Accumulation of non-functional $S$-haplotypes results in the breakdown of gametophytic self-incompatibility in tetraploid *Prunus*

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Running title: Breakdown of SI in Prunus

Keywords: Gametophytic self-incompatibility, S-RNase, S-locus, polyploidy, heteroallelic pollen

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ABSTRACT

The transition from self-incompatibility (SI) to self-compatibility (SC) is regarded as one of the most prevalent transitions in Angiosperm evolution, having profound impacts on the genetic structure of populations. Yet, the identity and function of mutations that result in the breakdown of SI in nature are not well understood. This work provides the first detailed genetic description of the breakdown of S-RNase mediated gametophytic self-incompatibility (GSI) in a polyploid species that exhibits genotype-dependent loss of SI. Genetic analyses of six natural sour cherry (Rosaceae, Prunus cerasus) selections identified seven independent, non-functional S-haplotypes with disrupted pistil component (stylar-S) and/or pollen component (pollen-S) function. A genetic model is developed and validated demonstrating that the breakdown of SI in sour cherry is due to the accumulation of a minimum of two non-functional S-haplotypes within a single individual. Our finding that sour cherry is SI when only one non-functional S-haplotype is present has significant evolutionary implications since non-functional S-haplotypes would be maintained in the population without causing an abrupt shift to SC. Furthermore, we demonstrate that heteroallelic sour cherry pollen is self-incompatible, which is counter to the well-documented phenomena in the Solanaceae where SC accompanying polyploidization is frequently due to the SC of heteroallelic pollen.
Gametophytic self-incompatibility (GSI) is a common genetic mechanism that promotes outcrossing in flowering plants (de Nettancourt 1977). In GSI, self-incompatibility (SI) is determined by a single, multi-allelic locus, called the $S$-locus, in which the compatibility of a cross is determined by the haploid genome of the pollen and the diploid genome of the pistil. Pollen tube growth is arrested if the pollen tube has an $S$-allele in common with one of the two $S$-alleles in the style. The $S$-locus contains a minimum of two genes, one controlling stylar-specificity and the other controlling pollen-specificity of the SI reaction. The stylar-$S$ in three plant families, the Solanaceae, Scrophulariaceae, and Rosaceae is a ribonuclease ($S$-RNase) (Anderson et al. 1986; McClure et al. 1989; Sassa et al. 1992; Xue et al. 1996), which is expressed in the pistil and specifically degrades RNA of incompatible pollen (McClure et al. 1990). The pollen-$S$ gene is an F-box gene named $S$-locus F-box (SLF) in Anthirrinum (Lai et al. 2002) and Prunus mume (Entani et al. 2003), PiSLF in Petunia inflata (Sijacic et al. 2004) and $S$-haplotype-specific F-box gene (SFB) in P. dulcis, P. avium, and P. cerasus (Ushijima et al. 2003; Yamane et al. 2003; Ikeda et al. 2004). The function of this F-box gene in the SI reaction remains unknown.

Within the Rosaceae, Prunus has emerged as the model GSI genus due to the small physical size of the $S$-haplotype region that facilitated map-based cloning of the pollen-$S$ (Entani et al. 2003; Ushijima et al. 2003). Four diploid Prunus species, sweet cherry (P. avium), almond (P. dulcis), and apricot (P. mume and P. armeniaca) have well-characterized GSI systems with over 50 $S$-RNases and 10 SFBs isolated and sequenced (Ushijima et al. 1998, 2003; Tao et al. 1999; Tamura et al. 2000; Sonneveld et al. 2001, 2003; Yaegaki et al. 2001; Ma and Oliveira
2002; Beppu et al. 2003; Romero et al. 2004; Wünsch and Hormaza 2004; de Cuyper et al. 2005). Within *Prunus*, cherry represents a natural diploid - tetraploid series with the tetraploid sour cherry arising through hybridization between sweet cherry and the tetraploid ground cherry (*P. fruticosa*) (Olden and Nybom 1968). Like sweet cherry, sour cherry exhibits an S-RNase-based GSI system (Yamane et al. 2001; Hauck et al. 2002; Tobutt et al. 2004); however, in contrast to sweet cherry, natural sour cherry selections include both SI and SC types (Lansari and Iezzoni 1990).

This genotype-dependent loss of SI in sour cherry indicates that genetic changes, and not polyploidy per se, cause the breakdown of SI. This is in contrast to the Solanaceae where polyploidy can result in the breakdown of SI (Livermore and Johnstone 1940; Stout and Chandler 1942; Pandey 1968). This breakdown of GSI accompanying polyploidy in Solanaceous species is a result of competitive interaction (Lewis 1943; Golz et al. 1999, 2001), in which pollen grains containing two copies of the same pollen-\(S\) allele (homoallelic pollen) are arrested if the cognate S-RNase is present in the style, while pollen grains containing two different pollen-\(S\) alleles (heteroallelic pollen) are compatible, regardless of the S-RNase composition of the style (Luu et al. 2001).

