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Does the genome of *Corylus avellana* L. contain sequences homologous to the self-incompatibility gene of *Brassica*?

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Abstract Self-incompatibility is a genetic mechanism enforcing cross-pollination in plants. Hazelnut (*Corylus avellana* L.) expresses the sporophytic type of self-incompatibility, for which the molecular genetic basis is characterized only in *Brassica*. The hypothesis that the hazelnut genome contains homologs of *Brassica* self-incompatibility genes was tested. The *S*-locus glycoprotein gene (*SLG*) and the kinase-encoding domain of the *S*-receptor kinase (*SRK*) gene of *B. oleracea* L. were used to probe blots of genomic DNA from six genotypes of hazelnut. Weak hybridization with the *SLG* probe was detected for all hazelnut genotypes tested; however, no hybridization was detected with PCR-generated probes corresponding to two conserved regions of the *SLG* gene. One of these PCR probes included the region of *SLG* encoding the 11 invariant cysteine residues that are an important structural feature of all *S*-family genes. The present evidence suggests that hazelnut DNA hybridizing to *SLG* differs significantly from the *Brassica* gene, and that the *S*-genes cloned from *Brassica* will not be useful for exploring self-incompatibility in hazelnut.

Key words Hazelnut · Filbert · *S*-locus glycoprotein · Sporophytic self-incompatibility

Introduction

Many plants possess structures or mechanisms that encourage outcrossing. One of the most effective outcrossing mechanisms known is self-incompatibility (SI), where a physiological barrier prevents self-fertilization in otherwise fertile hermaphrodites. In most cases, SI is determined by a single multi-allelic locus, called the *S*-locus (Matton et al. 1994). Any pollen expressing the same *S*-allele as the recipient pistil is rejected. Several forms of SI exist, among which gametophytic and sporophytic SI have been studied in most detail. In sporophytic self-incompatibility (SSI), pollen acceptance or rejection by the stigma is based on the (diploid) *S*-genotype of the pollen donor plant. In SSI, *S*-alleles can interact in pollen and in pistil, showing dominance, co-dominance, mutual weakening, or other interactions (Nasrallah and Nasrallah 1989).

The families Asteraceae, Brassicaceae, Convolvulaceae, Caryophyllaceae and *Corylus* of the Betulaceae exhibit SSI (Charlesworth 1988). Tapetal substances (including recognition molecules) transferred to the pollen-grain wall during microsporogenesis are believed to be responsible for the recognition of the pollen parent's *S*-phenotype on the recipient stigma (Heslop-Harrison et al. 1974). Incompatible pollen is arrested on the stigma surface, where pollen adhesion, hydration, germination, or penetration of the stigma is blocked. Callose is deposited in both the pollen grain/tube and the adjacent stigmatic papillae. These manifestations of SSI are very similar among different botanical families (Howlett et al. 1975; Boyle and Stimart 1986; Nasrallah and Nasrallah 1989).

The study of SSI at the molecular level is limited to the Brassicaceae (reviewed by Nasrallah and Nasrallah 1989, 1993; Dickinson et al. 1992; Hinata et al. 1993; Matton et al. 1994). At least two expressed genes are now known to reside at the *S*-locus in *Brassica: SLG* and *SRK* (Nasrallah and Nasrallah 1993). The term "*S*-haplotype" has been suggested as a replacement for "*S*-allele" to reflect this situation (Boyes and Nasrallah 1993). How *SLG* and *SRK* interact to effect pollen recognition remains unclear, but current evidence suggests that functional copies of both genes are required for the proper operation of SI (Nasrallah and Nasrallah 1993).

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The *S*-locus glycoprotein (*SLG*) encodes a secreted glycoprotein abundant in the cell walls of the stigmatic papillae, and shows precise temporal regulation coincident with the developmental acquisition of SI (Nasrallah et al. 1985a; Kandasamy et al. 1989). The *SLG* gene lacks introns (Nasrallah et al. 1988). Sequence comparisons among alleles have shown that the protein encoded by *SLG* contains both conserved and hypervariable regions. The conserved regions include a portion at the N-terminal end, and 11 invariant cysteine residues in a particular arrangement toward the C-terminal end (Nasrallah and Nasrallah 1989).

SRK contains seven exons, the first of which is virtually identical to *SLG* within a given *S*-haplotype (Stein et al. 1991). The seven exons of *SRK* encode this extracellular "*S*-domain", a membrane-spanning helix, and juxta-membrane, kinase catalytic and C-terminal domains. *SRK* encodes a functional serine/threonine kinase (Goring and Rothstein 1992), and *SRK* transcripts show temporal and tissue specificity similar to *SLG* (Stein et al. 1991).

