Rejection of S-Heteroallelic Pollen by a Dual-Specific S-RNase in *Solanum chacoense* Predicts a Multimeric SI Pollen Component

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ABSTRACT

S-heteroallelic pollen (HAP) grains are usually diploid and contain two different S-alleles. Curiously, HAP produced by tetraploids derived from self-incompatible diploids are typically self-compatible. The two different hypotheses previously advanced to explain the compatibility of HAP are the lack of pollen-S expression and the "competition effect" between two pollen-S gene products expressed in a single pollen grain. To distinguish between these two possibilities, we used a previously described dual-specific $S_{11/13}$-RNase, termed HVapb-RNase, which can reject two phenotypically distinct pollen (P_{11} and P_{13}). Since the HVapb-RNase does not distinguish between the two pollen types (it recognizes both), $P_{11}P_{13}$ HAP should be incompatible with the HVapb-RNase in spite of the competition effect. We show here that $P_{11}P_{13}$ HAP is accepted by $S_{11}S_{13}$ styles, but is rejected by the $S_{11/13}$-RNase, which demonstrates that the pollen-*S* genes must be expressed in HAP. A model involving tetrameric pollen-S is proposed to explain both the compatibility of $P_{11}P_{13}$ HAP on $S_{11}S_{13}$-containing styles and the incompatibility of $P_{11}P_{13}$ HAP on styles containing the HVapb-RNase.

SELF-INCOMPATIBILITY (SI) is a cell-cell recognition phenomenon used by higher plants to prevent inbreeding. In the most widespread type of SI [gametophytic SI (GSI)], the self-incompatibility phenotype is specified by a highly multiallelic *S*-locus, and the genotype of the haploid pollen determines its own incompatibility phenotype (De Nettancourt 1977, 2001). In the Solanaceae, the identity of the pollen component of the GSI is unknown, whereas the stylar product has been identified as an extracellular ribonuclease, *S*-RNase (McClure *et al.* 1989) expressed in the transmitting tissue of the style (Anderson *et al.* 1986). Gain-of-function experiments have shown that expression of an S-RNase transgene is necessary and sufficient to alter the SI phenotype of the pistil but does not change the pollen phenotype (Lee *et al.* 1994; Murfett *et al.* 1994; Matton *et al.* 1997), and thus the identity of the pollen-*S* gene must be different from the S-RNase (Kao and McCubbin 1997). S-RNases appear to contain two domains, an RNase activity domain essential for expression of the SI phenotype (Huang *et al.* 1994) and a recognition domain involved in the specificity of the cell-cell recognition phenomenon. In closely related S-RNases, such as the S_{11}- and S_{13}-RNases (Saba-El-Leil *et al.* 1994), the recognition domain includes the amino acids found in the so-called hypervariable (HV) regions (Ioeger *et al.* 1991). The HV regions of these two S-RNases differ by only four amino acids, and transgenic plants where these four residues in the S_{11}-RNase were replaced with those of the S_{13}-RNase displayed an S_{13} rather than an S_{11} phenotype (Matton *et al.* 1997). Curiously, replacement of only three of these four amino acids produced RNases, that are either nonfunctional (Matton *et al.* 2000) or have the unusual property of dual specificity (*i.e.*, able to reject both the phenotypically distinct P_{11} and P_{13} pollen; Matton *et al.* 1999).

The availability of this unique dual-specific S-RNase (termed HVapb-RNase) allowed us to reevaluate the S-heteroallelic pollen (HAP) effect (also known as competitive interaction in diploid HAP). In many diploid species with monofactorial GSI, naturally or artificially produced tetraploids often display self-compatibility (Lewis 1947; Brewbaker 1954; De Nettancourt 1977, 2001). Differences in reciprocal crosses between SI diploids and their tetraploid counterparts indicate that the breakdown of SI is due to the pollen and not the stylar component (De Nettancourt 1977). As first noted by Lewis (1947), only pollen that contains two different S-loci can bypass the SI barrier, an observation fully confirmed by recent molecular analyses in both *Lycopersicon peruvianum* (Chawla *et al.* 1997) and *Nicotiana alata* (Golz *et al.* 1999). The HAP effect requires

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only a second different S-locus whether carried by a centric fragment or not (van Gastel 1976; De Nettancourt 1977; Golz *et al.* 1999, 2000).

