Generation of Self-Incompatible *Arabidopsis thaliana* by Transfer of Two *S* Locus Genes from *A. lyrata*

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Transitions from cross-fertilizing to self-fertilizing mating systems have occurred frequently in natural and domesticated plant populations, but the underlying genetic causes are unknown. We show that gene transfer of the stigma receptor kinase SRK and its pollen-borne ligand SCR from one *S*-locus haplotype of the self-incompatible and cross-fertilizing *Arabidopsis lyrata* is sufficient to impart self-incompatibility phenotype in self-fertile *Arabidopsis thaliana*, which lacks functional orthologs of these genes. This successful complementation demonstrates that the signaling cascade leading to inhibition of self-related pollen was maintained in *A. thaliana*. Analysis of self-incompatibility will be facilitated by the tools available in this species.

Self-incompatibility (SI) is a widespread mechanism assuring cross-fertilization (outbreeding) in plant species. The family Brassicaceae (crucifers) includes self-incompatible and self-fertilizing species and has emerged as a model for investigating the evolution of mating systems in plants. In this family, SI is controlled by haplotypes of the *S* locus, whereby cells of the stigma epidermis recognize and inhibit self-related but not genetically unrelated pollen. Mathematical modeling (*1*), phylogenetic data (*2*), and mutational (*3*) studies argue that SI is the ancestral condition and that loss-of-function mutations in genes required for SI have occurred repeatedly to generate self-fertile (autogamous) species and inbreeding populations within a species. As a primary determinant of the outbreeding mating habit in crucifers, the *S* locus is key to understanding the evolutionary switch from SI to self-fertility in this family. Indeed, inactivation of *S*-locus genes may be the principal mutation underlying the switch to autogamy in the model plant *A. thaliana* (*4*). To test this hypothesis, we attempted to complement the self-fertile phenotype of *A. thaliana* by transformation with *S*-locus genes from *A. lyrata*, an obligate outbreeder that diverged from *A. thaliana* ~5 million years ago (*2*).

The *S* locus of crucifers is complex. In *Brassica* species (*5*) and in *A. lyrata* (*4*), the SI-specificity encoding region of this locus contains two genes, the *SRK* (*S*-locus receptor kinase) and *SCR* (*S*-locus cysteine rich protein) genes, which are the determinants of SI in stigma and pollen, respectively (*6*, *7*). A third gene found in the majority of *Brassica S* haplotypes (*5*) but absent in *A. lyrata* (*4*) is the *SLG* (*S*-locus glycoprotein) gene, which encodes a secreted protein that enhances the activity or stability of SRK (*5*, *6*). The highly polymorphic SRK and SCR proteins function as receptor and ligand (*8*, *9*) whose allele-specific binding triggers an intracellular cascade in the stigma epidermal cells that culminates in the failure of self pollen to hydrate and germinate on the stigma epidermis.

We transformed *A. thaliana* ecotype Columbia, which contains defective *SRK* and *SCR* genes (*4*), with the *SRKb* and *SCRb* alleles isolated from the *A. lyrata S*-locus haplotype *Sb* (*4*). Eight independent *SRKb* and nine independent *SCRb* transformants were generated (*10*). Primary (T1) transformants expressing either *SRKb* or *SCRb* were self-fertile. We chose three *SRKb* transformants that showed fewer pollen tubes (*10*) when pollinated with pollen from the transgenic *SCRb* plants, reasoning that these *SRKb* transformants had near-adequate levels of *SRKb* expression. Plants homozygous for each of *SRKb* and *SCRb* were obtained by self-pollination (*10*) and were crossed to generate plants harboring both the *SRKb* and *SCRb* transgenes, which we designate *SRKb/SCRb* plants (Fig. 1A).

