Chapter 5
What Genealogies of S-alleles Tell Us

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Abstract Drawing on examples from S-RNase-based self-incompatibility (SI), *S*-locus genealogies are used to infer the demographic history of lineages, the history of mating-system transitions in entire plant families and aspects of the evolution of the *S*-locus itself. Two lineages of Solanaceae suffered severe restrictions of *S*-locus diversity evident after millions of years. Broadly shared ancestral *S*-locus polymorphism is evidence that loss of this form of incompatibility was irreversible in the Solanaceae. Frequent and irreversible loss implies incompatibility is either declining in frequency through time, or that it confers an increased diversification rate relative to self-compatibility (SC). Differences in diversification rate among self-incompatible and self-compatible lineages likely cause the failure of current phylogenetic methods to correctly reconstruct the history of SI. Genealogies also show that origination of new S-RNases rarely occurs within the lifetimes of species. Surprisingly, genealogies of F-box genes purported to provide pollen specificity often do not correspond to those of their cognate S-RNases, indicating we have much to learn about how this system works and evolves.

Abbreviations

cpDNA	DNA from plant plastids
GSI	Gametophytic SI
mya	Million years ago
SC	Self-compatibility
SCR/SP11	*S*-locus cysteine-rich protein (the pollen *S*-determinant in Brassica)

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V.E. Franklin-Tong (ed.) *Self-Incompatibility in Flowering Plants – Evolution, Diversity, and Mechanisms*.

SFBB	*S*-locus F-box brothers
SI	Self-incompatibility
SLF/SFB	*S*-locus F-box (*SFB/SLF – S-Locus F-box*/*S-haplotype-specific F-box*-the pollen *S*-determinant in many GSI systems; *SLF* in *Antirrhinum* and *Petunia*; *SFB* in *Prunus*)
S-locus	Self-incompatibility locus
SRK	*S*-locus Receptor Kinase (the pistil *S*-determinant in Brassica)
S-RNase	S-ribonuclease

5.1 Introduction

The self-incompatibility (SI) locus of flowering plants has attracted the attention of population geneticists since extreme *S*-locus polymorphism was described by Emerson (1939) in natural populations of *Oenothera organensis*. Prior to molecular characterisations of the *S*-locus in several plant families, empirical studies focused on characterising the numbers of alleles in populations (reviewed in Lawrence 2000), while the main goal of theoretical treatments (Wright 1939, 1960, 1964; Fisher 1958, 1961; Moran 1962; Ewens and Ewens 1966) was to explain how selection could maintain the large numbers of alleles observed. However, it was appreciated very early on (Kingman 2000; W.J. Ewens personal communication) that the *S*-locus might also provide a unique historical perspective. The advent of *S*-locus sequence data from natural populations of self-incompatible plants (reviewed in Richman and Kohn 2000; Castric and Vekemans 2004) together with the extension of theoretical treatments of the *S*-locus to include coalescent approaches to loci under balancing selection (Takahata 1990, 1993; Takahata and Nei 1990; Clark 1993; Clark and Kao 1994; Vekemans and Slatkin 1994) has brought a resurgence of interest in the population genetics of the *S*-locus, including ways that it may be used to infer historical phenomena and to gain insight into longstanding evolutionary questions.

There are two primary reasons why the *S*-locus has attracted the attention of biologists. First, it is a system of self/non-self recognition and rejection. All such systems have inherent appeal in terms of their mechanisms of action and evolutionary properties. Second, the property that drives the evolution of the *S*-locus is negative frequency-dependent selection; alleles that are rare in a population have more potential mates, while those that are common have fewer. This is the force that Wright (1939) recognised could explain the extreme polymorphism discovered by Emerson (1939), and this is also the force that preserves historical information stretching much further back in time than it is possible to go using standard loci (Takahata 1990; Vekemans and Slatkin 1994). Selection that perpetually increases the frequency of rare alleles preserves polymorphism over very long periods of time. It is this property, extreme age of polymorphism, that is the focus of this chapter.

This chapter primarily uses examples from three angiosperm families, the Solanaceae, Plantaginaceae and Rosaceae, which use S-RNases as the stylar component

of specificity in the gametophytic SI (GSI) reaction (reviewed in Takayama and Isogai 2005); see also Chaps. 9 and Chap. 10 McClure, this volume. What is known from S-RNase-based systems is compared to information from the sporophytic *SRK*/*SCR*-based incompatibility in the Brassicaceae. Another, non-homologous, GSI system is known in the Papaveraceae, but sequence information from only five pistil *S*-locus alleles derived from two species of *Papaver* are currently available (Kurup et al. 1998).