In spite of more than 50 years of research since its first description, the HAP effect remains poorly understood. In particular, it is not known if it is caused by some peculiar features of a distinct pollen-*S* gene (still unknown), by some other component of the S-locus (McCubbin and Kao 1999), or by gene inactivation (Lewis 1961; van Gastel 1976), resulting in nonexpression of the pollen-S components. Current models for the biochemical role of pollen-S, however, cannot readily explain HAP compatibility. Immunocytochemical analyses showing that S-RNases can enter pollen tubes of any genotype (Luu *et al.* 2000) have provided experimental evidence for models involving RNase inhibitors (Thompson and Kirch 1992; Kao and McCubbin 1996). These models postulate that all S-RNases can enter a pollen tube and that their RNase activity is inhibited, except for that corresponding to the S-haplotype of the pollen. Models where the pollen-S and the RNase inhibitor are on the same molecule (Kao and McCubbin 1996) or on separate molecules (Luu *et al.* 2000) have both been proposed. All versions of the inhibitor model assume that pollen-S binding to the recognition domain of its cognate S-RNase is thermodynamically favored over binding to the RNase activity domain, so that it permanently precludes activity domain binding and permits RNase activity (Kao and McCubbin 1997). In HAP, the two pollen-S should each preferentially bind to the recognition domains of their respective S-RNases, leaving the RNases active. The inhibitor models thus predict incompatibility for HAP, in contrast with experimental observations.

We report here that $P_{11}P_{13}$ HAP is accepted by styles containing the S_{11}- and S_{13}-RNases but rejected by styles expressing the dual $S_{11/13}$ HVapb-RNase. This demonstrates that pollen SI components are functional in HAP, thus ruling out gene inactivation. We propose that pollen-S acts as a tetramer and that heterotetramers, such as would be produced in HAP, are unable to block inhibitor binding and thus produce compatible pollen.

MATERIALS AND METHODS

Solanum chacoense Bitt ($2n = 2x = 24$) plants of various S-constitutions were produced by crosses (Veronneau *et al.* 1992; Birhman *et al.* 1994; Van Sint Jan *et al.* 1996). Diploid genotypes V22 ($S_{11}S_{13}$), V28 ($S_{12}S_{13}$), and G4 ($S_{12}S_{14}$) were selected from crosses of two parental lines PI458314 ($S_{11}S_{12}$) and PI230582 ($S_{13}S_{14}$; Potato Introduction Station, Sturgeon Bay, WI), whereas L25 ($S_{11}S_{12}$) resulted from crosses between V22 and V28 (pollen parent). The dual-specific S-RNase that rejects both P_{11} and P_{13} pollen was produced by site-directed mutagenesis and is expressed as a transgene introduced into host plant G4 ($S_{12}S_{14}$; Matton *et al.* 1999). HVapb plants thus reject four different pollen haplotypes, the P_{12} and P_{14} via the endogenous S-RNases from the untransformed host and the P_{11} and P_{13} using the dual-specific HVapb-RNase. All plants with a dual-specific phenotype contain wild-type levels of the HVapb-RNase in the styles (Matton *et al.* 1999).

