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Expression of unilateral incompatibility in pollen of *Lycopersicon pennellii* is determined by major loci on chromosomes 1, 6 and 10

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Summary. We have previously described gene introgression from the wild nightshade *Solanum lycopersicoides* into tomato (*Lycopersicon esculentum*) through the use of either diploid or sesquidiploid hybrids (the latter consisting of two genomes of *L. esculentum* and one genome of *S. lycopersicoides*). Both types of intergeneric hybrids display pollen sterility, but workable ovule fertility. Unilateral incompatibility prevents their direct hybridization with staminate *L. esculentum*. Pollen of a self-compatible form of the related wild species *L. pennellii* is compatible with pistils of *L. esculentum* × *S. lycopersicoides* hybrids. This trait was backcrossed from *L. pennellii* to *L. esculentum* in order to develop bridging lines that could be used to obtain progeny from the intergeneric hybrids and to study the inheritance of bridging ability. In progeny of *L. esculentum* × *S. lycopersicoides* hybrids pollinated with *L. pennellii*-derived bridging lines, preferential transmission of *L. pennellii* alleles was observed for certain isozyme and RFLP markers on chromosomes 1, 6 and 10. The skewed segregations suggest linkage to three major pollen-expressed compatibility loci. This was confirmed by observations of pollen tube growth, which indicated that compatibility with pistils of the diploid intergeneric hybrid occurred only in bridging lines at least heterozygous for the *L. pennellii* markers on chromosomes 1, 6 and 10. Compatibility with the sesquidiploid hybrid required only the chromosome 1 and 6 loci, indicating an apparent effect of gene dosage on expression of incompatibility in the pistil. In an F_2 *L. esculentum* × *L. pennellii* population, preferential transmission of *L. pennellii* alleles was observed for the same markers on chromosomes 1 and 10, as well as other markers on chromosomes 3, 11, and 12, but not 6. The chromosome 1 pollen compatibility locus maps to or near the S-locus, which determines S-allele specificity. The results are discussed in relation to existing genetic models for unilateral incompatibility, including the possible involvement of the S-locus.

Key words: Tomato – *Solanum lycopersicoides* – Self-incompatibility – Incongruity – RFLPs – Isozymes

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Introduction

Unilateral incompatibility occurs when the pollen of one plant population is prevented from functioning on pistils of another population, while in the reciprocal cross no incompatibility is observed (deNettancourt 1977). This prefertilization barrier is manifested by the inhibition of pollen tube growth in stigma, style or ovaries. Unilateral incompatibility often occurs when self-compatible species are used as pollen parent in crosses with related, self-incompatible species. In tomato and its wild relatives (*Lycopersicon* spp.), unilateral incompatibility generally occurs between the red- and yellow-fruited, self-compatible species (subgenus *Eulycopersicon*), and the green-fruited, mostly self-incompatible species (Subgenus *Eriopersicon*) (Hogenboom 1972a). Intraspecific unilateral barriers have also been observed in crosses between different populations of the green fruited *L. hirsutum* (Martin 1963).

Several lines of evidence suggest a genetic and physiological relationship between unilateral and self-incompatibilities (de Nettancourt 1977). Lewis and Crowe (1958) proposed that unilateral incompatibility is a consequence of the evolution of self-compatibility from self-

incompatibility via step-wise mutations at the S-locus. They made a distinction between fully self-compatible ("SC") and recently evolved self-compatible ("Sc") species or biotypes. Pistils of SC species accept pollen of SI species, while the reciprocal cross fails. Pistils of Sc species accept pollen of SI but not SC, while pollen of Sc is compatible with pistils of either SC or SI. In *Lycopersicon,* the SC condition is exemplified by the domestic tomato *L. esculentum.* The Sc condition occurs in rare self-compatible biotypes of the normally self-incompatible wild species *L. pennellii* (Hardon 1967) and *L. peruvianum* (Rick 1986).

Classical genetic studies on the inheritance of unilateral incompatibility in *Lycopersicon* support a relationship with self-incompatibility, but indicate an involvement of other genes as well. Segregation for unilateral incompatibility in interspecific SC × SI crosses suggested control by two or more genes (McGuire and Rick 1954: Martin 1961, 1963, 1964, 1967). The involvement of the S-locus was supported by cosegregation of self- and unilateral incompatibilities (Martin 1961, 1968). Indeed, much of the breeding behavior of F_1 SC × SI and Sc × SI hybrids could be explained by the occurrence of self-fertility alleles at the S-locus; the latter are either recessive, as in SC species, or dominant, as in Sc species, to functional S-alleles (McGuire and Rick 1954; Hardon 1967). However, the failure of F_1 SC × SI hybrids to serve as pollen parent on SI plants containing different S-alleles could not be explained by mutations at the S-locus alone (McGuire and Rick 1954; Hardon 1967). Studies on unilateral incompatibility in *L. peruvianum* led Hogenboom (1972b, c) to conclude that the phenomenon is distinct from self-incompatibility and to propose an alternative genetic model, referred to as "incongruity" (Hogenboom 1973). In this model, the congruity or incongruity of interpopulational crosses is determined by "barrier genes" in pistils and corresponding "penetration genes" in pollen; these genes are envisaged to be distinct from those controlling self-incompatibility.