The *Brassica* genome also contains a number of sequences homologous to *SLG* (Nasrallah et al. 1985b), some of which are expressed. The intronless genes *SLR1* and *SLR2* encode secreted glycoproteins with significant sequence homology to *SLG*, and show a similar expression pattern (Trick and Heizmann 1992; Hinata et al. 1993). Sequence similarities include the 11 invariant cysteines in the same arrangement as in *SLG* and *SRK*. The functions of *SLR1* and *SLR2* are unknown, but they play no part in determining allelic identity, and neither is linked to the *S*-locus (Lalonde et al. 1989; Trick and Heizmann 1992).

The evolutionary relationship among *S*-genes is in dispute. Some scientists propose a common evolutionary origin, and others argue for multiple independent origins (Bateman 1952; Bell 1995; Read et al. 1995). Among families with gametophytic self-incompatibility (GSI), the *S*-gene product is an RNase in the Solanaceae, the Rosaceae, and perhaps the Campanulaceae (Sassa et al. 1992; Singh and Kao 1992; Stephenson et al. 1992). However, the *S*-gene products of *Papaver rhoeas* (Papaveraceae) and *Phalaris coerulescens* (Poaceae) are not RNases, and their mechanism of action is clearly different (Franklin-Tong et al. 1991; Foote et al. 1994; Li et al. 1994). Because only one family with SSI has been examined at the molecular level, no basis exists for judging which features are general aspects of SSI, and which are unique to crucifers.

We are interested in studying SI in *Corylus avellana* (hazelnut), partly as a basis of comparison to *Brassica* and partly for practical reasons. Hazelnut expresses SSI (Thompson 1979). All known hazelnut *S*-alleles are co-dominant in the pistil and show either co-dominance or simple dominance in the pollen (Mehlenbacher and Thompson 1988). To-date, 26 *S*-alleles have been identified (S. A. Mehlenbacher, Oregon State University, personal communication). The pollen-stigma interactions are typical of SSI (Hampson et al. 1993) but, in contrast to most species with SSI, hazelnut pollen is binucleate and potentially long-lived (Heslop-Harrison et al. 1986). In breeding programs, our present inability to overcome SI makes certain desirable crosses impossible, or compatible only in one direction. In the orchard, SI necessitates the planting of compatible pollenizers.

The objective of the present investigation was to determine whether the hazelnut genome contains homologs of the *Brassica* self-incompatibility genes *SLG* and *SRK*. We reasoned that, if the *S*-genes of the two species share a common ancestry or mechanism of action, they may show sequence similarity at the DNA level, and probes from *B. oleracea* L. might detect the hazelnut *S*-gene on genomic DNA blots. If successful, this approach may enable *Brassica* clones to be used as tools to advance the understanding of SSI in hazelnut.

Materials and methods

Plant material

Immature hazelnut leaves were collected from clones in a layer bed at the Oregon State University Vegetable Research Farm near Corvallis, Oregon, and from specimens growing in a greenhouse under natural daylengths. The cultivars and their *S*-allele genotypes were as follows: "Tonda di Giffoni" (S_2S_{33}), 'Barcelona' (S_1S_2), 'Butler' (S_2S_3), 'Gasaway' (S_3S_{26}), 'Hall's Giant' (S_5S_{15}), and 'Negret' ($S_{10}S_{22}$). *S*-allele designations are from Mehlenbacher and Thompson (1988), or S.A. Mehlenbacher, Department of Horticulture, Oregon State University (personal communication). These clones were chosen to represent a diversity of *S*-alleles and geographic origins, including England, Italy, Spain and the USA.

DNA was also extracted from three field-grown, self-incompatible inbred lines of broccoli (*B. oleracea* var. *italica* 161, 179 and 240-S-24), and from tobacco (*Nicotiana tabacum* 'Maryland Mammoth') growing in a greenhouse. The *S*-genotypes of the broccoli lines were unknown.

DNA extraction

A procedure based loosely on that of Radetzky (1990) was developed for use on hazelnut. Leaves (fresh, or previously frozen in liquid nitrogen and stored at −80 °C) were ground to a fine powder in liquid nitrogen in a mortar. The powder was added to pre-heated (65 °C) 2 × CTAB buffer (100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% CTAB, 1% β-mercaptoethanol) at a ratio of 10 ml·g^{-1} FWt of leaf in a 50-ml polypropylene centrifuge tube. The contents were promptly mixed, then homogenized for 15–20 s in a Tekmar homogenizer (Tekmar Company, Cincinnati, Ohio).

