DOI: 10.1534/genetics.105.049395

Accumulation of Nonfunctional *S*-Haplotypes Results in the Breakdown of Gametophytic Self-Incompatibility in Tetraploid Prunus

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Manuscript received August 10, 2005
Accepted for publication September 29, 2005

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, nonfunctional *S*-haplotypes with disrupted pistil component (stylar-*S*) and/or pollen component (pollen-*S*) function. A genetic model demonstrating that the breakdown of SI in sour cherry is due to the accumulation of a minimum of two nonfunctional *S*-haplotypes within a single individual is developed and validated. Our finding that sour cherry is SI when only one nonfunctional *S*-haplotype is present has significant evolutionary implications since nonfunctional *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 phenomenon 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 Antirrhinum (Lai *et al.* 2002) and in *Prunus mume* (Entani *et al.* 2003), *PiSLF* in *Petunia inflata* (Sijacic *et al.* 2004), and *S*-haplotype-specific F-box gene (*SFB*) in *Prunus dulcis*, *Prunus avium*, and *Prunus 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 *Prunus armeniaca*) have well-characterized GSI systems with >50 *S-RNase*s and 10 *SFB*s 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 (*Prunus 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 self-compatible (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

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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 to changes affecting the ability to carry out the incompatibility reaction, we took advantage of functional *S*-haplotypes shared between the sweet and the sour cherry and the full fertility of the reciprocal interspecific crosses. Four functional and seven nonfunctional *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 nonfunctional *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 Újfehértói fűrtős (UF)—and four sweet cherry cultivars—Chelan, Emperor Francis (EF), Gold, and Schmidt—were used (see supplemental 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 × EB, UF × Sure, UF × RS, and UF × Mont. *S*-haplotype segregation was examined from a total of 1200 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 the Michigan State University Experimental Stations in Clarksville, Traverse City, or Benton Harbor, Michigan.

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, composed of Pru-C2 and PCE-R (Yamane *et al.* 2001), was used for *S*-haplotype determination for all self- and interspecific seed. This primer pair could differentiate between most *S-RNase* alleles on the basis of 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) was used. In certain crosses, other *S-RNase*-allele-specific primers were used to verify *S-RNase* genotypes (Sonneveld *et al.* 2001, 2003). The S_a- and S_d-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 RS × EB, 17 from UF × Mont, and 22 from UF × RS were genotyped using allele-specific primers for each of the functional *SFB* alleles to verify cosegregation 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 PcSFB26-F (GATTTG CTTGCTTTTTAAATGTTACGG)/PcSFB26-R (CTTAATTCT TGTGTCAAGAACTTGCC) 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 *Hin*dIII or *Dra*I as previously described (Yamane *et al.* 2001). Predictions of the SI or SC phenotype for seedlings were made on the basis of 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 ($S_6S_{13'}\,S_{26}S_a$) was pollinated with pollen from the sweet cherry cultivar Gold (S_3S_6), all the progeny

TABLE 1

Segregation of pollen-derived *S*-haplotypes in interspecific crosses between sour cherry and sweet cherry

Parents (*S*-genotype)[a]	Population size	Segregation of paternal *S*-haplotypes: Observed ratio	Expected ratio[b]	χ^2 (*P*-value)
RS ($\underline{S_6}S_{13'}S_{26}S_a$) × Gold ($S_3\underline{S_6}$)	31	31:0 (S_3:S_6)	1:1	31.0 (<0.0001)
Mont ($\underline{S_6}\ S_{13'}S_aS_{null}$) × Gold ($S_3\underline{S_6}$)	55	55:0 (S_3:S_6)	1:1	55.0 (<0.0001)
UF ($S_{1'}\ \underline{S_4}S_dS_{null}$) × Schmidt ($S_2\underline{S_4}$)	66	66:0 (S_2:S_4)	1:1	66.0 (<0.0001)
Sure ($\underline{S_4}\ S_{13'}S_aS_{null}$) × EF ($S_3\underline{S_4}$)	30	30:0 (S_3:S_4)	1:1	30.0 (<0.0001)
EB ($\underline{S_4}S_{6m}S_aS_{null}$) × EF ($S_3\underline{S_4}$)	18	18:0 (S_3:S_4)	1:1	18.0 (<0.0001)
Cigány ($S_{6m2}\underline{S_9}S_{26}S_a$) × Chelan ($S_3\underline{S_9}$)	45	45:0 (S_3:S_9)	1:1	45.0 (<0.0001)
EB ($S_4\underline{S_{6m}}\ S_a\ S_{null}$) × Gold ($S_3\underline{S_6}$)	33	22:11 (S_3:S_6)	1:1	3.67 (0.0555)
Cigány ($\underline{S_{6m2}}S_9S_{26}S_a$) × Gold ($S_3\underline{S_6}$)	36	16:20 (S_3:S_6)	1:1	0.44 (0.5050)

[a] The *S*-haplotypes being tested are underlined.