Competitive interaction describes a specific example of a pollen-part mutation caused by the presence of two different functional pollen-\(S\) genes within a single pollen tube. However, numerous other types of mutations have been associated with the occurrence of SC from normally SI populations or individuals. Pollen-part mutations can also result from a structural alteration of the *SLF* or *SFB* gene (Ushijima et al. 2004; Sonneveld et al. 2005). Stylar-part mutants can result from a structural alteration of the *S-RNase* gene or its cis-acting promoter region (Yamane et al. 2003). Finally, SC can be caused by mutations affecting so-called
“modifier” genes that are required for pollen rejection but not for the allele specificity of the reaction (McClure et al. 1999).

We used a genetic approach to elucidate the basis for the breakdown of SI in sour cherry. Six diverse sour cherry selections representing the habitat range of the species were used, making it highly unlikely that these selections would contain similar mutations in modifier genes. To determine if the breakdown of SI is due to changes affecting the allele specificity of the SI reaction or changes affecting the ability to carry out the incompatibility reaction, we took advantage of functional S-haplotypes shared between sweet and sour cherry and the full fertility of the reciprocal inter-specific crosses. Four functional and seven non-functional S-haplotypes present in sour cherry are described. A model was developed and validated, confirming that SC in sour cherry is caused by the presence of two or more non-functional S-haplotypes within an individual. Furthermore, the demonstration that heteroallelic pollen is SI in sour cherry suggests that the pollen-S genes of Prunus and the Solanaceae may differ.

MATERIALS AND METHODS

Plant Material: Six SC sour cherry cultivars, ‘Cigány’, ‘Érdi Bőtermő’ (‘EB’), ‘Montmorency’ (‘Mont’), ‘Rheinische Schattenmorelle’ (‘RS’), ‘Surefire’ (‘Sure’), and ‘Újféhértói fürtős’ (‘UF’), and four sweet cherry cultivars, ‘Chelan’, ‘Emperor Francis’ (‘EF’), ‘Gold’, and ‘Schmidt’ were used (see supplementary Table 1 at http://www.genetics.org/supplemental). The S-alleles for the sweet cherry cultivars have been previously reported (Iezzoni et al. 2005). Initial S-allele characterizations for ‘Cigány’, ‘EB’, ‘Mont’, ‘RS’, ‘Sure’, and ‘UF’ have also been previously reported (Yamane et al. 2001). Triploid progeny were generated from reciprocal
interspecific crosses between sweet and sour cherry. Tetraploid progeny were generated from self-pollination of each of the sour cherry selections and the following sour cherry crosses: ‘RS’ x ‘EB’, ‘UF’ x ‘Sure’, ‘UF’ x ‘RS’, and ‘UF’ x ‘Mont’. S-haplotype segregation was examined from a total of 1,200 progeny from 25 different self- and hybrid populations. For the triploid progeny and a portion of the tetraploid progeny, genotyping was done using DNA extracted from mature seed. All other plant material was grown at Michigan State University Experimental Stations in Clarksville, Traverse City, or Benton Harbor, MI.

**DNA Extractions:** From leaves: DNA extractions were conducted as previously described (Hauck *et al.* 2002).

From seed: The testa was removed from the cherry seed and the remaining embryo and cotyledons were ground in liquid nitrogen and mixed in a buffer consisting of 1% CTAB, 150 mM Tris-HCl (pH 8.0), 20 mM EDTA, 800 mM NaCl, 0.25% SDS, and 1% β-mercaptoethanol. The DNA was purified by chloroform extraction and precipitated using isopropanol.

**S-RNase Genotyping:** The S-RNase gene specific primer set, Pru-C2 and PCE-R (Yamane *et al.* 2001), was used for S-haplotype determination for all self- and inter-specific seed. This primer pair could differentiate between most S-RNase alleles based on polymorphisms in the length of the second intron in the Prunus S-RNase. However, the $S_2$- and $S_{13}$ RNase alleles could not be reliably amplified using this primer pair. Instead, either PaS2-Fnew/PaS2-R (Sonneveld *et al.* 2003) or newly designed PcS13-F (AGC AAA CCT TCC CAC CAA C) / PcS13-R (AGG AGG GGT GTT CTT CCA GT) were used. In certain crosses, other S-RNase-allele specific primers were used to verify S-RNase genotypes (Sonneveld *et al.* 2001, 2003). The $S_{1a}$- and $S_{1d}$-haplotypes
can be differentiated using RFLP analysis, as described previously (Yamane et al. 2001). We previously aligned the amino acid sequences for the \( S_4, S_6, S_{13}, S_{26} \) and \( S_a \)-RNases from sour cherry (Hauck et al. 2002).