Tetraploids of genotypes L25 ($S_{11}S_{11}S_{12}S_{12}$), V28 ($S_{12}S_{12}S_{13}S_{13}$), and G4 ($S_{12}S_{12}S_{14}S_{14}$) were produced by leaf disc culture from the corresponding diploids as described (Veronneau *et al.* 1992), whereas tetraploids F20 ($S_{11}S_{11}S_{12}S_{13}$), F38 and F55 ($S_{11}S_{11}S_{13}S_{13}$), and F44 ($S_{12}S_{12}S_{12}S_{12}$) were selected from progeny of crosses between tetraploids L25 and V28 (pollen parent; Qin *et al.* 2001). Tetraploid plant 1022 ($S_{11}S_{11}S_{13}S_{13}$) was produced by leaf disc culture of a plant issued from a cross between V22 ($S_{11}S_{13}$) as pollen donor and SP10 ($S_{13}S_{13}$), an individual obtained by obligate selfing of parental line PI230582 ($S_{13}S_{14}$; Rivard *et al.* 1994). The genotype of all plants used was verified by Western analyses of stylar extracts using antibodies against S_{11}-, S_{12}-, and S_{13}-RNases (Matton *et al.* 1999; Qin *et al.* 2001), by Southern blot analyses, and by PCR analyses using S-allele-specific primers. All plants used were true tetraploids and not chimeric, as assessed by chloroplast number in stomatal guard cells (L1 layer), pollen size and chromosome number in pollen mother cells (L2 layer), and chromosome number in root meristems (L3 layer). All cytological analyses were performed as described (Cappadocia *et al.* 1984). Compared with their diploid relatives, most autotetraploids showed some reduction in pollen fertility, as generally reported (Singh 1993).

Crosses were performed under greenhouse conditions and were classified as compatible when almost all pollinations resulted in fruit formation and incompatible when no fruits developed. Because the nature of the study required a precise assessment of pollen tube behavior after pollinations, tube growth inside the styles was routinely monitored by UV fluorescence microscopy as described (Matton *et al.* 1997). Incompatibility defined by the pollinations corresponded in all cases to pollen tube growth arrest in the styles.

RESULTS

Tetraploids derived from self-incompatible diploids are known to produce compatible S-heteroallelic pollen (HAP; De Nettancourt 1977), and this was also observed with our *S. chacoense* tetraploids. As an illustration, the breeding behavior of tetraploids containing two different S-loci (plants G4, L25, V28, F38, F55, and 1022) or three (F20) is shown in Table 1. These plants all produce diploid pollen, about two-thirds of which contain two different S-alleles and are thus fully self-compatible (see pollinations along the diagonal). This behavior is in sharp contrast to the breeding behavior of diploid pollen containing only one type of S-allele, such as that produced by the S-homozygous tetraploid F44 (Table 1), which is incompatible with any plant containing the S_{12} allele. In agreement with all previous studies, the compatibility of these tetraploids is due to their pollen, as their styles continue to block haploid pollen containing corresponding S-alleles (Table 2). It is important to note that pollen produced by the plant V22 ($S_{11}S_{13}$) is rejected by all the tetraploids expressing both S_{11}- and S_{13}-RNases, by transgenic plants expressing the HVapb-RNase (an $S_{11/13}$ specificity), but is accepted by the untransformed host plant G4 ($S_{12}S_{14}$). Note also that the pollen produced by the HVapb plants behaves identically to the pollen produced by the untransformed host since transgene expression is restricted to the style.

TABLE 1

Breeding behavior of diploid pollen from tetraploids with various S-genotypes

		Pollen parent							
Plant style	Genotype	G4	L25	V28	F20	F38	F55	1022	F44
G4	$S_{12}S_{12}S_{14}S_{14}$	12/15	9/11	9/13	10/11	19/22	7/9	10/10	0/7
L25	$S_{11}S_{11}S_{12}S_{12}$	14/16	11/12	12/17	15/17	11/12	11/14	14/16	0/12
V28	$S_{12}S_{12}S_{13}S_{13}$	12/13	14/15	16/19	12/13	13/13	13/14	10/10	0/12
F20	$S_{11}S_{11}S_{12}S_{13}$	6/11	6/6	8/10	11/12	14/15	12/16	13/13	0/19
F38	$S_{11}S_{11}S_{13}S_{13}$	9/10	9/11	7/9	11/13	16/19	6/6	10/12	8/8
F55	$S_{11}S_{11}S_{13}S_{13}$	11/11	7/8	6/7	11/12	6/8	12/13	9/9	14/18
1022	$S_{11}S_{11}S_{13}S_{13}$	6/6	8/8	6/7	10/11	10/11	8/8	8/9	7/7
F44	$S_{12}S_{12}S_{12}S_{12}$	7/8	11/12	9/10	10/10	13/13	7/9	12/13	0/11

The pollen rejection phenotype, given as number of fruits set/number of flowers pollinated, is deemed incompatible when no fruits are set and compatible when the majority of pollinated flowers set fruit. All plants except F44 ($S_{12}S_{12}S_{12}S_{12}$) produce heteroallelic diploid pollen, which accounts for the observed compatibility.

