-p_n-p_b-p_{n+1})}{2N_6}\\
&= p_b - \left[\frac{c_1(n-1)}{4N_1} + \frac{c_2}{4N_2} + \frac{c_3}{N_3}\right]s(1-\sigma). \quad (14)
\end{aligned}$$

This last expression indicates that the number of S_b haplotypes transmitted through egg declines as the rate of receipt of self-pollen (s) increases and the viability of inbred offspring (σ) 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_b is

$$Tp_b' = (E + P)/2, \quad (15)$$

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_n, and p_{n+1} are defined in (2), (3), and (5), and p_a (frequency of S_a) corresponds to the right side of (4). T, the average viability among offspring, is

$$T = 1 - \left[\frac{c_1(n-1)}{2N_1} + \frac{c_3}{N_3} + \frac{c_6}{2N_6}\right]s(1-\sigma), \quad (16)$$

and the N_i give the fraction of compatible pollen received:

$$\begin{aligned}
N_0 &= (1-s)(1-p-p_n-p_a)\\
N_1 &= s/2 + (1-s)(1-p-p_{n+1})\\
N_2 &= (1-s)(1-p_n-p_a-p_{n+1})\\
N_3 &= s + (1-s)(1-p_{n+1})\\
N_4 &= (1-s)(1-p-p_{n+1})\\
N_5 &= (1-s)(1-p_n-p_a-p_{n+1})\\
N_6 &= s/2 + (1-s)(1-p_{n+1})\\
N_G &= (1-s)(1-2p). \quad (17)
\end{aligned}$$

Among offspring, the frequency (Tp') of a functional haplotype other than S_n corresponds to (9); the remaining haplotype frequencies are

$$Tp'_n = p_n/2 + \left[(n-1)\left(\frac{c_1}{N_1}+\frac{c_4}{N_4}\right)+\frac{c_3}{N_3}+\frac{c_6}{N_6}+\frac{G\left(\frac{n-1}{2}\right)}{N_G}\right] \times p_n(1-s)/2 \quad (18)$$

$$Tp'_a = p_a/2 + \left[(n-1)\left(\frac{c_1}{N_1}+\frac{c_4}{N_4}\right)+\frac{c_3}{N_3}+\frac{c_6}{N_6}+\frac{G\left(\frac{n-1}{2}\right)}{N_G}\right] \times p_a(1-s)/2 - \left[\frac{c_1(n-1)(1-3\sigma)}{4N_1}+\frac{c_3(1-2\sigma)}{N_3}+\frac{c_6(1-3\sigma)}{4N_6}\right]s/2 \quad (19)$$

$$Tp'_{n+1} = p_{n+1}/2 + \left[\frac{c_0(n-1)}{N_0}+\frac{G\left(\frac{n-1}{2}\right)}{N_G}\right] \times p_{n+1}(1-s)/2 - \frac{sc_6(1-\sigma)}{4N_6}, \quad (20)$$

for which

$$p(n-1) + p_n + p_a + p_{n+1} = p'(n-1) + p'_n + p'_a + p'_{n+1} = 1. \quad (21)$$

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 ($G = c_0 = 1/\binom{n}{2}$). Equations 9–11 indicate that if the viability of offspring derived by selfing is at least one-half that of outbred offspring ($\sigma > \frac{1}{2}$), the per-gene rate of increase of the nonfunctional haplotype S_b uniformly exceeds that of any of the existing functional haplotypes,

$$Tp'_b/p_b > Tp'/p,\ Tp'_n/p_n, \quad (22)$$

signifying that S_b increases to fixation upon its appearance in $\{S_i, S_n\}$ in any frequency.

Introduced in low frequency, haplotype S_b invades $\{S_i, S_n\}$ for $n < 5$ and for n sufficiently small to satisfy

$$sn^2(n-4) - (1-s)8(n-1)(n-2) + s(1-s)(n^3 - 2n^2 - 9n + 16) < 0. \quad (23)$$

For larger n, S_b increases only if σ 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 (σ) to ensure the invasion of S_b. In the limit as n becomes very large, S_b increases when rare in $\{S_i, S_n\}$ only if

$$\sigma^2 s - 3\sigma + 1 < 0. \quad (24)$$

Near the state of fixation of S_b, the mean viability of offspring (T) lies close to its minimum value:

$$1 - s + s\sigma. \quad (25)$$

This state resists the invasion of all original full-function haplotypes if

$$\sigma > \frac{1-s}{3-2s}. \quad (26)$$

Unlike the condition for the initial increase of S_b (A1), (26) is independent of n.

In a two-dimensional plot of σ (ordinate) against s (abscissa), condition (A1) demarcates the region above a monotonically increasing curve 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.