While the genetic basis of unilateral incompatibility is largely unknown, much is known about self-incompatibility. In the *Solanaceae,* self-incompatibility is of the gametophytic homomorphic type, and S-allele specificity is determined by a single locus, which has been mapped to chromosome 1 in *L. peruvianum* (Tanksley and Loaiza-Figueroa 1985). S-allele-associated style glycoproteins have been purified from several nightshades, including *Nicotiana alata* (Anderson et al. 1986) and *L. peruvianum* (Mau et al. 1986). In *N. alata,* the developmental and tissue-specific accumulation of both the glycoprotein and its corresponding mRNA coincide with the timing and site of pollen tube inhibition (Anderson et al. 1986; Cornish et al. 1987). S-allele-associated style glycoproteins in *N. alata* are ribonucleases (McClure et al. 1989); interestingly, no RNAse activity was detected in styles of the self-compatible *N. tabacum.* The identity of the S-gene product in pollen and the nature of its interaction with the stylar glycoprotein are as yet unknown.

The research reported herein resulted from our efforts to transfer traits from the wild nightshade *S. lycopersicoides* to *L. esculentum.* Hybrids between the species have been previously obtained (Rick 1951; Rick et al. 1986). However, their male sterility and unilateral incompatibility prevented until recently further hybridizations in either direction with *L. esculentum* (De Verna et al. 1987; Chetelat et al. 1989; Gradziel and Robinson 1989). Pollen of a self-compatible accession of *L. pennellii* is compatible with pistils of the intergeneric hybrids. This trait was backcrossed from *L. pennellii* to *L. esculentum* in order to develop efficient bridging lines. The objective of the present research was to determine the number and locations of *L. pennellii* genes conferring this bridging ability.

Materials and methods

Plant materials

The parental genotypes included *L. esculentum* cv 'UC82B' and 'VF36', *L. pennellii* accession LA716, which is self-compatible (Sc), and *S. lycopersicoides* accessions LA1964 and LA2408 (both self-incompatible). Seed of the wild species were kindly provided by the C. M. Rick Tomato Genetics Resource Center, at the University of California, Davis.

Sesquidiploid intergeneric hybrids (herein denoted LLS) were synthesized as described (Rick et al. 1986) and consisted of two genomes of *L. esculentum* and one genome of *S. lycopersicoides* (accession LA1964). Diploid intergeneric hybrids (denoted F_1LS) were synthesized as described (Chetelat et al. 1989) using *L. esculentum* cv 'VF36' or 'UC82B' and *S. lycopersicoides* accession LA2408. Backcross progenies of LLS (denoted LLS × BC_1LP) and F_1LS (denoted BC_1LS) were obtained by pollinating each with *L. pennellii*-derived bridging lines (Chetelat et al. 1989; J. W. DeVerna et al. unpublished).

The bridging lines included the F_1 *L. esculentum* × *L. pennellii* hybrid (denoted F_1LP) and various backcross derivatives (denoted BC_1LP, BC_2LP, etc). The recurrent parent in all backcrosses was *L. esculentum* cv 'UC82B'. Selections were made among segregating backcross populations for those individuals compatible with pistillate LLS and/or F_1LS.

An F_2 *L. esculentum* (cv 'VF36') × *L. pennellii* (LA716) population (denoted F_2LP), analyzed in collaboration with John Yoder at U. C. Davis, was used to map isozyme and DNA markers and to identify chromosomal regions showing non-Mendelian inheritance. Linkage analysis was performed using the LINKAGE-1 program (Suiter et al. 1983).

Pollinations and pollen tube growth studies

All controlled crosses were performed in the greenhouse using standard emasculation and pollination techniques. The compatibility reaction of crosses was determined by observing pollen tube growth in pistils 48 h after pollination (Martin 1959). Pollinations were judged incompatible when the growth of all pollen tubes was arrested in the upper half of the style (DeVerna et al. 1987). Conversely, compatible pollinations were those that resulted in one or more pollen tubes reaching the ovaries; plants