The presence of the transgenes was confirmed, and their expression was demonstrated by reverse transcriptase–polymerase chain reaction (RT-PCR) in T2 plants and in the *SRKb/SCRb* plants (*10*) (Fig. 1A). Pollination phenotype was determined at two stages of floral bud development (*11*), mature buds (stage 13) and young flowers (early stage 14), before anthers had extended above the stigma and deposited their pollen on the stigma epidermis (*10*). The inhibition of pollen from *SCRb* transformants on *SRKb* stigmas was recapitulated in the T2 generation (Table 1). In the *SRKb/SCRb* plants, a robust SI response was manifested by complete or near-complete inhibition of self pollen (Table 1,

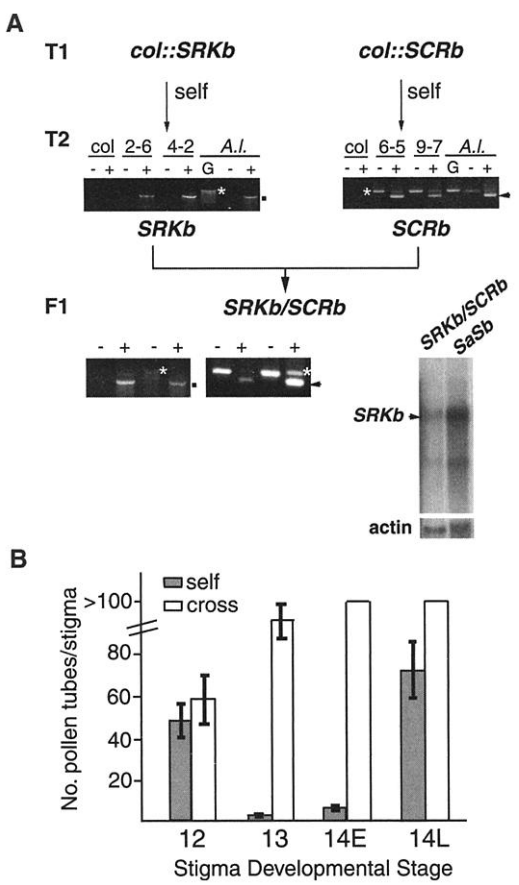

Fig. 1. Generation of *SRKb/SCRb* transformants and expression of SI in *A. thaliana*. (**A**) Generation and RNA analysis of *SRKb/SCRb* transformants starting from T1 *A. thaliana* ecotype Columbia plants transformed with *SRKb* (*col::SRKb*) or *SCRb* (*col::SCRb*). Results of RT-PCR (*10*) for representative T2 plants in each of two *SRKb* (plants 2-6 and 4-2) and two *SCRb* (plants 6-5 and 9-7) transgenic families, and for two *SRKb/SCRb* plants are shown. Controls: untransformed *A. thaliana* ecotype Columbia (col); *A. lyrata* (*A.l.*) RNA and genomic DNA (G). Amplification with (+) and without (–) reverse transcriptase. The dot and arrowhead mark the *SRKb* and *SCRb* RT-PCR products, respectively. The asterisks indicate PCR products derived from contaminating genomic DNA (*10*). The RNA gel blot shows *SRKb* transcripts in the pistils of *SRKb/SCRb* plants and *A. lyrata SaSb*. Hybridization with an actin probe served as a loading control (*10*). (**B**) Developmental regulation of SI in *SRKb/SCRb* stigmas from stage-12, -13, early -14 (14E), and late -14 (14L) buds. Numbers of pollen tubes produced per stigma in self- and cross-pollinations are the means (±SE) determined from analysis of two inflorescences in each of six *SRKb/SCRb* plants.

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Figs. 1B and 2A). The selective inhibition of self pollen was clearly demonstrated by simultaneous selfing and crossing of individual stigmas (Fig. 2B), a method we used to monitor the response of a single stigma to differing pollen genotypes (*10*). Furthermore, the pollen of *SRKb/SCRb* plants failed to grow on the stigmas of *SRKb*-expressing plants, and their stigmas inhibited pollen from *SCRb* transformants (Table 1). The specificity of these interactions was further demonstrated by interspecies pollinations with *A. lyrata* (*10*). Whereas untransformed *A. thaliana* stigmas allowed the development of *A. lyrata* pollen tubes irrespective of their *S*-locus genotype, *SRKb/SCRb* stigmas inhibited *A. lyrata* pollen from *Sb* homozygotes but not from plants homozygous for the *S*-locus haplotype *Sa* (Table 1, Fig. 2C), demonstrating that these transgenic plants had acquired *Sb* specificity.

