The different mechanisms of sporophytic self-incompatibility

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Flowering plants have evolved a multitude of mechanisms to avoid self-fertilization and promote outbreeding. Self-incompatibility (SI) is by far the most common of these, and is found in *ca.* 60% of flowering plants. SI is a genetically controlled pollen–pistil recognition system that provides a barrier to fertilization by self and self-related pollen in hermaphrodite (usually co-sexual) flowering plants. Two genetically distinct forms of SI can be recognized: gametophytic SI (GSI) and sporophytic SI (SSI), distinguished by how the incompatibility phenotype of the pollen is determined. GSI appears to be the most common mode of SI and can operate through at least three different mechanisms, two of which have been characterized extensively at a molecular level in the Solanaceae and Papaveraceae. Because molecular studies of SSI have been largely confined to species from the Brassicaceae, predominantly *Brassica* species, it is not yet known whether SSI, like GSI, can operate through different molecular mechanisms. Molecular studies of SSI are now being carried out on *Ipomoea trifida* (Convolvulaceae) and *Senecio squalidus* (Asteraceae) and are providing important preliminary data suggesting that SSI in these two families does not share the same molecular mechanism as that of the Brassicaceae. Here, what is currently known about the molecular regulation of SSI in the Brassicaceae is briefly reviewed, and the emerging data on SSI in *I. trifida*, and more especially in *S. squalidus*, are discussed.

Keywords: sporophytic self-incompatibility; S-haplotype; *Brassica*; *Ipomoea trifida*; *Senecio squalidus*

1. INTRODUCTION

SI is the most important and widespread mechanism promoting outbreeding and gene flow through pollen in flowering plants. SI is a genetically determined pollen–pistil recognition system that prevents self-fertilization and cross-fertilization between individuals sharing the same incompatibility type. SI therefore, prevents inbreeding at two levels: (i) by making selfing impossible and (ii) by preventing matings between close relatives that have inherited the same incompatibility type. It has been suggested that SI was a feature of the earliest flowering plants and may have played a significant role in their extraordinary evolutionary success by optimizing the benefits afforded by co-sexual flowers and insect pollination (Whitehouse 1950). Today it is estimated that SI is present in *ca.* 60% of all flowering plants (see Hiscock & Kües 1999).

Two genetic mechanisms of SI have been recognized: GSI and SSI. In GSI the incompatibility type of the pollen is controlled by its own haploid genotype, whereas in SSI, the pollen incompatibility type is controlled by the diploid (sporophyte) genotype of the parental anther in which it was produced. The well-known heteromorphic SI systems of distyly and tristyly, first described by Darwin (1877), are under sporophytic genetic control (reviewed by de Nettancourt 1977), but this review will focus only on so-called systems of ‘homomorphic’ SSI, where no such differences in floral morphology distinguish incompatibility

types. Details of the genetics and molecular basis of GSI are given in Franklin-Tong & Franklin (2003), where the important fact that GSI can operate through at least two different molecular mechanisms is highlighted. For SSI only one molecular mechanism has so far been fully characterized (for the Brassicaceae), so it is not yet known whether SSI can operate through other molecular pathways. Here, the molecular basis of SSI in the Brassicaceae will be briefly reviewed before assessing the evidence for alternative molecular mechanisms in other families with SSI, particularly the Asteraceae and Convolvulaceae. Recent data on the unique population genetics of SSI in *Senecio squalidus* (Asteraceae) will also be discussed before speculating on the evolution of SSI.

2. GENETIC DETERMINATION OF SSI

The genetics of SSI was first elucidated in two species from the Asteraceae: *Crepis foetida* (Hughes & Babcock 1950) and *Parthenium argentium* (Gerstel 1950). Later, SSI was described in species from the Brassicaceae (Bateman 1955). As with GSI, SSI is controlled by a single polymorphic locus, *S*. The fundamental genetic difference between SSI and GSI is that in SSI, the incompatibility phenotype of the pollen is determined by the diploid parental genome. Hence, pollen grains carry the products of two *S* (*S*) alleles, instead of one. Diploid sporophytic expression of SI allows dominance interactions to occur between *S*-alleles and such interactions can occur independently for pollen and stigma. This often results in very complex patterns of compatibility and incompatibility, with reciprocal differences in incompati-

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bility between individuals being a regular feature associated with differing dominance effects in pollen and stigma. Dominance interactions can also give rise to individuals homozygous for recessive *S*-alleles: an event that is impossible under GSI. With SSI, the total number of *S*-alleles needed to sustain a functional SI system is two, with the one dominant allele exerting its effect on both pollen and stigma. This is the situation with heteromorphic SI, where incompatibility and floral morphology are controlled by just two sporophytically expressed *S*-alleles, *S* and *s* with complete dominance (reviewed by de Nettancourt 1977). In the multiallelic 'homomorphic' SSI systems of the Brassicaceae and Asteraceae, between 30 and 40 *S*-alleles are typically found within natural populations (Lawrence 2000).

