Pollen recognition and rejection during the sporophytic self-incompatibility response: *Brassica* and beyond

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Many hermaphrodite flowering plants avoid self-fertilization through genetic systems of self-incompatibility (SI). SI allows a plant to recognize and to reject self or self-related pollen, thereby preserving its ovules for outcrossing. Genes situated at the *S*-locus encode the 'male' (pollen) and 'female' (pistil) recognition determinants of SI. In sporophytic SI (SSI) the male determinant is expressed in the diploid anther, therefore haploid pollen grains behave with a diploid *S* phenotype. In *Brassica*, the male and the female determinants of SSI have been identified as a peptide ligand and its cognate receptor, respectively, and recent studies have identified downstream signalling molecules involved in pollen rejection. It now needs to be established whether the *Brassica* mechanism is universal in species with SSI, or unique to the Brassicaceae.

Self-incompatibility (SI) is the most widespread mechanism preventing inbreeding and promoting out-breeding in flowering plants. The SI 'response' comprises recognition of self- or self-related pollen by cells of the pistil followed by rejection of the incompatible pollen through aborted development, either immediately after pollination on the stigma surface, or at a subsequent stage during pollen tube growth into the stigma or style [1]. In recent years, exciting progress has been made towards elucidating the molecular basis of SI in a variety of species and it is now clear that there are probably a multitude of different molecular mechanisms of SI [2]. In spite of this apparent mechanistic diversity, the genetic control of SI is fundamentally straightforward, with all SI systems falling into one of two distinct groups defined on the basis of whether the incompatibility phenotype of the pollen is determined by its own (haploid) genome, gametophytic SI (GSI, see Glossary) [3], or by that of its (diploid) parent, sporophytic SI (SSI). Like the majority of GSI systems, SSI is regulated by a single *S*-locus with multiple allelic forms (haplotypes). Because in SSI the pollen incompatibility phenotype is determined by its diploid parent, dominance relationships are possible between *S*-haplotypes 'in pollen' and pistil leading to the greater genetic complexity of SSI compared with GSI (Box 1).

GSI is the most common form of SI, with the widest phylogenetic distribution in angiosperms and is predicted to be the ancestral condition for the majority of angiosperms [4,5]. This means that SSI evolved more recently than either of the two molecularly well-characterized systems of GSI: RNase-mediated GSI and poppy GSI [3]. The scattered phylogenetic distribution of families with SSI – Brassicaceae, Asteraceae, Convolvulaceae, Betulaceae, Caryophyllaceae, Polemoniaceae and Malvaceae – supports the notion of multiple origins of SSI [2,6].

However, whereas molecular studies of GSI have sought to obtain molecular data from a variety of different families, molecular studies of SSI have focused almost exclusively on species in the Brassicaceae, particularly crop *Brassica* species [e.g. cabbages, kale and oilseed rape (canola)]. As a consequence, the molecular basis of SI in *Brassica* spp. and their relatives is better characterized than for any other plant, such that both the female and male determinants of SI have now been identified [7,8]. Nevertheless, little is known about molecular mechanisms operating in other families with SSI. To complement Noni Franklin-Tong's and Chris Franklin's review on GSI in this issue of *Trends in Plant Science* [3], our review will focus on SSI, highlighting recent work on *Brassica* in particular, and the initial findings from studies of the molecular basis of SSI in *Ipomoea trifida* (Convolvulaceae) and *Senecio squalidus* (Asteraceae).

Self-incompatibility genes of *Brassica*

In *Brassica*, the *S*-locus is extremely complex, spanning ~80–100 kb and containing as many as 17 genes [10]. Nevertheless, only two tightly linked highly polymorphic genes are required for *S*-haplotype-specific pollen recognition [7,11–14].

SRK (*S*-locus receptor kinase), the female determinant of SSI, encodes allelic forms of a receptor serine–threonine kinase expressed in the epidermal cells (papillae) of the

Glossary

ARC1: Armadillo-repeat-containing 1
GSI: gametophytic self-incompatibility
SC: self-compatibility or self-compatible
SCR: *S*-locus cysteine rich protein (synonym SP11)
SLG: *S*-locus glycoprotein
SP11: *S*-locus protein 11 (synonym SCR)
SRK: *S*-locus receptor kinase
SSI: sporophytic self-incompatibility
THL: thioredoxin-H-like protein

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Box 1. Genetic complexities of sporophytic self-incompatibility