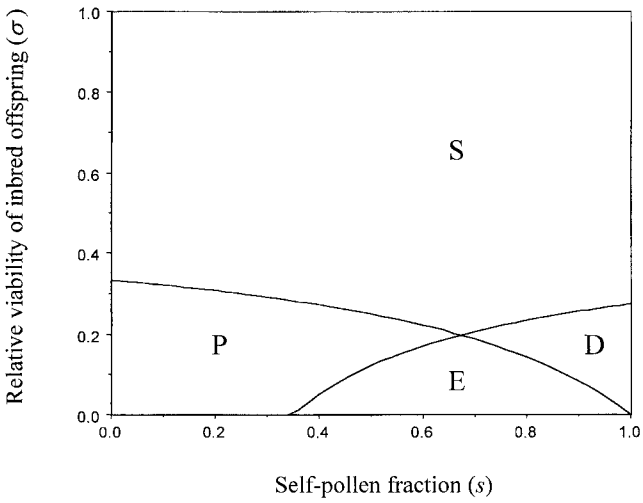

FIGURE 2.—Four evolutionary outcomes of the introduction of haplotype S_b into populations with $n = 10$ functional haplotypes. Values of the relative viability of inbred offspring (σ) and self-pollen fraction (s) corresponding to region S permit S_b to increase both near extinction and near fixation. 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.

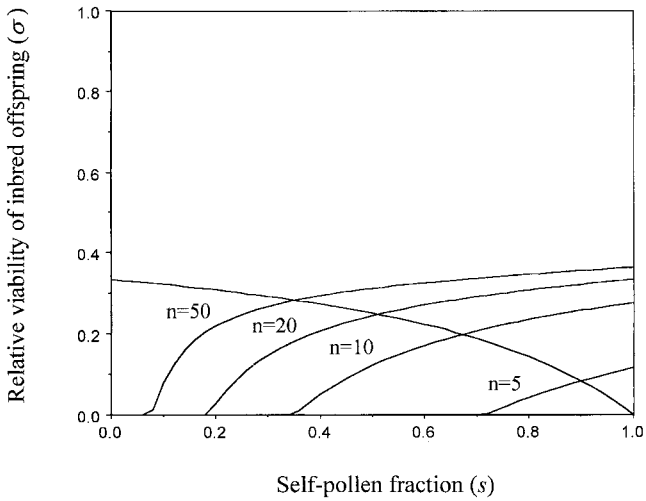

FIGURE 3.—Effect of the initial number of functional haplotypes (n) on the condition for initial increase of haplotype S_b. For $n < 5$, S_b uniformly invades, with the increasing curve corresponding to condition (A1) lying outside the range of valid parameters. As n increases, the curve moves upward and to the left, signifying greater stringency of conditions required for the invasion of S_b.

Polymorphic states: Parameter values corresponding to regions P and S ensure the increase of S_b upon its introduction in low frequency into $\{S_i, S_n\}$. Because the fixation of S_b is unstable in region P, the invasion of S_b implies convergence to a polymorphic state at which all full-function haplotypes ($\{S_i, S_n, S_b\}$) or all but the parental haplotype S_n ($\{S_i, S_b\}$) segregate. In region S, the fixation of S_b is locally stable, suggesting that the invasion of S_b 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_b 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_b) and decreasing curve (fixation of S_b; see Figure 2), the population converges to a state of partial self-compatibility, reflecting the maintenance of full-function haplotypes together with S_b.

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

We examine the conditions that permit S_b to invade $\{S_i\}$. In the absence of both S_n and S_{n+1}, S_b causes the rejection of no pollen and S_b pollen encounters rejection in no pistil. Only three genotypic classes exist: S_iS_j (frequency G), $S_iS_b(c_1)$, and $S_bS_b(c_3)$. In such populations, S_b 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_b invades for relative viabilities of inbred offspring (σ) 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 σ also increases with s and n. The minimum value of σ that permits the invasion of S_b 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_b invades in the absence of S_n but not in its presence. Under some conditions, S_b may initially decline in frequency upon its introduction into $\{S_i, S_n\}$, but then increase as its own presence causes the exclusion of S_n. In such cases, the introduction of S_b 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_b but not its fixation, implying that the population converges to a polymorphic state ($\{S_i, S_b\}$ or $\{S_i, S_n, S_b\}$), in which S_b segregates together with functional haplotypes. Expressions (11) and (12) indicate that the per-gene rate of increase of S_{n+1} exceeds that of S_b ($Tp'_{n+1}/p_{n+1} > Tp'_b/p_b$) 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 ($\sigma < \frac{1}{3}$). Because the instability of the fixation of S_b [violation of (26)] ensures $\sigma < \frac{1}{3}$, S_{n+1} invades all stable polymorphisms arising under region P, causing the extinction of S_b and restoring the population to full self-incompatibility.