[b] Observed ratios were tested for fit to the ratio expected if the shared *S*-haplotype is nonfunctional (1:1). If the shared *S*-haplotype were functional, it would not be inherited from the paternal parent.

contained the S_3-haplotype (Table 1). This indicates that Gold S_3 pollen was compatible in RS styles, whereas the Gold S_6 pollen was arrested by the presence of a functional S_6-RNase (Figure 1A). Likewise, S_6 and not S_3 pollen was selectively inhibited in Mont ($S_6S_{13'}\ S_aS_{null}$) styles, S_4 and not S_2 pollen was selectively inhibited in UF ($S_{1'}\ S_4S_dS_{null}$) styles, S_4 and not S_3 pollen was selectively arrested in Sure ($S_4S_{13'}\ S_aS_{null}$) and EB ($S_4S_{6m}S_aS_{null}$) styles, and S_9 and not S_3 pollen was selectively inhibited in Cigány ($S_{6m2}S_9S_{26}S_a$) 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 supplemental Table 2 (http://www.genetics.org/supplemental) for complete segregation of the *S*-genotypes in the triploid progeny from the interspecific reciprocal crosses between sweet and sour cherry.

Two stylar-part mutants are identified in sour cherry: Sweet cherry S_4 and S_9 pollen was selectively inhibited in EB ($S_4S_{6m}S_aS_{null}$) and Cigány ($S_{6m2}S_9S_{26}S_a$) styles, respectively, indicating that these sour cherry cultivars are able to carry out an SI reaction (Table 1). In contrast, S_6 pollen from the sweet cherry cultivar Gold successfully grew down the styles of these two selections, indicating that the S_6-RNases in these two cultivars are nonfunctional (Table 1). These nonfunctional stylar-part mutations, which can be distinguished on the basis of RFLP patterns (Yamane *et al.* 2001) and PCR amplification products (Yamane *et al.* 2003), are termed S_{6m} and S_{6m2} in EB and Cigány, respectively. We previously have shown that S_{6m} consists of a functional S_6-SFB but a nonfunctional S_6-RNase due to a 2600-bp insertion upstream from the S_6-*RNase* (Yamane *et al.* 2003).

Sweet cherry styles reject sour cherry pollen in an allele-specific manner: When RS ($S_6S_{13'}\ S_{26}S_a$) pollen was placed on Gold (S_3S_6) styles, the absence of progeny containing both the S_3- and S_6-haplotypes indicated that S_6-containing pollen from RS was selectively rejected by the S_6-RNase in Gold styles, regardless of what other *S*-haplotype was in the pollen (Table 2; Figure 1B). This establishes that the RS S_6-haplotype also exhibits S_6-pollen-specific rejection and is, therefore, fully functional. S_6-containing pollen of EB and Cigány was

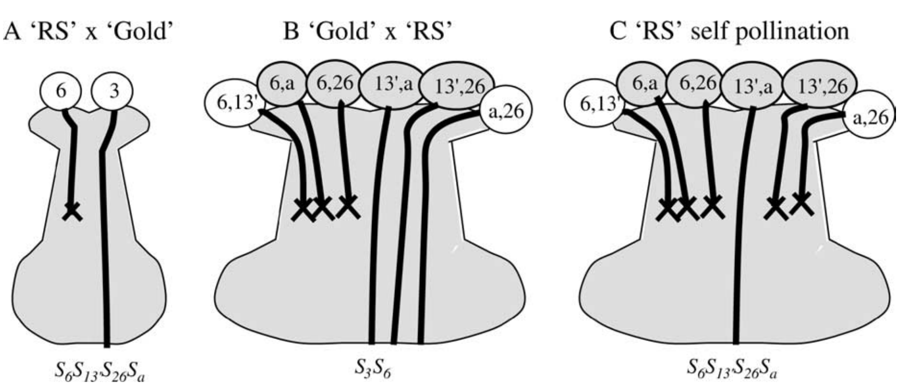

Figure 1.—Schematics of the interspecific crosses between RS ($S_6S_{13'}\ S_{26}S_a$) and Gold (S_3S_6) and the self-pollination of RS. (A) Pollination of RS styles with Gold pollen results in the rejection of all pollen containing the S_6-haplotype. Pollen containing the S_3-haplotype is successful. (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 nonhomologous 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 $S_{13'}S_a$.