A hallmark of SI in crucifers is that the ability of the stigma to reject self pollen is developmentally regulated and is first manifested in mature buds just before flower opening. Immature bud stigmas are self-compatible, which allows the generation of *S*-locus homozygotes. In *Brassica*, this regulation reflects the progressive increase in the amount of SRK to maximal levels in mature buds and young flowers (*12*). In older *Brassica* flowers, *SRK* transcript and protein levels decline, although they usually remain above the threshold required for maintenance of the SI response (*12*). SI phenotype was also developmentally regulated in the stigmas of *A. thaliana SRKb/SCRb* transformants (Figs. 1B and 2B), albeit over a narrower window than *Brassica* due to the accelerated developmental program of this species. Stigmas of immature buds were about equally receptive to self and cross (unrelated) pollen, whereas stage-13 to early stage-14 stigmas inhibited self pollen but remained receptive to cross pollen. Unlike *Brassica*, however, older *SRKb/SCRb* flowers regained a measure of receptivity to self pollen (Figs. 1B and 2B).

The developmental weakening of SI in *SRKb/SCRb* transformants resembles the pseudo-compatible response observed in some self-incompatible crucifer cultivars (*4*, *13*). *SCRb* transcript levels were about equivalent in *A. thaliana SRKb/SCRb* transformants and *A. lyrata SaSb* plants (data not shown). However, transgene-encoded *SRKb* transcript levels were only ~40% of those in *A. lyrata SaSb*, as determined by RNA gel blot analysis (Fig. 1A) (*10*), and may be reduced below a critical threshold in older flowers. In the future, it might be possible to increase the strength of the SI response in transgenic *A. thaliana* by using highly active stigma-specific promoters, a strategy that proved effective for expressing *SRK* in transgenic *Brassica* (*6*).

Plants offer several examples of dramatic interspecific trait differences that result from mutations at a small number of major genes (*14*, *15*). Our results show that acquisition of just two key *S*-locus recognition genes is sufficient to reverse the self-fertile phenotype of *A. thaliana* to the ancestral state of SI in mature buds and young flowers. The acquisition of *Sb* specificity in *A. thaliana SRKb/SCRb* transformants demonstrates that the shift to autogamy in *A. thaliana* resulted from inactivation of *S*-locus recognition genes. It appears that all other components of the SRK-mediated signal transduction pathway have been maintained in this species, possibly because they have essential functions unrelated to SI. In view of this result, the failure of a previous attempt at conferring a SI specificity on *A. thaliana* stigmas by transformation with a *Brassica SRK* gene (*16*) is difficult to explain. It might have been due to factors related to the evolution-