The samples were incubated for 60 min at 65 °C in a water bath, then 1 vol of chloroform: isoamyl alcohol (24:1 v:v) was added, and the phases mixed by rapid inversion. After centrifugation at 10 000 g for 10 min at 25 °C, the aqueous phase was carefully removed and 0.5 vol of 5 M NaCl added. DNA was precipitated with 0.6 vol of cold (−20 °C) isopropanol.

The solution was immeditately centrifuged (10 000 g, 10 min at 4 °C), and the supernatant discarded. The pellet was washed with 76% ethanol 10 mM ammonium acetate, air-dried for approximately 5 min and resuspended overnight in 0.7 ml of TE (10 mM Tris pH 8.0, 1 mM EDTA) at 4 °C. RNase A (Sigma, St. Louis, Missouri) was added to a final concentration of from 10 to 15 units per ml, and the samples incubated at 37 °C for ≥ 1 h.

DNA was extracted once with an equal volume of 1:1 Tris-equilibrated phenol:chloroform:isoamyl alcohol (24:1), and twice with 24:1 chloroform:isoamyl alcohol. The phases were separated by centrifugation for 10 min at about 14 000 g after each extraction. After

the final extraction, DNA was precipitated with 0.1 vol of 3 M sodium acetate (pH 5.2) and 1 vol of cold isopropanol. After centrifugation as before, the pellet was rinsed with cold 80% ethanol and drained. To rid the pellet of remaining ethanol, it was air-dried or left uncovered at 37 °C for 15–20 min (Lodhi et al. 1994). The DNA was re-suspended in an appropriate volume of TE (usually 100 µl) overnight at 4 °C, and stored at 4 °C or −20 °C.

The same procedure was used to isolate DNA from the herbaceous species, except that the homogenization step was omitted, and the initial precipitation of DNA from the aqueous phase was satisfactory with 1 vol of cold isopropanol alone.

Bacterial transformation, genomic DNA digests and Southern blotting

The pBOS5 plasmid, provided by Dr. June Nasrallah of Cornell University, contained a partial cDNA for *SLG* from the *B. oleracea* allele S_6 (SLG_6). This cDNA lacks only the portion encoding the extreme N-terminal end of the signal peptide. Standard protocols were used for bacterial transformation and gel-purification of the inserts (Sambrook et al. 1989).

Hazelnut (about 20 µg) or *Brassica* (about 10 µg) genomic DNA was digested with either *Hin*dIII, *Eco*RI or *Bam*HI (New England Biolabs, Beverly, Mass.) and electrophoresed through a 0.8% agarose gel using TAE buffer (Sambrook et al. 1989) at 120 V, or 25 V for overnight runs. DNA was blotted onto a Zetaprobe GT nylon membrane (Biorad, Hercules, Calif.), as described by Sambrook et al. (1989). Restriction enzyme-digested tobacco DNA or sheared, digested salmon-sperm DNA were included as negative controls.

Blots were probed separately with the 1.2-kb *B. oleracea* SLG_6 cDNA or PCR-amplified fragments thereof, or a soybean rRNA gene. At least two blots were tested with each probe. Approximately 50 ng of the desired DNA fragment was radiolabelled with 50 µCi of [α-^{32}P]dCTP, using the random primer labelling method (Feinberg and Vogelstein 1983). Pre-hybridization of the blot was carried out in 0.25 M Na_2HPO_4 (pH 7.2) and 7% SDS. The blot was hybridized overnight at 65 °C in the same solution with the labelled probe.

For low-stringency washes, the membranes were washed twice in 2 × SSC, 0.1% SDS for 20–30 min, once at room temperature and once at 65 °C. Under these conditions, an approximately 20–30% mismatch would be tolerated. For blots probed with the soybean rRNA gene, the blots were also washed successively with 1 × SSC, 0.5 × SSC, and 0.1 × SSC (all with 0.1% SDS) at 65 °C for 30–60 min each. Only a 3–4% mismatch is tolerated under these conditions. After washing, the blots were exposed to X-ray film (Fuji RX-6CU) for 5 days at −80 °C with two intensifying screens, except for the blots probed with the rRNA gene which were left on film for 1.5 h with no intensifying screen.

Blots to be re-probed were stripped by washing twice for 20–30 min each in a large volume of 0.1 × SSC 0.5% SDS at 95 °C. The stripped blots were checked by exposing them overnight at −80 °C with an intensifying screen.