TABLE 2

S-haplotypes of successful pollen types from interspecific crosses between sweet cherry and sour cherry selections

Parents (S-genotype)[a]	No. of progeny	Possible sour cherry pollen types: Successful	Possible sour cherry pollen types: Not detected
Gold ($S_3\underline{S_6}$) × RS ($\underline{S_6}S_{13'}S_{26}S_a$)	13	$S_{13'}S_{26}$, $S_{13'}S_a$	S_6S_{26}, S_6S_a, $S_6S_{13'}$, $S_{26}S_a$[b]
Gold ($S_3\underline{S_6}$) × EB ($S_4\underline{S_{6m}}S_aS_{null}$)	14	S_4S_a, S_4S_{null}, S_aS_{null}	$S_{6m}S_a$, $S_{6m}S_{null}$, S_4S_{6m}
Gold ($S_3\underline{S_6}$) × Cigány ($\underline{S_{6m2}}S_9S_{26}S_a$)	40	S_9S_a, S_9S_{26}, $S_{26}S_a$	$S_{6m2}S_a$, $S_{6m2}S_{26}$, $S_{6m2}S_9$
Gold ($S_3\underline{S_6}$) × Mont ($\underline{S_6}S_{13'}S_aS_{null}$)	15	$S_{13'}S_{null}$, $S_{13'}S_a$, S_aS_{null}	S_6S_{null}, S_6S_a, $S_6S_{1'}$
EF ($S_3\underline{S_4}$) × Sure ($\underline{S_4}S_{13'}S_aS_{null}$)	37	$S_{13'}S_{null}$, $S_{13'}S_a$, S_aS_{null}	S_4S_{null}, S_4S_a, $S_4S_{13'}$
EF ($S_3\underline{S_4}$) × UF ($S_{1'}\underline{S_4}S_dS_{null}$)	40	$S_{1'}S_{null}$, $S_{1'}S_d$, S_dS_{null}	S_4S_{null}, S_4S_d, $S_{1'}S_4$
EF ($S_3\underline{S_4}$) × EB ($\underline{S_4}S_{6m}S_aS_{null}$)	20	$S_{6m}S_{null}$, $S_{6m}S_a$, S_aS_{null}	S_4S_{null}, S_4S_a, S_4S_{6m}

[a] The S-haplotypes being tested are underlined.
[b] The $S_{26}S_a$ gamete type is rare, resulting in only 3% of the progeny in a fully compatible cross (see supplemental Figure 1 at http://www.genetics.org/supplemental/).

selectively rejected in Gold styles, indicating that the S_{6m}- and S_{6m2}-haplotypes in these selections have a functional pollen-S (Table 2). Likewise, S_6-containing pollen from Mont was selectively rejected in Gold styles, and S_4-containing pollen of Sure, UF, and EB was selectively rejected in EF (S_3S_4) 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 nonfunctional S-haplotypes: All of the progeny from the self-pollination of RS ($S_6S_{13'}$ $S_{26}S_a$) inherited the $S_{13'}$- and S_a-haplotypes, whereas the S_6- and S_{26}-haplotypes segregated 1:1 (present:absent) (Table 3). This can be explained by the arrest of pollen containing either the S_6- or S_{26}-haplotype, or both, and the self-compatibility of $S_{13'}S_a$-containing pollen (Figure 1C). Therefore, we conclude that both the S_6- and the S_{26}-haplotypes are fully functional, as pollen containing either of these S-haplotypes was incapable of

TABLE 3

Segregation of S-haplotypes following self-pollination of six sour cherry selections to determine the functionality of each S-haplotype