3. THE EFFECT OF DOMINANCE INTERACTIONS ON COMPATIBILITY RELATIONSHIPS BETWEEN INDIVIDUALS

In SSI systems, dominance interactions between *S*-alleles have the effect of increasing the number of compatible crosses possible within a population compared with a population containing all co-dominant *S*-alleles. When an *S*-allele is recessive, its effect is masked, and so it will not elicit an SI response between any two individuals that share it, thereby giving a compatible mating, which if the *S*-alleles were co-dominant would be incompatible (figure 1). Ockendon (1974) proposed that the relative frequencies of recessive and dominant *S*-alleles within a population will reflect a dynamic balance between reproductive assurance (favoured by recessive *S*-alleles) and the benefits afforded by outbreeding, namely avoidance of inbreeding depression (favoured by dominant *S*-alleles). The effect of different dominance scenarios on compatibility between individuals sharing a common recessive *S*-allele can be seen in figure 1.

4. MOLECULAR CONTROL OF SSI IN THE BRASSICACEAE

A key question for the pioneers of research into the cellular, and later, the molecular basis of SSI was how could a haploid pollen grain behave with a diploid *S*-phenotype? Two hypotheses were proposed: (i) *S*-alleles are expressed in meiocytes before the completion of meiosis (Pandey 1958), or (ii) *S*-alleles are expressed in the diploid cells of the anther tapetum and their products added to the pollen as a constituent of the pollen coating, which is derived from degradation of the tapetum late in pollen development (Heslop-Harrison 1968). Strong support for the latter hypothesis was presented by Heslop-Harrison *et al.* (1974) for *Iberis*, and Dickinson & Lewis (1973) for *Raphanus*, but conclusive evidence that the male determinant of SSI is located in the pollen coating was not forthcoming until Stephenson *et al.* (1997) performed a set of intricate pollen coat exchange experiments on *Brassica*. By applying extracted pollen coatings of various different *S*-genotypes to stigmatic papillae before pollination, Stephenson *et al.* (1997) were able to change the incompatibility behaviour of pollen grains applied to the treated stigmas. In this way pollen grains that should be incompatible with the stigma were rendered compatible

Figure 1. The effect of dominance interactions on reciprocal cross-compatibility of individuals sharing a common *S*-allele. Pollen grains are represented by circles. The haploid genotype of each pollen grain is indicated within each pollen grain (circle), and its diploid *S*-phenotype is given above. Compatible pollen grains are represented with an extending pollen tube. (a) All *S*-alleles are co-dominant and the cross is reciprocally incompatible. (b) The *S*₁ allele is dominant over the *S*₂ allele 'in pollen' (see § 3 for explanation) but co-dominant in stigma; the cross is therefore compatible in only one direction, when the *S*₁*S*₂ individual is the pollen parent. (c) The *S*₁ allele is dominant over the *S*₂ allele in both pollen and stigma; the cross is therefore reciprocally compatible because the *S*₂ is completely masked by the *S*₁ allele.

and pollen grains that should be compatible with the stigma were made incompatible. Detailed fractionation of protein components of the pollen coating identified an 'active fraction', consisting of a group of *ca.* 12 small (*M*_r 6–8 kD) cysteine-rich peptides termed PCPs (Doughty *et al.* 1998). Three abundant PCPs were cloned and shown to have a conserved structure built around four disulphide bridges formed by eight conserved cysteines. However,

Figure 2. Model for the mechanism of *S*-haplotype-specific pollen recognition and rejection in *Brassica*. In this example, *S*-haplotypes are all co-dominant. Proteins encoded by the same *S*-haplotype are given the same colour, i.e. *S*₁ proteins are red. The pollen SCR₁(SP11₁) protein is recognized by, and binds to the extracellular receptor domain of SRK₁, presumably inducing dimerization between SRK₁ proteins and transphosphorylation on serines and threonines in their kinase domains. Activation of the SRK complex, which includes SLG in most *S*-haplotype-specific interactions, initiates an intracellular signalling cascade within the stigmatic papilla cell that culminates in localized rejection of pollen. SRK and SCR/SP11 alone are required for *S*-haplotype-specific pollen recognition and rejection, but the strength of the SI response is enhanced by the presence of SLG in *S*-haplotypes that possess this protein. In *A. lyrata* (Brassicaceae) a SSI relative of *A. thaliana*, the *S*-locus appears to lack *SLG*, so SSI in this species is mediated entirely by orthologues of SRK and SCR/SP11. Signalling downstream of the activated SRK complex has yet to be characterized, however ARC1, an arm-repeat protein, that binds to the kinase domain of SRK in a phosphorylation-dependent manner is essential for SI, but as yet its function is unknown.

none of these cloned PCPs was linked to the *S*-locus (Doughty *et al.* 1998; J. Doughty, personal communication), leaving the identity of the male determinant of SSI unresolved.