**SFB Genotyping:** Thirty-three progeny from the cross between ‘RS’ x ‘EB’, 17 from ‘UF’ x ‘Mont’ and 22 from ‘UF’ x ‘RS’ were genotyped using allele-specific primers for each of the functional \( SFB \) alleles to verify co-segregation of the \( S-RNase \) and \( SFB \) alleles. Allele specific primers for \( SFB_4 \) (PaSFB4-F / PaSFB4-R) and \( SFB_6 \) (PaSFB6-F / PaSFB6-R) were used as previously described (Ikeda et al. 2005). The newly designed \( \text{PcSFB26-F} \) (GATTTGCTTGCTTTTTAAATGTTACGG) / \( \text{PcSFB26-R} \) (CTTAATTCTTGTGCTCAAGAACCCTTGC) were used for \( SFB_{26} \) genotyping.

**Model Testing:** The \( S-RNase \) genotypes for 92 mature seedlings with known pedigrees were determined using RFLP analyses following digestion with either \( \text{HindIII} \) or \( \text{DraI} \) as previously described (Yamane et al. 2001). Predictions of the SI or SC phenotype for seedling were made based on our developed hypothesis of the genetic control of SI and SC in sour cherry. The growth of self-pollen in each of the 92 seedlings was observed by aniline blue staining and UV-microscopy (Hauck et al. 2002).

**RESULTS**

**Sour Cherry Styles Reject Sweet Cherry Pollen in an \( S \)-allele Specific Manner:** The ability of sour cherry styles to arrest pollen in an \( S \)-haplotype specific manner was tested by crossing sour cherry and sweet cherry cultivars that have common \( S \)-haplotypes. When the sour cherry
cultivar ‘RS’ (S₆S₁₃S₂₆S₉) was pollinated with pollen from the sweet cherry cultivar ‘Gold’ (S₃S₆), all the progeny contained the S₃-haplotype (Table 1). This indicates that ‘Gold’ S₃ pollen was compatible in ‘RS’ styles, whereas the ‘Gold’ S₆-pollen was arrested by the presence of a functional S₆-RNase (Figure 1A). Likewise, S₆ and not S₃ pollen was selectively inhibited in ‘Mont’ (S₆S₁₃S₆S₉null) styles, S₄ and not S₂ pollen was selectively inhibited in ‘UF’ (S₁S₄S₆S₉null) styles, S₄ and not S₁ pollen was selectively arrested in ‘Sure’ (S₄S₁₃S₁S₉null) and ‘EB’ (S₄S₆mS₆S₉null) styles, and S₉ and not S₃ pollen was selectively inhibited in ‘Cigány’ (S₆m₂S₉S₂₆S₉) styles (Table 1). These results demonstrate that sour cherry retains the ability to reject pollen in an S-haplotype specific manner; therefore, SC must be caused by genetic changes affecting the specificity of the GSI reaction. See supplementary Table 2 (http://www.genetics.org/supplemental) for complete segregation of the S-genotypes in the triploid progeny from the inter-specific reciprocal crosses between sweet and sour cherry.

Two Stylar-Part Mutants Are Identified in Sour Cherry: Sweet cherry S₄ and S₉ pollen was selectively inhibited in ‘EB’ (S₄S₆mS₆S₉null) and ‘Cigány’ (S₆m₂S₉S₂₆S₉) styles, respectively, indicating that these sour cherry cultivars are able to carry out an SI reaction (Table 1). In contrast, S₆ pollen from the sweet cherry cultivar ‘Gold’ successfully grew down the styles of these two selections indicating that the S₆-RNases in these two cultivars are non-functional (Table 1). These non-functional stylar-part mutations, which can be distinguished based on RFLP patterns (Yamane et al. 2001) and PCR amplification products (Yamane et al. 2003), are termed S₆m and S₆m₂ in ‘EB’ and ‘Cigány’, respectively. We previously have shown that S₆m consists of a functional S₆-SFB but a non-functional S₆-RNase due to a 2600 bp insertion.
upstream from the S6-RNase (Yamane et al. 2003).