PCR conditions

The "Primers 2" computer program (Scientific and Educational Software, State Line, Pa.) was used to choose appropriate PCR primer pairs for amplifying target regions of interest from the pBOS5 plasmid. Primers were synthesized at the Central Services Laboratory, Oregon State University. The primer pairs (Table 1) amplified the following regions of the SLG_6 cDNA: a 148-bp fragment from nucleotides 384–532 (fragment A), a 102-bp fragment from nucleotides 725–827 (fragment B), a 362-bp fragment from nucleotides 840–1202 (fragment C). Figure 1 diagrams the relative positions of these fragments in the mature *S*-locus-specific glycoprotein (SLSG).

Satisfactory yield and purity of the desired fragments were obtained with 0.6 µg of template DNA and 2, 3 or 4 mM of $MgCl_2$ (depending on the primer pair used). The 100-µl reaction volume contained 200 µM of each dNTP and 0.75 µM of each primer, plus 2.5 units of Ampli*Taq* DNA polymerase in the supplied buffer. All reagents except the primers came from Perkin-Elmer (Norwalk, Conn.). Three controls were included with each reaction: no DNA polymerase, no primers, and no template. The samples were overlaid with mineral oil before PCR to prevent evaporation.

PCR was performed in a Thermolyne Temp-Tronic PCR machine (Barnstead-Thermolyne Corp., Dubuque, Iowa) as follows: 3 min at 94 °C; 39 cycles of 1 min at 92 °C plus 2 min at 55 °C plus 3 min at 72 °C. After the final cycle, the samples were held for 15 min at 72 °C.

Table 1 Sequences of PCR primers used to amplify selected portions of the *Brassica SLG* gene

Fragment A-	Forward:	CAG TAA CAA CGA CGC AA GTG
	Reverse.	CGC TTG ATG GAT CAT CTG AA
Fragment B-	Forward:	CCA ACA ACA GCA TCT ACT CG
	Reverse:	GAA GAC CAG AAC CTG TTC CA
Fragment C-	Forward:	CCA GTG CGA TAC ATA CAT AA
	Reverse:	TAA TTC CGC ATA TCG TCA AG

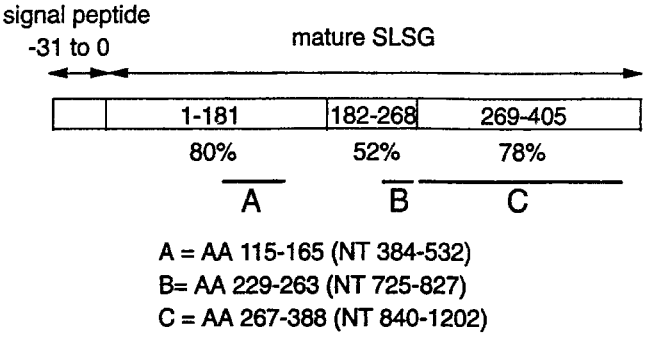


Fig. 1 Diagrammatic representation of the *Brassica S*-locus-specific glycoprotein (SLSG), showing amino-acid (AA) conservation of three different regions of the protein among different *S*-alleles (Nasrallah and Nasrallah 1989). The approximate positions of the protein regions encoded by the three PCR-generated nucleotide (*NT*) fragments used to probe DNA blots are shown below (not to scale)

Results

In initial experiments, faint bands were observed after 2 days of exposure with one intensifying screen when hazelnut genomic-DNA blots were probed with the *Brassica SLG* gene, while strong signals were observed with control *Brassica* DNA. To enhance detection of the bands from hazelnut, further DNA gel-blot hybridizations were carried out, using more hazelnut DNA, and increasing the exposure time to 5 days with two intensifying screens.

Figure 2 shows an example of a blot probed at low stringency with the 1.2-kb cDNA of *SLG*. Multiple bands were observed in the *Brassica* genotypes, consistent with previous reports that *SLG* is one member of a multigene family (Nasrallah et al. 1985b). Bands with the strongest hybridization in the *Brassica* lanes probably correspond to the *SLG* gene in these lines. Some RFLPs are present among the *Brassica* genotypes (Fig. 2, lanes 2, 9 and 12).