In sporophytic self-incompatibility (SSI), dominance interactions are possible between *S*-haplotypes because the pollen incompatibility phenotype is determined by the diploid genotype of its parent plant. This is possible because alleles of the pollen incompatibility gene are expressed in the diploid cells of the anther and their products transferred to the pollen coating. Dominance interactions between *S*-haplotypes can act independently in pollen and stigma and are not necessarily linear [1]. Figure I (a), (b) and (c) show the effect of different dominance scenarios on reciprocal cross-compatibilities between two individuals (left and right) sharing an *S*-haplotype (S_2). Pollen grains and pollen tubes are shown in yellow. Haploid pollen *S*-genotypes are shown within the pollen grains (yellow spheres) and their diploid *S*-phenotypes are indicated above each grain. (a) All three *S*-haplotypes are co-dominant making the cross incompatible in both directions. (b) The S_1 haplotype shows dominance over S_2 'in' the pollen but codominance in the stigma. Thus, the S_1S_2 individual is compatible with the S_2S_3 individual when it is the pollen parent (left) but incompatible as stigma parent (right). A similar reciprocal difference in incompatibility behaviour would arise if S_1 was dominant over S_2 in the stigma and codominant in the pollen. (c) The S_1 haplotype is dominant over S_2 in pollen and stigma so the cross is reciprocally incompatible. Dominance among *S*-haplotypes has several important consequences: (i) patterns of incompatibility among individuals can be extremely complex, with reciprocal incompatibility differences owing to dominance acting independently 'in pollen' and stigma (b); (ii) recessive *S*-haplotypes can achieve high frequencies in populations because their effects are masked by dominant *S*-haplotypes (c); (iii) some individuals can be homozygous for recessive *S*-haplotypes, a situation impossible under gametophytic SI (GSI). Dominance among *S*-haplotypes has no effect on prevention of self-fertilization, but it can lead to higher levels of compatibility between individuals that share *S*-haplotypes, particularly siblings. This can result in higher levels of biparental inbreeding than would be possible if all *S*-haplotypes were co-dominant [9]. As a consequence, high levels of dominance among *S*-haplotypes are thought only to evolve under special circumstances [9]. Thus, in *Brassica*, the well-characterized *S*-haplotypes (>80 in *Brassica oleracea* and 30 in *Brassica rapa*) exhibit considerably higher levels of codominance than dominance, and curiously, when dominance does occur it is usually confined to pollen [1,7,8]. Figure redrawn, with permission, from Ref. [6].

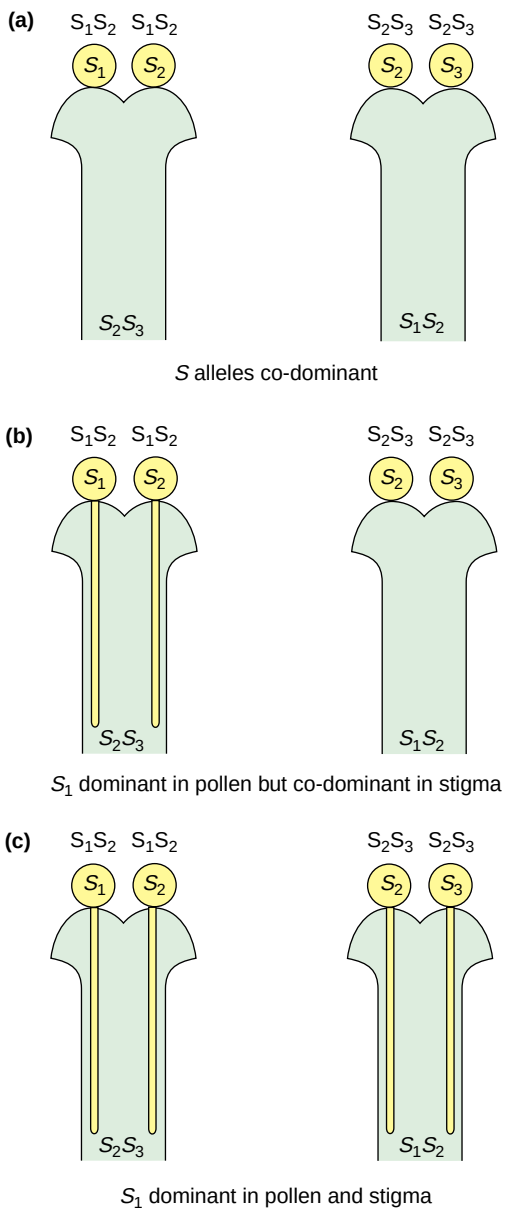

Figure I.

stigma [15,16]. Mature SRK spans the plasma membrane of papillae with the receptor (*S*) domain extending into the cell wall [16,17]. The majority of sequence variation between SRKs lies within the receptor domain where regions of hyper-variability are predicted to be responsible for *S*-specificity [6–8]. Transgenic gain-of-function experiments showed that SRK alone determines *S*-specificity in the stigma, although the ability of the stigma to reject incompatible pollen is enhanced by the presence of a second stigmatic *S*-gene (*S*-locus glycoprotein, *SLG*) [11].