Some years earlier, the female determinant of SSI was identified as an SRK that was expressed exclusively in stigmas and consisted of a conserved kinase domain, a trans-membrane domain and a variable extracellular receptor domain with 10–12 conserved cysteine residues arranged in a precise order (Stein *et al.* 1991; Nasrallah & Nasrallah 1993). By analogy with the well-characterized mechanisms of receptor kinase activation in animal sys-

tems, it was speculated that the pollen determinant of SSI was likely to be a ligand for the receptor domain of SRK (Nasrallah & Nasrallah 1993). Interestingly, *SRK* was first identified because of the homology of its receptor domain to another *S*-linked gene expressed specifically in stigmas, the *SLG* (Nasrallah *et al.* 1985; Nasrallah & Nasrallah 1993). For a given *S*-haplotype (allele) the receptor domain of SRK and SLG share *ca.* 90% amino acid sequence identity. This apparently close relationship between SRKs and SLGs of the same haplotype suggested that there might be some sort of concerted interaction between SRK, SLG and the pollen *S*-ligand (Kusaba *et al.* 1997). While this may be the case in some instances, SLG is clearly not essential for functional SSI because, in gain-of-function experiments, SRK on its own was shown to be sufficient to determine *S*-specificity in the stigma; the presence of SLG merely enhances the strength of the SSI response, as assessed by relative differences in incompatible pollen development (Takasaki *et al.* 2000). Furthermore, certain *Brassica* *S*-haplotypes lack an *SLG* gene altogether (Suzuki *et al.* 2000), as appears to be the situation in *Arabidopsis lyrata*, an SSI relative of *Arabidopsis thaliana* (Schierup *et al.* 2001; Nasrallah *et al.* 2002).

Extensive sequence analyses of the *S*-locus region between *SRK* and *SLG* of *Brassica rapa* (syn. *campestris*) eventually resulted in the independent identification of the male determinant of SSI as a small (*ca.* 8 kD) cysteine-rich pollen-specific protein (Schopfer *et al.* 1999; Takayama *et al.* 2000), similar to the PCPs previously identified by Doughty *et al.* (1998). The gene encoding this protein, designated *SCR* by Schopfer *et al.* (1999) and *SP11* by Takayama *et al.* (2000), is expressed sporophytically in cells of the anther tapetum, and also gametophytically in microspores and pollen (Takayama *et al.* 2000). Sporophytic expression of *SCR/SP11* in the tapetum was certainly predicted for the male determinant of SSI, but the significance of its gametophytic expression in microspores and pollen remains to be determined (see § 5).

Haplotype-specific interaction between SCR/SP11 and SRK was demonstrated independently using two different biochemical approaches (Kachroo *et al.* 2001; Takayama *et al.* 2001). This interaction was shown to induce transphosphorylation ('autophosphorylation') of serine and threonine residues in the kinase domains of, presumably dimerized, SRKs (Schopfer & Nasrallah 2000; Takayama *et al.* 2001). Before interaction with SCR/SP11, SRKs appear to be held in an inactive state through an interaction with two thioredoxin-h proteins (THL1 and THL2) (Cabrillac *et al.* 2001). A model for haplotype-specific recognition and rejection of incompatible pollen, as described in figure 2, is now widely accepted.

The next phase of SSI research will focus on identifying the downstream signalling events within the stigma that lead to pollen arrest. One *Brassica* SRK interactor essential for SSI has already been identified as an 'arm repeat' protein (ARC1) (Gu *et al.* 1998; Stone *et al.* 1999) that may act by signalling to components of the cytoskeleton. Further components of the SSI signalling pathway are expected to be identified swiftly in lines of SI *A. thaliana* engineered by transformation with *SRK* and *SCR/SP11* orthologues from its close SI relative *A. lyrata* (Nasrallah *et al.* 2002).