DNA from all the hazelnut cultivars hybridized to *SLG* (Fig. 2, lanes 4, 6–8, 10, 11). At the stringency used,

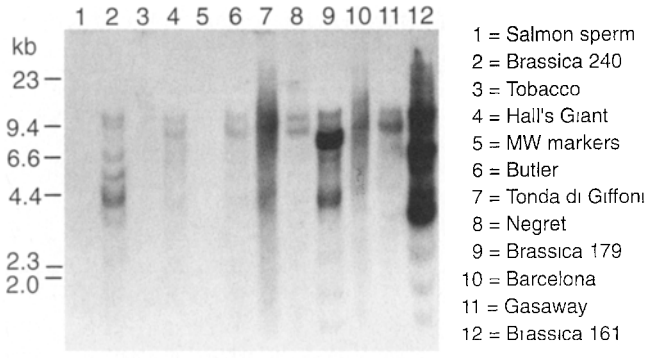


Fig. 2 Blot of *Hin*dIII-digested genomic DNA probed with *Brassica SLG*. Lanes were loaded with about 20 µg of hazelnut DNA or about 10 µg of *Brassica*, *Nicotiana*, or salmon-sperm DNA

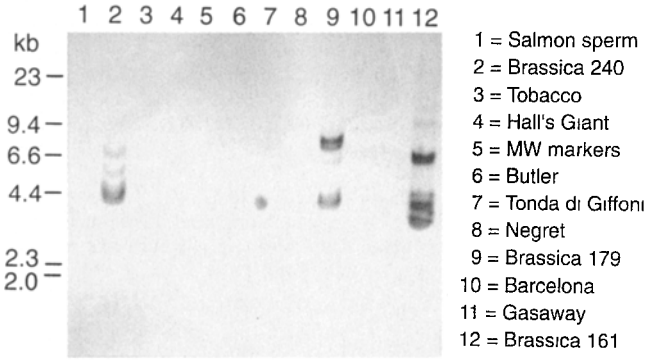


Fig. 3 Same blot as Fig 2, probed with fragment A (N-terminal fragment) of *SLG*

the hazelnut sequence is estimated to be 70–80% similar to that of the *Brassica* probe. Two bands of approximately 9.9 kb and 8.2 kb were common to 'Hall's Giant' (S_5S_{15}), 'Butler' (S_2S_3), 'Negret' ($S_{10}S_{22}$) and 'Gasaway' (S_3S_{26}). In 'Gasaway' (S_3S_{26}) and 'Barcelona' (S_1S_2), the 8.2-kb fragment is more prominent. An extremely faint band at about 4.2 kb was barely detectable in most hazelnut cultivars. The absence of any signal in the lambda MWt markers, salmon-sperm and tobacco DNA (Fig. 2, lanes 1, 3, 5) confirms that the probe is binding specifically.

To further characterize the nature of the hybridizing DNA in hazelnut, probes to specific portions of the *SLG* gene were used. The aim was to elucidate the segment of *SLG* that was hybridizing to the hazelnut sequence. For example, if the hazelnut sequence encodes a secreted glycoprotein or a receptor serine/threonine kinase, it may exhibit the common structural features of *S*-multigene family members, without necessarily being the hazelnut SI gene.

Three regions of *SLG* were chosen for PCR amplification and used as probes (see diagram in Fig. 1): (1) fragment A, coding for a portion of the N-terminal region A (amino acids 115–165 in the mature protein) that is conserved among *Brassica* Class-I alleles; (2) fragment B, corresponding to a short stretch of the "hypervariable" region B (amino acids 229–263); (3) fragment C, encoding a portion of the C-terminal region (amino acids 267–388) spanning the 11 conserved cysteine residues that are the "major structural hallmark" of all *S*-multigene family members (Nasrallah and Nasrallah 1993). Fragments A and C, but not B, were expected to hybridize with the *Brassica* DNA at low stringency.

The blot shown in Fig. 2 was probed sequentially with fragments A, B and C. Fragment A hybridized to all three *Brassica* positive controls (Fig. 3, lanes 2, 9, 12). No signals appeared in any lanes with hazelnut DNA, or with any of the negative controls. When the blot was probed with fragment B, no hybridization was observed, except for a very faint doublet in *Brassica* line 179 (data not shown). Presumably there is sufficient homology in this sequence with the SLG_6 hypervariable region for the probe to hybridize at low stringency. Probing with fragment C, the coding region spanning the cysteine array, likewise resulted in signals only in *Brassica* control lanes (Fig. 4). All three fragments therefore reacted as expected with the *Brassica* controls, and none hybridized to any of the negative controls. Probing the blot with a soybean rRNA gene demonstrated that the hazelnut DNA was capable of hybridizing with a conserved gene (Fig. 5).