Within regions S and D [satisfying (26)], the fully self-compatible state of fixation of S_b resists the invasion of any of the original full-function haplotypes. Expression (12) indicates that S_{n+1} increases when rare near such states [$c_3 = 1$, 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_{n+1} express incompatibility against all pollen and the entire population derives from seeds set by S_bS_b individuals alone.

Region S also admits stable polymorphisms comprising S_b together with full-function haplotypes for values of σ close to the minimum (A1) required for the invasion of $\{S_i, S_n\}$ by S_b (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_{n+1} near any polymorphism that arises in region S will result in the exclusion of the single mutant S_b 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_n, S_n persists in the population while S_b declines to extinction upon the introduction of S_{n+1}. Through this pathway, the number of full-function *S*-haplotypes can increase from n to $n + 1$. Because S_n declines to extinction upon the invasion of S_b under all but a small set of parameter combinations (see preceding section), we conclude that the number of full-function *S*-haplotypes can increase, but only under restrictive conditions.

Within regions D and E, S_b declines in frequency upon its appearance as a rare mutant in $\{S_i, S_n\}$. However, S_b may in fact succeed in invading after an initial decline if its introduction causes the extinction of S_n. As discussed in the preceding section, the parameter region that permits the invasion of S_b in the absence of S_n (27) but not in its presence (A1) is quite small. For $n < 21$, this region entails $\sigma < \frac{1}{3}$, which would ensure that the appearance of the double mutant S_{n+1} into polymorphic, partially self-compatible, states would restore full SI by excluding S_b. For larger values of n, $\sigma > \frac{1}{3}$ 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 $\sigma > \frac{1}{3}$, our preliminary results indicate that S_{n+1} excludes S_b in this situation as well.

Results of our analytical and numerical studies of the fate of S_b, bearing a pollen-part mutation, indicate that with the delimited exceptions noted, emergence of a new *S*-haplotype and restoration of full SI occur primarily in region P (Figure 2). In this region, the appearance of the single mutant (S_b) results in convergence to a polymorphic state of partial self-compatibility, with the parental haplotype (S_n) almost always excluded. Upon the subsequent appearance of the double mutant (S_{n+1}), the single mutant declines to extinction, resulting in the generation of a new *S*-haplotype 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_n uniformly exceeds that of S_a [$Tp'_n/p_n > Tp'_a/p_a$ for $\sigma > \frac{1}{3}$; from (18) and (19)], which ensures the extinction of S_a. Alternatively, S_a drives S_n to extinction if inbred offspring have at least one-half the viability of outbred offspring ($Tp'_a/p_a > Tp'_n/p_n$ for $\sigma > \frac{1}{2}$). Comparison of (9) and (19) after the extinction of S_n and before the entrance of S_{n+1} ($c_0 = c_2 = c_4 = c_5 = c_6 = 0$) indicates that $\sigma > \frac{1}{2}$ in fact ensures the extinction of all functional haplotypes ($Tp'_a/p_a > Tp'/p$).

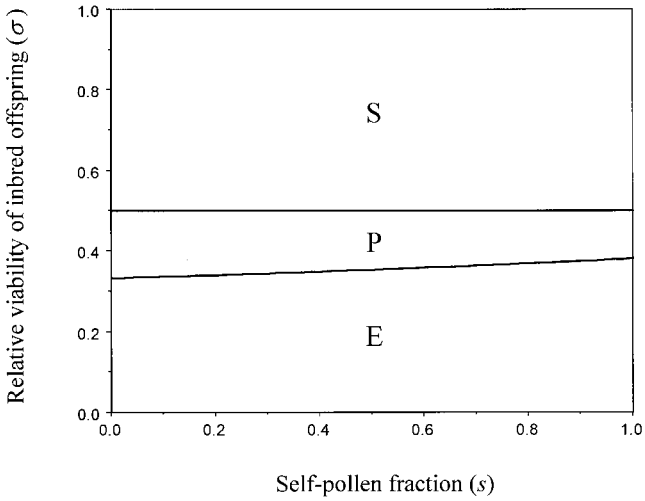

FIGURE 4.—Three evolutionary outcomes of the introduction of haplotype S_a. For values of σ greater than one-half (region S), haplotype S_a invades and excludes all functional haplotypes, causing a permanent and total loss of self-incompatibility. Parameter combinations in region P give rise to a stable polymorphism including haplotype S_a and all functional haplotypes, which corresponds to the permanent coexistence of self-compatible and self-incompatible genotypes. This state resists the invasion of haplotype S_{n+1}, which bears a mutation that would restore full SI by complementing the mutation of S_a. Extinction of S_a occurs in region E.