Parent (S-genotype)	No. of progeny observed	Segregation of S-haplotypes: S-haplotype	Observed ratio	Expected ratio[a]	χ^2 (P-value)
RS	54	S_6	28:26	1:1	0.07 (0.7855)
($S_6S_{13'}S_{26}S_a$)		$S_{13'}$	54:0	1:1	54.0 (<0.0001)
		S_{26}	23:31	1:1	1.19 (0.2763)
		S_a	54:0	1:1	54.0 (<0.0001)
Cigány	59	S_{6m2}	59:0	1:1	59.0 (<0.0001)
($S_{6m2}S_9S_{26}S_a$)		S_9	24:35	1:1	2.05 (0.1521)
		S_{26}	36:23	1:1	2.86 (0.0906)
		S_a	59:0	1:1	59.0 (<0.0001)
EB	25	S_4	9:16	1:1	1.96 (0.1615)
($S_4S_{6m}S_aS_{null}$)		S_{6m}	25:0	1:1	25.0 (<0.0001)
		S_a	20:5	1:1	9.00 (0.0027)
Sure	64	S_4	35:29	1:1	0.56 (0.4533)
($S_4S_{13'}S_aS_{null}$)		$S_{13'}$	64:0	1:1	64.0 (<0.0001)
		S_a	63:1	1:1	60.1 (<0.0001)
UF	102	$S_{1'}$	102:0	1:1	102.0 (<0.0001)
($S_{1'}S_4S_dS_{null}$)		S_4	60:42	1:1	3.18 (0.0747)
		S_d	98:4	1:1	86.6 (<0.0001)
Mont	135	S_6	72:63	1:1	0.60 (0.4386)
($S_6S_{13'}S_aS_{null}$)		$S_{13'}$	131:4	1:1	119.4 (<0.0001)
		S_a	131:4	1:1	119.4 (<0.0001)

[a] A 1:1 ratio is expected if the shared S-haplotype is fully functional, resulting in pollen rejection. A shared nonfunctional 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.

self-fertilization, whereas the $S_{13'}$- and S_a-haplotypes were nonfunctional. $S_{13'}$ was also determined to be a nonfunctional *S*-haplotype on the basis of 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 nonfunctional *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 on the basis of the 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 indicate 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 an *SFB* probe (Yamane *et al.* 2001).

In UF, S_4 is the only fully functional *S*-haplotype, whereas $S_{1'}$ and S_d are nonfunctional *S*-haplotypes (Table 3). Preliminary sequence and genetic analyses indicate that $S_{1'}$ is a pollen-part mutant (N. R. Hauck, unpublished results). The nonfunctional 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 results). These two *S*-haplotypes can be differentiated on the basis of *Hin*dIII *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 S_6S_{26} pollen is viable is provided by the fully compatible cross UF × RS where 11 of 59 progeny inherited S_6S_{26} pollen from RS (see supplemental Figure 1 at http://www.genetics.org/supplemental/). RS S_6S_{26} pollen was always rejected by Gold (S_3S_6) styles and self-styles, presumably due to the presence of the S_6-RNase in the Gold and the S_6- and S_{26}-RNases in RS (Tables 2 and 3). Rejection of the RS S_6S_{26} 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 (S_9 and S_{26}), and EB, which contains at least two functional pollen-*S* genes (S_4 and S_{6m}) (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 S_4-, S_{6m}-, or S_{6m2}-haplotypes was also arrested in styles of sweet cherry cultivars containing the S_4- or S_6-haplotypes, respectively (Table 2 and see supplemental Table 2 at http://www.genetics.org/supplemental/). Additionally, previous work with the SI sour cherry cultivar Crisana (*S-RNase* phenotype: $S_1S_4S_d$) demonstrated that it contains two fully functional *S*-haplotypes (S_1 and S_4) and all Crisana pollen was rejected in sweet cherry styles known to contain functional S_1- and S_4-RNases (Hauck *et al.* 2002).

The SI of heteroallelic pollen in sour cherry could be due to either 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 dominant/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 nonfunctional *S*-haplotypes ($S_{1'}$, S_{6m}, S_{6m2}, $S_{13'}$, S_a, S_d, and S_{null}) (Hauck *et al.* 2002; Yamane *et al.* 2003; Tobutt *et al.* 2004; this work) have been identified. A comparison of the SI and SC selections revealed that the SI selections contained only one nonfunctional *S*-haplotype, whereas the SC selections contained two to four nonfunctional *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 nonfunctional *S*-haplotypes.

To test this model, we genotyped 92 seedlings from four crosses among five sour cherry selections. All seedlings that contained only one nonfunctional *S*-haplotype ($n = 17$) were SI and all seedlings that contained two or more nonfunctional and noncomplementary *S*-haplotypes ($n = 75$) were SC (Table 4 and see supplemental Table 3 at http://www.genetics.org/supplemental/). Since the nonfunctional 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.