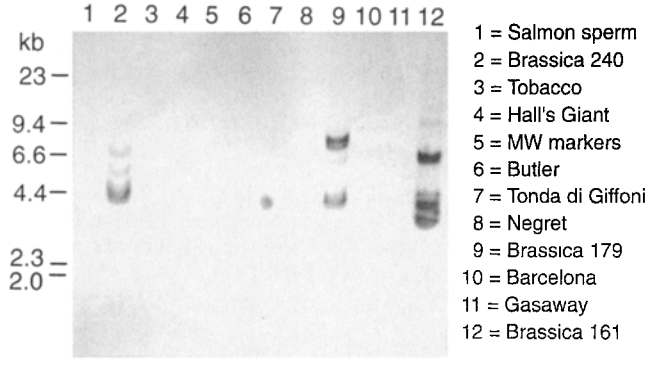
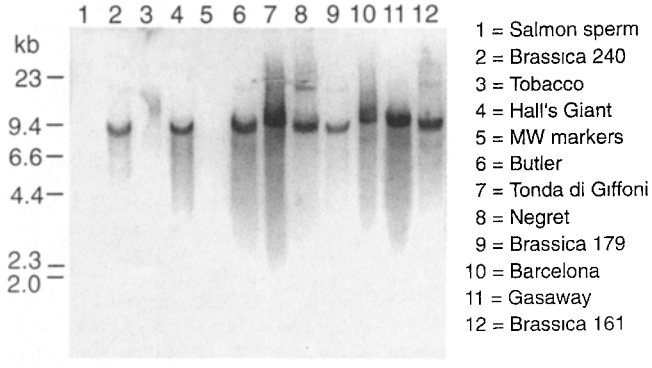


Fig. 4 Same blot as Fig. 2, probed with fragment C (cysteine-region fragment) of *SLG*

Fig. 5 Same blot as Fig 2, probed with soybean rRNA gene



Discussion

The common position of bands hybridizing to *SLG* among hazelnut genotypes with different *S*-alleles and widely different geographic origins was very surprising. Because hazelnut cultivars are clonally propagated and strongly outcrossing, one would expect them to be at least as heterozygous as the three different inbred lines of broccoli. The positions of bands hybridizing to the *SLG* probe were also common to all hazelnut cultivars when *Eco*RI or *Bam*HI was used to digest the DNA. The hybridizing sequence in hazelnut therefore seemed to be very conserved among genotypes, in contrast to the abundant polymorphisms around the *S*-locus of *Brassica* (Nasrallah et al. 1985b).

None of the PCR-generated probes hybridized to DNA from any of the hazelnut genotypes. The most likely explanation for this observation is that the hazelnut sequence is hybridizing to some region of *SLG* outside the areas spanned by fragments A, B and C, and either contains no sequence similarity to *Brassica* within these regions, or else the sequences diverge by more than 30% at the nucleotide level. The three fragments cover only about half of the *Brassica SLG*-coding sequence. However, fragments A and C comprise parts of two *SLG* regions showing conservation among *Brassica S*-alleles. Conserved regions are believed to be important for the gene's function, especially the cysteine array. The fact that these appear to be absent in the hazelnut DNA that hybridized to the *SLG* probe suggests that the hazelnut DNA sequences differ from *SLG* in important ways.

Certain other explanations for the lack of hybridization to hazelnut DNA can be discounted from control data. For example, it is unlikely that hybridization signals fell below the detection limit when using shorter probes (the fragments), because *Brassica* bands as faint as those of hazelnut using *SLG* as a probe were detectable with the fragments (e.g., compare the intensity of the three or four highest-molecular-weight bands in line 240 when probed with *SLG* (Fig. 2, lane 2) and with fragment C (Fig. 4, lane 2)). The specific activities of the probes were probably similar.

A weakened signal due to repeated probing is also unlikely. Other blots, probed few or no times previously, reacted the same way to the different fragments as the blot shown. In addition, equally faint bands in *Brassica* had not disappeared during consecutive hybridizations. Furthermore, the blot shown here was re-probed with *SLG* at the end of the experiment, and the same two bands noted earlier in the hazelnut lanes were still easily distinguishable.

The absence of polymorphisms in the hazelnut DNA when probed with *SLG* raises the question of whether the hazelnut sequence could be a conserved *S*-multigene family member (e.g., an *SLR*-like molecule, another secreted glycoprotein, or a transmembrane receptor kinase). Such genes have been found in several plant species (e.g., *Daucus*) and may be involved in intercellular signalling (Nasrallah and Nasrallah 1993). However, the precise arrangement of cysteines is considered to be a distinguishing feature of the *S*-multigene family, so fragment C should have hybridized to any such genes in hazelnut. The cysteine region is either absent in the *SLG*-hybridizing hazelnut sequence, or else it differs by more than about 30% from the *Brassica* probe.