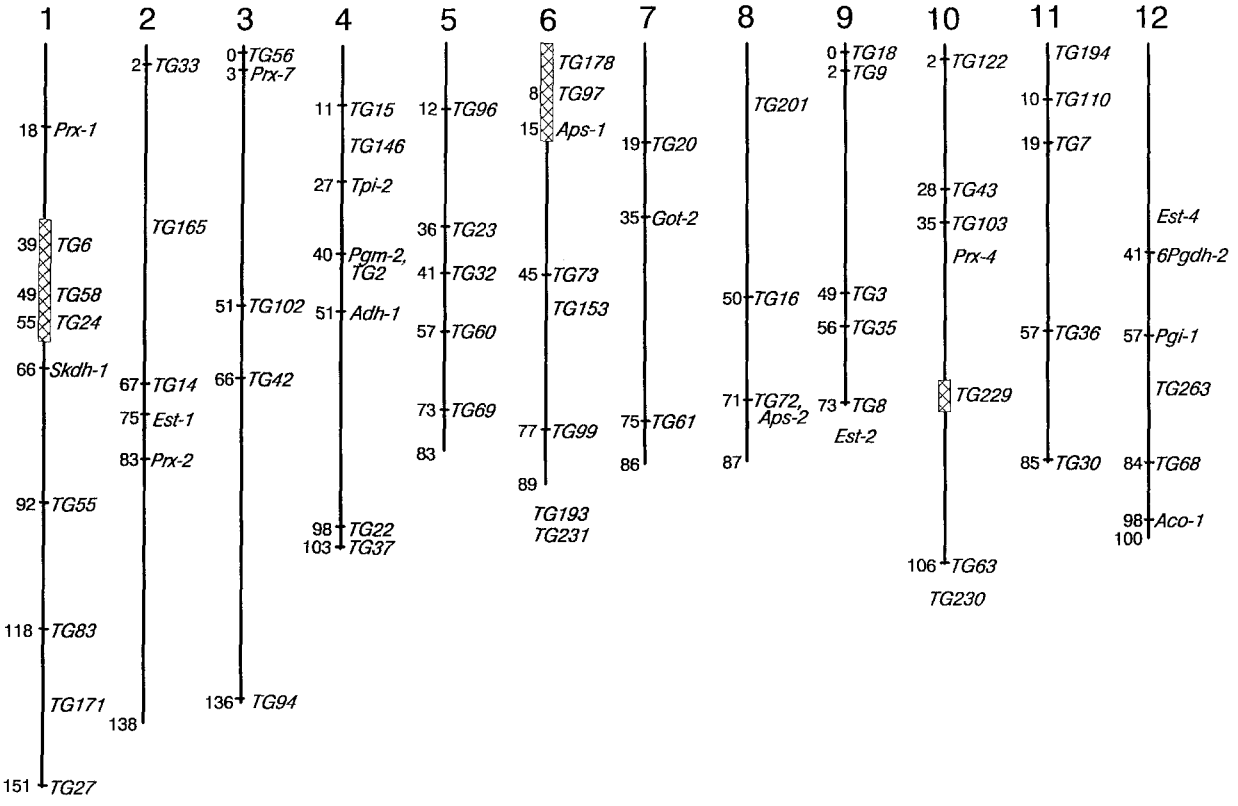

Fig. 1. Linkage map of RFLP and isozyme markers used in this study. Map positions are from Young and Tanksley (1989); approximate location of some markers was inferred from Tanksley and Mutschler (1990) or from our own linkage data. *Shaded regions* represent approximate location of *L. pennellii* loci associated with bridging ability

heterozygous for compatibility loci produced mixtures of compatible and incompatible pollen. At least three pistils were examined per cross.

Isozyme analysis

Isozyme analysis was performed as described (Chetelat et al. 1989) on BC_1LS, $LLS \times BC_1$ LP progenies, bridging lines and F_2LP. *L. pennellii* alleles could be distinguished from those of *L. esculentum* at 17 isozyme loci: *Aco-1, Adh-1, Aps-1, Aps-2, Est-1, Est-2, Est-4, Got-2, 6Pgdh-2, Pgi-1, Pgm-2, Prx-1, Prx-2, Prx-4, Prx-7, Skdh-1* and *Tpi-2*. The *L. pennellii* alleles for *Got-2, 6Pgdh-2, Pgm-2* and *Tpi-2* could not be distinguished from those of *S. lycopersicoides*, which segregated in BC_1LS and $LLS \times BC_1LP$ populations. Together, these markers cover 10 of the 12 tomato chromosomes (Chetelat 1989) (Fig. 1).

RFLP analysis

Total DNA was isolated as described by Bernatzky and Tanksley (1986), except that the extraction buffer contained sodium bisulfite (3.8 g/l), and the CsCl purification step was omitted. DNA (5–10 µg per sample) was digested with *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII or *Xba*I (Bethesda Research Laboratories) according to manufacturer's instructions, except that 4 m*M* spermidine was included in the digestion buffer. Restriction fragments were electrophoresed in 1% agarose gels as described by Bernatzky and Tanksley (1986) and transferred to GeneScreen Plus membranes (New England Nuclear) according to manufacturer's specifications.

Probes consisted of random tomato genomic (TG) clones of known map location (Young et al. 1989; Tanksley and Mutschler 1990) provided by Steve Tanksley at Cornell University, Ithaca, New York. The probes were radiolabelled with [^{32}P] dATP and dCTP by the random hexamer primer method (Feinberg and Vogelstein 1983) using whole plasmids or inserts amplified by the polymerase chain reaction (PCR).

Inserts were amplified using pUC/M13 universal primers (Promega catalog number Q5391 and Q5421). The reaction mixture contained reaction buffer, 200 µ*M* of each dNTP, 10 p*M* of each primer, 20 ng of template DNA, 1.25 units of *Taq* polymerase and water for a final volume of 100 µl. Amplifications were carried out for 34 cycles, each consisting of 1 min at 92 °C, 40 s at 50 °C and 1 min at 72 °C. Unincorporated dNTPs were removed by centrifugation through Millipore filters (catalogue number UFC3 THK00) according to manufacturer's specifications.

Hybridization and wash conditions were as described by Bernatzky and Tanksley (1986) except that the hybridization solution contained 5% dextran sulfate. Washed filters were exposed to Kodak X-Omat AR X-ray film with intensifying screens for 1–7 days at −80 °C. After autoradiography, filters were strip-washed in 0.4 *N* NaOH at 42 °C according to manufacturer's instructions.