The male determinant of SSI, a small (6 kDa) cysteine-rich pollen protein, was identified independently by groups in the USA and Japan, and therefore, somewhat confusingly, it bears two names: SCR, for *S*-locus cysteine-rich protein [12], and SP11, for *S*-locus pollen protein 11 [13]; for simplicity, SCR will be used from now on. The discovery of *SCR* was a significant moment in SI research because it was the first (and so far only) pollen *S* determinant to be characterized for any SI system, confirming the long-held belief that the male and female determinants of SI are encoded by different genes at the *S* locus [1]. Alleles of *SCR* exhibit far greater polymorphism (19.5–94.0% amino acid identity) than alleles of *SRK* do (65.0–98.0% amino acid identity), but all possess a conserved glycine, and usually eight conserved cysteines, presumably responsible for a similar three-dimensional structure in the mature protein [8]. Recent support for this prediction has come from structure-based sequence alignments and homology

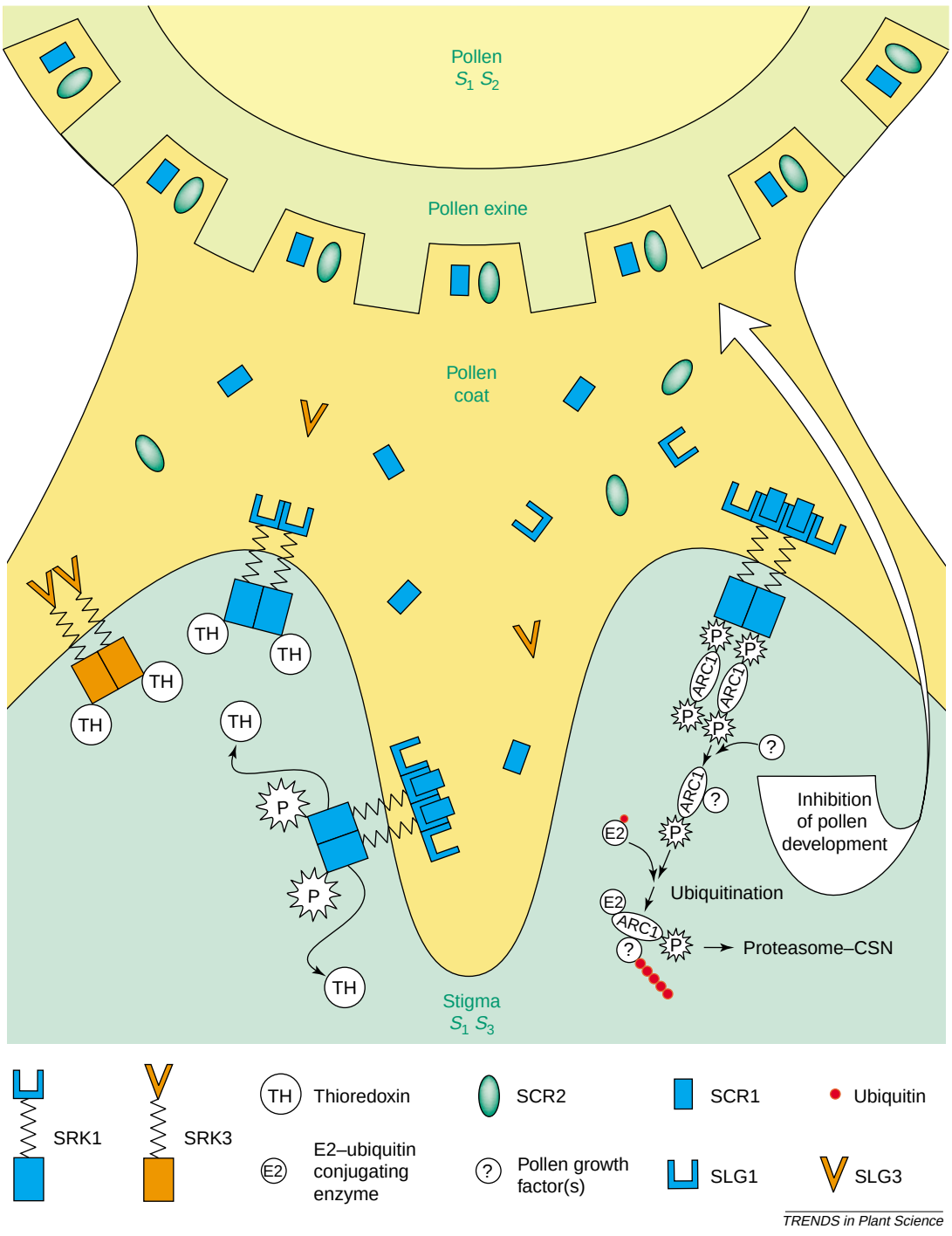

Figure 1. Model for the mechanism of sporophytic self-incompatibility (SSI) in *Brassica*. *S*-locus receptor kinase (SRK), the female determinant of SSI is a serine–threonine receptor kinase that spans the plasma membrane of stigmatic papilla cells. *S*-locus cysteine-rich protein (SCR), the male determinant of SSI is the cognate ligand of SRK. SLG is a secreted glycoprotein with high sequence similarity to the receptor region of SRK; evidence indicates that in most cases *S*-locus glycoprotein (SLG) is not essential for SSI although it has been shown to form part of the SCR–SRK interaction complex. *S*-gene products from the same *S*-haplotype are depicted in the same colour. In this example, pollen and stigma share gene products of the S_1 haplotype and therefore are incompatible. Pollen recognition involves a haplotype-specific interaction between SCR1 and the receptor domain of SRK1, which 'activates' the kinase domain. In an 'inactive' state, SRK molecules appear to associate as dimers or oligomers with thioredoxins bound to their kinase domains. Association with the thioredoxin is thought to prevent autophosphorylation. Activation of SRK following SCR binding is accompanied by dissociation of the thioredoxins and autophosphorylation on serine and threonine residues in the kinase domain. A stigma-specific arm-repeat motif-containing protein, ARC1, interacts in a phosphorylation-dependent manner with the kinase domain of SRK and functions as an E3 ubiquitin ligase. Therefore, ARC1 is thought to be responsible for directing localized inhibition of self-pollen development by initiating the degradation of pollen growth promoting factors via the ubiquitin pathway, which targets ubiquitinated proteins to the proteasome–COP9 signalasome (CSN) for degradation. Figure adapted from Ref. [39].

modeling of allelic forms of SCR that confirm a common three-dimensional structure and a 'protruding' hypervariable loop that is proposed to be responsible for allele-specific binding to cognate SRKs [18]. In the majority of *S*-haplotypes, *SCR* is expressed sporophytically in diploid cells of the anther tapetum (which breaks down to form the pollen coating) and gametophytically in haploid microspores, but in pollen recessive *S*-haplotypes,

SCR expression is exclusively sporophytic in the tapetum [19]. Interestingly, recent immunocytochemical studies show that even when *SCR* is expressed in microspores, mature SCR protein always becomes incorporated into the pollen coating [20]. This indicates that there are two pathways for targeted translocation of SCR to the pollen coating, one sporophytically controlled and the other gametophytically controlled. The reasons for and consequences of the different patterns of *SCR* expression await clarification.