Two characteristics of the molecular variation found among S-RNases exemplify the extreme age of *S*-locus polymorphism. First, alleles from the same individual can be extremely divergent. In the Solanaceae, two stylar S-RNase alleles found in the same obligately heterozygous individual often differ at more than 50% in their amino acid residues (Richman et al. 1995). Second, phylogenies of *S*-alleles show abundant evidence of shared ancestral polymorphism (Ioerger et al. 1990; Richman et al. 1996b; Richman and Kohn 2000; Castric and Vekemans 2004). Alleles drawn from different species and genera cluster together in phylogenetic reconstructions, indicating that the allelic lineages they represent were present in the common ancestor of the species sampled. This pattern of shared ancestral polymorphism confirms theoretical expectations that negative frequency-dependent selection will preserve polymorphism for very long periods of time. In the Solanaceae, much of the observed *S*-locus polymorphism arose prior to the most recent common ancestor of all of the species whose *S*-alleles have been examined (Igic et al. 2004, 2006). This ancestor is thought to have occurred 35–45 million years ago (mya) (Ioerger et al. 1990; Paape et al. 2008). Similar ancient polymorphism exists at the sporophytic *S*-locus of the Brassicaceae (reviewed in Castric and Vekemans 2004).

Polymorphism that persists for tens of millions of years can be used to answer questions about the locus itself, the history of lineages that carry it and the consequences of its loss. Because the *S*-locus enforces outcrossing, its evolution and loss is intimately tied to breeding system transitions between outcrossing and partial or complete selfing. The historical information preserved within *S*-locus polymorphism can be used to trace, with an unusual degree of certainty, the history of such transitions across entire plant families. This provides a unique tool with which to address longstanding problems in biology such as whether selfing lineages are shorter lived than outcrossing ones (Stebbins 1957, 1974; Takabayashi and Morrell 2001), and whether complex biological traits such as self-incompatibility, once lost, can be regained in the same form.

5.2 Long-Term Demographic Information from the *S*-locus

The earliest studies comparing small samples of S-RNase sequences from different Solanaceae (e.g. species of *Petunia*, *Nicotiana* and *Solanum* sect. *Lycopersicon*) found strong evidence of ancestral polymorphism shared among the taxa sampled (Ioerger et al. 1990). However, studies that surveyed *S*-locus variation in natural populations (Richman et al. 1995, 1996a) also found variation in the level of ancestral polymorphism preserved in different genera (Richman et al. 1996b). In

particular, members of the closely allied genera *Physalis* and *Witheringia* have *S*-alleles that represent only three lineages that predate their most recent common ancestor (Richman et al. 1996b; Richman and Kohn 1999; Lu 2001; Stone and Pierce 2005), while all other genera of Solanaceae sampled have *S*-alleles from many more ancient lineages (Richman 2000; Richman and Kohn 2000; Savage and Miller 2006). Richman et al. (1996b) compared the restricted *S*-allele lineage diversity in *Physalis crassifiolia* to the more diverse S-allele assemblage sampled from *Solanum carolinense*. Using a coalescent approach, they showed that the long-term population size of *P. crassifolia* was one or two orders of magnitude smaller than that of *S. carolinense*. This contrasts with current estimates of population size derived from *S*-allele numbers. *P. crassifolia* populations currently harbour far more alleles at the *S*-locus than are found in populations of *S. carolinense*.

While the study of Richman et al. (1996a) used coalescence-based methods as a tool to explore deep historical demographic events, species-specific genealogies of *S*-alleles do not conform standard expectations of a birth–death process (Uyenoyama 1997). In particular, *S*-allele genealogies are more star-like than expected, exhibiting rapid diversification near the base of the genealogy with a subsequent apparent slowdown in the rate of allelic diversification. Explanations for this observation vary. Uyenoyama (1997) advanced the hypothesis that deleterious recessives hitchhiking on the obligately heterozygous *S*-locus retard the apparent diversification rate. Richman and Kohn (1999) found evidence that more divergent alleles were preserved when ecological factors reduce the effective population size and the number of *S*-alleles maintained. Whatever the cause, the more star-like than expected genealogies of S-alleles require that caution be exercised when applying coalescence-based methods to this locus.

Nevertheless, *S*-allele sequences from species of *Physalis* and *Witheringia* provide an extremely strong phylogenetic signal. To date, a total of 93 *S*-allele sequences from three species of *Physalis* (Richman et al. 1996a; Richman and Kohn 1999; Lu 2001) and two of *Witheringia* (Richman and Kohn 2000; Stone and Pierce 2005) have been published and all fall within only three lineages that predate the most recent common ancestor of these genera. A simple question to ask is, how old is this *S*-locus bottleneck? Clearly this event occurred prior to the most recent common ancestor of *Physalis* and *Witheringia*, but more recently than the divergence of these genera from any that do not show restriction of *S*-locus sequence variation. Paape et al. (2008) assayed *S*-alleles from several members of the subtribe Iochrominae (Solanaceae), a group found to be sister to the clade containing *Physalis* and *Witheringia* in a large molecular phylogenetic analysis of Solanaceae (Olmstead et al. in press). Even with a limited sample, *S*-alleles from the Iochrominae represent several lineages not observed in *Physalis* and *Witheringia* (Fig. 5.1). This means that the restriction of the *S*-locus had to have occurred after the most recent common ancestor of the group containing the Iochrominae, *Physalis* and *Witheringia*, but before the most recent common ancestor of *Physalis* and *Witheringia*. Using cpDNA sequence information calibrated with fossil data, Paape et al. (2008) estimated that the most recent common ancestor of *Physalis* and *Witheringia* occurred some 14 mya while their most recent common ancestor with the Iochrominae occurred