Twenty-four BC_1LS plants were scored for the following 42 RFLP markers: TG's 3, 6, 7, 8, 9, 14, 15, 16, 18, 20, 22, 23, 24, 27, 32, 33, 35, 36, 60, 61, 63, 68, 72, 96, 97, 99, 102, 103, 122, 153, 165, 171, 178, 192, 193, 194, 201, 229, 230, 231, 246, and 263. BC_4F_3 bridging lines, which segregated for compatibility with LLS, were scored for the following 36 RFLP markers: TG's 2, 6, 7, 8, 9, 14, 15, 18, 23, 28, 30, 32, 36, 37, 42, 55, 56, 58, 61, 63, 68, 69, 72, 73, 83, 94, 96, 97, 102, 103, 108, 110, 122, 146, 178, and 229. The map locations of all markers are given in Fig. 1.

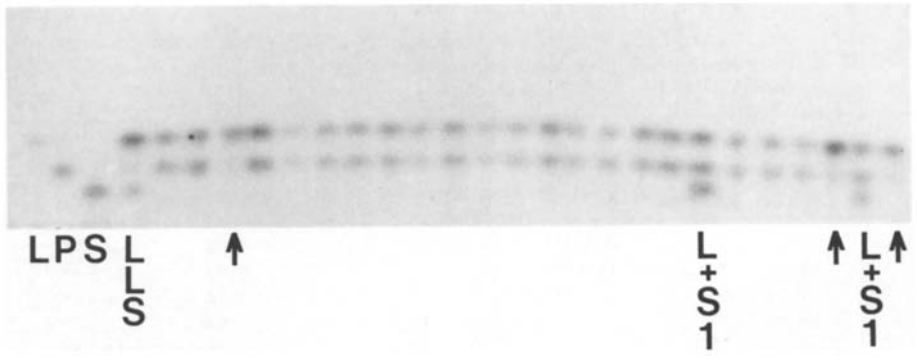

Fig. 2. Starch gel showing segregation for *Skdh-1* in progeny of sesquidiploid *L. esculentum* × *S. lycopersocoides* hybrids pollinated with BC_1 *L. esculentum* × *L. pennellii* bridging lines. Control lanes include *L. esculentum* (*L*), *L. pennellii* (*P*), *S. lycopersicoides* (*S*), and the sesquidiploid hybrid (*LLS*). Progeny lanes include two alien addition lines each carrying an extra *S. lycopersicoides* chromosome 1 (*L* + *S1*), and three putative recombinants (*arrows*, see text for explanation)

Results

Development of L. pennellii-derived bridging lines

The male sterility but workable female fertility of F_1LS[1] and LLS made it necessary to overcome the unilateral incompatibility these hybrids normally express towards *L. esculentum* pollen. Although bud pollination of LLS was successful in bypassing the stylar barrier, an inhibition of pollen tube growth in the ovaries prevented fertilization (data not shown). Attempts at "stigma complementation" (Gradziel and Robinson 1989) did not improve pollen germination or tube growth. Treatment of LLS pistils with 0.005% *p*-chlorophenoxyacetic acid resulted in the formation of abundant fruit, none of which contained viable seeds or culturable embryos.

Pollen of *L. pennellii* is compatible with pistils of the intergeneric hybrids. This gametophytic trait is expressed in F_1LP, and segregates in the BC_1. Out of 53 BC_1LP plants tested, 18 were compatible as pollen parent with LLS; this segregation is consistent with that expected for two pollen-expressed genes ($X^2 = 1.81$). Progeny resulting from pollinations of LLS with pure *L. pennellii* are themselves unilaterally incompatible with staminate *L. esculentum,* a trait inherited from *L. pennellii.* On the other hand, a proportion of the progeny resulting from LLS × F_1LP and LLS × BC_1LP crosses are compatible with pollen of *L. esculentum.* Of 101 LLS × BC_1LP progeny, 75 were compatible with pollen of *L. esculentum,* based on fruit and seed set, while 26 were incompatible. Of 25 LLS × F_1LP progeny, 5 were compatible with *L. esculentum* pollen. Bilaterally compatible progeny were also obtained from F_1LS through crosses with either F_1LP or BC_1LP (Chetelat et al. 1989).

[1] See *Materials and methods* for definition of all symbols referring to hybrids

Table 1. Joint segregation for *Skdh-1* and *Prx-1* in progeny of *L. esculentum* × *S. lycopersicoides* hybrids pollinated with *L. pennellii*-derived bridging lines. Data were pooled from progeny of sesquidiploid and diploid *L. esculentum* × *S. lycopersicoides* hybrids. Only progeny of BC_1 bridging lines heterozygous (+/p) for both *Skdh-1* and *Prx-1* are included

		Prx-1		
		+/+	+/p	Total
Skdh-1	+/+	1	9	10
	+/p	16	142	158
	Total	17	151	158

Recombination frequency:
Skdh-1–S^P = 10/168 = 0.060 = 6%
Prx-1–S^P = 17/168 = 0.101 = 10.1%
Skdh-1–*Prx-1* = 27/168 = 0.161 = 16.1%[a]

