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Recombination in sesquidiploid hybrids of *Lycopersicon esculentum* × *Solanum lycopersicoides* and derivatives

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Summary. Sesquidiploid hybrids of *L. esculentum* (L) × *S. lycopersicoides* (S) were backcrossed to L via *L. pennellii* (P) as a bridging species in order to detect and measure recombination. Although use of P injected its traits into the populations, the investigated traits were proven to originate from S. The appearance of S traits in diploids in the immediate progeny of sesquidiploids but mainly of derived alien addition types proved the occurrence of recombination at rates varying from 1.6% to 16%. In subsequent BC's, these traits were inherited in dominant Mendelian fashion, except for deviations favoring recurrent parent alleles, sometimes with highly significant deviations from 1:1. Inheritance was investigated in BC and F_2 ex BC for 13 traits with strong phenotypic modifications of morphological, physiological, and isozymic nature. Monogenic determination was confirmed in most instances by tight linkages. For most of the traits, small progenies allowed only rough estimates of linkage intensities, but for *Wa* (gene for White anthers, universal in S), a test cross with four markers on chromosome 8 established its locus 2 cM distal to *dl*, proximally on 8L. Also noteworthy is the linkage of *Dls*, a gene determining sensitivity of flowering to long days, close to *sp*, situated subterminally on 6L. For the majority of traits, these manifestations of linkage proved that the appearance of S traits resulted from recombination, not alien chromosome substitution – a conclusion also reinforced by observations of chromosome pairing in alien addition types and diploid derivatives. Recombined S alleles have loci in various chromosome positions. Although they were discovered on the shorter chromosomes (nos. 6–12), hybridization barriers precluded tests with the longer chromosomes. Thus, no evidence was found for restriction of recombination to certain chromosomes or chromosomal regions. The prospects therefore appear favorable for deriving valuable traits from the S parent.

Key words: Tomato – *Lycopersicon esculentum* – *Solanum lycopersicoides* – Intergeneric hybridization – Recombination

Introduction

In recent decades, hybridization with wild *Lycopersicon* spp. has played a major role in tomato improvement. Although such crosses have provided many valuable traits, other desirable characters have been identified in closely related *Solanum* spp. Amongst the latter, *S. lycopersicoides* (diploid genome designated SS) can be hybridized with the cultivated tomato (*Lycopersicon esculentum*) (LL), such hybrids having been first reported by Rick (1951). Unique, useful traits already identified in SS are cold tolerance, and resistance to virus diseases, root rots, *Botrytis* mold, and various insect pests. The 2× hybrids (LS) were sterile, and the 4× hybrids (LLSS), although somewhat more fertile, did not hybridize with LL. Thirty-five years later, renewed efforts, entailing crosses between LL and newly synthesized, rather fertile LLSS hybrids yielded two sesquidiploid (LLS) hybrids, which succeeded as a vehicle to transfer genes from SS to LL (Rick et al. 1986). Hybridizations of LLS with LL failed, but succeeded with *L. pennellii* (PP) to produce alien addition lines (intergeneric trisomics) as well as diploid progeny, which included some obvious recombinants (DeVerna et al. 1987a, b; Rick et al. 1987).

Despite its extremely high sterility, the 2× LS hybrid exhibits considerable bivalent chromosome pairing. In pachytene, the chromosomes appear to be completely associated (Menzel 1962; Menzel and Price 1966). This observed association needs careful interpretation because non-homologous pairing has often been observed at this stage in induced chromosomal deficiencies of to-

mato (Khush and Rick 1968) and in aneuploids and heterozygous chromosomal structural variants of maize (McClintock 1933). Pairing in later meiotic stages, usually reflecting true homology, is considerably reduced, a mean of 10 bivalents per cell were observed by Menzel (1962) and 7.4 by Rick (1951), and the extent of chiasma formation is lessened in the observed bivalents. In terms of conventional cytogenetics, such chromosomal association reflects earlier crossover events. A moderate extent of recombination should therefore be anticipated in these hybrids.