In most *S*-haplotypes, a third polymorphic gene, *SLG* (*S*-locus glycoprotein) is present at the *S*-locus. *SLG*, which encodes a stigma-specific secreted glycoprotein localized to cell walls of papilla cells, was the first *S*-linked gene identified in *Brassica* [21,22]. Sequence similarity between *SLG* and a putative receptor kinase gene in maize, *ZmPK1* [23], led to the discovery of *SRK* [15]. For a given *S*-haplotype, *SLG* and the receptor (*S*) domain of *SRK* can share as much as 98% nucleotide sequence identity [8,15]. This led to the prediction that SRK and SLG might form a receptor complex, but loss-of-function experiments showed that SLG was not essential for haplotype-specific pollen recognition even though the presence of SLG with SRK increased the strength of the incompatibility response [11]. Evidence of a role for SLG in SSI comes from biochemical analysis of self-compatible (SC) lines of *Brassica* showing normal *SRK* expression, but negligible *SLG* expression [24]. This suggested that in these lines, SLG might be required to stabilize SRK and facilitate its accumulation to physiologically relevant levels in the stigma [24] – a role somewhat similar to CLAVATA2 (CLV2) during its interaction with the receptor kinase, CLV1 [24,25]. However, more recently, three *Brassica rapa* *S*-haplotypes have been identified with no *SLG* [26], confirming that, at least for these three *S*-haplotypes, SLG is not required for SSI. SLG therefore has a somewhat ambiguous role in *Brassica* SSI with an effect in some *S*-haplotypes but not in others. It is commonly observed that certain *S*-haplotypes are 'stronger' than others, in that they reject incompatible pollen earlier than 'weaker' *S*-haplotypes do, usually before germination, compared to after pollen tube extension in the weaker *S*-haplotypes [27]. It will be interesting to determine whether SLG is responsible for this phenomenon.

Self-pollen recognition involves interaction between SRK and SCR

Expression of *SRK*, *SCR* and *SLG* is tightly regulated, being undetectable in small flower buds and reaching maximal levels just before anthesis when flowers become SI. Thus, when the flower opens it is primed to discriminate between self- and cross-pollen. In the stigma before pollination, SRK appears to exist as dimers or oligomers in association with two thioredoxin-H-like proteins (THL1 and THL2) [28–30] (Figure 1). Interaction of THL1 and THL2 with the kinase domain of SRK *in vitro* is phosphorylation-independent and the interaction between SRK and THL1 has been demonstrated to inhibit autophosphorylation of SRK oligomers [30]. It is therefore predicted that *in vivo*, the interaction with THL1 (and probably also THL2) maintains SRK in an 'inactive' state [30]. Transition of the SRK complex to an 'activated' state *in vivo* takes place rapidly in the presence of pollen grains or isolated pollen coating of the same *S* haplotype [20,30] through a haplotype-specific interaction between SCR and the receptor domain of SRK [31,32]. This interaction results in the rapid autophosphorylation of serine and threonine residues in the kinase domain of SRK [32], an immediate consequence of which is hypothesized to be the dissociation of THL1 and THL2 from the kinase domain [30], although this has yet to be demonstrated *in vivo* (Figure 1). This activated form of SRK is then predicted to initiate a signalling cascade within papilla cells through the interaction of its kinase domain with specific cytosolic proteins that target the incompatible pollen grain for rejection.