+, Alleles of *L. esculentum* or *S. lycopersicoides*; p, allele of *L. pennellii*; S^P, putative *L. pennellii* S-allele
[a] Assumes that the one *Skdh-1*$^{+/+}$ *Prx-1*$^{+/+}$ plant is the result of a double crossover between the markers

Isozyme and RFLP analysis of backcross L. esculentum × *S. lycopersicoides derivatives*

Progenies of LLS and F_1LS pollinated with BC_1LP bridging lines were scored for *L. pennellii* allozymes at 17 loci. Segregation for *S. lycopersicoides* alleles from the female parent was also observed, and are grouped with *L. esculentum* alleles in the "+" category. Analysis of these progeny revealed an excess of *L. pennellii* alleles (p) for *Skdh-1* and *Prx-1,* both on chromosome 1 (Table 1, Fig. 2). A test for homogeneity indicated the data from the two populations could be pooled. Since all the BC_1LP bridging lines were heterozygous +/p for *Prx-1* and *Skdh-1,* the expected segregation in LLS × BC_1LP

Table 2. Monogenic segregations for selected isozyme and RFLP markers in progeny of F_1 *L. esculentum* × *S. lycopersicoides* hybrids pollinated with *L. pennellii*-derived bridging lines (BC_1LS), and F_2 *L. esculentum* × *L. pennellii* (F_2LP). For each chromosome showing skewed segregations, three markers are listed, in the order in which they occur on their respective chromosomes. Chi-square values are reported only if significant

Locus	Chromosome	BC_1LS			F_2LP			
		+/+	+/p	χ^2 (1:1)	+/+	+/p	p/p	χ^2 (1:2:1)
Prx-1	1	4	57	46.0***	5	12	33	44.9***
TG24	1	1	23	18.4***	5	37	24	11.9**
Skdh-1	1	1	41	36.2***	5	32	14	6.49*
TG66	3	–	–		10	31	24	6.17*
TG102	3	12	12		9	23	25	11.1**
TG42	3	–	–		11	31	22	
TG97	6	0	23	21.0***	17	26	12	
TG178	6	0	24	22.0***	16	34	9	
TG153	6	4	20	10.7***	14	38	14	
TG122	10	14	10		1	33	33	30.6***
TG103	10	10	14		8	31	28	12.3**
TG229	10	1	23	18.4***	8	32	26	9.88**
TG194	11	–	–		13	40	13	
TG7	11	11	12		6	30	27	14.1***
TG36	11	10	14		18	21	17	
6Pgdh-2	12	–	–		6	33	26	12.3**
TG111	12	–	–		4	31	26	15.9***
Pgi-1	12	38	26		3	35	27	18.1***

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$
+, Allele of *L. esculentum* or *S. lycopersicoides*; p, allele of *L. pennelli*

and $F_1LS \times BC_1LP$ progenies would be 1 +/+:1 +/p. The significant excess of +/p genotypes suggests linkage of the markers to a locus from *L. pennellii* required for compatibility of pollen with pistils of the intergeneric hybrids. If so, then the recombination rate between the compatibility locus and *Prx-1* or *Skdh-1* is equal to the percentage of +/+ genotypes for each marker (Table 1). The results indicate the compatibility locus is situated between *Prx-1* and *Skdh-1*, 10.1 cM from the former and 6.0 cM from the latter.

RFLP analysis was used to confirm the isozyme data for chromosome 1 and to identify additional loci showing preferential transmission of *L. pennellii* alleles. Twenty-four BC_1LS plants were scored for 42 RFLP markers representing all 12 chromosomes. As expected, an excess of *L. pennellii* alleles was observed for TG24, situated between *Skdh-1* and *Prx-1* (Table 2) on chromosome 1. Segregations for certain markers on chromosome 6 (TG97, TG178) and chromosome 10 (TG229) also showed an excess of +/p heterozygotes (Table 2).

Cosegregation of molecular markers and pollen compatibility in bridging lines

Twelve BC_1LP bridging lines, selected from a random BC_1 population on the basis of their compatibility with pistillate LLS and F_1LS, were scored for the following isozyme markers: *Aco-1, Est-1, Got-2, 6Pgdh-2, Pgi-1, Pgm-2, Prx-1, Prx-2, Prx-7, Skdh-1* and *Tpi-2*. Segregation ratios approximating the expected 1 +/+:1 +/p were observed for all markers except *Skdh-1* and *Prx-1*, which segregated 1 +/+:11 +/p and 1 +/+:10 +/p, respectively.

Table 3. Cosegregation of isozyme markers and pollen compatibility in BC_3F_2, BC_4F_2, and BC_4F_3 *L. esculentum* × *L. pennellii* bridging lines. Pollen compatibility was determined by observing pollen tube growth in pistils of sesquidiploid *L. esculentum* × *S. lycopersicoides* hybrids

Skdh-1	*Prx-1*	Pollen compatibility	
		Compatible	Incompatible
+/+	+/+	0	9
+/p	+/p	24	10
p/p	p/p	28	13
p/p	+/p	1	2
+/p	p/p	2	1

By using the isozyme markers *Skdh-1* and *Prx-1* as an indirect selection criterion, the putative pollen compatibility locus was backcrossed into *L. esculentum*. A total of 90 BC_3F_2, BC_4F_2 and BC_4F_3 plants were scored for *Skdh-1* and *Prx-1* and tested for pollen compatibility with LLS (Table 3). Nine plants homozygous +/+ for *Skdh-1* and *Prx-1* were, as expected, incompatible with LLS. Of the 81 plants heterozygous (+/p) or ho-