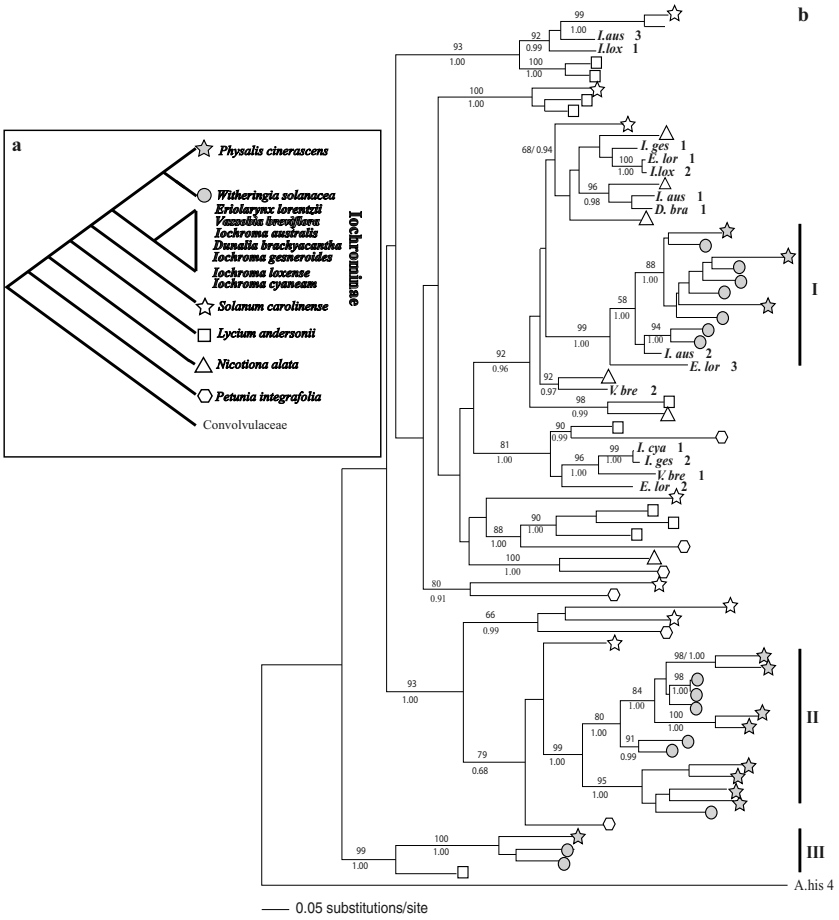

Fig. 5.1 (**a**) The phylogeny of Solanaceae species from which S-alleles were sampled. All nodes in the phylogeny have >90% bootstrap support in the large phylogenetic analysis of Olmstead et al. (in press). (**b**) Maximum-likelihood phylogeny of 72 S-alleles. Symbols correspond to alleles from taxa in the species phylogeny. All alleles from the genera *Physalis* and *Witheringia* are restricted to one of the three lineages indicated by Roman numerals. The 14 alleles sampled from various Iochrominae species (*boldface*) represent several lineages not found in *Physalis* or *Witheringia*, which predate the divergence of *Solanum* from the other genera sampled. The *S*-allele DNA phylogeny was constructed in PAUP* v4.0 (Swofford 2002). Bootstrap scores are indicated above branches and posterior probabilities >80% generated by Mr. Bayes v3.0 (Ronquist and Huelsenbeck 2003) are below branches. Only a subset of alleles known from *Physalis*, *Witheringia* and other Solanaceae were used to simplify the computation and presentation of the data. For additional details, see Paape et al. (2008) from which the figure is redrawn

approximately 18 mya. The restriction of *S*-locus variation had to have occurred between these two dates, exemplifying the great age of the historical information available from the *S*-locus.

A more difficult question than the age of the restriction in *S*-locus variation in *Physalis* and *Witheringia* is what could have caused it. Because of the power of

negative frequency-dependent selection to preserve variation, the *S*-locus is particularly resistant to all but the most severe types of bottleneck events. For instance, a population with constant size of only 100 individuals nevertheless maintains six alleles at equilibrium (Wright 1939). A weak bottleneck followed by allelic turnover that further reduced the number of *S*-lineages appears unlikely, given the great strength of selection preserving alleles when their number is below equilibrium, and also given the slow pace of allelic turnover observed elsewhere in the Solanaceae. Extreme types of bottleneck events would apparently be required to explain the fact that only three ancient lineages are represented among the *S*-alleles of *Physalis* and *Witheringia*. A founder event involving perhaps as few as two individuals would seem the only likely way for such a restriction to arise, but also would require the lack of further gene flow between source and founder populations. Subsequent gene flow would almost certainly introduce additional lineages of *S*-alleles. Whatever its cause, information to date suggests that lineages rarely survive such restrictions and remain self-incompatible. Observations of species whose *S*-alleles represent many ancient lineages, the common finding, imply that no ancestor of those species suffered a similar restriction at the *S*-locus.