5. MOLECULAR DETERMINATION OF DOMINANCE

Dominance interactions between *S*-alleles are a key element in the genetics of SSI. With the identification of two or three tightly linked genes, *SRK*, *SCR/SP11* and *SLG*, at the *S*-locus, allelic forms of the *S*-locus are now referred to as haplotypes, and the term allele reserved for variants of the specific genes themselves. Based on sequence data, *SRK*s, *SLG*s and more recently *SCR/SP11*s can be placed in one of two haplotype classes, class I or class II (see Shiba *et al.* 2002). Class I *S*-haplotypes are generally dominant or co-dominant, whereas class II *S*-haplotypes are all characteristically recessive to class I haplotypes in pollen (Nasrallah & Nasrallah 1993). Such pollen recessive haplotypes can attain high frequencies in populations by virtue of the masking effect of dominant *S*-haplotypes (Uyenoyama 2000). Class I *SRK* and *SLG* allele sequences are *ca.* 80% identical at the amino acid level but share only *ca.* 60% amino acid identity with *SRK* and *SLG* alleles from class II haplotypes. Class I and class II *SCR/SP11* alleles show a similar relationship, but are far more polymorphic than either *SRK* or *SLG*: sequence identities among class I *SCR/SP11* alleles ranging from 19.5% to 76.1% (Shiba *et al.* 2002). Class II *SCR/SP11* alleles are relatively more conserved (sequence identities ranging from 63.2% to 94.6%) but highly diverged from class I *SCR/SP11* alleles (Kusaba *et al.* 2002; Shiba *et al.* 2002).

Recent studies have attempted to assess the molecular basis of dominance through expression studies of *SCR/SP11* and *SRK*. Class I *SCR/SP11* alleles are characteristically expressed both sporophytically (in tapetum) and gametophytically (in microspores and pollen), whereas class II *SCR/SP11* alleles are only expressed sporophytically (Kusaba *et al.* 2002; Shiba *et al.* 2002). However, in heterozygous combinations of class I and class II *SCR/SP11* alleles, expression of the pollen recessive *SCR/SP11* allele was suppressed in cells of the tapetum, while expression of the class I *SCR/SP11* allele was unaffected. This demonstrates that dominance of *SCR/SP11* alleles is regulated at the RNA level, presumably by some form of epigenetic gene silencing (Kusaba *et al.* 2002; Shiba *et al.* 2002).

In *Brassica*, dominance interactions between *S*-alleles are less common in the stigma than they are in pollen. Hatakeyama *et al.* (2001) investigated the role of *SRK* on dominance relationships in the stigma using five *S*-homozygotes carrying a construct of *SRK*₂₈: an *SRK* derived from the stigma dominant *S*₂₈ haplotype. They showed that the dominance relationship between the *SRK*₂₈ transgene and each of the endogenous *S*-haplotypes was identical to that normally observed between these *S*-haplotypes in untransformed plants, indicating that *SRK* alone was sufficient to determine dominance in the stigma. Furthermore, expression levels of the endogenous (recessive) *SRK* alleles were relatively unaffected by the presence of the dominant *SRK*₂₈ transgene, indicating that dominance in the stigma is not regulated by differential expression of *SRK* alleles. Dominance in the stigma must therefore be controlled at a different level to dominance in the pollen, perhaps at the level of interaction between *SRK* and *SCR/SP11*, or the relative abilities

of dominant and recessive *SRK* alleles to recruit downstream signalling factors such as ARC1 (Hatakeyama *et al.* 2001).

6. SSI IN OTHER FAMILIES

SSI has been identified in six families other than the Brassicaceae: the Asteraceae, Convolvulaceae, Betulaceae, Caryophyllaceae, Sterculiaceae and Polemoniaceae (discussed by Goodwillie 1997). Among these families SSI has been investigated at a molecular level only in the Convolvulaceae and the Asteraceae (see §§ 7 and 8). Intriguingly, in the Polemoniaceae, there are reports of both SSI and GSI in *Linanthus parviflorus* (Goodwillie 1997) and *Phlox drummondii* (Levin 1993), respectively. To our knowledge these are the only reported cases of the presence of two genetically distinct SI systems within the same family. From the SI literature it has always appeared that SI species from a given family share the same genetic mode of SI (Hiscock & Kues 1999). Even so, there are convincing reports of the presence of a cryptic GSI system operating alongside the SSI system in the Brassicaceae and Asteraceae (reviewed by Lewis 1994) but these genetic analyses have largely been ignored by commentators on SI, perhaps because they confuse and obscure the distinct genetic boundary between GSI and SSI.