The dual-specific HVapb-RNase provides a unique tool with which to distinguish between gene inactivation and competition models for the HAP effect. If the HAP effect were caused by gene inactivation (Lewis 1961; van Gastel 1976), the $P_{11}P_{13}$ HAP would be as compatible with HVapb plants as with V22 ($S_{11}S_{13}$). In contrast, if competition between P_{11} and P_{13} pollen-S components present together in diploid pollen takes place, the $P_{11}P_{13}$ HAP pollen should be rejected by HVapb plants (just like normal haploid pollen) since our dual-specific HVapb-RNase rejects both P_{11} and P_{13} pollen. As shown in Table 3, no fruits are formed when pollen from plants with an $S_{11}S_{11}S_{13}S_{13}$ genotype (F38, F55, or 1022) is tested on styles of HVapb plants, and microscopic examination of these pollinated styles confirms full rejection of $P_{11}P_{13}$ HAP at midstyle (not shown). Since plants F38, F55, and 1022 all have different genetic backgrounds, indicating that HAP rejection is not restricted to a particular genotype, we conclude that the pollen components of the SI system must be fully expressed in HAP.

TABLE 2

Breeding behavior of haploid pollen from plants with various S-genotypes

		Pollen parent		
Plant style	Genotype	V22	G4 (2×)	HVapb
G4(4×)	$S_{12}S_{12}S_{14}S_{14}$	15/19	0/17	0/14
L25	$S_{11}S_{11}S_{12}S_{12}$	13/13	9/10	11/12
V28	$S_{12}S_{12}S_{13}S_{13}$	14/16	9/10	11/12
F20	$S_{11}S_{11}S_{12}S_{13}$	0/32	8/9	11/11
F38	$S_{11}S_{11}S_{13}S_{13}$	0/11	8/8	8/10
F55	$S_{11}S_{11}S_{13}S_{13}$	0/14	12/13	7/8
1022	$S_{11}S_{11}S_{13}S_{13}$	0/12	12/12	10/11
F44	$S_{12}S_{12}S_{12}S_{12}$	13/13	11/11	8/9
V22	$S_{11}S_{13}$	0/23	15/16	23/24
G4(2×)	$S_{12}S_{14}$	14/14	0/9	0/9
HVapb	$S_{12}S_{14}S_{11/13}$	0/38	0/14	0/32

Data for the HVapb dual-specific RNase are pooled from five independent transgenic lines.

The genetic analysis also demonstrates that the dual-specific HVapb-RNase alone is responsible for HAP rejection. First, there is nothing unusual about plants F38, F55, and 1022, as their HAP is self-compatible (Table 1), compatible on V22 styles (Table 3), and their styles reject pollen from V22 (Table 2). Second, there are no breeding differences between the five independent transgenic plants expressing the HVapb-RNase, as $P_{11}P_{13}$ HAP was fully rejected by their pistils (Table 3). All the HVapb plants used here express wild-type levels of their transgene S-RNase (Matton *et al.* 1999) and accept all other HAP combinations such as $P_{11}P_{12}$, $P_{12}P_{14}$, or $P_{12}P_{13}$ (Table 1). In addition, HVapb transgenic plants that do not express the transgene behave like the untransformed host G4 and do not reject HAP (not shown). Last, the rejection of the $P_{11}P_{13}$ HAP is unrelated to expression of more than two different S-RNases in the style, as neither tetraploid F20 (Table 1) nor a transgenic plant of $S_{12}S_{14}$ genotype expressing an additional S_{11}-RNase (Matton *et al.* 1997) reject $P_{11}P_{13}$ HAP (not shown).