Only one study has found restricted *S*-locus variation outside of *Physalis* and *Witheringia*, and this bottleneck seems certain to have been caused by a founder event associated with long-distance dispersal. Miller et al. (2008) examined *S*-alleles from several self-incompatible species of *Lycium* from southern Africa. The genus *Lycium* is found primarily in the new world, and phylogenetic analyses (Levin and Miller 2005; Levin et al. 2007) strongly suggest that it originated in South America. However, *Lycium* species are also found on several oceanic islands as well as southern Africa. African species are monophyletic within the genus, suggesting a single colonisation event brought *Lycium* to the Old World. Fewer *S*-allele lineages that pre-date the genus *Lycium* are represented among African *Lycium* S-alleles than are found among samples of the same size from several New World species (Miller et al. 2008). In fact, current data are consistent with the idea that as few as three individuals could have made up the founding Old World population, though that estimate could rise with additional sampling. The restriction of *S*-locus variation in Old World *Lycium* is estimated to have occurred less than 10 mya. However, it pre-dates the diversification of the monophyletic assemblage of more than 30 African species of *Lycium,* providing another stunning example of the time-depth of information preserved at the *S*-locus.

5.3 Implications of Shared Ancestral Polymorphism

5.3.1 Tracing the History of Mating System Change

In addition to demographic information, ancestral *S*-locus polymorphism provides important historical implications about breeding systems that extend back in time to the most recent common ancestor of all SI Solanaceae. For instance, species

of *Brugmansia, Lycium*, *Nicotiana, Petunia, Physalis, Solanum*, and *Witheringia* have been assayed for *S*-locus polymorphism (Igic et al. 2006 and references therein). Each one possesses *S*-alleles representing multiple lineages that were present in the common ancestor these genera. This implies a continuous history of self-incompatibility from the time of that common ancestor to the present (Igic et al. 2004, 2006).

What justifies the assertion that all ancestors of sampled SI species were themselves SI going all the way back to the most recent common ancestor of those species? When SI is lost, polymorphism at the *S*-locus is rendered selectively neutral and is expected to collapse in 4 N_e generations (Hudson 1990), or sooner if loss of SI is caused by a selective sweep of a non-functional S-allele. Once polymorphism is lost, the GSI system cannot be regained because, with fewer than three different alleles, all individuals are mutually incompatible (Wright 1939). Even if it were possible to regain the system following complete collapse of *S*-locus polymorphism, such an occurrence would leave an indelible mark on the *S*-locus; a lineage whose entire complement of *S*-alleles forms a monophyletic clade relative to *S*-alleles from other taxa. This has never been observed in the Solanaceae. In addition, once SI is lost, mutations in other genes whose products are required for self-incompatibility to operate are expected to accumulate. Little or no polymorphism at the *S*-locus, together with multiple loss of function mutations, are commonly observed in SC taxa recently derived from SI ones (Stone 2002; Igic et al. 2008).

Character states of ancestral taxa, and transition rates among character states, are usually reconstructed based solely upon the character state distribution among extant taxa (Pagel 1999). Compared to this situation, shared ancestral polymorphism provides an unparalleled degree of certainty regarding the character states of ancestors. Igic et al. (2004, 2006) used evidence from shared ancestral polymorphism as an aid in reconstructing the history of self-incompatibility throughout the Solanaceae. The Solanaceae comprises some 2,600 species, an estimated 40% of which are SI (Whalen and Anderson 1981; Igic and Kohn 2006). SI and SC taxa are broadly intermixed on the family phylogeny, as is common in large families with self-incompatibility (Heilbuth 2000; Ferrer and Good-Avila 2007; see also Chap. 4, this volume). When only the self-incompatibility status of extant taxa are considered, standard methods (Pagel 1999) of reconstructing character state transition rates find strong statistical support for multiple gains of SI within the Solanaceae, rejecting the hypothesis that SI has never been regained once lost. However, when inference from shared ancestral *S*-locus polymorphism is used to unite all Solanaceae whose *S*-alleles have been sampled with a continuous history of SI, the hypothesis that the rate of transition from SC to SI in the Solanaceae is zero cannot be rejected (Igic et al. 2006). Within the Solanaceae, self-incompatibility follows Dollo's law (Gould 1970) that a complex character, once lost, is never regained.

Dollo's law is subject to several interpretations (Bull and Charnov 1985). The meaning here is not that SI, once lost, is never regained by some new mechanism, but that RNase-based SI has not been regained once lost. Across the angiosperms, it is clear that multiple gains of various forms of incompatibility have occurred (Weller

et al. 1995; Igic et al. 2008). Nevertheless, the rate of gain of incompatibility systems is very far exceeded by the frequency of loss.

A similar phylogenetic analysis concerning the history of sporophytic SI in the Asteraceae (Ferrer and Good-Avila 2007) also finds many cases of closely related extant taxa with alternative SI and SC character states; see also Chap. 4, this volume. No molecular information concerning the basis of SI is available for Asteraceae. Ferrer and Good-Avila (2007) used standard reconstruction procedures (Pagel 1999) to conclude that multiple gains of incompatibility occurred in the family, precisely the conclusion that is reached in the Solanaceae in the absence of information from shared ancestral polymorphism (Igic et al. 2004, 2006). Since molecular evidence of shared ancestral polymorphism in the Solanaceae controverts the conclusion of multiple origins of SI, the finding of multiple origins of SI within the Asteraceae, based solely on the distribution of character states among extant taxa, must be viewed with caution.