**Sweet Cherry Styles Reject Sour Cherry Pollen in an Allele Specific Manner:** When ‘RS’ (S6S13’ S26Sa) pollen was placed on ‘Gold’ (S3S6) styles, the absence of progeny containing both the S3- and S6-haplotypes indicated that S6-containing pollen from ‘RS’ was selectively rejected by the S6-RNase in ‘Gold’ styles, regardless of what other S-haplotype was in the pollen (Table 2; Figure 1B). This establishes that the ‘RS’ S6-haplotype also exhibits S6 pollen specific rejection and is, therefore, fully functional. S6-containing pollen of ‘EB’ and ‘Cigány’ was selectively rejected in ‘Gold’ styles indicating that the S6m- and S6m2-haplotypes in these selections have a functional pollen-S (Table 2). Likewise, S6-containing pollen from ‘Mont’ was selectively rejected in ‘Gold’ styles, and S4-containing pollen of ‘Sure’, ‘UF’, and ‘EB’ were selectively rejected in ‘EF’ (S3S4) styles (Table 2). This demonstrates that sour cherry pollen containing a functional pollen-S from an S-haplotype that is identical to the one in sweet cherry is always rejected. This allele-specific pollen rejection occurred regardless of the other S-haplotype present in the diploid pollen.

**Self-Pollinated Progeny of Sour Cherry Segregate for Functional and Non-Functional S-Haplotypes:** All of the progeny from the self-pollination of ‘RS’ (S6S13’ S26Sa) inherited the S13’- and S6-haplotypes; whereas, the S6- and S26-haplotypes segregated 1:1 (present: absent) (Table 3). This can be explained by the arrest of pollen containing either the S6- or S26-haplotype, or both, and the self-compatibility of S13’ S6a containing pollen (Figure 1C). Therefore, we conclude that both the S6- and S26-haplotypes are fully functional, as pollen containing either of these S-haplotypes was incapable of self-fertilization, whereas the S13’- and S6a-haplotypes were non-
functional. $S_{13}$ was also determined to be a non-functional $S$-haplotype based upon self-pollinations of ‘Sure’ and ‘Mont’ (Table 3). $S_{13}$ was previously shown to have a functional stylar component in crosses with sweet cherry containing an $S_{13}$-allele (Tobutt et al. 2004); therefore, we predict that the mutation affects the pollen component. $S_a$ was also confirmed to be a non-functional $S$-haplotype from self-pollinations of ‘Cigány’, ‘EB’, ‘Sure’, and ‘Mont’ (Table 3). Finally, $S_{26}$ was also confirmed to be a fully functional $S$-haplotype based on self-pollination of ‘Cigány’ (Table 3).

For four of the sour cherry selections (‘EB’, ‘Sure’, ‘Mont’, and ‘UF’), only three different $S$-haplotypes could be identified (Yamane et al. 2001). Segregation data presented in this study indicates that each $S$-haplotype was present in a single copy (Table 3). Therefore the fourth $S$-haplotype is hypothesized to be $S_{null}$, containing a deletion of the $S$-locus since no RFLP fragment associated with $S_{null}$ was visualized with either an $S$-RNase or $SFB$ probe (Yamane et al. 2001).

In ‘UF’, $S_4$ is the only fully functional $S$-haplotype, whereas $S_{1'}$ and $S_d$ are non-functional $S$-haplotypes (Table 3). Preliminary sequence and genetic analyses indicate that $S_{1'}$ is a pollen-part mutant (N.R. Hauck, unpublished). The non-functional $S_a$- and $S_d$-haplotypes likely represent different mutations of a common $S$-haplotype, since partial $S$-RNase and $SFB$ sequences of the $S_a$- and $S_d$-haplotypes are identical (N.R. Hauck, unpublished). These two $S$-haplotypes can be differentiated based upon HindIII $S$-RNase fragments ($S_a$, 6.4 kb; $S_d$, 6.2 kb) (Yamane et al. 2001).

**Heteroallelic Sour Cherry Pollen is SI:** The presence of two fully functional $S$-haplotypes ($S_6$ and $S_{26}$) in ‘RS’ allowed us to test whether heteroallelic pollen is SI or SC. Evidence that ‘RS’
S6S26 pollen is viable is provided by the fully compatible cross ‘UF’ x ‘RS’ where 11 out of 59 progeny inherited S6S26 pollen from ‘RS’ (see supplementary Figure 1 at http://www.genetics.org/supplemental). ‘RS’ S6S26 pollen was always rejected by ‘Gold’ (S3S6) styles and self-styles, presumably due to the presence of the S6-RNase in the ‘Gold’ and the S6- and S26-RNases in ‘RS’ (Table 2 and 3). Rejection of the ‘RS’ S6S26 pollen containing two functional pollen-S alleles in both ‘Gold’ and ‘RS’ styles indicates that heteroallelic pollen is SI in sour cherry.