In the remaining parameter range ($\frac{1}{3} < \sigma < \frac{1}{2}$), S_a increases when rare in $\{S_i, S_n\}$ only under (24), the limiting condition for the invasion of the pollen-part mutant S_b for arbitrarily large n. Consequently, the invasion of S_a requires more stringent conditions (higher viability of inbred offspring) than the invasion of S_b. Whether S_a increases when rare is independent of n, unlike S_b.

These results indicate that the system exhibits three qualitative behaviors: substitution of S_a 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_a directs a rejection response against no pollen currently in the population, although it continues to express the S_n specificity in pollen. In the absence of S_n, S_a is indistinguishable from a haplotype that lacks all self-incompatibility function. Comparison of Figures 2 and 4 illustrates that the rejection of S_a pollen by pistils carrying S_n opposes the introduction of the single mutant S_a and transforms its evolutionary fate.

Polymorphic states: Numerical iteration of the system of recursions in the absence of S_{n+1} indicates that for values of $\sigma < \frac{1}{2}$ but sufficiently large to ensure the initial invasion of S_a into $\{S_i, S_n\}$ (24), the population converges to a stable polymorphism comprising all haplotypes ($\{S_i, S_n, S_a\}$). 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 σ 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 $\{S_i, S_n, S_a\}$ 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; Holsinger *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 dissimiliar 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 *S*-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 Holsinger 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_b bears a mutation in the pollen component that accepts disablement only from a novel *S*-RNase that is not yet present in the population. When rare, S_b occurs both in homozygotes (frequency c_3) and in heterozygotes, which also bear S_n (c_2) or any of the other full-function haplotypes [total frequency $(n-1)c_1$]. Homozygotes may transmit a copy of a particular S_b 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 $(1-2\sigma)$ against the c_3 term in the expression for the transmitted frequency of S_b (11) reflects the classical twofold cost of outcrossing: relative to outbred offspring, inbred offspring survive at a lower rate (σ) but have a twofold higher probability of carrying the haplotype. In heterozygotes, S_b occurs in one-half of the transmitted egg cells, but in all compatible self-pollen. The factor $(1-3\sigma)$ against the c_1 and c_2 terms in (11) reflects that heterozygotes transmit threefold more copies of S_b to inbred offspring than outbred offspring. Haplotype S_a 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_a 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 $\binom{n}{2}$ common genotypes in the population. Because S_a encodes the pollen specificity of S_n, exported pollen bearing S_a encounters incompatible pistils at the same rate as pollen bearing a full-function haplotype. Exported pollen bearing S_b, 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_b (A1) converges to that for S_a (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_b, 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 S_iS_b individuals (frequency c_1) to that of one of the S_b haplotypes in S_bS_b (frequency c_3). 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. S_bS_b individuals may transmit the focal S_b 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_b haplotype is

$$s\sigma + (1 - s)/2. \quad (28)$$

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

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

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 → Glu), although a more basic residue (Asp → 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 ($A_1B_1 \rightarrow A_1B_2^* \rightarrow A_2B_2^*$, 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_2^* initially does not affect recognition, it becomes functionally significant upon the appearance of A_2, which enables pistils to discriminate between pollen that express B_1 and B_2^*. Pathway II involves neutral variation first in *A*, followed by a functional mutation in *B* ($A_1B_1 \rightarrow A_2^*B_1 \rightarrow A_2^*B_2$). Unlike B_1, B_2 distinguishes between A_1 and A_2^*.

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_2^*, 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 refuses disablement from A_1 while B_1 accepts disablement from A_2^*. Consequently, it is the ancestral form (A_1B_1) that replaces the derived form ($A_2B_2^*$) in pathway I and the derived form ($A_2^*B_2$) 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_c) that shares several biochemical properties with *S*-RNases, with the significant exception of ribonuclease activity (Bernatzky and Miller 1994; Kowyama *et al.* 1994). Isolation and characterization of the gene that encodes this glycoprotein (Royo *et al.* 1994) revealed that S_c 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_c 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_c serves as an example of a mutant with impaired SI function that appears to have established itself in stable polymorphism with functional *S*-alleles.

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 disablement, even at the cost of incurring substantial inbreeding depression. If the new equilibrium state of the population maintains full-function haplotypes together with such a 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 *S*-haplotype, generally without an increase in the number of *S*-haplotypes. The full-function double mutant (S_{n+1}) causes the extinction of the self-compatible intermediate (S_h) from which it descends. With the exception of cases in which the invasion of S_h fails to exclude its ancestral *S*-haplotype (S_n), the generation of the new haplotype represents a specificity shift within an *S*-haplotype lineage (extinction of S_n before invasion of S_{n+1}), but not a bifurcation of that lineage (coexistence of S_n and S_{n+1}). We conjecture that the rate of branching of *S*-haplotype lineages may depend critically on population structure. For example, distinct *S*-haplotypes 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.

