LETTERS TO THE EDITOR

How Can Two-Gene Models of Self-Incompatibility Generate New Specificities?

The unsolved problem of how the pollen and pistil components of angiosperm self-incompatibility (SI) are inherited has a long history (Lewis, 1960). A recent paper provides evidence, based on transgenic experiments in *Solanum chacoense*, that a single amino acid difference between proteins encoded by two very similar SI-specifying (*S*) alleles can result in plants that reject the pollen of both alleles (Matton et al., 1999). The authors propose that this result may help solve the difficult problem of how new *S* allele specificities could arise over evolutionary time if there are separate (but linked) loci for pollen and pistil specificities. The purpose of the present Letter is to suggest, however, that this proposal is implausible.

The first step in the proposed pathway from one allele (S_x) to a new functional allele (S_y) could be a change in the pistil component of recognition, from an allele that recognizes its own corresponding pollen specificity (S_x), to a dual-function allele (designated S_{xyF} for specificities *x* and *y* in the female function) that recognizes both S_y and S_x pollen. The S_y component of such a dual-recognition allele should be effectively "neutral" to the extent that no corresponding S_{yM} (male) function would preexist in the population. As pointed out by Matton et al. (1999), the S_{xyF} allele could therefore persist in the population, and would not suffer the evident disadvantage (in a two-gene system) that a changed pollen or pistil specificity, without a change in the other component, would simply cause self-compatibility (Charlesworth, 1995). Matton et al. (1999) propose that this first change, to dual specificity, might later be followed by changes in the

male function, creating a new specificity haplotype with female and male alleles S_{xyF} and S_{yM}, respectively. Finally, loss of the dual specificity by replacement of S_{xyF} by S_{yF} could lead to a fully functional system of SI based on the novel *S* haplotype, $S_{yF}-S_{yM}$. (The opposite order of the changes, i.e., pollen reaction changed first, followed by changed pistil reaction, would also be possible, and everything below can also be applied to this version.)

On closer examination, this attractive scenario appears less easy to accept. Consider a two-locus model, as hypothesized by Matton et al. (1999). In a population in which the first change has occurred, so that the population contains both the initial $S_{xF}-S_{xM}$ haplotype and the new $S_{xyF}-S_{xM}$ one, the requisite change to generate S_{yM} must subsequently happen in the very same haplotype that carries the S_{xyF} allele at the female function gene. Otherwise, if the S_{yM} allele appeared in a different haplotype, say the S_a haplotype, the new "*y*-type" specificity would encounter two disadvantages. Table 1 shows how the model of Matton et al. (1999) would behave in this case and illustrates the difficulties. First, $S_{aF}-S_{yM}$ plants would have the disadvantage of being self-compatible; the disadvantage that new specificities cause loss of self-incompatibility thus appears at this stage of the evolutionary process, rather than at the first step, and is not eliminated by allowing dual-specificity alleles. Second, the new S_{yM} pollen would be incompatible with unrelated plants carrying S_{xyF}, leading to lower fertility for this pollen type than for other pollen types. (S_{xM} would also manifest this problem, but would confer self-incompatibility.) Thus, S_{yM} would be a cross-

incompatibility allele, not a new SI allele, and would be more likely to be eliminated from the population than to be selectively advantageous. In view of the two disadvantages of the $S_{aF}-S_{yM}$ haplotype, it seems that S_{yM} could be an evolutionary successful mutation only if it were to occur in the haplotype that carries the S_{xyF} allele. It is therefore unnecessary to discuss mechanisms by which the two "*y*-type" components might subsequently be brought together into a single $S_{yF}-S_{yM}$ haplotype.

Can we then envisage the evolution of an $S_{yF}-S_{yM}$ haplotype by assuming that the S_{yM} mutation arises from the $S_{xyF}-S_{xM}$ haplotype? This would produce self-incompatibility and cross-compatibility, as required, but the difficulties are not eliminated. We still have a process requiring three successive mutations all within the same haplotype (loci affected shown in bold):

$$\boldsymbol{S_{xF}} - S_{xM} \rightarrow \boldsymbol{S_{xyF}} - S_{xM} \qquad (1)$$

$$S_{xyF} - \boldsymbol{S_{xM}} \rightarrow S_{xyF} - \boldsymbol{S_{yM}} \qquad (2)$$

$$\boldsymbol{S_{xyF}} - S_{xM} \rightarrow \boldsymbol{S_{yF}} - S_{yM} \qquad (3)$$

Note that the second of these mutations must create a male determinant that is rejected by the pistil *y* specificity of the haplotype (but not by its *x* specificity, because the new haplotype ultimately generated by these mutations should not be rejected by pistils with *x* specificity). The other two mutations (steps 1 and 3), moreover, must both occur in the same gene. This might be plausible, given the large amounts of time available for self-incompatibility to evolve, but it is difficult to accept that this process could occur for each new specificity, given the very high numbers of specificities in some species (sometimes

LETTERS TO THE EDITOR

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	$S_{xF} \Rightarrow S_{xyF}$	$\Downarrow$	
		$S_{xyF} - S_{xM}$	Self-incompatible (specificity *x*), cross-compatible with all non-*x* alleles
2	$S_{aM} \Rightarrow S_{yM}$	$\Downarrow$	
		$S_{aF} - S_{yM}$	Self-compatible, pistil cross-compatible with all non-*a* alleles, but pollen incompatible with S_{xyF}
3	$S_{aF} \Rightarrow S_{yF}$	$\Downarrow$	
		$S_{yF} - S_{yM}$	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.

Deborah Charlesworth
Institute of Cell, Animal and
Population Biology (ICAPB)
University of Edinburgh
Ashworth Lab. King's Buildings
W. Mains Rd., Edinburgh EH9 3JT, UK

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