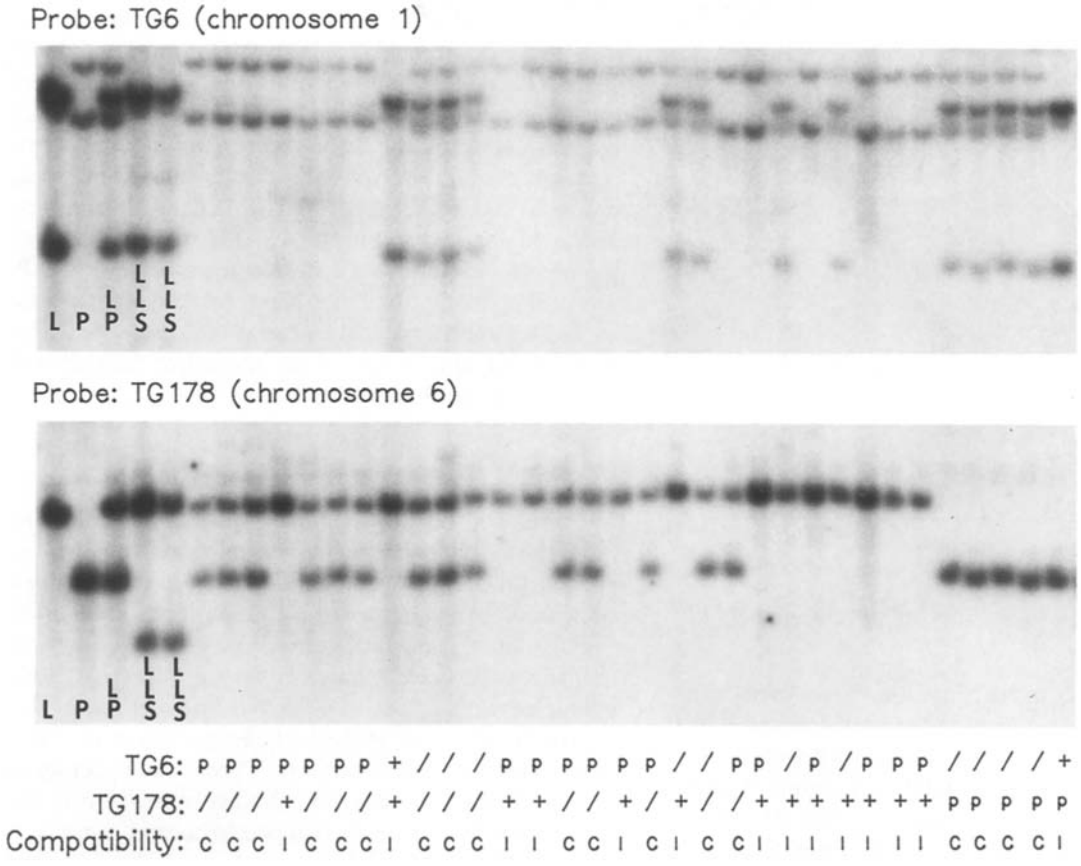

Fig. 3. Southern blot analysis of BC_4F_3 *L. esculentum* × *L. pennellii* bridging lines, showing cosegregation of RFLP markers with Pollen compatibility. The genomic clones TG6 (chromosome 1) and TG178 (chromosome 6) were hybridized with *Eco*RV-(TG6) or *Eco*RI-(TG178) digested genomic DNA from: *L. esculentum* (*L*), *L. pennellii* (*P*), F_1 *L. esculentum* × *L. pennellii* (*LP*), sesquidiploid *L. esculentum* × *S. lycopersicoides* hybrids (*LLS*) and BC_4F_3 bridging lines (unlabelled). Genotypes of bridging lines and compatibility of their pollen with pistils of sesquidiploid *L. esculentum* × *S. lycopersicoides* hybrids are indicated beneath the lanes. + Homozygous for *L. esculentum* allele, *p* homozygous for *L. pennellii* allele, / heterozygous, *C* compatible pollen, *I* incompatible pollen. *Note:* for simplicity, not all hybridizing DNA fragments are shown

mozygous (p/p) for the *L. pennellii* alleles at both isozyme loci, 55 were compatible and 26 incompatible with LLS. Such a large proportion of incompatible plants cannot be explained by recombination, since this would require rare double crossovers between the isozyme markers and the pollen compatibility locus. The ratio of compatible to incompatible plants approximates a 3:1 segregation ($X^2 = 2.19$), suggesting the presence of a second, unlinked, pollen compatibility gene from *L. pennellii*.

To map the second pollen compatibility locus, segregating BC_4F_3 families were tested for compatibility with LLS and scored for 36 RFLP markers on all 12 chromosomes. Backcrossing had eliminated the *L. pennellii* alleles at most loci, although the plants still segregated for certain markers on chromosomes 1, 3, 6, 9, and 11. The only markers which cosegregated with pollen compatibility were those on chromosome 1 (TG6, TG58) and chromosome 6 (TG97, TG178). Plants at least heterozygous (+/p or p/p) for the *L. pennellii* alleles of markers on both chromosomes were all compatible with LLS (Fig. 3). Plants homozygous +/+ for markers on one or both chromosomes were all incompatible with LLS. Therefore, the chromosome 1 and 6 loci are both required, and together are sufficient, for compatibility of pollen with pistils of LLS.