Interestingly, SCR protein alone cannot initiate an incompatibility response when applied directly to stigmas, apparently because it is unable to diffuse through the stigmatic cuticle [20]. This indicates that an additional pollen factor is required to ensure that contact is made between SCR and SRK, perhaps a pollen cutinase or one of several pollen serine esterases needed for correct pollen tube development and penetration of the *Brassica* stigma [33,34].

Mechanism of self-pollen rejection

Rejection of incompatible pollen on the *Brassica* stigma is precisely controlled – a single papilla cell will simultaneously permit development of a compatible pollen grain while rejecting an incompatible grain situated immediately next to it [35]. The 'rejection' process is also rapid because differences in development (hydration, germination and stigma penetration) between compatible and incompatible pollen grains are observed within 10–20 min of pollination [35,36]. Furthermore, 'rejection' within this time period is reversible because incompatible pollen grains can be 'resurrected' by moving them to a stigma with which they are compatible [36]. These physiological observations need to be accommodated by any hypotheses concerning the mechanism of self-pollen rejection.

A strong candidate for the initiator of the cytosolic signalling cascade that directs self-pollen rejection is a stigma-specific Armadillo repeat motif-containing protein, ARC1 [37]. Yeast two-hybrid assays demonstrated that ARC1 interacts strongly and specifically with the kinase domain of SRK in a phosphorylation-dependent manner [37], and the antisense loss-of-function experiments correlated reduced *ARC1* expression profiles with a reduced ability of stigmas to reject incompatible pollen effectively [38]. Recent analyses of ARC1 identified a U-box motif within the protein, and biochemical assays demonstrated U-box-associated E3 ubiquitin ligase activity and the ability of ARC1 to promote ubiquitination of stigma proteins during rejection of incompatible pollen [39]. This suggests that rejection of self-pollen might involve directed degradation of stigmatic proteins that support pollen germination and/or pollen tube growth (Figure 1). Stigmatic glycoproteins that promote pollen tube growth have been identified in species of *Nicotiana* [40], but similar proteins have yet to be identified in *Brassica*. Identification of ARC1 substrates will therefore be an

essential next step in dissecting the process of pollen rejection. An elegant series of experiments using reporter constructs of ARC1 showed that these ubiquitinated substrates are targeted, together with ARC1, to the proteasome–COP9 signalosome (CSN) for probable degradation [39]. When expressed in tobacco suspension-cultured cells, ARC1 shuttled between the nucleus and the cytosol, but in the presence of an 'activated' SRK, ARC1 moved into the proteasome–CSN, presumably accompanied by its ubiquitinated substrate(s). In support of this assumption, inhibition of proteolytic activity in the proteasome significantly disrupted SI, suggesting that the ubiquitin pathway is directly involved in rejecting incompatible pollen [39] (Figure 1).

Intriguingly, ubiquitin-mediated protein degradation has recently been implicated in the mechanism of RNase-mediated gametophytic SI with the identification of a pollen-specific gene encoding polymorphic alleles of an F-box protein tightly linked to the *S*-locus [41] and a Ring-finger protein that interacts with S-RNases in yeast two-hybrid assays [42] (discussed by Franklin-Tong and Franklin [3]). Thus, for the first time, a mechanistic link has been established for GSI and SSI, which might have implications for the evolution of SSI systems.

A significant recent breakthrough in SSI research came with the cloning of *SRK* and *SCR* orthologues in *Arabidopsis lyrata*, a close SI relative of SC *Arabidopsis thaliana*. Transformation of *A. thaliana* with these genes rendered it self-incompatible [43] even though previous transformations with *Brassica SRK* had failed to transfer the female SI phenotype to *A. thaliana* [44]. Transformation with the orthologous *SRK*/*SCR* gene pair from the more closely related *A. lyrata* must therefore have allowed a better genetic match between the *S* genes and their downstream effectors. This remarkable achievement shows that in spite of diverging from its closest SI relative ~5 million years ago, SC *A. thaliana* has retained the necessary biochemical machinery required to reject self-pollen [43]. However, the induced SI phenotype of the *A. thaliana* SI transformants is short-lived, relative to true SI *A. lyrata* and *Brassica*, breaking down swiftly as the flowers matures [43]. Even with this age-dependent phenotype, SI *Arabidopsis* should still prove to be an important tool for elucidating the mechanism of pollen rejection, particularly the targets for ubiquitinization by ARC1. Many *ARC1*-like genes as well as other genes from the ubiquitin degradation pathway are present within the *Arabidopsis* genome [39]. Gene knockout lines should be available for these genes and can readily be introgressed into the SI line, so it might be only a matter of time before the pathway to pollen rejection is determined, ironically, in self-compatible *A. thaliana*.