The kinase domain of *SRK* (clone provided by Drs. J. Nasrallah and J. Stein of Cornell University, Ithaca, N.Y.) hybridized weakly with the 8.2-kb band in hazelnut, but only in 'Hall's Giant' and 'Butler' (Hampson et al., unpublished data). Any kinase related to SI in hazelnut should be present in all these cultivars, because all are self-incompatible. Bands hybridizing to *SRK* in hazelnut appeared in different positions from those hybridizing to *SLG* when *Bam*HI was used to digest the DNA (data not shown).

The identity and significance of the *SLG*-hybridizing sequences in hazelnut DNA remain uncertain. However, present evidence suggests that these sequences are unlikely to be the *S*-gene of hazelnut. *SLG* was the only probe that hybridized to all hazelnut cultivars, and it provided a weak signal. Furthermore, the two bands detected by *SLG* were at the same position in all genotypes tested. Considering the diversity of *S*-alleles, in terms of the genetic backgrounds, geographic origins and phenotypes represented by the hazelnut cultivars, the absence of variation is remarkable. Such conservation around a putative *S*-locus is even more difficult to explain in view of work on other species with SSI or GSI, where variability both within and around the *S*-locus is high (Singh and Kao 1992; Hinata et al. 1993). The hazelnut DNA also failed to hybridize with two regions of *SLG* believed to be important to its function in SI, including one that is an invariant structural feature among all *S*-multigene family genes.

The evidence presented in this paper suggests that the *S*-genes cloned from *Brassica* will not be useful for exploring the mechanism of SSI in hazelnut. Furthermore, the SSI genes of *Brassica* and *Corylus* appear to differ significantly. If the hazelnut DNA seen hybridizing to *SLG* here is the *S*-gene of hazelnut, its nucleotide sequence must differ substantially from that of *Brassica* in two regions showing high conservation among *Brassica S*-alleles. Alternatively, regions such as the cysteine array may be entirely absent from the hazelnut *S*-gene.

If the hazelnut DNA hybridizing to *SLG* is not the hazelnut *S*-gene, the actual *S*-gene of hazelnut must be sufficiently different to elude detection by *Brassica S*-gene probes even at low stringency. Such differences are consistent with the idea that the SSI genes of *Brassica* and *Corylus* have either diverged greatly during evolution, or are of independent origin.

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References

Bateman AJ (1952) Self-incompatibility systems in angiosperms. I Theory. Heredity 6.285–310

Bell PR (1995) Incompatibility in flowering plants: adaptation of an ancient response. Plant Cell 7:5–16

Boyes DC, Nasrallah JB (1993) Physical linkage of the *SLG* and *SRK* genes at the self-incompatibility locus of *Brassica oleracea*. Mol Gen Genet 236:369–373

Boyle TH, Stimart DP (1986) Self-incompatibility and interspecific incompatibility: relationships in intra- and inter-specific crosses of *Zinnia elegans* Jacq. and *Z. angustifolia* HBK (Compositae). Theor Appl Genet 73 305–315

Charlesworth D (1988) Evolution of homomorphic sporophytic self-incompatibility. Heredity 60:445–453

Dickinson HG, Crabbe MJC, Gaude T (1992) Sporophytic self-incompatibility systems: *S* gene products. Int Rev Cytol 140:525–561

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Foote HCC, Ride JP, Franklin-Tong VE, Walker EA, Lawrence MJ, Franklin FCH (1994) Cloning and expression of a distinctive class of self-incompatibility (*S*) gene from *Papaver rhoeas* L. Proc Natl Acad Sci USA 91:2265–2269

Franklin-Tong VE, Atwal KK, Howlett EC, Lawrence MJ, Franklin FCH (1991) Self-incompatibility in *Papaver rhoeas*: there is no evidence for the involvement of stigmatic ribonuclease activity. Plant Cell Environ 14:423–429

Goring DR, Rothstein SJ (1992) The *S*-locus receptor kinase gene in a self-incompatible *Brassica napus* line encodes a functional serine/threonine kinase. Plant Cell 4:1273–1281

Hampson CR, Azarenko AN, Soeldner A (1993) Pollen-stigma interactions following compatible and incompatible pollinations in hazelnut. J Am Soc Hort Sci 118.814–819