DISCUSSION

Model for pollen-S action: Any model for GSI must now explain the normal compatibility of HAP, as well as its incompatibility with the cognate dual-specific S-RNase, as shown here. To develop a working model, however, two additional observations must be taken into account. First, screens for compatible pollen produced after mutagenesis have uncovered a variety of pollen part mutants, some of which contained what was referred to as an additional S-allele while others apparently lack any S-allele (Pandey 1967; van Gastel 1976; De Nettancourt 1977; Golz *et al.* 1999). Clearly, while an additional S-allele (pollen-S) could be analogous to

TABLE 3

Rejection of $P_{11}P_{13}$ heteroallelic pollen by a dual recognition specificity S-RNase

Plant style	Genotype	G4(4×)	L25	V28	F20	F38	F55	1022	F44
V22	$S_{11}S_{13}$	11/12	13/13	15/19	10/13	23/25	17/19	21/23	11/12
G4(2×)	$S_{12}S_{14}$	11/13	8/9	9/9	16/20	11/12	13/15	14/14	0/13
HVapb	$S_{12}S_{14}S_{11/13}$	12/12	15/15	10/10	25/30	0/46	0/20	0/19	0/15

The pollen donors are tetraploids and their genotypes are shown in Table 1. Data are given as number of fruits set/number of flowers pollinated. Data for the HVapb dual-specific RNase are pooled from five independent transgenic lines.

HAP, deletion of pollen-S must be different. Thus, any model for SI must predict a compatible pollen phenotype either when two different pollen-S are expressed or when none is expressed. Second, as discussed above, at least part of the function of the SI system inside pollen tubes is likely to involve RNase inhibitors (RI). Although not yet reported for plants, RI are well known in animal systems (Hofsteenge 1997).

We recently proposed a model for GSI with two pollen components, one a general RI that can inactivate any S-RNase and the other an S-allele-specific product that maintains the activity of a specific S-RNase inside the pollen tube by blocking RI binding (Luu *et al.* 2000). Separation of the pollen-S blocker from the general RI was proposed to explain the compatibility of pollen mutants possibly lacking pollen-S (Pandey 1967; van Gastel 1976; Golz *et al.* 1999). Interestingly, Dr. T. Sims recently identified in *Petunia hybrida*, by the two-hybrid system, a nonpolymorphic S-RNase-binding protein with a RING-HC domain that could represent a possible candidate for the general inhibitor (T. Sims and M. Ordanic, unpublished results).

The multimeric nature of pollen-S: From the results shown here, we deduce that only a multimeric pollen-S blocker can explain all aspects of the HAP phenotype. First consider the incompatibility reaction of haploid P_{11} pollen growing in an $S_{11}S_{13}$ style (Figure 1A). The RI components are drawn as shaded arcs to mimic the structure of the mammalian RNase inhibitor (Hofsteenge 1997), the P_{11} blockers as small shaded circles, and the S-RNases as large white ovals. S-RNases enter the pollen tubes from the styles (Luu *et al.* 2000) and, in this illustration, we assume that eight S-RNases of any type present in the style will enter. We also assume that there are sufficient blockers in a pollen tube to bind to their cognate S-RNases, and therefore the P_{11} pollen tube contains eight P_{11} tetramers in addition to the S_{11}- and S_{13}-RNases. All the S_{11}-RNases bind the P_{11} blocker (favored thermodynamically over RI binding; Kao and McCubbin 1997). Since blocker binding precludes RI binding, S_{11}-RNase remains active and incompatibility results. The RI binds the S_{13}-RNase because no P_{13} blocker is present in the P_{11} pollen, but inhibition of the S_{13}-RNase activity has no effect on the incompatibility phenotype since the active S_{11}-RNase causes pollen rejection.