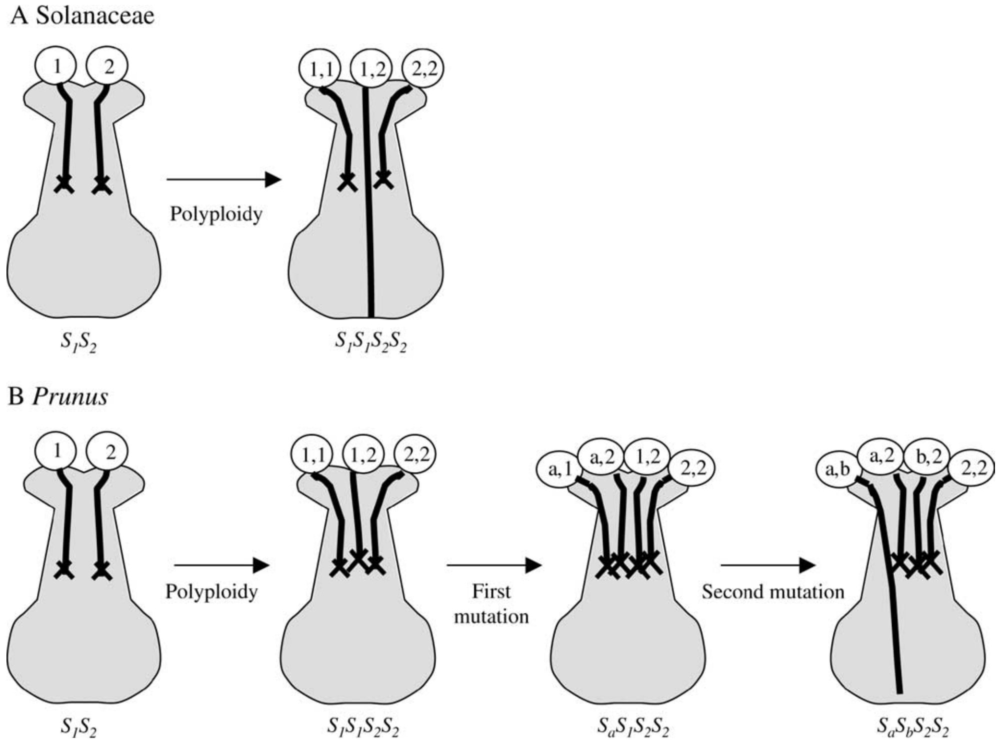

Figure 2.—Schematic of the affects of polyploidy on GSI in (A) the Solanaceae and (B) Prunus. In the Solanaceae, polyploidy directly causes the conversion from SI to SC due to the compatibility of heteroallelic pollen. In Prunus, polyploidy does not directly result in a breakdown of SI. Rather, SC requires the loss-of-function for a minimum of two *S*-haplotype-specificity components. Polyploidization creating tetraploid sour cherry presumably resulted from the mating of a 2*n* gamete from sweet cherry and an *n* gamete from tetraploid ground cherry (Iezzoni and Hancock 1984).

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 (S_4, S_6, S_9, and S_{26}). Instead, all mutations affected the allele specificity of the reaction by disrupting 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 nonfunctional *S*-haplotype also has significant evolutionary implications in that nonfunctional *S*-haplotypes could be maintained in the population without causing an abrupt shift to SC.

Molecular characterization of five of the seven nonfunctional *S*-haplotypes that are completed or in progress, reveal structural changes of the *S*-haplotype (Yamane *et al.* 2003; N. R. Hauck, unpublished results). The S_{6m}-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 results). The S_{null} presumably resulted from a deletion that encompasses the *S-RNase* and *SFB* genes. The molecular characterizations of the

TABLE 4

Number of nonfunctional *S*-haplotypes and the SI or SC phenotypes for 92 sour cherry seedlings

No. of nonfunctional *S*-haplotypes in each Seedling	No. of seedlings analyzed	Phenotype of seedlings	
		No. SI	No. SC
1	17	17	0
2	17	3[a]	14
3	37	0	37
4	21	0	21

[a] Three progeny with *S*-genotype $S_4S_6S_aS_d$ were determined to be SI, despite having two nonfunctional *S*-haplotypes. Partial *S-RNase* and *SFB* sequences from the S_a- and S_d- haplotypes are identical (N. R. Hauck, unpublished results), 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 nonfunctional SFB, whereas S_d has a nonfunctional S-RNase and a functional SFB. In this case, $S_4S_6S_aS_d$ 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_d.

S_{6m2}-, S_{d}-, 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_{a}-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. First, 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). Second, the pollen-*S* allele in Prunus, *SFB*, exhibits a higher degree of sequence diversity than the pollen-*S* allele, *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.

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 U.S. Department of Agriculture, Cooperative State Research, Education, and Extension Service–National Research Initiative, Plant Genome, Bioinformatics and Genetics Resources Program.

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Communicating editor: D. Charlesworth