This article reports observations and analyses of segregation in derivatives of the original hybridizations of LLS × PP. Selected diploid and trisomic progeny of the BC_1 were further backcrossed to LL, the persistent strong unilateral barrier characteristic of such hybrids requiring the latter as female parent. These materials proved ideal for the purposes of this investigation. The wide intergeneric gap between the parents guarantees a wealth of genetic differences. Amongst these traits, allozymes offer manifold advantages, as experienced in many previous investigations. Another key advantage is the dominance typical of many wild traits in crosses with domesticates. Although the wild parent undoubtedly harbors recessives, the abundance of character differences, including co-dominant allozymes, ensures an adequate supply of useful dominant markers for our purposes. Further, as manifest in our results, relatively unsophisticated observations in progenies of modest size can often yield highly significant results.

As observed in our initial studies (DeVerna et al. 1987a; Rick et al. 1987), genetic recombination is manifest in the appearance of certain simple dominant S traits in the 2× offspring of LLS. The first such trait detected was white anthers (*Wa*), exhibited in not only the triplo-8 progeny but also in a single diploid individual. We had the same experience with *Pgi-1*^{s}, marking chromosome 12, as well as with several other traits.

Materials and methods

By its very nature, genetic recombination can be tested only in segregating progenies. For this purpose we established the following pedigrees. The LLS sesquidiploids (GH266 and 8619-1) were hybridized as pistillate parents with PP, LA716, a self-pollinating accession from Atico (Arequipa), Perú (Rick et al. 1987). Selected diploid and aneuploid progeny from this cross were mated as staminate parents in successive backcrosses to LL, cv 'VF36' (LA490), a consistently good performer under our field and greenhouse conditions. All of the hybridizations were performed in the greenhouse on flowers emasculated in the standard fashion. All selected BC derivatives were routinely selfed, and selected progenies grown as needed.

The segregating winter generations were grown in the greenhouse, the summer populations in the field. All seeds were treated with 2.6% sodium hypochlorite solution for 30 min, rinsed, then sown in standard fashion in the greenhouse in nursery flats of a light organic soil mix, and later transplanted into larger containers for greenhouse culture or to field cultures.

Since PP serves as the recurrent *Lycopersicon* parent of BC_1, it is critical to discriminate between P and S traits. In most instances this distinction was clear because the morphological differences between the two parents are vast. In others, linkage with S traits established the source. Examples of S traits that are unknown in the P parent (as described in the Results) are white anthers (*Wa*), daylength sensitivity (*Dls*), bifurcate inflorescence (*Bif*), fimbriate (*Fmb*), frilly (*Frl*), and laciniate (*Lac*) leaf margins, Rugose leaf surface (*Rug*), and the allozymes corresponding to *Got-3*^{s}, *Got-4*^{s}, and *Pgi-1*^{s}. Proof of the S origin of such traits is also manifest in their co-segregation with extra chromosomes in the progeny of alien addition lines. Such segregation, as well as linkage with other marker genes segregating in the same populations, also served to verify the monogenic nature of the segregations.

The standard aceto-carmine smear technique as modified by Khush and Rick (1963) was utilized for chromosome determination. The pertinent allozyme phenotypes and electrophoretic methodology are presented by Chetelat et al. (1987).

Results

Chromosome 6

Amongst the progeny of LLS × PP (BC_1) appeared a diploid individual (9069-1) that displayed a markedly deviating leaf phenotype. Its leaf margins were deeply incised and somewhat more wavy than those of LP. Since this individual was not trisomic, an assignment to a chromosome could not be made. It was hybridized with LL, and in the consequent BC_2, grown in the winter greenhouse, we observed much variation in leaf phenotype. A classification for deviant versus normal L leaf type was attempted with a tally of 39 normal and 33 laciniate, although under the low light conditions, identification of classes was not unequivocal. In the same progeny, a strong repulsion with *sp* (determinate habit from the L parent) and coupling with the tightly linked *B* (β-orange fruit pigment from the S parent) was also apparent, lending credence to a monogenic segregation for a dominant S gene symbolized *Lac*.