The multimeric nature of the blocker is irrelevant for the incompatibility phenotype of normal (haploid) pollen but is essential to explain the compatibility of HAP (see next section for the choice of tetramers over dimers). When $P_{11}P_{13}$ HAP grows in $S_{11}S_{13}$ styles (Figure 1B), S_{11}- and S_{13}-RNases enter the pollen tube as before. Once again, blockers will compete with the RI for binding to their cognate S-RNases. However, even if HAP produces the same number of P_{11} (small circles) or P_{13} blockers (small squares) as would haploid, the random assembly of monomers into tetramers would produce homotetramers and heterotetramers in binomial proportions, similar to the 1:4:6:4:1 ratio observed for lactate dehydrogenase tetramers (Markert 1963). In this case, only 1 out of the 16 blocker tetramers in $P_{11}P_{13}$ HAP would be a P_{11} homotetramer and thus only one of the S_{11}-RNases entering the pollen tube would remain active. If heterotetramers were inactive, the other S_{11}-RNases would be inhibited because the hybrid blockers would no longer outcompete RI binding. The same argument holds for assembly of a P_{13} homotetramer blocker and its binding to the S_{13}-RNase. Therefore, only a fraction (one-quarter) of the amount of RNase active in haploid pollen (Figure 1A) would be active in HAP (Figure 1B). Is this reduction in active RNase sufficient to cause compatibility? It is generally accepted that a minimum threshold of S-RNase is required for pollen rejection. The threshold idea is derived from experiments in transgenic *P. inflata* (Lee *et al.* 1994) and data from natural SC Japanese pear mutants (Hiratsuka *et al.* 1999), where S-RNase expression at one-third the normal level results in self-compatibility. Thus, a reduction in the amount of active S-RNases to one-quarter normal levels could indeed result in HAP compatibility.

How, then, might the dual-specific HVapb-RNase reject HAP? Since this S-RNase can bind either P_{11} or P_{13} (Matton *et al.* 1999), it is unlikely to discriminate between any of the heterotetramers in $P_{11}P_{13}$ pollen (Figure 1C). Blocker binding then would be unaffected by the formation of heterotetramers and this RNase would remain fully active and reject the pollen. For clarity, we have drawn only the HVapb RNase in Figure 1C, although it must be kept in mind that these pollen tubes will also contain S_{12}- and S_{14}-RNases, which are present