5.3.2 Diversification Rate Differences and Character State Reconstruction

Two questions arise from the observation that shared ancestral polymorphism provides evidence for irreversible loss of self-incompatibility at family level. First, if SI is frequently lost but never regained within the Solanaceae, is SI becoming less frequent through time? If not then SI species must, on average, have a higher diversification rate (defined as the speciation rate minus the extinction rate) than SC taxa. The second question is, why do methods of estimating character state transition rates and ancestral states (Pagel 1999) fail when only the character states of extant taxa are used? The answer to this second question is likely to be related to the answer to the first, as will be seen below.

Using simple macroevolutionary models that assume irreversible transitions from SI to SC, Igic et al. (2004, 2008) showed that the frequency of SI species will decline unless the diversification rate associated with self-incompatibility is greater than the sum of the diversification rate associated with self-compatibility plus the transition rate of species from SI to SC states. A remaining challenge is to calculate character state specific rates of diversification and the SI to SC transition rate from detailed phylogenetic data. Doing so will test whether SI is being maintained due to some macro-evolutionary advantage it provides. Stebbins (1974) argued that the transition from outcrossing enforced by self-incompatibility to predominant selfing was the most commonly repeated evolutionary pathway in flowering plants. In his view, this line of evolution was often a short-term solution to some ecological challenge but tended to be an evolutionary dead end (Stebbins 1957), an assertion that has proven difficult to test (Takabayashi and Morrell 2001). Macro-evolutionary approaches such as those outlined above will provide useful evidence in this regard.

If SI does provide an increased diversification rate relative to SC, it is unlikely to do so by increasing the speciation rate of taxa that possess it. Selfing taxa usually show greater levels of inter-population genetic differentiation (Hamrick and

Godt 1989), and would therefore be expected to speciate more readily than outcrossing taxa. However, enforced outcrossing might reduce the rate of extinction relative to selfing taxa, due to increased levels of genetic variation preserved within outcrossing populations (Hamrick and Godt 1989; Charlesworth and Charlesworth 1995; Glémin et al. 2006).

Why do commonly used methods of ancestral state and transition rate estimation fail? A little-appreciated assumption of the models underlying these methods is that the character states do not themselves affect the diversification rate (Igic et al. 2006; Maddison 2006). If diversification rates differ among character states, current methods will tend to overestimate the transition rate towards the state that provides the greater diversification rate (Maddison 2006). This could explain the substantial transition rates from SC to SI that are inferred in the Solanaceae and Asteraceae when evidence from shared ancestral polymorphism is not used in the analyses (Igic et al. 2004, 2006; Ferrer and Good-Avila 2007).

5.4 The Pace of New Allele Formation

Another area of *S*-locus evolution where genealogical approaches can provide insight concerns the evolution of new *S*-alleles. Different pollen and pistil expressed genes encode specificity in all incompatibility systems that have been characterised at the molecular level. This brings up the knotty problem of how new alleles arise, because a mutation changing the specificity of, for instance, the pollen would result in a haplotype that is self-compatible and potentially lost from the population if self-compatibility is selected against. Mutations in both the female and the male components of the system would apparently be required to form a new specificity (Charlesworth 2000). Several interesting proposals for how new alleles can arise have been suggested (Matton et al. 1999; Uyenoyama and Newbigin 2000; Uyenoyama et al. 2001, Chookajorn et al. 2004), but all require that polymorphism within an allele be maintained either within or between populations while the appropriate mutations are accumulating. Negative frequency-dependent selection, while tending to preserve extreme polymorphism among alleles, is expected to have the opposite effect on polymorphism within alleles, reducing polymorphism below levels found at standard loci. If n alleles are maintained in a population, the population effective size of each allele is $1/n$ times the effective size of the population (Clark 1993) and the expected level of polymorphism is reduced accordingly. In fact, the extreme difference in within- vs. among-allele polymorphism can be used to confirm that balancing selection is acting on a locus, as has been done for the mating-type loci of fungi (May et al. 1999).

Genealogical approaches can ask at least two questions relevant to the formation of new *S*-alleles. First, what is the level of polymorphism within functionally equivalent alleles either within or between populations? Since polymporphism is necessary for the transitional stages of new allele formation under current models, polymorphic alleles could represent opportunities to study how this process occurs.

Second, what is the tempo of new allele formation relative to species formation? If, species-specific monophyletic clades of alleles are common, then new alleles are frequently arising within the lifespan of species. If, on the other extreme, the closest relative of each allele is found in some other species, then new allele formation would appear to be extremely rare relative to the lifespan of species, and finding transitional stages in the process of new allele formation would require interspecific comparisons (Sato et al. 2006; Surbanovski et al. 2007).