None of the BC_4F_3 bridging lines were compatible with F_1LS, however. Segregation for TG229 in BC_1LS suggested that an *L. pennellii* gene on chromosome 10, not present in the BC_4F_3 lines, is also required for compatibility with F_1LS. To test this, a BC_2LP population of 52 plants was scored for RFLP markers on chromosomes 1 (TG24), 6 (TG178) and 10 (TG229), and tested for pollen compatibility with F_1LS. Only plants heterozygous +/p for all three RFLP markers were compatible with F_1LS (Table 4). One triple heterozygote was not compatible, suggesting recombination between one of the markers and its linked compatibility locus.

Table 4. Cosegregation of RFLP markers and pollen compatibility in BC_2 *L. esculentum* × *L. pennellii* bridging hybrids. Pollen compatibility was determined by observing pollen tube growth in pistils of diploid F_1 *L. esculentum* × *S. lycopersicoides* hybrids

Chr 1 TG24	Chr 6 TG178	Chr 10 TG229	Pollen compatibility	
			Compatible	Incompatible
+/+	+/+	+/+	0	4
+/p	+/+	+/+	0	5
+/+	+/p	+/+	0	6
+/+	+/+	+/p	0	5
+/p	+/p	+/+	0	1
+/p	+/+	+/p	0	9
+/+	+/p	+/p	0	18
+/p	+/p	+/p	3	1
Totals			3	49

Marker-facilitated introgression of pollen compatibility loci

The three pollen compatibility loci have been introgressed into *L. esculentum* using both isozyme and RFLP markers in order to develop bridging lines with a minimal amount of unlinked *L. pennellii* DNA. Isozyme markers, which offer greater speed and lower cost than RFLPs, are available for each of the three loci. *Prx-1* and *Skdh-1* are on opposite sides of the chromosome 1 compatibility locus, and *Aps-1* is tightly linked to the chromosome 6 locus marked by TG97 (Fig. 1). As for chromosome 10, an analysis of gene distances in our F_2LP population indicates that *Prx-4* is proximal to the compatibility locus marked by TG229 on chromosome 10 (recombination frequency = 20%) (Fig. 1). These markers have been used as a prescreen in segregating backcross populations to identify putative bridging lines containing all three *L. pennellii* chromosomal segments; pollen tube studies were performed on a small number of plants in each generation to confirm their compatibility with the intergeneric hybrids. To date, BC_5 bridging lines containing all three loci have been developed by this method.

RFLP and isozyme analysis of F_2 L. esculentum × L. pennellii

L. pennellii and F_1LP are unilaterally incompatible with staminate *L. esculentum* (Hardon 1967). It was therefore desirable to determine if the chromosome 1, 6 and/or 10 pollen compatibility loci are required to overcome this unilateral incompatibility as well. An F_2LP population of 67 plants was scored for 112 isozyme and RFLP loci covering each of the 12 chromosomes. Markers on chromosomes 1, 3, 10, 11 and 12 showed skewed segregation (Table 2). By comparing the deviations from Mendelian inheritance for several markers per chromosome, the approximate position of the loci causing segregation distortion can be determined (Table 2). The chromosome 1 and 10 data suggest involvement of the same loci in overcoming the unilateral incompatibility of both F_1LP and F_1LS hybrids. However, the chromosome 10 situation is complex, since TG122 (on the short arm) showed the most dramatic skewing in F_2LP, while TG229 (on the long arm) was most skewed in BC_1LS. The markers on chromosome 6 did not show distorted segregation in F_2LP, unlike BC_1LS. On the other hand, markers on chromosome 3, 11 and 12 showed an excess of *L. pennellii* alleles in F_2LP, while segregation was normal or near normal for the same markers in BC_1LS.

Discussion

The results of the present studies provide, to our knowledge, the first data on the linkage relations of pollen-expressed unilateral incompatibility loci, and suggest an association between unilateral and self-incompatibilities. Segregation for molecular markers was monitored in progeny of *L. esculentum* × *S. lycopersicoides* hybrids pollinated with *L. pennellii*-derived bridging lines. This approach facilitated analysis of segregation in the male and female gametes separately. Three major loci from *L. pennellii*, determining compatibility of pollen with the intergeneric hybrids, were mapped to chromosomes 1, 6 and 10. Observations of pollen tube growth confirmed that the preferential transmission of *L. pennellii* alleles was due to the strict incompatibility of pollen containing the *L. esculentum* allele at any one of these loci. Other potential causes of segregation distortion (e.g., small sample size, pollen or embryo lethality, etc) were thereby ruled out. The chromosome 1 locus is situated between *Prx-1* and *Skdh-1*, at or near the S-locus; the estimated map distance between *Prx-1* and the pollen compatibility locus was 10.1 c*M*, quite close to that reported for the *Prx-1*- -S interval (9.9 cM, Tanksley and Loaiza-Figueroa 1985).