Molecular basis of dominance

Any proposed mechanisms of SSI have to explain the dominance hierarchies that define SSI and recent studies are providing exciting and unexpected insights into the molecular basis of dominance, the most significant being that dominance among *SRK* alleles is controlled in a totally different way to dominance among *SCR* alleles. A study of the expression of pairs of dominant and recessive *SRK* alleles in heterozygotes showed that expression levels of the two alleles were roughly equal [45]. Comparable levels of expression were also observed when these *SRK* alleles were combined in co-dominant combinations or as homozygotes, showing that dominance among *SRK*s must be determined post-transcriptionally [45]. By contrast, comparisons of *SCR* expression in dominant and recessive heterozygous combinations revealed that in the presence of a dominant *SCR* allele, recessive *SCR* alleles were not expressed, indicating that dominance is controlled at the level of transcription [19,46]. In accordance with the linear dominance hierarchies observed among pollen-recessive *S*-haplotypes, some *SCR* alleles can be dominant or recessive, with corresponding changes in expression, dependent on their allelic partner [46]. Similar patterns of expression were observed for dominant and recessive alleles of *SCR* orthologues in SI *A. lyrata* [47]. In *Brassica* and probably therefore in other species from the Brassicaceae, dominance among *SRK* alleles is thus determined at the protein level, either by relative amounts of SRK or by the relative strengths with which different SRKs interact with molecules downstream in the signalling pathway [45]. However, dominance among *SCR* alleles has a reversible epigenetic basis. This remarkable finding neatly explains why *S*-haplotypes show independent dominance interactions in pollen and stigma.

Co-evolution of *SRK* and *SCR*

Patterns of *SRK* and *SCR* sequence variation among *S*-haplotypes of *Brassica oleracea* and *B. rapa* suggest that the intimate relationship between *SRK* and *SCR* was established in a common ancestor long before these two species diverged and that these genes subsequently co-evolved as non-recombining gene pairs [14,48]. Recent experimental proof of this has come from pollination assays using *S*-haplotypes of *B. oleracea* and *B. rapa* with similar *SRK* and *SCR* sequences. In spite of slight amino acid sequence differences in their respective SRKs and SCRs, these *S*-haplotypes were shown to encode the same *S*-specificity [14,49]. The *B. oleracea* S_7-haplotype and the *B. rapa* S_{46}-haplotype, for instance, which have *SRK* alleles and *SCR* alleles that are 95.0% and 96.1% identical, respectively, at the amino acid level, were shown by pollination assays to be functionally identical. This was cleverly done by producing an allotetraploid hybrid of *B. rapa* (Br. $S_{46}S_{46}$) and *B. oleracea* (Bo. $S_{15}S_{15}$) with a chimeric heterozygous genotype, BrS_{46} BoS_{15}, that showed reciprocal incompatibility with a *B. oleracea* S_7-homozygote and full compatibility, as either pollen or pistil parent, with a range of other *B. oleracea* *S*-homozygotes [49]. Two further *S*-haplotype pairs from *B. oleracea and B. rapa* were similarly shown to share the same *S*-specificities: BoS_{64} and BrS_{41} and BoS_{24} and BrS_{36} [50], indicating that their *SRK*and *SCR* gene pairs have maintained the same functional recognition sequences since the divergence of *B. oleracea* and *B. rapa* 2 million to 3 million years ago [48] (Figure 2). In spite of *SRK* and *SCR* sequences being similar in these *S*-haplotypes, the genomic sequences around them are different, indicating that these sequences diversified after speciation [49]. Sequence heterogeneity around *SRK* and

