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Table 1. Step-Wise Process leading to New SI Specificity[a]

Step No.	Mutation	Haplotype	Phenotype
		$S_{xF}-S_{xM}$	Self-incompatible (specificity *x*), cross-compatible with all non-*x* alleles
1	$S_{xF}\Rightarrow S_{xyF}$	⇓	
		$S_{xyF}-S_{xM}$	Self-incompatible (specificity *x*), cross-compatible with all non-*x* alleles
2	$S_{aM}\Rightarrow S_{yM}$	⇓	
		$S_{aF}-S_{yM}$	Self-compatible, pistil cross-compatible with all non-*a* alleles, but pollen incompatible with S_{xyF}
3	$S_{aF}\Rightarrow S_{yF}$	⇓	
		$S_{yF}-S_{yM}$	Self-incompatible (specificity *y*), cross-compatible with all non-*y* alleles, complete new specificity

[a] An evolutionary model for SI is depicted. The model assumes separate but tightly linked pollen and pistil genes; note that the second mutation does not occur in the same haplotype as the first (but in a haplotype with pistil allele S_{aF}).

as many as a hundred or more; e.g., Bernatzky et al., 1988; Okazaki et al., 1997).

Given the mounting evidence that separate pollen and pistil genes exist in a self-incompatible Brassica species (Schopfer et al., 1999), along with the clear implication of two-gene systems in fugal incompatibility (see Casselton, 1997, 1998), there is a pressing need to solve the puzzle of how new specificities arise. It seems, however, that the possibility of dual specificities does not provide an easy solution to this puzzle.

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REFERENCES

Bernatzky, R., Anderson, M.A., and Clarke, A.E. (1988). Molecular genetics of self-incompatibility in flowering plants. Dev. Genet. **9,** 1–12.

Casselton, L.A. (1997). Molecular recognition in fungal mating. Endeavour **21,** 159–163.

Casselton, L.A. (1998). Molecular genetics of mating recognition in Basidiomycete fungi. Microbiol. Mol. Biol. Rev. **62,** 55–70.

Lewis, D. (1960). Genetic control of specificity and activity of the S antigen in plants. Proc. Roy. Soc. Lond. B **151,** 468–477.

Matton, D.P., Luu, D.T., Xike, Q., Laublin, G., O'Brien, M., Maes, O., Morse, D., and Cappadocia, M. (1999). Production of an S RNase with dual specificity suggests a novel hypothesis for the generation of new S alleles. Plant Cell **11,** 2087–2098.

Okazaki, K., Kusaba, M., Ockendon, D., and Nishio, T. (1997). Characterization of *S*-tester lines in *Brassica oleracea*: polymorphism of restriction fragment length of *SLG* homologues and isoelectric points of *S*-locus glycoproteins. Theor. Appl. Genet. **98,** 1329–1334.

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

Evolutionary Dynamics of Dual-Specificity Self-Incompatibility Alleles

Allelism is one of the most striking characteristics of the *S* locus, which controls self-incompatibility (SI) of flowering plants. The deceptively simple biology of SI requires some degree of allelism: styles reject those pollen grains that express an *S* allele that they themselves express. Even though a population expressing gametophytic SI can theoretically persist with only three *S* alleles, natural populations generally contain many more.

How do new *S* alleles evolve? Despite progress in the identification of genes involved in SI, answers to this apparently straightforward question remain elusive. Attempts to change the specificity of an *S* allele by mutation or meiotic recombination have been unsuccessful. The most likely explanation for this failure is that the *S* locus contains at least two genes: a style gene that encodes a factor to disable incompatible pollen and a pollen gene that encodes a factor to control recognition of the disabling style factor. Because mutations that alter allelic specificity

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while preserving allelic recognition are unlikely to arise simultaneously in both genes, *S* alleles have probably arisen by stepwise changes, first in one gene and then in the other, with self-incompatibility presumably not an intermediate state. The conceptual challenge has therefore been to describe a pathway in which a new specificity might evolve such that each step maintains allelic recognition and all intermediates are self-incompatible.