We thank Ruth Shaw and two anonymous reviewers for their constructive and stimulating comments. E.N. receives funding from the Australian Research Council. U.S. Public Health Service grant GM 37841 to M.K.U. provided support for this study.

LITERATURE CITED

Anderson, M. A., E. C. Cornish, S.-L. Mau, E. G. Williams, R. Hoggart *et al.*, 1986 Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. Nature **321:** 38–44.

Bernatzky, R., and D. D. Miller, 1994 Self-incompatibility is codominant in intraspecific hybrids of self-compatible and self-incompatible *Lycopersicon peruvianum* and *L. hirsutum* based on protein and DNA marker analysis. Sex. Plant Reprod. **7:** 297–302.

Boyes, D. C., M. E. Nasrallah, J. Vrebalov and J. B. Nasrallah, 1997 The self-incompatibility (*S*) haplotypes of Brassica contain highly divergent and rearranged sequences of ancient origin. Plant Cell **9:** 237–247.

Broothaerts, W., G. A. Janssens, P. Proost and W. F. Broekart, 1995 cDNA cloning and molecular analysis of two self-incompatibility alleles from apple. Plant Mol. Biol. **27:** 499–511.

Charlesworth, D., 2000 How can two-gene models of self-incompatibility generate new specificities? Plant Cell **12:** 309–310.

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

de Nettancourt, D., 1977 *Incompatibility in Angiosperms*. Springer-Verlag, Berlin.

Dodds, P. N., C. Ferguson, A. E. Clarke and E. Newbigin, 1999 Pollen-expressed *S*-RNases are not involved in self-incompatibility in *Lycopersicon peruvianum*. Sex. Plant Reprod. **12:** 76–87.

Fisher, R. A., 1958 *The Genetical Theory of Natural Selection*, Ed. 2. Dover, New York.

Fisher, R. A., 1961 A model for the generation of self-sterility alleles. J. Theor. Biol. **1:** 411–414.

Franklin, F. C. H., M. J. Lawrence and V. E. Franklin-Tong, 1995 Cell and molecular biology of self-incompatibility in flowering plants. Int. Rev. Cytol. **158:** 1–64.

Golz, J. F., A. E. Clarke and E. Newbigin, 2000 Mutational approaches to the study of self-incompatibility: revisiting the pollen-part mutants. Ann. Bot. **85** (Suppl. A): 95–103.

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.

Huang, S., H.-S. Lee, B. Karunanadaa and T.-h. Kao, 1994 Ribonuclease activity of *Petunia inflata* S proteins is essential for rejection of self-pollen. Plant Cell **6:** 1021–1028.

Ioerger, T. R., A. G. Clark and T.-h. Kao, 1990 Polymorphism at the self-incompatibility locus in Solanaceae predates speciation. Proc. Natl. Acad. Sci. USA **87:** 9732–9735.

Ioerger, T. R., J. R. Gohlke, B. Xu and T.-h. Kao, 1991 Primary structural features of the self-incompatibility protein in Solanaceae. Sex. Plant Reprod. **4:** 81–87.

Ishimizu, T., T. Endo, Y. Yamaguchi-Kabata, K. T. Nakamura, F. Sakiyama *et al.*, 1998 Identification of regions in which positive selection may operate in S-RNase of Rosaceae: implication for *S*-allele-specific recognition sites in S-RNase. FEBS Lett. **440:** 337–342.

Kakeda, K., N. D. Jordan, A. Conner, J. P. Ride, V. E. Franklin-Tong *et al.*, 1998 Identification of residues in a hydrophilic

loop of the *Papaver rhoeas* S protein that play a crucial role in recognition of incompatible pollen. Plant Cell **10:** 1723–1731.

Kawata, Y., F. Sakiyama, F. Hayashi and Y. Kyogoku, 1990 Identification of two essential histidine residues of ribonuclease T2 from *Aspergillus oryzae*. Eur. J. Biochem. **187:** 255–262.

Kheyr-Pour, A., S. B. Bintrim, T. R. Ioerger, R. Remy, S. A. Hammond *et al.*, 1990 Sequence diversity of pistil S-proteins associated with gametophytic self-incompatibility in *Nicotiana alata*. Sex. Plant Reprod. **3:** 88–97.

Kimura, M., 1959 Conflict between self-fertilization and outbreeding in plants. Natl. Inst. Genet. (Mishima) Annu. Rep. **9:** 87–88.

Kowyama, M., C. Kunz, I. Lewis, E. Newbigin, A. E. Clarke *et al.*, 1994 Self-compatibility in a *Lycopersicon pervianum* variant (LA2157) is associated with a lack of style S-RNase activity. Theor. Appl. Genet. **88:** 859–864.