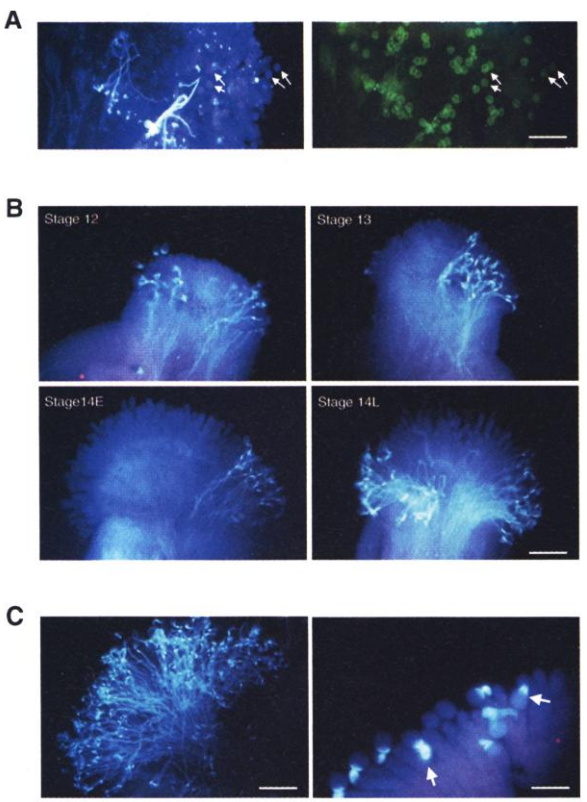

Fig. 2. Pollination assays of *SRKb/SCRb* stigmas. (**A**) Pollination test of an early stage-14 *SRKb/SCRb* stigma. The stigma was pollinated with pollen from untransformed *A. thaliana* on its left side and self pollen on its right side. The two images of the same stigma were taken with different filters and show aniline blue fluorescence of pollen tubes (left) and autofluorescence of pollen grains (right). Incompatible pollen grains adhere poorly and are normally dislodged from the stigma surface upon processing for microscopy (Fig. 1B), unless mild conditions are used (*10*), as was the case for this stigma. Pollen tubes grow on the cross-pollinated side, but no tubes are formed on the self-pollinated side. Some pollen grains (arrows) are visible in both images and provide landmarks for comparison of the images. Bar, 30 μm. (**B**) Single-stigma self- or cross-pollination test of *SRKb/SCRb* stigmas from stage-12, 13, early -14 (14E), and late -14 (14L) buds. The left half of each stigma was self-pollinated, and the right half was cross-pollinated with pollen from untransformed *A. thaliana*. The SI response is manifested by absence of pollen tubes on the self-pollinated half of stage-13 and stage-14E stigmas. Bar, 50 μm. (**C**) Interspecies pollination of *A. thaliana* stigmas with pollen from an *A. lyrata SbSb* plant. Left, compatible response of an untransformed control. Bar, 50 μm. Right, incompatible response of an *SRKb/SCRb* transformant, in which short pollen tubes (arrows) that do not invade the stigma epidermis were produced. Bars, 20 μm.

Table 1. Pollination responses in crosses among transgenic lines (*col::SRKb*, *col::SCRb*, *col::SRKb/SCRb*), with untransformed *A. thaliana* ecotype Columbia (*col*), and with *A. lyrata Sa* and *Sb* homozygotes. +, >100 pollen tubes per stigma; –, 0 to 10 pollen tubes per stigma. Based on pollinations of stage-13 and early stage-14 stigmas (*10*).

Stigma parent	Pollen/parent					
	col	*col::SRKb*	*col::SCRb*	*col::SRKb/SCRb*	*A. lyrata*	
					SaSa	*SbSb*
col	+	+	+	+	+	+
col::SRKb	+	+	–	–	+	–
col::SCRb	+	+	+	+	+	+
col::SRKb/SCRb	+	+	–	–	+	–
*A. lyrata SaSa**					–	+
*A. lyrata SbSb**					+	–

*See (*14*).

ary divergence of *Brassica* and *Arabidopsis*, such as aberrant maturation of the *Brassica* SRK protein in *A. thaliana* stigmas or its inability to interact productively with *Arabidopsis*-derived downstream targets.

The large number of genetically well-characterized *S* haplotypes that are available in *Brassica* species has been critical for identification of the SRK and SCR SI recognition proteins. However, the relatively laborious transformation methods and rudimentary state of genome studies in *Brassica* make further studies of the SI response difficult. The availability of *A. thaliana* strains that express SI provides new opportunities for exploiting the tools of this tractable model plant for structure-function studies of SRK and SCR as well as for the genetic and molecular dissection of the SRK-mediated signal transduction pathway.

References and Notes

1. D. Charlesworth, S. I. Wright, *Curr. Opin. Genet. Dev.* **11**, 685 (2001).
2. M. Koch, B. Haubold, T. Mitchell-Olds, *Mol. Biol. Evol.* **17**, 1483 (2000).
3. J. B. Nasrallah, S. J. Rundle, M. E. Nasrallah, *Plant J.* **5**, 373 (1994).
4. M. Kusaba *et al.*, *Plant Cell* **13**, 627 (2001).
5. J. B. Nasrallah, *Curr. Opin. Plant Biol.* **3**, 368 (2000).
6. T. Takasaki *et al.*, *Nature* **403**, 913 (2000).
7. C. R. Schopfer, M. E. Nasrallah, J. B. Nasrallah, *Science* **286**, 1697 (1999).
8. A. P. Kachroo, C. R. Schopfer, M. E. Nasrallah, J. B. Nasrallah, *Science* **293**, 1824 (2001).
9. S. Takayama *et al.*, *Nature* **413**, 534 (2001).
10. Materials and methods are available as supporting material on *Science* Online.
11. D. R. Smyth, J. L. Bowman, E. M. Meyerowitz, *Plant Cell* **2**, 755 (1990).
12. J. C. Stein, R. Dixit, M. E. Nasrallah, J. B. Nasrallah, *Plant Cell* **8**, 429 (1996).
13. T. Hodgkin, *Theor. Appl. Genet.* **53**, 81 (1978).
14. S. White, J. Doebley, *Trends Genet.* **14**, 327 (1998).
15. A. Frary *et al.*, *Science* **289**, 85 (2000).
16. Y. M. Bi, N. Brugiere, Y. Cui, D. R. Goring, S. J. Rothstein, *Mol. Gen. Genet.* **263**, 648 (2000).