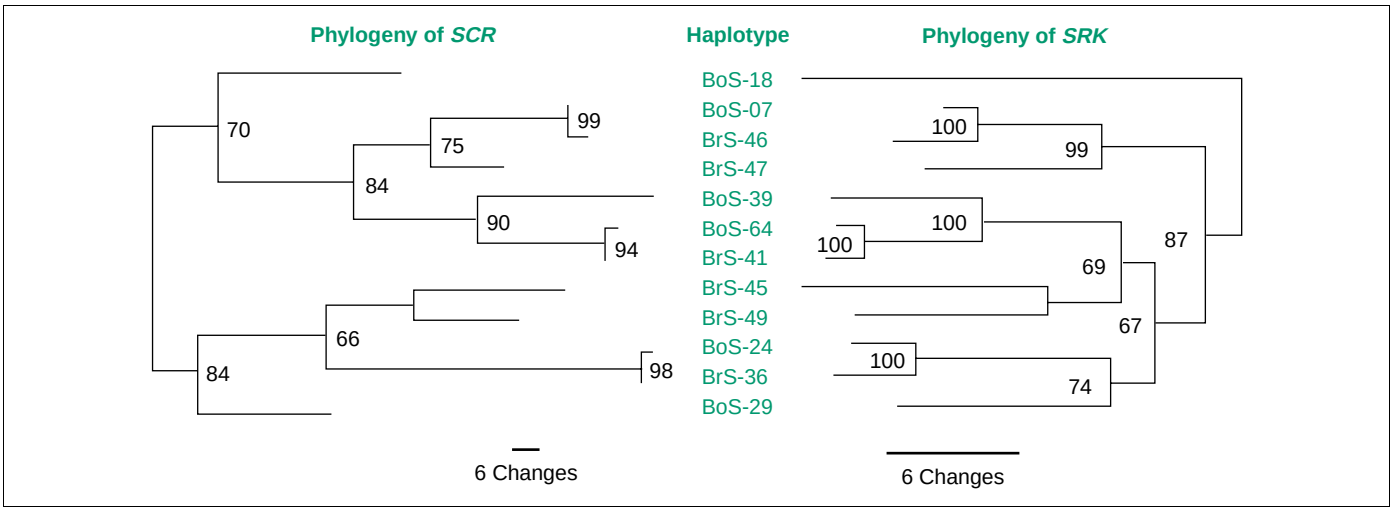

Figure 2. Co-evolution of *Brassica* self-incompatibility (SI) genes *SCR* and *SRK*. The two unrooted trees show the phylogenetic relationships of *SCR* alleles and *SRK* alleles from the same *Brassica S*-haplotypes. In spite of slight variation, the topologies of the two trees are almost mirror images, suggesting co-evolution of *SRK* and *SCR* alleles of the same haplotype. Stringent maximum likelihood analyses confirmed that *SRK* and *SCR* (*SP11*) gene pairs probably diverged at the same time [14]. BoS haplotypes are from *Brassica oleracea* and BrS haplotypes are from *Brassica rapa*. Functional analyses (using pollination tests) of the three pairs of *S*-haplotypes from *B. oleracea* and *B. rapa* with highly similar *SCR* and *SRK* alleles (BoS-07/BrS-46, BoS-64/BrS-41 and BoS-24/BrS-36) showed that each haplotype pair encodes the same *S*-specificity [49,50]. This indicates that *SCR/SRK* gene pairs evolved before the divergence of *B. oleracea* and *B. rapa* and confirmed that *SCR/SRK* gene pairs have co-evolved. The number shown at each node indicates the bootstrap value of the OTU (operational taxonomic unit) cluster connecting at each node. The scale of each tree is indicated by a bar. Figure redrawn, with permission, from Ref. [14].

SCR appears to be a feature of all *S*-haplotypes and has probably favoured the maintenance of tight linkage between these two genes by discouraging recombination [14,49]. Interestingly, the same cannot be said for *SLG* and *SRK*, which appear to have recombined in several *S*-haplotypes in spite of sequence heterogeneity in their flanking regions [51]. The absolute requirement of *SRK* and *SCR*, compared to the dispensability of *SLG* for SSI, supports a model of co-evolution of *SRK* and *SCR* at the *S*-locus [14,49]. Further evidence for co-evolution of *SRK* and *SCR* has come from an elegant phylogenetic analysis of twelve *SRK*/*SCR* gene pairs that produced almost identical tree topologies for *SRK* and *SCR* sequences [14] (Figure 2).

The ancient co-evolved nature of *SRK*/*SCR* gene pairs is further highlighted by sequence comparisons between pollen-recessive *S*-haplotypes and *S*-haplotypes that generally behave in a co-dominant manner. Based on their *SLG* (and later *SRK*) nucleotide sequences, pollen-recessive *S*-haplotypes have been referred to traditionally as class-II *S*-haplotypes because these sequences differ extensively from those of the *SLG*s and *SRK*s of other *S*-haplotypes (which are grouped together as class-I *S*-haplotypes) [52,53]. Recent analyses of *SCR* nucleotide sequences revealed a similar dichotomy for class-I and class-II *S*-haplotypes, again endorsing the predicted ancient origin of *SRK*/*SCR* gene pairs [14,19]. Divergence of *S*-haplotype gene sequences into two distinct monophyletic clades indicates that new *S*-haplotypes only arise from pre-existing *S*-haplotypes with the same type of pollen dominance behaviour [48]. This is consistent with the finding that the regulatory elements of class-I and class-II *SCR* alleles are fundamentally different [19,46].

Beyond *Brassica* – how many other molecular mechanisms for sporophytic self-incompatibility are there?