Lawrence, M. J., 2000 Population genetics of the homomorphic self-incompatibility polymorphisms in flowering plants. Ann. Bot. **85** (Suppl. A): 221–226.

Lee, H.-S., S. Huang and T.-h. Kao, 1994 *S* proteins control rejection of incompatible pollen in *Petunia inflata*. Nature **367:** 560–563.

Lewis, D., 1949 Structure of the incompatibility gene II. Induced mutation rate. Heredity **3:** 339–355.

Lewis, D., 1954 Comparative incompatibility in angiosperms and fungi. Adv. Genet. **6:** 235–285.

Lewis, D., and L. K. Crowe, 1957 Unilateral interspecific incompatibility in flowering plants. Heredity **12:** 233–256.

Lush, M. W., and A. E. Clarke, 1997 Observations on pollen tube growth in *Nicotiana alata* and their implications for the mechanism of self-incompatibility. Sex. Plant Reprod. **10:** 27–35.

Matton, D. P., O. Maes, G. Laublin, Q. Xike, C. Bertrand *et al.*, 1997 Hypervariable domains of self-incompatibility RNases mediate allele-specific pollen recognition. Plant Cell **9:** 1757–1766.

Matton, D. P., D. T. Luu, X. Qin, G. Laublin, M. O'Brien *et al.*, 1999 Production of an S RNase with dual specificity suggests a novel hypothesis for the generation of new *S* alleles. Plant Cell **11:** 2087–2097.

Matton, D. P., D. T. Luu, D. Morse and M. Cappadocia, 2000 Reply: establishing a paradigm for the generation of new *S*-alleles. Plant Cell **12:** 312–315.

Maynard Smith, J., 1971 The origin and maintenance of sex, pp. 163–171 in *Group Selection*, edited by G. C. Williams. Aldine-Atherton, Chicago.

Maynard Smith, J., 1978 *The Evolution of Sex*. Cambridge University Press, Cambridge, UK.

McClure, B. A., J. E. Gray, M. A. Anderson and A. E. Clarke, 1990 Self-incompatibility in *Nicotiana alata* involves degradation of pollen rRNA. Nature **347:** 757–760.

McCubbin, A. G., and T.-h. Kao, 1999 The emerging complexity of self-incompatibility (*S*-) loci. Sex. Plant Reprod. **12:** 1–5.

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, J. B., T.-h. Kao, M. L. Goldberg and M. E. Nasrallah, 1985 A cDNA clone encoding an *S*-locus-specific glycoprotein from *Brassica oleracea*. Nature **318:** 263–267.

Nasrallah, J. B., J. C. Stein, M. K. Kandasamy and M. E. Nasrallah, 1994 Signaling the arrest of pollen tube development in self-incompatible plants. Science **266:** 1505–1508.

Parry, S., E. Newbigin, C. Craik, K. T. Nakamura, A. Bacic *et al.*, 1998 Structural analysis and molecular model of a self-incompatibility RNase from wild tomato. Plant Physiol. **116:** 463–469.

Rick, C. M., 1986 Reproductive isolation in the *Lycopersicon peruvianum* complex, pp. 477–495 in *Solanaceae: Biology and Systematics*, edited by W. G. D'arcy. Columbia University Press, New York.

Royo, J., C. Kunz, Y. Kowyama, M. Anderson, A. E. Clarke *et al.*, 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.

Saba-El-Leil, M. K., S. Rivard, D. Morse and M. Cappadocia, 1994 The *S11* and *S13* self-incompatibility alleles in *Solanum chacoense* Bitt. are remarkably similar. Plant Mol. Biol. **24:** 571–583.

Schopfer, C. R., M. E. Nasrallah and J. B. Nasrallah, 1999 The male determinant of self-incompatibility in *Brassica*. Science **286:** 1697–1700.

Steinbach, 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., J. Doughty, S. Dixon, C. Elleman, S. Hiscock *et al.*, 1997 The male determinant of self-incompatibility in *Brassica oleracea* is located in the pollen coating. Plant J. **12:** 1351–1359.

Suzuki, G., N. Taki, T. Hirose, K. Fukui, T. Nishio *et al.*, 1999 Genomic organization of the *S* locus: identification and characterization of genes in *SLG/SRK* region of *S9* haplotype of *Brassica campestris* (syn. *rapa*). Genetics **153:** 391–400.

Takasaki, T., K. Hatakeyama, G. Suzuki, M. Watanabe, A. Isogai *et al.*, 2000 The *S* receptor kinase determines self-incompatibility in *Brassica* stigma. Nature **403:** 913–916.