In a recent research article in THE PLANT CELL, Matton et al. (1999) describe an experimentally produced style factor that rejects pollen bearing either of two *S* alleles. The authors argue that such a dual-specificity style factor may play a pivotal role in the generation of new *S* alleles, and suggest a pathway in which all intermediates are self-incompatible. Here, we consider the evolutionary fate of new *S* alleles that arise by this pathway and argue that selection would eliminate them from the population. We propose alternative scenarios that would permit the maintenance of new *S* alleles.

In *Solanum chacoense*, the species studied by Matton et al. (1999), the style factor is an extracellular ribonuclease (the S RNase) and the pollen factor is an unknown molecule commonly called pollen S. In the following discussion, we refer to the genes that encode these factors as *A* and *B*, respectively, and designate particular alleles by integer subscripts. For example *S* allele S_1 corresponds to haplotype A_1B_1, in which the pollen S encoded by B_1 causes recognition of the S RNase encoded by A_1. We assume that selection disfavors self-fertilization and removes from the population mutations that disrupt recognition between *A* and *B* of the same haplotype. It is important to note that allele and haplotype are not used here as synonymous terms: mutations that change a haplotype but preserve allelic recognition may segregate in the population as neutral variants. Positive selection to maintain such intermediates need not be invoked as Matton et al. (1999) appear to do.

Mutually distinct *S* alleles may arise through coordinated mutations in *A* and *B*. For example, haplotype A_1B_1 may give rise to A_2B_2 through mutation in *A* followed by mutation in *B* (pathway I: $A_1B_1 \rightarrow A_1B_2 \rightarrow A_2B_2$) or in the reverse order (pathway II: $A_1B_1 \rightarrow A_2B_1 \rightarrow A_2B_2$). The model of Matton et al. (1999) resembles pathway I, with the addition of an extra step in which a (dual-specificity) style factor recognizes two different pollen factors. In our nomenclature, we represent this dual-specificity factor as $A_{1,2}$ and the proposed pathway as $A_1B_1 \rightarrow A_{1,2}\ B_1 \rightarrow A_{1,2}\ B_2 \rightarrow A_2B_2$. By regarding $A_{1,2}$ as a neutral variant of A_1, we subsume this pathway under pathway I.

In pathway I, positive selection of gametophytic SI requires that A_1 be recognized by both B_1 and B_2 (i.e., A_1 is a dual-specificity style factor) and that B_2 recognize both A_1 and A_2 (i.e., B_2 is a dual-specificity pollen factor). Because A_2 and B_1 have never occurred in the same haplotype, selection has not constrained their interaction. Consequently, B_1 pollen tubes may fail to recognize the A_2 style factor, permitting compatibility between A_1B_1 pollen and styles carrying A_2B_2. In contrast, because B_2 arose in an A_1 haplotype, styles expressing A_1 reject A_2B_2 pollen.

Alternatively, in pathway II, A_2 is retained only if B_1 recognizes A_2 in addition to A_1, and B_2 is retained only if it recognizes A_2. Because A_1 and B_2 have never occurred in the same haplotype, A_2B_2 may possibly fertilize a style carrying A_1B_1, whereas the converse may not occur.

Table 1 summarizes the compatibility relationships among the haplotypes in the two pathways. Both pathways show asymmetric compatibility between pairs of haplotypes: it is the original haplotype A_1B_1 that can pollinate styles expressing the derived form A_2B_2 in pathway I, whereas the converse holds in pathway II.

A simple argument shows that, in the absence of any selective forces other than the expression of gametophytic SI, haplotypes that escape rejection by haplotypes that they themselves reject drive the latter to extinction. First, consider that half of the gene pool in any generation is derived from parental egg cells and half from parental pollen cells. Each gene can be expected, assuming Mendelian segregation of mating type alleles, to transmit one copy of itself to the offspring generation through an egg

Table 1. Cross-Compatibility between Haplotypes Expressed in Style and Pollen

Pathway I

	Pollen		
Style	A_1B_1	A_1B_2	A_2B_2
A_1B_1		−[a]	−
A_1B_2	−		−
A_2B_2	+[b]	−	

Pathway II

	Pollen		
Style	A_1B_1	A_2B_1	A_2B_2
A_1B_1		−	+
A_2B_1	−		−
A_2B_2	−	−	

[a] (−) denotes incompatibility.
[b] (+) denotes compatibility.