Additional evidence that the breakdown of GSI in sour cherry is not caused by the SC heteroallelic pollen is provided by the self-pollinations of ‘Cigány’, which contains two fully functional S-haplotypes (S9 and S26), and ‘EB’, which contains at least two functional pollen-S genes (S4 and S6m) (Table 3). Similar to ‘RS’, self-pollination of ‘Cigány’ and ‘EB’ resulted in the rejection of pollen containing these S-haplotypes. ‘Cigány’ and ‘EB’ pollen containing the S4-, S6m- or S6m2-haplotypes was also arrested in styles of sweet cherry cultivars containing the S4- or S6-haplotypes, respectively (Table 2 and see supplementary Table 2 at http://www.genetics.org/supplemental). Additionally, previous work with the SI sour cherry cultivar ‘Crisana’ (S-RNase phenotype: S1S4Sd) demonstrated that it contains two fully functional S-haplotypes (S1 and Sd) and all ‘Crisana’ pollen was rejected in sweet cherry styles known to contain functional S1- and Sd-RNases (Hauck et al. 2002).

The SI of heteroallelic pollen in sour cherry could either be due to the absence of competitive interaction or the presence of genetic dominance/recessive relationships between pollen-S alleles similar to that exhibited by the sporophytic SI system in Brassica (Thompson and Taylor 1966). Although the crosses made cannot conclusively distinguish between these two possibilities, we obtained no data consistent with dominance/recessive relationships among the
six functional pollen-\(S\) alleles identified (\(S_4, S_6, S_{6m}, S_{6m2}, S_9\) and \(S_{26}\)) as allele-specific pollen rejection occurred regardless of the other \(S\)-haplotype present in the diploid pollen (Table 2).

**Model Development and Testing:** Taken together, our data indicate that the breakdown of GSI in sour cherry is caused by the accumulation of stylar-part and pollen-part mutants affecting multiple \(S\)-haplotypes (Figure 2). In sour cherry, four functional (\(S_4, S_6, S_9,\) and \(S_{26}\)) and seven non-functional \(S\)-haplotypes (\(S_1', S_{6m}, S_{6m2}, S_{13}, S_a, S_d,\) and \(S_{null}\))(this work, and Hauck *et al.* 2002; Yamane *et al.* 2003; Tobutt *et al.* 2004) have been identified. A comparison of the SI and SC selections revealed that the SI selections contained only one non-functional \(S\)-haplotype, whereas the SC selections contained two to four non-functional \(S\)-haplotypes. From this, we developed the “one-allele match” model, in which a match between a functional pollen-\(S\) gene product in the pollen and its cognate functional \(S\)-RNase in the style would result in an incompatible reaction. A similar reaction would occur regardless of whether the pollen contained a single functional pollen-\(S\) gene, or two different functional pollen-\(S\) genes. The absence of a functional match would result in a compatible reaction; thus, for successful self-fertilization, pollen must contain two non-functional \(S\)-haplotypes.

To test this model, we genotyped 92 seedlings from four crosses among five sour cherry selections. All seedlings that contained only one non-functional \(S\)-haplotypes (\(n=17\)) were SI and all the seedlings that contained two or more non-functional and non-complementary \(S\)-haplotypes (\(n=75\)) were SC (Table 4 and see supplementary Table3 at http://www.genetics.org/supplemental). Since the non-functional \(S_a\) and \(S_d\)-haplotypes likely represent different mutations of a common \(S\)-haplotype, we hypothesize that \(S_a\) and \(S_d\) have complementary pistil-\(S\) and pollen-\(S\) mutations, resulting in a functional \(S\)-haplotype. Therefore,
these results validate the one-allele-match model for the genetic control of SC and SI in sour cherry.

**DISCUSSION**

The transition from SI to SC has occurred repeatedly and has had a profound impact on angiosperm evolution; yet the genetic and molecular basis of this transition is not well understood. This study provides the first detailed genetic analysis of GSI breakdown in a diploid-polyploidy series involving multiple independent S-haplotype mutations. In each case the mutations were not in “modifier” genes that would cause a disruption in the ability to carry out the SI reaction as four S-haplotypes were found to be fully functional (S4, S6, S9, and S26). Instead, all mutations affected the allele specificity of the reaction by disrupting either pistil-S and/or pollen-S function.

The one-allele match model suggests a fundamental difference in the effect of polyploidy on SI between the Solanaceae and *Prunus*. In the Solanaceae, polyploidy is a direct cause of SC as a result of competitive interaction (Figure 2A), whereas in *Prunus*, polyploidy does not directly cause SC since heteroallelic pollen retains its SI phenotype. Rather, in sour cherry, mutations of the stylar- and pollen- specificity components have occurred and then accumulated to result in SC (Figure 2B). Our finding that sour cherry is SI despite the presence of one non-functional S-haplotype also has significant evolutionary implications in that non-functional S-haplotypes could be maintained in the population without causing an abrupt shift to SC.