Takayama, S., H. Shiba, M. Iwano, H. Shimosato, F.-S. Che *et al.*, 2000 The pollen determinant of self-incompatibility in *Brassica campestris*. Proc. Natl. Acad. Sci. USA **97:** 1920–1925.

Tsai, D.-S., H.-S. Lee, L. C. Post, K. M. Krelling and T.-h. Kao, 1992 Sequence of an S-protein of *Lycopersicon peruvianum* and comparison with other solanaceous S-proteins. Sex. Plant Reprod. **5:** 256–263.

Uyenoyama, M. K., 1988 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.

Uyenoyama, M. K., 1995 A generalized least-squares estimate for the origin of sporophytic self-incompatibility. Genetics **139:** 975–992.

Uyenoyama, M. K., 2000 Evolutionary dynamics of self-incompatibility alleles in Brassica. Genetics **156:** 351–359.

Uyenoyama, M. K., and E. Newbigin, 2000 Evolutionary dynamics of dual-specificity self-incompatibility alleles. Plant Cell **12:** 310–312.

Wright, S., 1939 The distribution of self-sterility alleles in populations. Genetics **24:** 538–552.

Xue, Y., R. Carpenter, H. G. Dickinson and E. S. Coen, 1996 Origin of allelic diversity in *Antirhinnum* S locus RNases. Plant Cell **8:** 805–814.

Communicating editor: R. G. Shaw

APPENDIX

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

$$Tc_0' = \left[\frac{c_4}{N_4} + \frac{G(n-2)}{N_G}\right](1-s)p_n/2 + \left[\frac{c_0(n-2)}{N_0} + \frac{c_2}{N_2} + \frac{c_5}{N_5}\right](1-s)p/2$$

$$Tc_1' = \left[\frac{c_2}{2N_2} + \frac{c_3}{N_3} + \frac{c_6}{2N_6}\right](1-s)p + \left[\frac{c_0}{N_0} + \frac{G(n-2}{N_G}\right](1-s)p_b/2 + \frac{c_1\{s\sigma/2 + (1-s)[p_b + p(n-2)]\}}{2N_1}$$

$$Tc_2' = \frac{c_0(n-1)(1-s)p_b}{2N_0} + \frac{c_2[s\sigma/2 + (1-s)p_b]}{2N_2}$$

$$Tc_3' = \frac{c_1(n-1)[s\sigma/2 + (1-s)p_b]}{2N_1} + \frac{c_2[s\sigma/2 + (1-s)p_b]}{2N_2} + \frac{c_3[s\sigma + (1-s)p_b]}{N_3}$$

$$Tc_4' = \left[\frac{c_4(n-2)}{N_4} + \frac{c_5}{N_5} + \frac{c_6}{N_6}\right](1-s)p/2 + \left[\frac{c_0}{N_0} + \frac{c_1}{N_1} + \frac{G(n-2)}{N_G}\right](1-s)p_{n+1}/2$$

$$Tc_5' = \frac{c_4(n-1)(1-s)p_n}{2N_4}$$

$$+\left[\frac{c_0(n-1)}{N_0}+\frac{c_2}{N_2}\right](1-s)p_{n+1}/2$$

$$Tc_6'=\left[\frac{c_1(n-1)}{2N_1}+\frac{c_2}{2N_2}+\frac{c_3}{N_3}\right](1-s)p_{n+1}$$

$$TG'=\left[\frac{c_0}{N_0}+\frac{c_1}{N_1}+\frac{c_4}{N_4}+\frac{G(n-3)}{N_G}\right](1-s)p$$

in which p, p_n, p_b, p_{n+1}, T, and the N_i appear in (2) through (8).

Haplotype S_b uniformly invades $\{S_i, S_n\}$ under (23). For parameter values (larger n or s) violating (23), S_b increases for σ sufficiently large such that

$$\begin{aligned}(ns\sigma)^3(n-2)-(ns\sigma)^2(n-2)[n(7-2s)-9(1-s)]\\ +ns\sigma[sn^2(7n-16)+(1-s)2(n-1)(6n^2-24n+25)\\ +s(1-s)(9n^2-44n+50)]\\ -2(n-1+s)[sn^2(n-4)-(1-s)8(n-1)(n-2)\\ +s(1-s)(n^3-2n^2-9n+16)]>0.\end{aligned}\tag{A1}$$