Raspé and Kohn (2007) examined S-RNases from *Sorbus aucuparia* (Rosaceae, subfamily Maloideae) from the Pyrenees Mountains and compared them to sequences recovered previously from a population in Belgium (Raspé and Kohn 2002). Of the alleles recovered, 10 were found in both populations. Nine out of the ten alleles showed no nucleotide polymorphism, despite the 1,000 km separation of the two populations. The tenth showed a single nucleotide change, but this could not be confirmed as only a single sequence was recovered from the original Belgian population. These findings confirm the theoretical prediction that polymorphism within alleles will be rare (Clark 1993). In addition, phylogenetic analysis finds that nearly every allele from *Sorbus aucuparia* is more closely related to an allele from another genus (other Maloideae assayed are members of the genera *Crataegus*, *Malus* or *Pyrus*) than to other alleles in this species (Fig. 5.2). The same is true of the other Maloideae. Monophyletic clades of alleles drawn from single species are rare, represented by only two intra-specific sister pairs, neither of which is strongly supported by bootstrap analysis (Fig. 5.2; Raspé and Kohn 2007). This implies that stylar S-alleles have rarely diversified since the origination of the species in which they now occur, or that whenever a new allele does arise, it displaces its progenitor allele, leaving no apparent diversification (Uyenoyama et al. 2001).

The data from the Maloideae contrast with findings in the Brassicaceae, where within-specificity polymorphism has been documented in *Brassica oleracea* (Miege et al. 2001) and where studies of similar alleles in closely related species are beginning to unlock stages in the development of new specificities (Sato et al. 2006). In addition, we know from studies of Solanaceae that diversification rates of S-alleles increase following bottlenecks (Richman 2000; Paape et al. 2008; Miller et al. 2008), as is expected with greater selection when the population is below equilibrium allele number. The slow pace of recent diversification among the Maloideae may reflect demographic stasis and reduced selection for new alleles. To date both the small number of SI systems that have been characterised at the molecular level, and the limited sampling of natural populations that has been done within each of them, restrict our ability to draw firm conclusions about how and how rapidly new alleles arise.

5.5 Remaining Issues of S-RNase Evolution

Some major questions remain unanswered concerning the evolution of stylar S-RNases. Most pressing is the apparent disparity in patterns of diversification seen in the Solanaceae and Plantaginaceae relative to what is observed in the Rosaceae. The

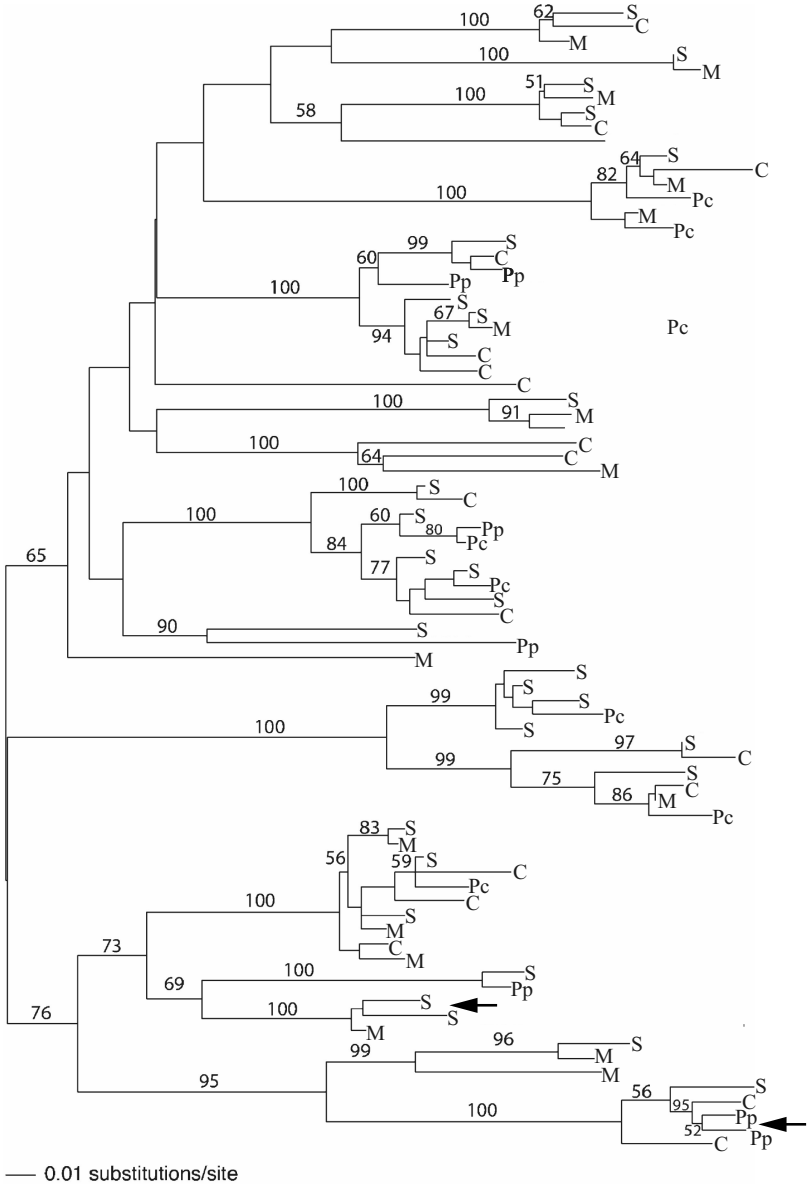

Fig. 5.2 Midpoint-rooted maximum-likelihood phylogeny of 80 S-alleles from the Maloideae (redrawn from Raspé and Kohn 2007). Arrows indicate the only two instances in which sister alleles are drawn from the same species. Neither sister relationship receives strong bootstrap support