Heslop-Harrison J, Knox RB, Heslop-Harrison Y (1974) Pollen-wall proteins: exine-held fractions associated with the incompatibility response in Cruciferae. Theor Appl Genet 44:133–137

Heslop-Harrison Y, Heslop-Harrison JS, Heslop-Harrison J (1986) Germination of *Corylus avellana* L. (hazel) pollen: hydration and the function of the oncus. Acta Bot Neerl 35:265–284

Hinata K, Watanabe M, Toriyama K, Isogai A (1993) A review of recent studies on homomorphic self-incompatibility. Int Rev Cytol 143:257–296

Howlett BJ, Knox RB, Paxton JD, Heslop-Harrison J (1975) Pollen-wall proteins: physicochemical characterization and role in self-incompatibility in *Cosmos bipinnatus*. Proc Roy Soc Lond Ser B 188.167–182

Kandasamy MK, Paolillo DJ, Faraday CD, Nasrallah JB, Nasrallah ME (1989) The *S*-locus-specific glycoproteins of *Brassica* accumulate in the cell wall of developing stigma papillae. Dev Biol 134:462–472

Lalonde BA, Nasrallah ME, Dwyer KG, Chen C-H, Barlow B, Nasrallah JB (1989) A highly conserved *Brassica* gene with homology to the *S*-locus-specific glycoprotein structural gene. Plant Cell 1:249–258

Li X, Nield J, Hayman D, Langridge P (1994) Cloning a putative self-incompatibility gene from the pollen of the grass *Phalaris coerulescens* Plant Cell 6:1923–1932

Lodhi MA, Ye G-N, Weeden NF, Reisch BI (1994) A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13

Matton DP, Nass N, Clarke AE, Newbigin E (1994) Self-incompatibility: how plants avoid illegitimate offspring. Proc Natl Acad Sci USA 91.1992–1997

Mehlenbacher SA, Thompson MM (1988) Dominance relationships among *S*-alleles in *Corylus avellana* L. Theor Appl Genet 76·669–672

Nasrallah JB, Nasrallah ME (1989) The molecular genetics of self-incompatibility in *Brassica*. Annu Rev Genet 23·121–139

Nasrallah JB, Nasrallah ME (1993) Pollen-stigma signaling in the sporophytic self-incompatibility response. Plant Cell 5:1325–1335

Nasrallah JB, Doney RC, Nasrallah ME (1985a) Biosynthesis of glycoproteins involved in the pollen-stigma interaction of incompatibility in developing flowers of *Brassica oleracea* L. Planta 165:100–107

Nasrallah JB, Kao T-H, Goldberg ML, Nasrallah ME (1985b) A cDNA clone encoding an *S*-locus-specific glycoprotein from *Brassica oleracea*. Nature 318:263–267

Nasrallah JB, Yu S-M, Nasrallah ME (1988) Self-incompatibility genes of *Brassica oleracea*: expression, isolation, and structure. Proc Natl Acad Sci USA 85:5551–5555

Radetzky R (1990) Analysis of mitochondrial DNA and its inheritance in *Populus* Curr Genet 18:429–434

Read SM, Newbigin E, Clarke AE, McClure BA, Kao T-H (1995) Disputed ancestry: comments on a model for the origin of incompatibility in flowering plants. Plant Cell 7· 661–664

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning. a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sassa H, Hirano H, Ikehashi I (1992) Self-incompatibility-related RNases in styles of Japanese pear (*Pyrus serotina* Rehd.). Plant Cell Physiol 33:811–814

Singh A, Kao T-H (1992) Gametophytic self-incompatibility: biochemical, molecular genetic, and evolutionary aspects. Int Rev Cytol 140:449–483

Stein JC, Howlett B, Boyes DC, Nasrallah ME, Nasrallah JB (1991) Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. Proc Natl Acad Sci USA 88.8816–8820

Stephenson AG, Winsor JA, Richardson TE, Singh A, Kao T-H (1992) Effects of style age on the performance of self and cross pollen in *Campanula rapunculoides*. In: Ottaviano E, Mulcahy DL, Sari Gorla M, Bergamini Mulcahy G (eds) Angiosperm pollen and ovules. Springer-Verlag, New York Berlin Heidelberg, pp 117–121

Thompson MM (1979) Genetics of incompatibility in *Corylus avellana* L. Theor Appl Genet 54:113–116

Trick M, Heizmann P (1992) Sporophytic self-incompatibility systems· *Brassica S* gene family. Int Rev Cytol 140:485–524