What these reports indicate is that the genetic boundaries between GSI and SSI may not be so rigid as is generally believed, and it may be possible for one SI system to evolve into the other simply by a switch in the timing of expression of the pollen *S*-gene. This was first suggested by Lundqvist (1990, 1994) who identified a system of SSI in *Cerastium arvense* and *Stellaria holostea* (Caryophyllaceae) with traces of gametophytic control in the pollen and no evidence of the dominance interactions usually so characteristic of SSI in other families. 'Fast' *S*-alleles showed sporophytic expression of the *S*-phenotype whereas 'slow' *S*-alleles followed a gametophytic pattern of expression. Lundqvist believed that this flexibility in the timing of pollen *S*-gene expression in the Caryophyllaceae provided critical evidence that SSI could evolve from GSI by a permanent switch in the timing of pollen *S*-gene expression. The recent finding of dual sporophytic and gametophytic expression of class I *SCR/SP11* alleles in *Brassica* (Takayama *et al.* 2000) may be relevant to Lundqvist's hypothesis, because gametophytic expression of class I *SCR/SP11* alleles could be relictual, reflecting the evolution of the present SSI system from a more ancient system of GSI that also used *SCR* and *SRK*. Interestingly, almost identical patterns of gene expression have been observed for an *Antirrhinum* gene, *AhSLF-S2*, which is a strong candidate for the male determinant of S-RNase-mediated GSI (Lai *et al.* 2002).

To gain more insights into the complexity and evolution of SI systems it is important to study more SI species at a molecular level in families that have hitherto received little attention. In recent years two such families have been targeted for molecular studies of SSI: the Convolvulaceae, using *Ipomoea trifida* (Kowyama *et al.* 2000) and the Asteraceae, using *S. squalidus* (Hiscock 2000b).

7. SSI IN *IPOMOEA TRIFIDA*

Ipomoea trifida (Convolvulaceae), a close diploid relative of hexaploid sweet potato (*I. batatas*), possess a very strong system of SSI characterized by rapid arrest of pollen development before emergence of a pollen tube (Kowyama *et al.* 1994). Preliminary investigations into the molecular basis of SSI in *I. trifida* focused on attempting to identify orthologues of *Brassica* *SRK* and *SLG* genes (Kowyama *et al.* 1995). Four distinct cDNAs were identified in pools of stigma mRNAs showing *ca.* 40% amino acid identity to the *Brassica* *SLG* and receptor region of *SRK*. One clone, *IRK1*, was expressed exclusively in stigma tissues and showed a pattern of developmental regulation suggestive of a female *S*-gene, with optimal expression at, or just after, anthesis. Segregation analysis, however, showed that *IRK1* was not linked to the *S*-locus (Kowyama *et al.* 1996). In an approach similar to that used originally to identify *SLG* and *S*-RNases in *Brassica* and *Nicotiana*, respectively, Kowyama *et al.* (2000) then used two-dimensional gels to detect polymorphic stigma-specific proteins associated with defined *S*-alleles. Four acidic *ca.* 70 kDa stigma proteins with variable isoelectric points (pIs ranging from 4–6) were identified, each associated with one of four different *S*-alleles. The genes encoding these proteins were cloned and shown to encode allelic forms of the same putative enzyme, a non-metallo *SCAD* (Kowyama *et al.* 2000). These *SCAD* alleles shared an overall amino acid identity of 95–98% and were expressed specifically in stigmas with maximal expression coinciding with anthesis, when SI becomes fully functional. These characteristics made *SSP* a strong candidate for the female *S*-gene but segregation analysis failed to confirm complete linkage to the *I. trifida* *S*-locus: recombination analysis placing *SSP ca.* 1.2 cM from the *S*-locus (Kowyama *et al.* 2000). Current attempts to identify further *S*-linked markers in *I. trifida* are now focused on using amplified fragment length polymorphism analysis of stigma cDNAs from different *S*-haplotypes (Kowyama *et al.* 2000; Y. Kowyama, personal communication).

8. SSI IN *SENECIO SQUALIDUS* (ASTERACEAE)

Senecio squalidus (Oxford ragwort) was chosen as the model species for our studies of SSI in the Asteraceae because it has a fairly short life cycle (usually two to three generations are possible per year) and is easy to cultivate and propagate vegetatively through cuttings. Of equal importance was the unusual population biology of *S. squalidus*. All British populations of *S. squalidus* are believed to be descended from an original 'collection' of a small number of plants collected from the slopes of Mount Etna (Sicily) and propagated in the Oxford Botanic Garden during the early 1700s (Harris 2002). Plants from this original stock escaped from the gardens and spread across Britain as the railway system expanded during the nineteenth century, the clinker beds providing ample ideal habitats. Such a founder effect would have no doubt created a population bottleneck coinciding with a reduced diversity of *S*-alleles. With a sporophytic SI system this low *S*-allele diversity might have been predicted to impose severe constraints on mating but this is clearly not the case, because *S. squalidus* has proved to be an

extremely successful colonist. It was therefore reasoned that a population genetic study of SSI in *S. squalidus* might provide important more general insights into the evolution and maintenance of SSI systems. Work on SSI in *S. squalidus* is therefore split into two main areas of investigation: (i) genetics and population genetics of SSI, and (ii) molecular determination of the mechanism of SSI. I will now summarize progress to date in these two areas.