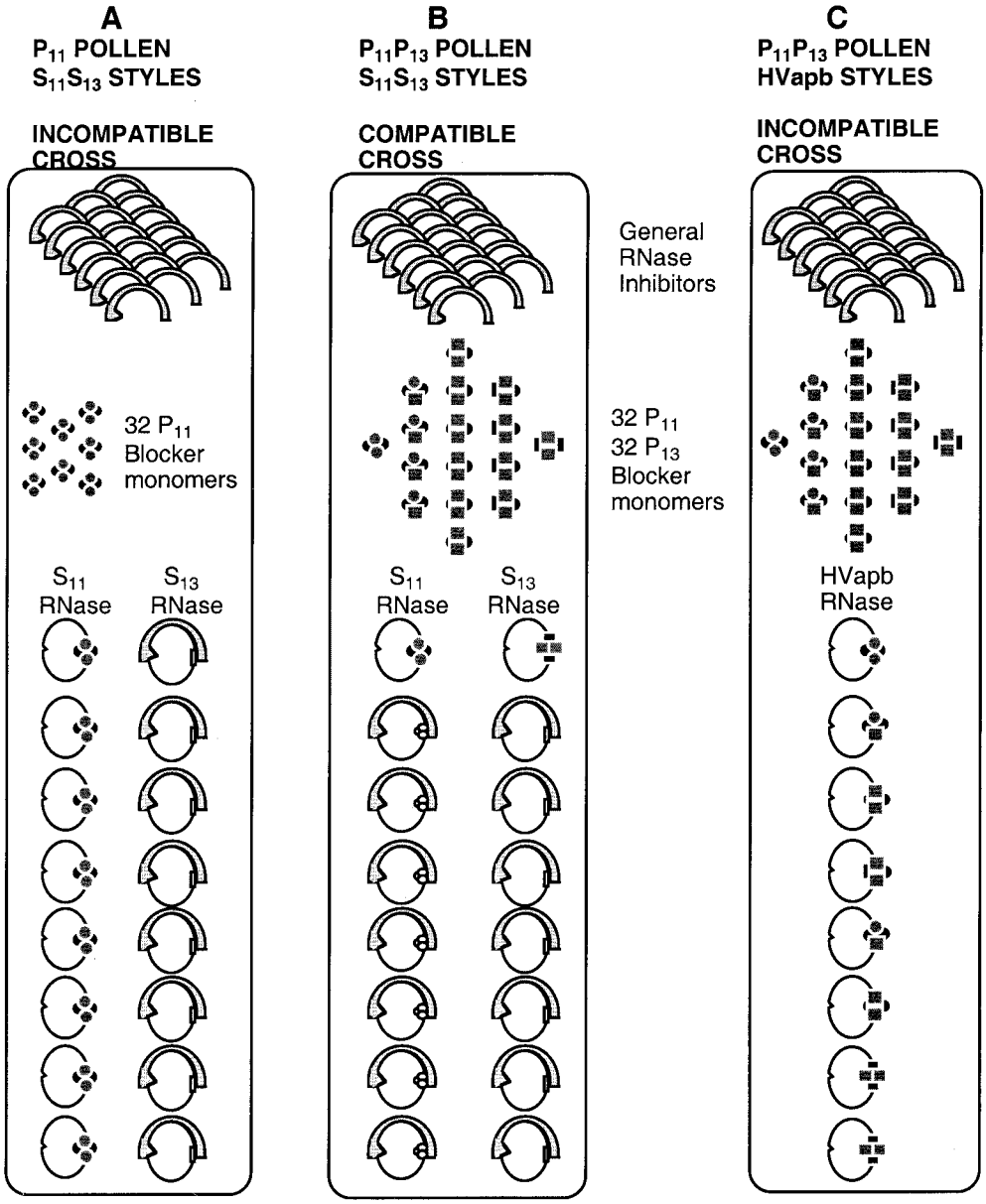

FIGURE 1.—A model for GSI derived from the HAP effect. (A) Haploid P_{11} pollen growing in an $S_{11}S_{13}$ style is incompatible because the ribonuclease inhibitors (RI; shaded arcs) are prevented from binding to the S_{11}-RNases (open ovals at left), which have entered the pollen tubes from the styles, by the P_{11} blockers (small shaded circles) present in the pollen tubes. Since P_{13} blockers are absent, RI binds S_{13}-RNases (open ovals at right). (B) S_{11}- and S_{13}-RNases from an $S_{11}S_{13}$ style enter diploid $P_{11}P_{13}$ HAP, but RI binding cannot be fully prevented because a binomial distribution of tetramer types results in a lower number of P_{11} and P_{13} homotetramers than would be found in either haploid pollen type. (C) The dual-specific HVapb-RNase does not discriminate between P_{11} and P_{13} blockers and thus binds all of the heterotetrameric blockers. This results in incompatibility because RI is prevented from binding.

in the styles of the transformed plants at the same (wild-type) levels as the HVapb-RNase.

Theoretical support for a tetrameric pollen-S: To buttress the intuitive argument provided above, we have also analyzed the predictions of a mathematical formulation for the amount of pollen-S, which takes into account the possibility of fractional activity of heteromers relative to homomers (b) and relative expression of pollen-S in diploid compared to pollen (a). In the expression defined below for k-mers, the amount of active pollen-S in diploid pollen (x_d) is a function of the amount of pollen-S normally expressed in haploid pollen (x_h):

$$x_d = 2ax_h\,((1/2)^{k-1} + b(1 - (1/2)^{k-1})). \qquad (1)$$

This equation takes into account situations where pollen-S expression levels are less than in haploid plants ($a < 1$) as well as cases where heteromers are partially active ($0 < b < 1$). In the section above, we assumed that expression of pollen-S was the same in diploid and in haploid pollen ($a = 1$) and that heteromers are totally inactive ($b = 0$).