Solanaceae and Plantaginaceae appear to harbour similar high levels of nucleotide diversity and shared ancestral polymorphism at the S-RNase locus, though the Plantaginaceae is currently less well-sampled (Xue et al. 1996; Vieira and Charlesworth 2002). Compared to these families, S-RNases from the Rosaceae have lower levels of nucleotide diversity and S-RNases from two subfamilies (Maloideae and Amygdaloideae (*Prunus*)) form reciprocally monophyletic clades (Igic and Kohn 2001; Ma and Oliviera 2002; Steinbachs and Holsinger 2002). Population allele numbers in species of Rosaceae and Solanaceae appear similar, and so smaller population sizes appear unlikely to have caused the increased turnover seen in Roasaceae *S*-allele genealogies. In addition, the Rosaceae appears to be no older than the Solanaceae (Wikström et al. 2001) so increased time is an unlikely cause of increased turnover.

The genealogies of Maloideae and Amygdaloideae *S*-RNases also differ from one another. Those from *Prunus* show very little phylogenetic structure. That is, *S*-RNase genealogies from species of *Prunus* are extremely star- or comb-like, with very few internal branches achieving statistical support from bootstrap analyses (Nunes et al. 2006). More structure is seen in genealogies of Maloid *S*-RNases where genealogical relationships among alleles are often strongly supported (Fig. 5.2; Raspé and Kohn 2007). These puzzling findings suggest that *S*-RNase evolution in the Rosaceae, particularly in *Prunus*, differs in some important respects from the Solanaceae and Plantaginaceae. One possibility is increased levels of recombination in the Rosaceae. Recombination reduces apparent coalescence time and could both increase the observed rate of turnover and reduce the internal structure of gene genealogies (Schierup et al. 2001). To date, however, evidence for recombination at the *S*-RNase locus is mixed (Vieira et al. 2003; Nunes et al. 2006) and no study has demonstrated increased rates of recombination in the Rosaceae relative to other families using S-RNase-based gametophytic SI.

5.6 Pollen Specificity Genes

While the pistil *S*-RNases have been studied now for more than two decades, only recently has the pollen specificity component of these systems begun to be elucidated (Entani et al. 2003; Ikeda et al. 2004; Qiao et al. 2004; Sijacic et al. 2004; Ushijima et al. 2003, 2004; Sassa et al. 2007). Expectations for the gene for the pollen specificity component of any self-incompatibility system are the same as those for the pistil component: tight linkage to the cognate locus, unusually high nucleotide polymorphism, long coalescence time, and evidence at the molecular level for positive selection. In addition, genealogies of female and male specificity genes should be concordant, reflecting long coevolutionary histories. Recombination events between pollen and style genes should result in self-compatible haplotypes, expected to be lost from the population if SI is selected for.

In the Brassicaceae, where *SRK* and *SCR* (also known as *SP11*) genes specify pistil and pollen specificity, respectively (reviewed in Takayama and Isogai 2005; see

also Chaps. 6 and 7, this volume), these expectations are met. High levels of synonymous and non-synonymous nucleotide polymorphism are seen in both genes, though polymorphism may be somewhat higher in *SCR*/*SP11* than *SRK* (Sato et al. 2002). This could reflect a greater proportion of the pollen than the pistil gene under diversifying selection. Importantly, Sato et al. (2002) found that the genealogies of twelve pollen and twelve pistil alleles were largely congruent. The hypothesis of strict co-evolution of linked pollen and pistil genes, expected for the *S*-locus, could not be rejected.

The recent discoveries implicating F-box proteins as the pollen specificity component of the *S*-locus in all three families that utilise S-RNases (Entani et al. 2003; Ikeda et al. 2004; Qiao et al. 2004; Sijacic et al. 2004; Ushijima et al. 2003, 2004; Sassa et al. 2007; see also Chaps. 9 and 10, this volume) held the promise of rapid gains in understanding the co-evolution of pistil and pollen components in this system. Phylogenetic clustering and structural similarity of S-RNases relative to other plant RNases led to the conclusion that *S*-RNase-based incompatibility is homologous among the Solanaceae, Plantaginaceae and Rosaceae (Igic and Kohn 2001; Steinbachs and Holsinger 2002). The fact that all three families are reported to use *S*-locus *F*-box genes as the pollen specificity component of the SI reaction (see Chap. 9, this volume) would appear to bolster the case for homology of these systems. The alternative, that convergent evolution led to the independent evolution of *S*-RNase/F-box incompatibility systems in different families and/or subfamilies is possible, but we so far lack good candidates for independent ancestral RNases (Igic and Kohn 2001; Steinbachs and Holsinger 2002).