Molecular characterization of five of the seven non-functional S-haplotypes that are completed or in progress, reveal structural changes of the S-haplotype (Yamane et al. 2003 and N. R. Hauck, unpublished results). The S6m-haplotype has a transposon-like element insertion in
the putative promoter region of the $S_6$-RNase (Yamane et al. 2003). The coding sequence of the pollen part mutant $SFB_1$ contains a 615-bp Ds-like element, while the coding sequence of the pollen part mutant $SFB_{13}$ contains a nonsense mutation (N. R. Hauck, unpublished). The $S_{null}$ presumably resulted from a deletion that encompasses the $S$-RNase and $SFB$ genes. The molecular characterizations of the $S_{6m2}$, $S_a$ and $S_d$-haplotypes are not yet complete. However, we predict that at a minimum the $S_d$-haplotype will have an ~2 kb deletion within the $S$-haplotype region that is not present in the $S_d$-haplotype.

Phylogenetic analyses of $S$-RNases from the Solanaceae, Scrophulariaceae, and Rosaceae support the conclusion of a common evolutionary origin for $S$-RNase-mediated GSI (Igic and Kohn 2001; Steinbachs and Holsinger 2002). The finding that the pollen-$S$ in these three families is an F-box protein, implicates ubiquitination as a common mechanism for $S$-RNase degradation (Kao and Tsukamoto 2004). Yet, the SI of heteroallelic pollen in sour cherry suggests that the pollen-$S$ differs between $Prunus$ (Rosaceae) and the Solanaceae. Two other lines of evidence support this contention. Firstly, sweet cherry pollen carrying the mutated $SFB_3$, characterized by the complete deletion of a functional $SFB_3$, is viable and SC (Sonneveld et al. 2005). However, in the Solanaceae, loss of the pollen-$S$ gene is predicted to be lethal to the pollen (Golz et al. 1999, 2001). Secondly the pollen-$S$ alleles in $Prunus$, $SFB$, exhibit a higher degree of sequence diversity than the pollen-$S$ alleles, $SLF$, in Antirrhinum and Petunia (Ikeda et al. 2004; Kao and Tsukamoto 2004). Further insight will require an understanding of the biochemical interactions involving the pollen-$S$ and stylar-$S$ genes in both the Solanaceae and $Prunus$. 
Acknowledgements: We thank A. Sebolt for technical assistance and H. Sassa, N. Jiang, and S. van Nocker for their critical reviews. This work was partially supported by a grant from the USDA, CSREES-NRI, Plant Genome, Bioinformatics and Genetics Resources Program.

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Table 1: Segregation of pollen-derived S-haplotypes in inter-specific crosses between sour cherry and sweet cherry.

<table>
<thead>
<tr>
<th>Parents (S-genotype)</th>
<th>Population</th>
<th>Segregation of paternal S-haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size</td>
<td>Observed Ratio</td>
</tr>
<tr>
<td>RS ($S_6 S_{13} S_{26} S_a$) x Gold ($S_3 S_6$)</td>
<td>31</td>
<td>31 : 0 ($S_3 : S_6$)</td>
</tr>
<tr>
<td>Mont ($S_6 S_{13} S_a S_{null}$) x Gold ($S_3 S_6$)</td>
<td>55</td>
<td>55 : 0 ($S_3 : S_6$)</td>
</tr>
<tr>
<td>UF ($S_4 S_{13} S_a S_{null}$) x Schmidt ($S_2 S_4$)</td>
<td>66</td>
<td>66 : 0 ($S_2 : S_4$)</td>
</tr>
<tr>
<td>Sure ($S_4 S_{13} S_a S_{null}$) x EF ($S_3 S_4$)</td>
<td>30</td>
<td>30 : 0 ($S_3 : S_4$)</td>
</tr>
<tr>
<td>EB ($S_4 S_{6m} S_a S_{null}$) x EF ($S_3 S_4$)</td>
<td>18</td>
<td>18 : 0 ($S_3 : S_4$)</td>
</tr>
<tr>
<td>Cigány ($S_{6m2} S_9 S_{26} S_a$) x Chelan ($S_3 S_9$)</td>
<td>45</td>
<td>45 : 0 ($S_3 : S_9$)</td>
</tr>
<tr>
<td>EB ($S_4 S_{6m} S_a S_{null}$) x Gold ($S_3 S_6$)</td>
<td>33</td>
<td>22 : 11 ($S_3 : S_6$)</td>
</tr>
<tr>
<td>Cigány ($S_{6m2} S_9 S_{26} S_a$) x Gold ($S_3 S_6$)</td>
<td>36</td>
<td>16 : 20 ($S_3 : S_6$)</td>
</tr>
</tbody>
</table>

$^a$ The S-haplotypes being tested are indicated in bold.

