Reply: Establishing a Paradigm for the Generation of New *S* Alleles

The major problem with the evolution of new recognition specificities in a two-gene system (e.g., host–pathogen, or self-incompatibility [SI] specificities) remains the coevolution of both partners. For plant–pathogen recognition systems, the two genes are present in two different organisms, and there is an obvious selective advantage for a plant that can successfully defend itself against pathogen attack. Many resistance genes are arranged in multiple-copy tandem arrays, suggesting that the evolution of new resistance specificities involves intra- and intergenic recombinations (Parniske et al., 1997; Song et al., 1997). In sporophytic SI a similar mode of evolution of *S* alleles is believed to occur (Nasrallah, 1997). In contrast, the single-copy gene that encodes the S RNase in the gametophytic SI system is highly polymorphic and embedded in highly variable flanking sequences, suggesting that point mutations rather than recombination are likely to be involved in generating new *S* alleles (Coleman and Kao, 1992; Matton et al., 1995). The *S* locus which controls the gametophytic SI phenotype is thought to contain at least two genes, because gain-of-function experiments in *Petunia*, *Nicotiana* and *Solanum* spp clearly show that S RNase expression does not affect the pollen phenotype (Lee et al., 1994; Murfett et al., 1994; Matton et al., 1997, 1999). To date, the nature of the pollen component in gametophytic SI is unknown. Lastly, it is not clear why attempts to produce new *S*-allele specificities by chemical or physical mutagenesis result in nonfunctional alleles, yet widely divergent *S*-allele sequences are found in natural populations.

If mutations usually produce nonfunctional alleles, how then are new gametophytic SI specificities generated? We have previously reported the sequence of two closely related S RNases (S_{11} and S_{13}) whose sequences differ by only ten amino acids, four of which are located in the hypervariable (HV) domains (Saba-El-Leil et al., 1994). We have also found that alteration of the four HV-region amino acids from an S_{11} type to an S_{13} type is sufficient to transform the phenotype from S_{11} to S_{13} (Matton et al., 1997). The similarity between the S_{11} and S_{13} sequences suggested that both are derived from the same ancestral sequence, or even that one may have arisen from the other by an accumulation of point mutations. To address the issue of *S*-allele evolution, we have thus produced and studied the incompatibility behavior of potential intermediates in such a process. One intermediate, termed the *HVapb* allele (Table 1), has shown the unexpected property of dual specificity because it can recognize and reject two phenotypically distinct pollen types (Matton et al., 1999). We remind the reader that dual specificity, having also been found in proteins involved in plant–pathogen recognition, is not unique to SI. For example, the Arabidopsis resistance gene *RPM1* shows dual specificity towards the *avrB* and *avrRpm1* avirulence genes from *Pseudomonas syringae* (Grant et al., 1995), and a polygalacturonase inhibiting protein (PGIP) has been found to exhibit specific binding to two different fungal polygalacturonases (Leckie et al., 1999). The observation of dual specificity in such widely disparate examples of cell–cell recognition suggests that this phenomenon may be not only widespread but of functional significance.

We have proposed that dual specificity may be involved in evolution of new *S* alleles. Starting with a two-component system, our model first proposes one or a series of point mutations that produce dual specificity in one component. This means that this component has maintained its original specificity but has also acquired the potential to react with a different partner. Next, one or a series of point mutations altersw the partner so that it is recognized only by the new specificity in the first component. Lastly, additional point mutations in the first component could result in its inability to recognize its original partner. Because the dual-specificity intermediate is able to recognize both the original and the mutated partner, SI behavior is not lost during these mutational steps. Maintenance of an SI phenotype is required to explain why compatible al-leles do not seem to accumulate in SI populations. The key

Table 1. HV Domain Sequences and Phenotypes of Natural and Mutated S RNases

S RNase	HVa Region Sequence	HVb Region Sequence	Phenotype
S_{11}	KPKLTYNYFSDKML	IDQASARKDQP	S_{11}
HVapb	....N..F.....	.....L....	S_{11} and S_{13}
HVa	....N.KF.....	.....	None
HVab	....N.KF.....	.....L....	S_{13}
S_{13}	....N.KF.....	.....L....	S_{13}

LETTERS TO THE EDITOR

feature of the model is to free the incompatibility system from the burden of immediate co-evolution of new specificities in both stylar and pollen parts, and thus allows point mutations to accumulate sequentially rather than simultaneously. It is important to note, however, that not all mutations lead to dual specificity. For example, *HVa* (Table 1) is another possible intermediate allele in the hypothetical evolutionary scheme linking S_{11} with S_{13}, but genetic analyses show this allele to be a compatible alternative to *HVapb* (Table 2).