The third backcross, grown under more favorable field conditions, yielded definitive segregations, the laciniation appearing more pronounced in the LL background than in the mixed genotypes of the earlier generations. An F_2 from +/*Lac* BC_3 was scorable for three phenotypes (Fig. 1c) with a total segregation of 11 +/+ : 28 presumed +/*Lac* : 9 presumed *Lac*/*Lac*. Any remaining doubts about monogenic segregation were dispelled by tight linkages observed between *Lac* of the co-segregating *sp* and *B*. From BC_3 data for field-grown populations summarized in Table 1, the estimated distances and near-absence of presumed double crossover classes + + + and *Lac sp B* established the relationship of these loci as *Lac*-2.5-*sp*-4.9-*B* near the distal end of the long arm of chromosome 6. The orientation of this seg-

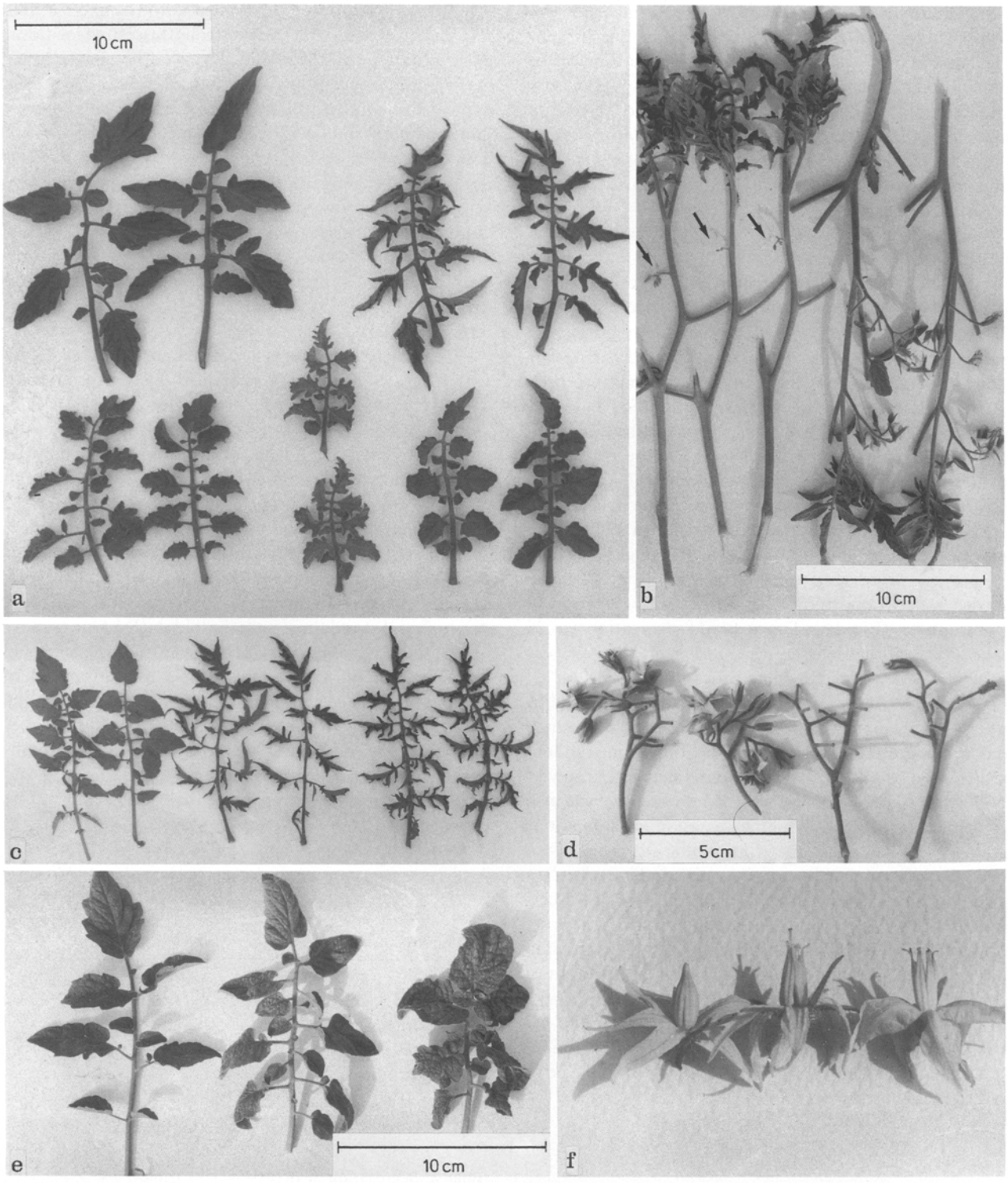

Fig. 1a–f. Phenotypes of dominant monogenic traits derived from *S. lycopersicoides*. **a** Leaf modifications. Upper left pair: normal (+) segregant; upper right, +/*Lac*; lower left, +/*Fmb*; lower center, +/*Frl* in alien addition triplo-7; lower right, +/*Frl* in diploid. **b** Three left stems, +/*Dls* (*arrows* point to abortive inflorescences); two right stems, +/+. **c** Left leaf pair, +/+; center presumed +/*Lac*; right, presumed *Lac*/*Lac*. **d** Inflorescences of +/*Bif*. **e** Left leaf, +/+; center, presumed +/*Rug*; right, presumed *Rug*/*Rug*. **f** Left flower, +/+ center, *dl*/*dl; right: dl*/*dl*^{s}, +/*Wa*

ment within the arm is uncertain, however, since previous tests found *sp* and *B* too close to determine their order.

The discovery of *Lac* in this part of the tomato genome is reminiscent of the situation with *Pts*, a similarly situated wild gene of somewhat similar phenotype and derived from *L. cheesmanii* f. *minor* (Rick 1980). The two differ, however, in the less intense linkage of *Pts* with *sp* and greater subdivision and much more obtuse segments of the *Pts* leaf.

Descendents of 9145-3, a BC_1 individual with one unidentified extra chromosome, displayed polymorphy for control of flower development. Inflorescences of the parent plant were aborted during the summer months of 1986. The BC_2 was grown in the greenhouse during the subsequent winter, when all plants flowered freely. The same restriction of long days on flowering has also been observed consistently in SS, LS, and LLSS, but LLS develops flowers yet is totally sterile under the same conditions. As in *L. chilense, L. hirsutum,* and certain races of *L. cheesmanii, peruvianum,* and *pimpinellifolium,* long