$^b$ Observed ratios were tested for fit to the ratio expected if the shared S-haplotype is non-functional (1:1). If the shared S-haplotype were functional, it would not be inherited from the paternal parent.
Table 2: S-haplotypes of successful pollen types from inter-specific crosses between sweet cherry and sour cherry selections.

<table>
<thead>
<tr>
<th>Parents (S-genotype)(^a)</th>
<th>No. of progeny</th>
<th>Possible Sour Cherry Pollen Types</th>
<th>Successful</th>
<th>Not detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold (S(_3)S(<em>6)) x RS (S(<em>6)S(</em>{13})-S(</em>{26})S(_a))</td>
<td>13</td>
<td>S(<em>{13})-S(</em>{26}), S(_{13})-S(_a)</td>
<td>S(<em>6)S(</em>{26}), S(_6)S(<em>a), S(<em>6)S(</em>{13}), S(</em>{26})S(_a)(^b)</td>
<td></td>
</tr>
<tr>
<td>Gold (S(_3)S(_6)) x EB (S(<em>4)S(</em>{6m})S(<em>a)S(</em>{null}))</td>
<td>14</td>
<td>S(_4)S(_a), S(<em>4)S(</em>{null}), S(<em>a)S(</em>{null})</td>
<td>S(_6m)S(_a), S(<em>6m)S(</em>{null}), S(<em>4)S(</em>{6m})</td>
<td></td>
</tr>
<tr>
<td>Gold (S(_3)S(_6)) x Cigány (S(_6m)2S(<em>9)S(</em>{26})S(_a))</td>
<td>40</td>
<td>S(_9)S(<em>a), S(<em>9)S(</em>{26}), S(</em>{26})S(_a)</td>
<td>S(_6m2)S(_a), S(<em>6m2)S(</em>{26}), S(_6m2)S(_9)</td>
<td></td>
</tr>
<tr>
<td>Gold (S(_3)S(_6)) x Mont (S(<em>6)S(</em>{13})-S(<em>a)S(</em>{null}))</td>
<td>15</td>
<td>S(<em>{13})-S(</em>{null}), S(_{13})-S(_a), S(<em>a)S(</em>{null})</td>
<td>S(<em>6)S(</em>{null}), S(_6)S(_a), S(_6)S(_1)</td>
<td></td>
</tr>
<tr>
<td>EF (S(_3)S(_4)) x Sure (S(<em>4)S(</em>{13})-S(<em>a)S(</em>{null}))</td>
<td>37</td>
<td>S(<em>{13})-S(</em>{null}), S(_{13})-S(_a), S(<em>a)S(</em>{null})</td>
<td>S(<em>4)S(</em>{null}), S(_4)S(_a), S(<em>4)S(</em>{13})</td>
<td></td>
</tr>
<tr>
<td>EF (S(_3)S(_4)) x UF (S(_1)-S(_4)S(<em>d)S(</em>{null}))</td>
<td>40</td>
<td>S(<em>1)-S(</em>{null}), S(_1)-S(_d), S(<em>d)S(</em>{null})</td>
<td>S(<em>4)S(</em>{null}), S(_4)S(_d), S(_1)-S(_4)</td>
<td></td>
</tr>
<tr>
<td>EF (S(_3)S(_4)) x EB (S(<em>4)S(</em>{6m})S(<em>a)S(</em>{null}))</td>
<td>20</td>
<td>S(<em>6m)S(</em>{null}), S(_6m)S(_a), S(<em>a)S(</em>{null})</td>
<td>S(<em>4)S(</em>{null}), S(_4)S(_a), S(<em>4)S(</em>{6m})</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) The S-haplotypes being tested are indicated in bold.

\(^b\) The S\(_{26}\)S\(_a\) gamete type is rare, resulting in only 3% of the progeny in a fully compatible cross (See supplementary Figure 1 at http://www.genetics.org/supplemental).
Table 3: Segregation of S-haplotypes following self-pollination of six sour cherry selections to determine the functionality of each S-haplotype.