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Materials and Methods

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Evolution of the Gene Network Underlying Wing Polyphenism in Ants

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Wing polyphenism in ants evolved once, 125 million years ago, and has been a key to their amazing evolutionary success. We characterized the expression of several genes within the network underlying the wing primordia of reproductive (winged) and sterile (wingless) ant castes. We show that the expression of several genes within the network is conserved in the winged castes of four ant species, whereas points of interruption within the network in the wingless castes are evolutionarily labile. The simultaneous evolutionary lability and conservation of the network underlying wing development in ants may have played an important role in the morphological diversification of this group and may be a general feature of polyphenic development and evolution in plants and animals.

Polyphenism, which is the ability of a single genome to produce two or more alternative morphologies in response to an environmental cue, is an ecologically important and phylogenetically widespread feature of plants and animals (*1*). Yet almost nothing is known about its developmental genetic basis. Wing polyphenism in ants is a dramatic case: Depending on environmental cues, the regulatory gene network underlying wing development either produces a queen with fully functional wings or is halted to produce a wingless soldier or worker (*2*, *3*). We examined the expression of several ant genes orthologous to those from the wing-patterning network in *Drosophila melanogaster* (Fig. 1) to determine how the expression of this network changes during the development and evolution of winged and wingless ant castes.

The wing-patterning network has been largely conserved across holometabolous insects (*4*) for 300 million years (*5*). Therefore, we predicted that this network would be conserved in reproductive castes (queens and males), which produce wings. Fossil and phylogenetic evidence strongly supports a single origin of wing polyphenism in ants: A wingless worker caste was present in the earliest known

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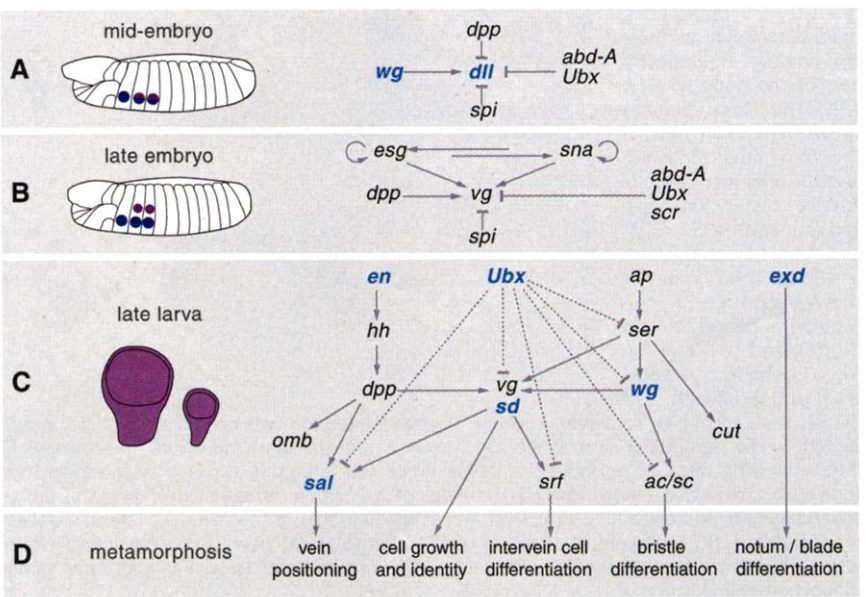

Fig. 1. The wing-patterning network in *D. melanogaster*. During embryogenesis (**A**), interacting signaling molecules and transcription factors establish a cluster of about 20 ectodermal cells as precursors of both the leg and wing imaginal discs (*11*) (blue/purple circles). (**B**) A second set of interacting gene products then divides these cells into separate clusters that give rise to three pairs of leg (blue) and two pairs of wing (purple) imaginal discs (*11*). During the last larval instar (**C**), the wing precursor cells proliferate into full-sized imaginal discs (purple). A third set of interacting gene products then patterns these discs, imparts a wing-specific identity, and activates downstream target genes that pattern detailed structures, such as veins and bristles (**D**) (*18*). Genes examined in this study are shown in blue. Dashed lines indicate regulatory interactions specific to the hindwing disc, arrowheads indicate activation, and bars indicate repression.