days in the May–September period in our latitude induce bud abortion, but do not interfere with initiation of inflorescences. In the following season, we grew a small F_2 progeny from selfing a segregant in BC_2, which afforded a first opportunity for observing segregation of this character (Fig. 1 b). The observed segregation was 28 sensitive:9 normal flowering. Monogenic control was proven by the fortunate coincidence of linkage with *sp*, for, of the seven *sp* segregants, all save one were insensitive. Also presumed recombinants were three insensitive indeterminate individuals. The deviation from random segregation was 4.3 with a corresponding contingency χ^2 of 9.5***. A strong coupling linkage with *Lac* was also evident, although extraneous segregation in populations so little removed from the wild parent did not allow a clear classification. All of the *sp Dls*$^+$ individuals were also B^+, but, of course, fruit color could not be ascertained in the *Dls* classes because inhibition of flowering prevented fruit set. Thus, as tiny as this family is, the data are sufficient to establish a linkage with *sp* on chromosome 6, thereby adding to the list of wild alleles known in this region. Further backcrosses of *Dls* into pure LL background should provide lines useful for experimentation on the development and physiology of this type of photoperiodic control of flowering.

Chromosome 7

A triplo-7 BC_1 plant (GH592) was selected as the parent to be hybridized with LL to produce BC_2. As with all of the BC_1, the strict unilateral barrier permitted such crosses only if LL were used as the pistillate parent. A progeny of 85 individuals was grown from this mating, including three triplo-7 and 82 diploids, hence an appreciable male transmission of the extra chromosome. All of the trisomics exhibited the typical autotrisomic features of foreshortened plant parts, strong fasciation of floral parts, and typical leaf modifications, although the latter were considerably exaggerated in the respects that leaves tended to be much smaller and convexly recurved both longitudinally and laterally. The same, though somewhat less extreme, leaf phenotype could also be identified in four diploid segregants, revealing that it is determined by a gene on S chromosome 7, and can be transmitted to diploid progeny via genetic recombination. This leaf phenotype – relatively distinct from that of the aforementioned *Lac* and other leaf shape modifiers inherited from S (Fig. 1 a) – is designated *Frl* (frilly). This distinction was verified by its affiliation with chromosome 7.