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

$$Tc_0'=\left[\frac{c_0(n-2)}{N_0}+\frac{c_2}{N_2}+\frac{c_5}{N_5}\right](1-s)p/2$$
$$+\left[\frac{c_1}{N_1}+\frac{c_4}{N_4}+\frac{G(n-2)}{N_G}\right](1-s)p_n/2$$

$$Tc_1'=\left[\frac{c_2}{2N_2}+\frac{c_3}{N_3}+\frac{c_6}{2N_6}\right](1-s)p$$
$$+\left[\frac{c_4}{N_4}+\frac{G(n-2)}{N_G}\right](1-s)p_a/2$$
$$+\frac{c_1\{s\sigma/2+(1-s)[p_a+p(n-2)]\}}{2N_1}$$

$$Tc_2'=\left[\frac{c_1(n-1)}{2N_1}+\frac{c_3}{N_3}+\frac{c_6}{2N_6}\right](1-s)p_n$$

$$Tc_3'=\frac{c_1(n-1)[s\sigma/2+(1-s)p_a]}{2N_1}+\frac{c_3[s\sigma+(1-s)p_a]}{N_3}$$
$$+\frac{c_6[s\sigma/2+(1-s)p_a]}{2N_6}$$

$$Tc_4'=\left[\frac{c_4(n-2)}{N_4}+\frac{c_5}{N_5}+\frac{c_6}{N_6}\right](1-s)p/2$$
$$+\left[\frac{c_0}{N_0}+\frac{G(n-2)}{N_G}\right](1-s)p_{n+1}/2$$

$$Tc_5'=\frac{c_0(n-1)(1-s)p_{n+1}}{2N_0}+\left[\frac{c_4(n-1)}{N_4}+\frac{c_6}{N_6}\right](1-s)p_n/2$$

$$Tc_6'=\frac{c_4(n-1)(1-s)p_a}{2N_4}+\frac{c_6[s\sigma/2+(1-s)p_a]}{2N_6}$$

$$TG'=\left[\frac{c_0}{N_0}+\frac{c_1}{N_1}+\frac{c_4}{N_4}+\frac{G(n-3)}{N_G}\right](1-s)p$$

in which p, p_n, and p_{n+1} are defined in (2), (3), and (5); p_a corresponds to the right side of (4); T is given in (16); and the N_i are given in (17).

High viability of inbred offspring ($\sigma > \frac{1}{2}$) ensures that S_a, introduced in any frequency into $\{S_i, S_n\}$, excludes the functional haplotypes, while low viability ($\sigma < \frac{1}{3}$) ensures the exclusion of S_a (see RESULTS). For values of σ in the remaining range ($\frac{1}{2} > \sigma > \frac{1}{3}$) that permit the invasion of S_a into $\{S_i, S_n\}$ (24), the population converges to the polymorphic state $\{S_i, S_n, S_a\}$ described by

$$c_0=\frac{p_n[T(1+s-2s\sigma)-(1-s)]}{Ts(1-\sigma)(n-1)}$$

$$c_1=\frac{4(1-T)(1-2\sigma)[s/2+(1-s)(1-p)]}{(n-1)s(1-\sigma)^2}$$

$$c_2=\frac{p_n(1-s)(1-T)}{Ts(1-\sigma)}$$

$$c_3=\frac{(1-T)(3\sigma-1)}{s(1-\sigma)^2}$$

$$G=\frac{2(1-2p)\{s(1-\sigma)^2-(1-T)[(1-s)(3-5\sigma)+2s(1-\sigma)^2]\}}{(n-1)(n-2)s(1-\sigma)^2}$$

$$p_a=\frac{T(3\sigma-1)-s\sigma^2}{(1-s)(1-\sigma)}$$

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

in which $p(n-1)+p_n+p_a=1$ and T is a root of

$$ns(1-\sigma)Q_1-(1-T)Q_2=0,\tag{A2}$$

in which

$$\begin{aligned}Q_1=&T^2[(1-s)+2s(3\sigma-1)](3-s-2\sigma)\\ &-(1-s)^2(1-2\sigma)\\ &-2T\{1-s+2s(1-\sigma)\sigma^2-s(1-s)[(1-\sigma)^2+\sigma^2(1-2\sigma)]\}\end{aligned}$$

$$\begin{aligned}Q_2=&2T^2[(1-s)+2s(3\sigma-1)][(1-\sigma)(3-s-2\sigma)-2\sigma^2(1-s)]\\ &-T\{-2s(1-3\sigma+s\sigma^2)+(1-2\sigma)[4+s(1-3\sigma)\\ &\quad-2\sigma^2(4-3\sigma-4\sigma^2)+s^3(5-5\sigma-2\sigma^2-4\sigma^3)]\}\\ &-(1-s)[2(1-\sigma)(1-s+s^2\sigma^2)-s(1-s)(3\sigma-1)(1-2\sigma)].\end{aligned}$$

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

$$\frac{(1-s)(1-\sigma)+s\sigma^2}{3\sigma-1}>T>\frac{s\sigma^2}{3\sigma-1},$$
$$\frac{s(1-\sigma)^2+(1-s)(3-5\sigma)}{2s(1-\sigma)^2+(1-s)(3-5\sigma)}.\tag{A3}$$