<table>
<thead>
<tr>
<th>Parent (S-genotype)</th>
<th>No. of Progeny</th>
<th>Segregation of S-haplotypes</th>
<th>( \chi^2 ) (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed S-haplotype</td>
<td>Observed Ratio</td>
</tr>
<tr>
<td>RS</td>
<td>54</td>
<td>( S_6 )</td>
<td>28 : 26</td>
</tr>
<tr>
<td>( (S_6S_{13'}S_{26}S_a) )</td>
<td></td>
<td>( S_{13'} )</td>
<td>54 : 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( S_{26} )</td>
<td>23 : 31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( S_a )</td>
<td>54 : 0</td>
</tr>
<tr>
<td>Cigány</td>
<td>59</td>
<td>( S_{6m2} )</td>
<td>59 : 0</td>
</tr>
<tr>
<td>( (S_{6m2}S_9S_{26}S_a) )</td>
<td></td>
<td>( S_9 )</td>
<td>24 : 35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( S_{26} )</td>
<td>36 : 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( S_a )</td>
<td>59 : 0</td>
</tr>
<tr>
<td>EB</td>
<td>25</td>
<td>( S_4 )</td>
<td>9 : 16</td>
</tr>
<tr>
<td>( (S_4S_{6m}S_aS_{null}) )</td>
<td></td>
<td>( S_{6m} )</td>
<td>25 : 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( S_a )</td>
<td>20 : 5</td>
</tr>
<tr>
<td>Sure</td>
<td>64</td>
<td>( S_4 )</td>
<td>35 : 29</td>
</tr>
<tr>
<td>( (S_4S_{13'}S_aS_{null}) )</td>
<td></td>
<td>( S_{13'} )</td>
<td>64 : 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( S_a )</td>
<td>63 : 1</td>
</tr>
<tr>
<td>UF</td>
<td>102</td>
<td>( S_1' )</td>
<td>102 : 0</td>
</tr>
<tr>
<td>( (S_1'S_4S_aS_{null}) )</td>
<td></td>
<td>( S_4 )</td>
<td>60 : 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( S_d )</td>
<td>98 : 4</td>
</tr>
<tr>
<td>Mont</td>
<td>135</td>
<td>( S_6 )</td>
<td>72 : 63</td>
</tr>
<tr>
<td>( (S_6S_{13'}S_aS_{null}) )</td>
<td></td>
<td>( S_{13'} )</td>
<td>131 : 4</td>
</tr>
</tbody>
</table>
A 1:1 ratio is expected if the shared $S$-haplotype is fully functional resulting in pollen rejection. A shared non-functional $S$-haplotype would not result in pollen rejection; therefore, the shared $S$-haplotype would be transmitted to the progeny at a higher frequency than expected.
Table 4: Number of non-functional S-haplotypes and the SI or SC phenotypes for 92 sour cherry seedlings.

<table>
<thead>
<tr>
<th>No. of Non-Functional S-haplotypes in each Seedling</th>
<th>No. of Seedlings Analyzed</th>
<th>Phenotype of Seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. SI</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Three progeny with S-genotype $S_dS_dS_aS_d$ were determined to be SI, despite having two non-functional S-haplotypes. Partial S-RNase and SFB sequences from the $S_a$- and $S_d$- haplotypes are identical (N.R. Hauck, unpublished), suggesting that the $S_a$ and $S_d$ represent different mutations of a common S-haplotype. We are currently testing the possibility that $S_a$ has a functional S-RNase and a non-functional SFB; whereas, $S_d$ has a non-functional S-RNase and a functional SFB. In this case, $S_dS_dS_aS_d$ individuals would be predicted to be SI under the one-allele-match model since $S_aS_d$ pollen would be rejected due to a match between a functional $S_a$-RNase and SFB<sub>d</sub>. 
Figure Legends

**Figure 1:** Schematic representations of the inter-specific crosses between ‘RS’ ($S_6S_{13}S_{26}S_a$) and ‘Gold’ ($S_3S_6$) and the self-pollination of ‘RS’. (A) Pollination of ‘RS’ styles with ‘Gold’ pollen results in the rejection of all pollen containing the $S_6$-haplotype. Pollen containing the $S_3$-haplotype is successful. (B) Pollination of ‘Gold’ styles with ‘RS’ pollen results in the rejection of all pollen containing the $S_6$-haplotype. Any pollen that does not contain the $S_6$-haplotype is successful. Because sour cherry exhibits homologous and occasional non-homologous pairing (Beaver and Iezzoni 1993), all possible chromosome pairing configurations are considered. Pollen types formed by homologous pairing are shaded. (C) Self-pollination of ‘RS’ results in rejection of all pollen containing either $S_6$ or $S_{26}$, or both. The only successful pollen is $S_{13}S_a$.

**Figure 2.** Schematic representation of the affects of polyploidy on GSI in (A) the Solanaceae and (B) *Prunus*. In the Solanaceae, polyploidy directly causes the conversion from SI to SC due to the compatibility of heteroallelic pollen. In *Prunus*, polyploidy does not directly result in a breakdown of SI. Rather, SC requires the loss-of-function for a minimum of two $S$-haplotype specificity components.
Figure 1

(A) ‘Sch’ x ‘Gold’

(B) ‘Gold’ x ‘Sch’

(C) ‘Sch’ self pollination
Figure 2

(A) Solanaceae

(B) Prunus

First mutation

Second mutation

Polyploidy

First mutation

Second mutation

Polyploidy