(a) *Genetics and population genetics of SSI in S. squalidus*

Full diallel crosses of F_1 progenies derived from forced selfs confirmed that SI in *S. squalidus*, as for other members of the Asteraceae, was sporophytic and controlled by a single, potentially polymorphic *S*-locus (Hiscock 2000a,b). In the four parental plants selected for generating F_1 self-progenies, just four *S*-alleles were identified, even though the plants were obtained from different Oxford localities, suggesting that the prediction of low numbers of *S*-alleles in British populations of *S. squalidus* might be correct. This was confirmed by an extensive population genetic analysis of *S*-allele diversity in an Oxford population that identified just six *S*-alleles (Brennan *et al.* 2002) in 26 individuals. Estimates of *S*-allele diversity in a further seven UK populations predicted that the entire UK population of *S. squalidus* may contain no more than 7 to 11 *S*-alleles (Brennan *et al.* 2003a).

A further intriguing finding of these population genetic studies was the high level of dominance interactions observed between *S*-alleles within populations. Individuals within a given population could be assigned an *S*-genotype based solely on the expression of just one designated *S*-allele showing complete dominance in pollen and stigma (Brennan *et al.* 2002). Such high levels of *S*-allele dominance are unusual and have not been recorded in population genetic studies of species from the Brassicaceae. Only in wild populations of *I. trifida* have correspondingly high levels of dominance previously been recorded (Kowyama *et al.* 1994; Brennan *et al.* 2003a). As discussed earlier, high levels of dominance interactions between *S*-alleles have the effect of increasing compatibility between SSI individuals within a population. If the number of *S*-alleles in a population is low, dominance will increase mating potential between individuals sharing recessive *S*-alleles, so an increase in dominance is predicted to be an evolutionary response to reduced *S*-allele frequency (Byers & Meagher 1992). Our data suggest that *S. squalidus* may be the empirical proof of this theoretical prediction, but comparisons are needed of *S*-allele frequency and dominance interactions between UK and Sicilian *S. squalidus* populations before we can be sure.

Genetic studies of SSI in *Senecio* also highlighted many crossing 'anomalies', many of which could not be fitted into a sporophytic model of SI (Hiscock 2000b; D. Tabah and S. Hiscock, unpublished data). These crossing anomalies usually resulted in compatibility when incompatibility was predicted and probably reflect the activity, in different genetic backgrounds, of 'modifier loci' unlinked to the *S*-locus. Two types of crossing anomaly have been noted in *S. squalidus*: (i) anomalies that can be explained by changes in the dominance relationships between *S*-alleles in pollen and/or stigma; and (ii) anomalies that fit

no sporophytic SI explanation and where compatibility must result from a silencing of the *S*-alleles (D. A. Tabah and S. J. Hiscock, unpublished data). It is possible that this second class of crossing anomaly may be produced by the activity of a modifier similar or equivalent to the diallelic gametophytic locus, *G*, first identified in SSI *Brassica* and *Raphanus* by Lewis and co-workers (Lewis *et al.* 1988; Zuberi & Lewis 1988). This is because all plants remained strongly self-incompatible even though their *S*-alleles were apparently silent in crosses with individuals sharing one or two *S*-alleles. Segregation studies are currently being carried out to test for such a *G*-gene system in *S. squalidus* but so far the compatibility relationships are proving extremely complicated and difficult to interpret. Whatever the genetic basis or bases of crossing anomalies in *S. squalidus*, their direct effect on the mating system is to increase compatibility between SI individuals within a population, and so provide a further means of ameliorating the effect of low numbers of *S*-alleles on mating potential.

The predicted population bottleneck experienced by *S. squalidus* during its introduction and early invasion of the UK might have been predicted to favour selection of individuals with weakened SSI or mutations leading to self-compatibility and therefore increased reproductive assurance. Indeed, breakdown of SSI leading to self-compatibility in *Aster furcatus* appears to have been induced by insufficient *S*-allele diversity in a declining population (Reinartz & Les 1994). However, our extensive studies of SSI in eight UK populations of *S. squalidus* have identified just two partially self-compatible individuals, indicating that selection does not appear to be favouring a breakdown of SSI in UK *S. squalidus* (Brennan *et al.* 2002, 2003b; A. Brennan, S. Harris and S. Hiscock, unpublished data). It thus seems probable that high levels of dominance between *S*-alleles and the activity of modifier loci may be sufficient to maintain a fully functional SSI system in *S. squalidus*, by reducing the potential negative reproductive assurance consequences arising from having so few *S*-alleles (Brennan *et al.* 2002, 2003a,b). Notwithstanding these genetic characteristics of the SSI system, ecological and life-history characteristics of *S. squalidus*, such as its perennial (albeit short-lived) habit, its long flowering season and production of multiple inflorescences per individual, must also contribute significantly to increasing mate availability and therefore gene flow in this strongly self-incompatible colonist (Brennan *et al.* 2003b).