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cell, whereas transmission through pollen depends on access to compatible mates.

Let p_i denote the frequency of the *S* locus haplotype *i* within any given generation; p_i', the frequency of *i* in the subsequent generation, will then be

$$p_i' = p_i + \frac{1}{2}\sum_j t_{ij}P_j, \quad (1)$$

where t_{ij} denotes the rate of production of pollen bearing S_i by pollen incompatibility class *j* (i.e., $p_i = \Sigma_j\, t_{ij}$). P_j represents the pollination success of class *j.* Because pollen incompatibility class is determined under gametophytic SI by the *S* allele carried by the pollen itself, t_{ii} corresponds to p_i, the frequency of S_i in pollen, with t_{ij} equal to zero for all *i* different from *j.* Equation 1 reduces under gametophytic SI to

$$p_i' = p_i(1 + P_i)/2. \quad (2)$$

We use p_i to denote the frequency of the i^{th} haplotype among the *k* haplotypes derived from and including the original *S* allele A_1B_1. For example, in pathway I these haplotypes include A_1B_1, A_1B_2, and A_2B_2, so that *k* equals three and *i* ranges between one and three. Suppose that pollen carrying a certain haplotype (arbitrarily designated α) can fertilize styles carrying at least one haplotype in this group, but that the reciprocal cross is incompatible. Some number of other *S* alleles, fully functionally distinct from this group of haplotypes and from each other, also segregate in the population, each with frequency *q.*

Equation 2 determines evolutionary changes in the frequencies of all haplotypes:

$$q' = q(1 + P_q)/2 \quad (3)$$

$$p_\alpha' = p_\alpha(1 + P_\alpha)/2 \quad (4)$$

$$p_i' = p_i(1 + P_i)/2, \quad \text{for } 1 \leqslant i \leqslant k, \text{ and } i \neq \alpha. \quad (5)$$

If haplotype α can nonreciprocally fertilize a group of styles that includes at least one other haplotype derived from A_1B_1, its pollination success exceeds that of other haplotypes in the group ($P_\alpha > P_i$). Consequently, as long as this advantage in transmission through pollen accrues to haplotype α, it increases relative to other members of the group ($p_\alpha'/p_i' > p_\alpha/p_i$ for $1 \leqslant i \leqslant k$, and $i \neq \alpha$.).

Evolution favors style component mutations that *expand* the set of pollen factor alleles rejected by the style factor and favors pollen component mutations that *restrict* the style factors recognized. In pathway I, haplotype A_1B_1 is expected to cause the extinction of the new haplotypes, whereas in pathway II, the derived haplotype A_2B_2 is expected to replace A_1B_1. This analysis suggests that, in the pathway proposed by Matton et al. (1999), the new haplotype A_2B_2 can enter the population only if the original haplotype A_1B_1 were no longer present. In the absence of A_1B_1, however, the new haplotype A_2B_2 would simply segregate as a neutral variant of the intermediate A_1B_2 rather than constitute a functionally distinct *S* allele.

During the course of evolution, mutations in both the pollen and style components may arise, undergoing extinction or substitution as a consequence of genetic drift and selection. Preliminary studies of our model indicate that the rate of fission of *S* allele lineages, corresponding to the coexistence of functionally distinct *S* haplotypes derived from a common ancestral haplotype, depends strongly on population structure. In particular, subdivision into a number of partially isolated demes in which alternative descendant haplotypes may undergo substitution and subsequent evolution enhances the rate of *S* allele diversification.

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REFERENCE

Matton, D.P., Luu, D.T., Qin, X., Laublin, G., O'Brien, M., Maes, O., Morse, D., and Cappadocia, M. (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.