However, several aspects of current findings regarding *S*-locus F-box genes differ very markedly from what would be expected if specificity determining F-Box and *S*-RNase genes had the same evolutionary history (see Chap. 10, this volume). Multiple F-box loci are found at the *S*-locus of each family or subfamily known to use *S*-RNase-based incompatibility (Sassa et al. 2007; Wheeler and Newbigin 2007). This is not surprising given that over 600 F-box loci found are found in the genome of *Arabidopsis thaliana* (Wang et al. 2004). What is surprising is that sequences of F-box genes from the *S*-loci of *Petunia* (Solanaceae), *Antirrhinum* (Plantaginaceae), *Malus* and *Pyrus* (Rosaceae:Maloideae) and *Prunus* (Rosaceae:Amygdaloideae) form family- or subfamily-specific monophyletic clades in phylogenetic reconstructions (Sassa et al. 2007; Wheeler and Newbigin 2007). This means that the locus implicated as encoding pollen specificity in a particular group is more closely related to the other F-box genes at its *S*-locus than to the F-box genes purported to specify pollen mating type in other groups. This contrasts sharply with S-RNases where, for instance, Plantaginaceae and Solanaceae S-RNases form a monophyletic clade relative to other plant RNases (Igic and Kohn 2001; Steinbachs and Holsinger 2002).

Second, levels of polymorphism of the F-box genes thought encode pollen specificity are surprisingly low in some taxa, while in others they seem to conform to expectations of long coalesence times. In *Prunus* a *S*-linked F-Box locus (*SFB*) has been identified that has similar levels of polymorphism as the S-RNase locus, and which shows evidence of positive selection at the molecular level (Ikeda et al. 2004;

Nunes et al. 2006). Loss-of-function mutations in the *SFB* gene are associated with pollen-part self-compatibility in several studies (Ushijima et al. 2004; Sonneveld et al. 2005, Hauck et al. 2006) However, while distances among *Prunus* S-RNase alleles and those among corresponding *SLF* alleles are correlated, genealogies of *Prunus* pistil and purported pollen alleles do not strictly correspond (Nunes et al. 2006), perhaps due to rare recombination events.

In the subfamily Maloideae (Rosaceae). F-box loci currently called *S*-locus F-box brothers (*SFBB*) occur. These loci have levels of polymorphism similar to one another and to S-RNases from Maloideae. It is unclear which locus is involved in pollen specificity and Sassa et al. (2007) suggest that these loci may act in concert to determine mating type. The species of Rosaceae used for studies of self-incompatibility are all trees, making confirmatory experimental molecular approaches difficult.

In contrast to the Rosaceae, the implicated F-Box genes of the Solanaceae and Plantaginaceae have reduced levels of nucleotide polymorphism. This occurs despite the fact that S-RNases of these two families have higher levels of nucleotide polymorphism than do those of *Prunus* or the Maloideae. The contrast in levels of polymorphism between pistil and putative pollen genes is particularly striking in *Antirrhinum* (Plantaginaceae). Levels of both amino acid and synonymous DNA site divergence (Ks) are at least an order of magnitude lower at the *S*-locus F-box (*SLF*) locus than the S-RNase locus (Xue et al. 1996; Zhou et al. 2003; Sassa et al. 2007; Wheeler and Newbigin 2007). Together these facts imply that the histories of the implicated F-Box genes and their S-RNase cognates are markedly different. These findings are so far from expectations that it is difficult to make sense of them at present, but several possibilities have been suggested.

First, the fact that each purported pollen-specifying *S*-locus F-box gene is most closely related to other F-Box genes in its own genome might suggest independent evolution of S-RNase-based self-incompatibility in different groups. However, because the pollen F-box genes within a family or subfamily often do not have evolutionary histories that correspond to their cognate S-RNases, it can hardly be expected that pollen specifying F-Box genes from different families would necessarily group together. Either some or all of the implicated F-box genes do not function as the pollen specificity component, or the way that these evolve is quite unexpected. Considerable experimental evidence implicates the *SLF* loci of *Antirrhinum* and *Petunia* as the pollen specificity components of the incompatibility reaction (Qiao et al. 2004; Sijacic et al. 2004; Hua et al. 2007; see Chap. 9, this volume). However, definitive experiments, such as transformation from one specificity to another, have yet to be done. If these loci specify incompatibility, then the challenge is to explain why they have different apparent histories than their cognate S-RNases. One possibility could be that inter-locus recombination among F-box genes homogenises sequences among loci, leaving only the variation that encodes specificity. Another possibility is that over time different F-box loci get recruited to operate in pollen specificity, so that F-box genes in different families and sub-families are unrelated.

5.7 Conclusions

Genealogical analysis of the pistil components of both the sporophytic self-incompatibility system of the Brassicaeae and the gametophytic S-RNase-based system in the Solanaceae, Plantaginaceae and Rosaceae has confirmed many of the predictions that arise from negative frequency-dependent selection. In particular, predictions of long coalescence times and abundant shared ancestral polymorphism are met, though the tempo of allele formation and turnover varies somewhat among groups. In the Solanaceae, S-RNase variation had been useful in studies of the ancient demographic histories of lineages, in answering longstanding questions of mating system evolution and in crystallising the realisation that current methods of estimating character evolution are prone to error, particularly when the character analysed affects the diversification rate. In the Brassicaceae, the pollen specificity component evolves in a similar fashion to the pistil gene and there is good correspondence between the pollen and pistil gene trees, indicating a long co-evolutionary history (see Chap. 6, this volume). In RNase-based incompatibility, F-box genes implicated as specifying mating type in pollen evolve differently than their stylar cognates in some or all cases. This unexpected finding should instigate a great deal of future research.

Acknowledgements The author thanks B. Igic, E. Newbigin, and T. Paape for useful discussions. Support was provided by NSF DEB-0639984 to J.R.K.

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