(b) Molecular determination of SSI in *S. squalidus*

As a first step towards identifying putative *S. squalidus* genes involved in SSI it seemed logical to look for stigma-expressed orthologues of the *Brassica* *SRK* gene. Southern blot analyses using *Brassica* *SRK*₆₃ as a probe revealed the presence of *SRK*-like sequences within the *Senecio* genome (Hiscock *et al.* 2003). Potential *SRK*-like cDNAs were then amplified from *S. squalidus* stigma mRNAs using RT-PCR with degenerate primers designed to conserved regions of *Brassica* *SRK*s. Sequencing these PCR products identified three *SRK*-like cDNAs, designated *SSRLK1*, *SSRLK2* and *SSRLK3* (Hiscock *et al.* 2003). At the amino acid level, *SSRLK*s were ca. 35–46% identical to *Brassica* *SRK*s and *IRK1*, an *S*-receptor-like kinase from *I. trifida*

Figure 3. Phylogenetic (parsimony) tree showing relationship between the predicted amino acid sequences of three *Senecio squalidus* *S*-receptor-like kinase (*SSRLK*) cDNAs (underlined) and amino acid sequences of corresponding regions of *Brassica* *SRK*s (*Bo SRK*s) and the putative protein encoded by an *Ipomoea trifida* *SRK*-like gene, *IRK1* (*ItIRK1*). *AtLRK* is a leucine-rich receptor kinase from *Arabidopsis thaliana* and *ZmPK1*, *ZmSERK1* and *ZmSERK2* are receptor kinases from *Zea mays*. The *S. squalidus* *SSRLK*s form a distinct monophyletic group, as do the *Brassica* *SRK* sequences. The parsimony tree is constructed for 295 characters (characters are unordered, of equal weight, six are constant, 71 variable, 218 parsimony informative). Branch lengths are given as the mean number of substitutions. Adapted from Hiscock *et al.* (2003).

(figure 3). Preliminary RFLP analyses suggest that none of the three *SSRLK*s is linked to the *S*-locus of *S. squalidus*, as was also shown for *SRK*-like sequences from *I. trifida* (Kowyama *et al.* 1996). Furthermore, expression of all three *SSRLK*s is not specific to stigmas, with each *SSRLK* showing some expression in vegetative tissues (Hiscock *et al.* 2003), again suggesting that *SSRLK*s are unlikely to be involved in SSI in *S. squalidus*.

As an alternative route to identifying putative *S*-genes in *Senecio*, stigmatic protein profiles of plants segregating for two defined *S*-alleles, *S*₁ and *S*₂, were analysed using isoelectric focusing. Two protein bands with basic isoelectric points of 8.2 and 7.1 were identified associated with the *S*₁ and *S*₂ alleles, respectively (Hiscock *et al.* 2003). These proteins were designated SSPs 1 and 2, respectively, and in small (10–15 individuals) segregating populations, each SSP remained associated with its respective *S*-allele, suggesting that the *SSP* gene may be linked to the *S*-locus. Purification followed by N-terminal amino acid sequencing revealed SSP1 and SSP2 to represent allelic forms of the same 35–40 kDa glycoprotein. Full-length cDNAs have been obtained for the *SSP1* and *SSP2* genes using 3' and 5' RACE and sequencing has shown them to encode putative proteins of 308 amino acids (35 kDa). Northern blot analysis showed that *SSP1* and *SSP2* are expressed exclusively in stigmas and flower buds, expression beginning ca. 1–2 days before anthesis, when SI is becoming fully functional (Hiscock *et al.* 2003). This pattern of tissue specificity and developmental regu-

Figure 4. Simplified phylogenetic tree showing the main angiosperm clades and the scattered distribution of families with SSI (underlined). (Based on Soltis *et al.* 1999).

lation makes *SSP* a promising candidate for the female determinant of SSI in *S. squalidus*. Segregation studies are currently being carried out on *SSP1* and *SSP2* using an *EcoR1* RFLP to determine linkage of *SSP* to the *S*-locus.

