The Plant Cell, Vol. 12, 309, March 2000, www.plantcell.org © 2000 American Society of Plant Physiologists

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):

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

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

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

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

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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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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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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}

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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.

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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

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Jack collection of insertional mutants (Campisi et al., 1999). The T-DNA insertion was located 466 bp downstream of the putative start codon, and was predicted to create a null mutation (Figure 1). Plants heterozygous for the T-DNA insertion appeared wild type, whereas homozygotes had a number of distinctive features reminiscent of the *rev* mutant. The most prominent characteristic was a failure in the development of all types of apical meristem: lateral shoot meristems in the axils of cauline and rosette leaves were often completely absent, or replaced by a solitary leaf. These effects were most evident in higher order shoots, but in some cases, the primary shoot meristem also failed, terminating growth in a cluster of filamentous structures. Compared to wild-type plants, the mutant showed a dramatic reduction in branching at maturity, delayed senescence, and enlarged revolute leaves. Defects in the floral meristem, moreover, resulted in enlarged floral organs, altered organ numbers, or the replacement of floral organs by filamentous structures.

The similarity between the *rev* phenotype and that of the *mup24.4* insertion mutant raised the possibility that the two genes were allelic. To test this hypothesis, we isolated the *MUP24.4* sequence from *rev-1* mutants and wild-type plants of ecotype Nossen (the background in which the *rev-1* mutation had been isolated). The *MUP24.4* sequence from *rev-1* exhibited eight single-base changes compared to that from wild type Nossen (and 9 differences compared to wild type Columbia, reflecting a single base polymorphism between Nossen and Columbia in the 5th intron). Of these eight changes, one was upstream of the putative start codon, four were present in putative introns, and two were present in the 3' untranslated region. The eighth change was a G-to-A substitution predicted to disrupt the splice site at the junction between the eleventh intron and the twelfth exon (Figure 1), thereby implicating *MUP24.4* as the *REV* gene. To confirm this possibility, homozygotes for the *mup24.4* T-DNA insertion were crossed to *rev-1* homozygotes: all F1 plants from this complementation test exhibited the *rev* phenotype.

The *REV* gene consists of 18 exons, which are predicted to encode an HD-ZIP protein of 842 amino acids, the sequence of which is identical to that of IFL1 (Zhong and Ye, 1999). (We predict, however, that translation starts two codons prior to the ATG suggested by Zhong and Ye [1999] in their published protein sequence). Thus, the *ifl1* mutations are, in fact, alleles of *rev.*

The finding that *IFL1* is *REV* might help explain the deficiencies of fiber differentiation in the mutant. Lignified fiber cells are essential in providing support for the plant stem, and are thought to develop in response to the polar auxin flow that originates at the shoot tips (Aloni, 1987). IFL1 was proposed to act either by regulating polar auxin flow or by regulating the genes involved in the transduction of hormonal signals that trigger fiber differentiation (Zhong and Ye, 1999). *REV* is considered to be essential for apical meristem development. Since the auxin stream that induces fiber differentiation derives from shoots, it seems reasonable to suggest that defects in shoot meristem development would alter the polar auxin flow, and as a consequence, influence fiber differentiation.

The precise role of *REV* remains elusive. It has been suggested that *REV* promotes the growth of apical meristems (including floral meristems) at the expense of nonapical (cambial) meristems (Talbert et al., 1995). It is not yet clear, however, whether expression data support such a role. Strong expression of *REV* has been detected in interfascicular regions and developing vascular tissue, but in situ expression analysis of apical meristems has not yet been reported. REV is a group III HD-ZIP protein and shares high sequence similarity (and organization) with the proteins encoded by three other *Arabidopsis* genes: *Athb8*, *Athb9*, and *Athb14* (Sessa et al., 1998). It is possible, therefore, that these genes act together in the same developmental process. In support of this suggestion, *Athb8* shows an expression pattern similar to that of *REV* and is transcribed in the procambial regions of vascular bundles (Baima et al., 1995). Thus, to gain a full understanding of *REV* function and its contribution to plant architecture, it will

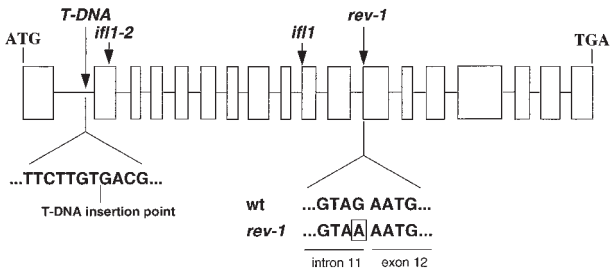

Figure 1. Structure of the *REVOLUTA* Gene.

The coding sequence between the start (ATG) and stop (TGA) codons is 4200 bp in length and consists of 18 exons and 17 introns. Exons are represented by boxes and introns by single lines. Arrows indicate the position of lesions that give rise to mutant alleles. Positions of the *ifl1* alleles are taken from Zhong and Ye (1999).

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be necessary to study the gene in conjunction with the other homologs.

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ACKNOWLEDGMENTS

We thank Marsha Pilgrim, Luc Adam, and Roderick Kumimoto for identification of the T-DNA insertion in *MUP24.4.*

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