The authors of the two Letters to the Editor that appear in this issue of THE PLANT CELL have questioned some aspects of this model, although they do not contest the idea of an SI system remaining functional (i.e., incompatible) during the generation and evolution of new *S* alleles. In regard to the comments of Charlesworth, we agree with her assessment that any mutations leading to a new *S*-allele specificity must occur in a single haplotype. If this were not the case, independent segregation of the pollen and stylar components would result in breakdown of SI. We disagree, however, with her suggestion that our model, which involves three mutational events, would be less likely than a more direct model involving only two. After all, the frequency of two mutations occurring separately per gene per replication would be twice the frequency of a single mutation, whereas the frequency of two mutations occurring simultaneously would be the square of the frequency of a single mutation. Our model does involve more steps, but because the requirement for simultaneity has been eliminated, even several additional steps would be more likely to occur than a simultaneous change in both stylar and pollen parts. The dual-specificity component is at first neutral, as pointed out by Charlesworth, and arises by point mutations as a natural variant that retains the specificity of the original allele. Thus there would be no selection against an initial spreading in the population of an allele that confers dual-specificity incompatibility, and the increase in the number of individuals harboring such an allele would in turn increase the likelihood of a further mutational event occurring in the same haplotype.

A second series of objections to our model has been raised by Uyenoyama and Newbigin. We agree with their analysis demonstrating that, in ideal populations, an allele which is rejected by two haplotypes will fare less well than an allele rejected by only one haplotype. We also agree with their assessment that a population divided into semi-isolated groups would provide a protected niche for either the original or the mutated specificity. We disagree, however, with two aspects of their assessment. First, they posit no requirement for a positive selective force maintaining allelic recognition and assume that selection disfavors self-fertilization (presumably through inbreeding depression). In our view, a positive selective force is necessary to block the appearance and spread of pollen containing a nonfunctional allele throughout the population. Much as a stone dropped in water produces ripples radiating outward, the propagation of nonfunctional alleles from a focal point cannot be prevented fast enough by inbreeding depression. Because natural populations do not generally maintain nonfunctional alleles, an additional selective pressure must thus be invoked. Second, we disagree with Uyenoyama and Newbigin's interpretation of the SI recognition system in such a way as to suggest that evolution favors both pollen which becomes less recognizable by styles, and styles that recognize more pollen types. Such a view, rather analogous to the incongruity model proposed by Hogenboom (1973), is at odds with the apparent lack of nonfunctional alleles in natural populations. In contrast, our model posits a positive selective force by which the individual's pollen is recognized by its own styles (i.e., *functional pairs* of stylar and pollen components are maintained), thereby preventing breakdown of the SI system.

In summary, different models for the evolution of new *S*-allele specificities can be derived if different assumptions are made. Within the context of our assumption that SI behavior is conserved during the evolution of new alleles, we believe that dual specificity can function as a paradigm. Ultimately, a rigorous evaluation of the underlying assumptions of our model will require the detailed knowledge of the various components of the ribonuclease-based gametophytic SI system. It may be that several different mechanisms, of which dual specificity is only part, may contribute to the evolution of new *S*-allele specificities in natural populations.

Table 2. Incompatibility of the HVa S RNase as Assessed by Genetic Crosses

	No. Plants (Fruits/Pollinated Flowers)	
Phenotype[a]	$S_{11}S_{12}$ Pollen Donor	$S_{13}S_{14}$ Pollen Donor
Incompatible	0	0
Partially compatible[b]	3 (15/27)[c]	4 (31/51)
Compatible	30 (301/306)	29 (256/262)

[a] Genotype of the host plants is $S_{12}S_{14}$, and they are all self-incompatible.

[b] Partially incompatible plants are those with intermediate levels of pollen rejection.

[c] Plants partially incompatible with S_{11} pollen are different from those partially incompatible with S_{13} pollen.

LETTERS TO THE EDITOR

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INTERFASCICULAR FIBERLESS1 Is the Same Gene as *REVOLUTA*

The recently cloned *INTERFASCICULAR FIBERLESS1* (*IFL1*) gene encodes a homeodomain–leucine zipper protein (HD-ZIP) that spatially regulates fiber differentiation in Arabidopsis (Zhong and Ye, 1999). Mutations of the *IFL1* gene are recessive and highly pleiotropic. In *ifl1* mutants, normal interfascicular fibers are absent from the inflorescence stem and the differentiation of both xylary fibers and vessel elements in vascular bundles is disrupted. They further display long pendant stems, dark green leaves, delayed senescence, and fewer lateral branches (Zhong et al., 1997; Zhong and Ye, 1999). These morphological characteristics are similar to those of plants with a defect in *REVOLUTA (REV)*, a gene that influences aerial architecture by regulating the relative growth of apical versus non-apical meristems (Alvarez, 1994; Talbert et al., 1995).

We recently discovered a putative homeobox gene, *MUP24.4*, within P1 clone MUP24 (GenBank accession number AB005246). Plants carrying a T-DNA insertion in the *MUP24.4* sequence were then obtained by PCR-based screening of DNA pools from the