The quantity of active pollen-S in HAP must be $< x_h$ for compatible crosses (with $S_{11}S_{13}$ plants) and $\geq x_h$ in incompatible crosses (with HVapb plants). To visualize the main conclusions of this model, we calculated the range of values of pollen-S expression (a) that satisfy these two requirements for various values of (k) and (b) (Table 4). Note that $b = 1$ for HVapb plants, since the dual-specific RNase cannot distinguish between P_{11} and P_{13}. Two important conclusions can be unequivocally drawn from this analysis. First, the pollen-S cannot be a monomer (Table 4). Second, heteromers cannot be as active as homomers, as no value of a can produce compatibility with $S_{11}S_{13}$ and incompatibility with HVapb plants if $b = 1$.

TABLE 4

Relative levels of pollen-S expression (a) in diploid *vs.* haploid pollen as predicted from Equation 1

k-mer	Expression levels of pollen-S required: For compatibility in $S_{11}S_{13}$ plants	Expression levels of pollen-S required: For incompatibility in HVapb plants	Conclusion
Monomer ($k = 1$)	$a < ½$	$a \geq ½$	Impossible
Dimer ($k = 2$)			
$b = 0$	$a < 1$	$a \geq ½$	Possible
$b = 1$	$a < ½$	$a \geq ½$	Impossible
Tetramer ($k = 4$)			
$b = 0$	$a < 4$	$a \geq ½$	Possible
$b = 1$	$a < ½$	$a \geq ½$	Impossible

The analysis also allows us to describe the conditions required for dimeric or tetrameric blocker activity. Were pollen-S dimeric, its expression in HAP would be restricted to $½ \leq a < 1$. Only two values of b are shown, but it is clear that as the activity of the heteromers (b) increases, the a value must decrease. If pollen-S were tetrameric, a wider range of pollen-S expression levels is permitted ($½ \leq a < 4$). Thus, if pollen-S is a dimer, this model would require a reduced expression in diploid pollen. While pollen-S is as yet unknown and cannot be assayed, reduction of allele expression in polyploids has been reported for some genes (Birchler and Newton 1981). However, we do not as yet have any evidence for a reduction in the levels of S-RNases (presumably tightly linked to the pollen-S gene at the S-locus) in tetraploids. Epigenetic silencing, resulting from the increase in ploidy level, could also account for a reduced level of pollen-S, although this phenomenon is usually restricted to the silencing of one of the original alleles (Scheid *et al.* 1996). Furthermore, it is unclear why gene silencing would preferentially occur in HAP as opposed to homoallelic pollen. We therefore conclude that the blocker is probably a tetramer.

The model proposed here supports the proposal that the S-RNase-based GSI evolved from an RNase-based defense mechanism (Kao and McCubbin 1996). Indeed, extracellular S-like RNases not involved in GSI have been described and their role in host defense has been suggested (Lee *et al.* 1992). If extracellular S-RNases can enter pollen tubes indiscriminately (Luu *et al.* 2000), then inhibitors for S-like RNases must have been necessary in pollen. GSI could indeed have derived stepwise from an ancestral self-compatible system as proposed (Uyenoyama 1988). Initially, pollen tube RI would block the cytotoxicity of extracellular S-like RNases. These extracellular S-like RNases may already have evolved polymorphisms, which, if selected for a role in pathogen defense, would be neutral for pollen RI binding. Thus, we see self-incompatibility as having arisen by the development of an allele-specific recognition domain on a pollen protein that binds a particular stylar RNase and blocks RI binding.

Finally, our model suggests that the term "competition effect" may not accurately reflect the mechanism of HAP compatibility. Earlier interpretations of the phenomenon were that two different pollen components competed with each other for some limiting factor (De Nettancourt 1977). In our view, the reduced activity of heteromers compared to homomers points to pollen-S itself as the limiting factor.

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LITERATURE CITED

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