The well developed genetic maps of tomato (Chetelat 1989, Tanksley and Mutschler 1990) and the presence of extensive intra and interspecific variation in mating systems make *Lycopersicon* an excellent genus in which to study sexual incompatibility genes. In addition to the S-locus, a second gene, apparently required for the expression of SI, though not S-allele specificity, was mapped to chromosome 2 using morphological markers (Martin 1961; Rick 1963). Rick (1982) detected possible linkage between SI and *B*, a gene on chromosome 6 controlling fruit color. In an F_2 population resulting from a cross between two self-compatible accessions of *L. hirsutum*, segregation for SI/SC was observed; RFLP analysis suggested the independent breakdown of SI in these two accessions was not due to mutation at the S-locus (Rick and Chetelat 1991).

Another significant finding of these studies is a possible link between gene dosage and strength of the stylar unilateral incompatibility response: while all three loci are required for compatibility with the diploid hybrid (F_1LS), only the chromosome 1 and 6 loci are required for compatibility with the sesquidiploid (LLS). This interpretation is supported by our observations that none of the bridging lines developed so far can overcome the apparently stronger incompatibility of pure *S. lycopersicoides* or *L. pennellii.*

An analysis of segregation in F_2 *L. esculentum* × *L. pennellii* revealed an excess of *L. pennellii* alleles for loci on chromosome 1, 3, 10, 11 and 12. These segregation distortions may reflect linkage to gametophytic factors required for compatibility with F_1LP, although no pollen tube studies were performed to confirm this. The results suggest that *L. pennellii* loci on chromosomes 1 and 10 are required for compatibility with both F_1LP and F_1LS hybrids. The skewed segregations for markers on chromosomes 3, 11 and 12 may reflect linkage to *L. pennellii* loci not required for compatibility with the intergeneric hybrids, or having only minor effects. Alternatively, these aberrant segregations may be attributed to *L. pennellii* factors affecting pre- or post-zygotic stages other than pollen tube growth. Thus, the data suggest there are shared, and possibly unshared, pollen compatibility genes functioning in Sc and SI species. Skewed segregations for isozyme markers on chromosomes 1 (*Skdh-1* and *Prx-1*) and 12 (*Pgi-1* and *6Pgdh-2*) have been previously reported in tomato interspecific F_2 populations (Quiros et al. 1986; Gadish and Zamir 1987). Pollen dilution studies suggested that pollen competition was not responsible for the abnormal segregations in F_2 *L. esculentum* × *L. pennellii* progeny (Gadish and Zamir 1987). However, this does not rule out the involvement of unilateral incompatibility, since reduced pollen competition would not be expected to weaken the incompatibility response.

A question raised by the present study is the genetic control of unilateral incompatibility in pollen versus pistil. The genetic determinants of pollen compatibility do not appear to be sufficient for functional unilateral incompatibility in the pistil since (1) bridging lines containing the *L. pennellii* chromosome 1, 6 and 10 loci are fully compatible with staminate *L. esculentum,* and (2) progeny of LLS and F_1LS include individuals compatible with staminate *L. esculentum.* On the other hand, certain loci may be necessary for expression in both pollen and pistil: of the 12 possible *S. lycopersicoides* monosomic alien addition lines in an *L. esculentum* background, only those containing an extra chromosome 1 or 6 are consistently incompatible with *L. esculentum* pollen (J. W. DeVerna et al. unpublished).

The linkage data presented here support the hypothesis that unilateral incompatibility is controlled by an interaction between expression at the S-locus and other genes. The probable involvement of the S-locus, if true, would contradict the "incongruity" model, although "penetration gene(s)" tightly linked to S could also explain the results (Hogenboom 1973). The finding that genes on chromosomes 6 and 10 (and possibly others) also play an essential role, argues against the hypothesis of Lewis and Crowe (1958) that mutations at the S-locus are the sole cause of unilateral incompatibility.

The bridging lines described herein may have broad utility for the transfer of nuclear-encoded traits and cytoplasmic genomes. By virtue of their compatibility with *L. esculentum* × *S. lycopersicoides* hybrids, these bridging lines have enabled the synthesis of both diploid backcross progeny and alien addition lines – germ plasm useful for trait introgression and for basic studies on genome organization. Preliminary tests indicate the bridging lines are also compatible with recently synthesized *L. esculentum* × *S. rickii* sesquidiploid hybrids (DeVerna et al. 1990; R. T. Chetelat et al. unpublished); if confirmed, this would permit the first reported gene transfer from *S. rickii* to tomato. These bridging lines may also be useful for the introgression of traits from *L. peruvianum,* whose hybrids with *L. esculentum* typically display early embryo abortion (Taylor 1986; Poysa 1990). Lastly, bridging lines that overcome the unilateral incompatibility of pure *L. pennellii,* and possibly other *Lycopersicon* spp., could be developed by methods similar to those described herein, permitting the transfer of alien cytoplasms into *L. esculentum* (Mutschler 1990). Knowledge of the chromosomal location of compatibility loci would be particularly valuable in this regard. For example, one strategy would be to use RFLP markers to synthesize segmental substitution lines which contain the *L. pennellii* alleles for those markers on chromosomes 1, 3, 10, 11 and 12 that showed skewed segregation in F_2LP.

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