Species in the Brassicaceae, such as *A. lyrata* and *Raphanus sativus* use the same molecular mechanism for SSI as *Brassica*, but is this also true for species in other families? Recently initiated molecular studies of SSI in the Convolvulaceae, Asteraceae and Betulaceae will soon provide a definitive answer to this question [6]. Initial investigations in the sweet potato relative *Ipomoea trifida* (Convolvulaceae), Oxford ragwort [*Senecio squalidus* (Asteraceae)] and hazelnut [*Corylus avellana* (Betulaceae)] understandably sought to identify orthologues of *SRK* (and *SLG*) [54–58]. In light of the number of *SRK*-like sequences now known to be present in the *Arabidopsis* genome, it is not surprising that *SRK*/*SLG*-like genes were duly identified, but only in *I. trifida* and *S. squalidus* have these genes been cloned and characterized. One *SRK*-like gene from *I. trifida* was expressed exclusively in flower tissues – stigmas and anthers – but segregation studies revealed that it was not linked to the *S*-locus [57]. Other *SRK*-like genes amplified from *I. trifida* stigmatic RNAs were likewise dismissed as unlikely candidates for a role in SSI [54,57]. Similarly, three *SRK*-like genes identified in *S. squalidus* were found not to be expressed exclusively in stigmas, so were also unlikely candidates for stigma *S* genes – an assumption endorsed by their lack of polymorphism in different *S* genotypes [55]. These data therefore strongly suggest that *Ipomoea* and *Senecio* do not use the *Brassica* SRK/SCR system of SSI.

How then is SSI controlled in these diverse families? Does each possess its own unique molecular mechanism of SSI or is there a system of SSI, such as RNase-mediated GSI, that is shared between them? To identify potential female *S* determinants one 'tried and tested' approach is to screen for polymorphic stigma proteins that associate with particular *S*-genotypes. This method, used first to identify *Brassica SLG* and the *Nicotiana* S-RNase, has been applied to *I. trifida* and *S. squalidus*. In *I. trifida*, an acidic protein (~70 kDa) showing charge polymorphisms associated with different *S* homozygotes was identified and

cDNA clones obtained for four allelic variants [58]. These alleles were found to encode putative short chain alcohol dehydrogenases (SCADs) that were 95–98% identical at the amino acid level. Expression of the *SCAD* gene in mature stigmas just before anthesis was consistent with a potential role in SI, but RFLP (random fragment length polymorphism) mapping placed the *SCAD* 1.2 cM from the *S*-locus, making it an unlikely candidate for the female *S*-determinant [58]. Current work on *I. trifida* is using AFLP (amplified fragment length polymorphism) and AMF (AFLP-based mRNA fingerprinting) analysis to construct a saturated map of the *S*-locus and several tightly linked markers are now available for a gene walk through the *S*-locus. To date, 17 genes have been identified in the *Ipomoea S*-locus region and, importantly, none of these genes shares any homology with the *Brassica S*-genes (Yasuo Kowyama, pers. commun.). Hopefully it will not be long before candidate *S*-genes are identified in *Ipomoea* allowing a second SSI system to be dissected at the molecular level.

What then of the Asteraceae? Analyses of stigmatic proteins in *Senecio squalidus* have identified a family of polymorphic basic proteins (pI 7–9) of ~35 kDa, different variants of which associate with specific *S* genotypes [55]. Four alleles of this stigma *S*-associated protein (SSP) gene have been cloned from different *S*-genotypes and are 95–98% identical at the amino acid level. *SSP* is expressed specifically in stigmas with maximal expression commencing just before anthesis, as predicted for a potential female *S*-gene, and *in situ* hybridization shows that *SSP* is expressed exclusively in stigmatic papilla cells (S.J. Hiscock, unpublished). *SSP*, which bears no resemblance to either *SRK* or *SLG*, is therefore a strong candidate for female *S*, but exhaustive mapping analysis is needed before its degree of linkage to the *S*-locus can be established.

Given that the Convolvulaceae and Asteraceae are more closely related to each other than either is to the Brassicaceae [59], it will be interesting to discover whether they share the same mechanism of SSI or whether each possesses its own unique system of SSI. So, is the SRK/SCR system unique to the Brassicaceae? To answer this question, molecular studies need to be initiated in more families with SSI. *C. avellana* (Betulaceae) still needs more extensive molecular investigation, and the Caryophyllaceae, which is the most basal eudicot family with SSI, and the Malvaceae, which is the closest family with proven SSI to the Brassicaceae, would be particularly interesting to study at a molecular level.

Outlook

The next few years should be exciting for SI research. With pollen and stigma recognition components of SSI identified in *Brassica*, the next major goal will be fully characterizing the biochemical mechanism of targeted pollen rejection. Exciting progress is now being made in this area through characterization of ARC1 and the generation of transgenic SI *Arabidopsis*. Therefore there is the possibility that the mechanism of pollen rejection might be elucidated in a normally self-compatible plant. Progress is also being made towards the characterization of other mechanisms of SSI in *Ipomoea* and *Senecio*, which should lead to new and exciting insights into the evolution and diversity of SSI systems.

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