9. CONCLUSIONS

The Brassicaceae, Asteraceae and Convolvulaceae all possess SSI but are not closely related (figure 4). Indeed, the Brassicaceae is more closely related to the Rosaceae, whereas the Convolvulaceae is a sister family to the Solanaceae (Soltis *et al.* 1999): both Rosaceae and Solanaceae exhibiting well-documented S-RNase-based GSI systems (Franklin-Tong & Franklin 2003). Strong phylogenetic evidence has recently been put forward for a monophyletic origin of S-RNase-based GSI in the Rosaceae, Solanaceae and Scrophulariaceae, implying that this form of SI evolved before the divergence of the Rosid and Asterid clades of higher eudicots (Igic & Kohn 2001). If this is correct then SSI in the Brassicaceae (Rosid clade), Convolvulaceae (Asterid) and Asteraceae (Asterid) must be a derived character that evolved more recently and, most probably, independently in each of these three families. Indeed evidence from molecular studies of SSI in *I. trifida* (Kowyama *et al.* 2000) and *S. squalidus* (Hiscock *et al.* 2003) provide strong evidence for novel mechanisms of SSI in these two families because stigmatically expressed orthologues of *Brassica SRK* appear not to be involved in the SSI response. These findings suggest that at least the SSI system of the Brassicaceae probably evolved independently, but until the determinants of SSI in *I. trifida* and *S. squalidus* have been fully characterized, the homology between the SSI systems of the Convolvulaceae and Asteraceae cannot be determined.

The suggestion that there may be different, independently evolved, mechanisms of SSI operating in the Brassicaceae, Convolvulaceae and Asteraceae gains some circumstantial support from comparative observations of the incompatibility response in *Brassica*, *I. trifida* and *S. squalidus*. In *I. trifida*, incompatible pollen grains never produce a pollen tube and therefore possess the 'tightest' form of SI yet observed (Kowyama *et al.* 2000). In *Bras-*

sica, incompatible pollen grains generally undergo limited development before inhibition. Usually incompatible pollen grains germinate to produce a short pollen tube that ceases development soon afterwards (Ockendon 1972), although in some 'strong' *Brassica* S-haplotypes the incompatibility response resembles that of *I. trifida*, with pollen arrest occurring before germination, perhaps following only limited hydration (Dickinson 1995). In contrast to both *I. trifida* and *Brassica*, incompatible pollen tubes of *S. squalidus* tend to undergo far more development before inhibition; indeed many incompatible pollen tubes even penetrate the stigma before arrest (Hiscock *et al.* 2002). Interestingly, in *S. squalidus* when incompatible tubes penetrate the stigma, inhibition of further pollen tube growth is accompanied by a swelling of the pollen tube tip, somewhat reminiscent of the GSI inhibition response in species from the Solanaceae (Hiscock *et al.* 2002). Thus, at a phenotypic level, the stigmatic inhibition that defines the SSI response appears to be subtly different in *I. trifida*, *Brassica* and *S. squalidus*. Whether these subtle phenotypic differences reflect basic differences in the genes regulating SSI in these three species has yet to be determined. Elucidation of the molecular control of SSI in *Ipomoea* and *Senecio* is an exciting prospect for the future of SSI research that will provide crucial insights into the evolution of SSI.

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Discussion

D. Charlesworth (*Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh, UK*). Domi-

nance relationships, and weak SI are not exclusive to Asteraceae, but are also found in *Arabidopsis lyrata* (Brassicaceae). The ‘textbook examples’ are in part enriched for systems with few complexities, while more systems with complications remain unpublished. Dr Rieseberg mentioned earlier in the meeting that complications have prevented his publishing his results from *Helianthus*.

S. Hiscock. This is certainly so. Only when you start looking at SSI in wild species, do you realize how complicated and unpredictable such systems can be. Many years of manipulation by plant breeders has made the *Brassica* SSI system extremely predictable.

A. G. Stephenson (*Department of Biology, Pennsylvania State University, PA, USA*). With the variation in dominance/recessiveness, modifiers etc. should we not consider SI systems as subtle regulators of the breeding system rather than a qualitative trait?

S. Hiscock. *Senecio* is strongly self-incompatible based on hundreds of crosses, but matings with close relatives do not follow GSI–SSI expectations, suggesting more subtle regulation involving modifiers.

GLOSSARY

- GSI: gametophytic self-incompatibility
 PCP: pollen coat protein
 RACE: rapid amplification of cDNA ends
 RFLP: restriction fragment length polymorphism
 SCAD: short-chain alcohol dehydrogenase
 SCR: S-cysteine-rich protein
 SI: self-incompatibility
 SLG: S-locus glycoprotein
 SP11: S-pollen protein 11
 SRK: S-receptor kinase
 SSI: sporophytic self-incompatibility
 SSP: S-locus-associated stigma protein
 SSRK: *Senecio* S-receptor-like kinase