The GH592 parent also carried the expected *Got-2* and *Got-3* S alleles. As explained by DeVerna et al. (1987 b), this aneuploid was triply heterozygous for L, P, and S alleles of *Got-2*, but since both P and S allozymes migrate to the same advanced position, they cannot be distinguished by position alone. For *Got-3*, this problem does not exist, because the P allozyme is indistinguishable from that of L, and the S band is clearly retarded. The joint segregation for chromosome number and for *Frl* and *Got-3* is summarized in Table 2. Nine plants were $+/Got\text{-}3^s$, the remainder, $+/+$. Of the nine, two were triplo-7 $+/Frl$ and three were diploid $+/Frl$. One triplo-7 $+/Frl$ did not possess the $Got\text{-}3^s$ allele, and the same held for four diploid $+/Frl$. Thus, $Got\text{-}3^s$ is transmitted to diploid progeny at about the same rate as S alleles in the previous examples. The single triplo-7 lacking $Got\text{-}3^s$ probably possessed an extra recombinant L/S chromosome. The virtually independent segregation of *Frl* and *Got-3* suggests that they are probably rather distantly situated.

Table 1. Linkage segregation in $BC_3 + sp + \times \frac{Lac + B}{+ sp +}$

Phenotype			F	Phenotype			F
Lac	+	*B*	87	*Lac*	*sp*	*B*	1
Lac	+	+	3	*Lac*	*sp*	+	4
+	+	*B*	0	+	*sp*	*B*	6
+	+	+	0	+	*sp*	+	102

Lac–sp 2.5 cM
sp–B 4.9 cM
Lac–B 6.4 cM

Table 2. Segregation in BC_2 of $++ \times$ triplo-7 $\frac{Frl\ Got\text{-}3^s}{+\ +}$ $\frac{}{+\ +}$

	Phenotype				Total
	++	*Frl*+	+*Got-3*^{s}	*Frl Got-3*^{s}	
2n	69	2	4	3	78
2n+1	0	1	0	2	3

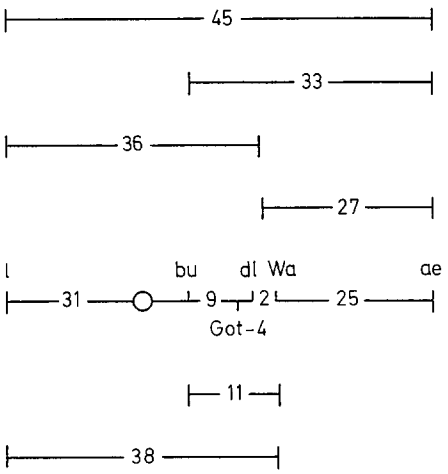

Fig. 2. Map positions of markers on chromosome 8 as approximated from data in Table 5. Locus of *Got-4* from Bernatzky and Tanksley (1986)

Chromosome 8

The first evidence of L/S recombination was detected for this chromosome in the segregation of *Wa*, the gene determining the white anther trait that is universal in *S. lycopersicoides*. As explained previously (DeVerna et al. 1987a, b; Rick et al. 1987), localization to chromosome 8 was evident from the first observation of this striking character in triplo-8 individuals. The nearly constant association with triplo-8 and its absence in other alien addition types provided incontrovertible proof of its locus on chromosome 8, and, along with data presented here, for monogenic control (DeVerna et al. 1987a). The appearance in BC_1 of a single diploid *Wa* individual indicated either alien substitution of S chromosome 8 or genetic exchange between L and S chromosomes.

Our tests of *Wa* included a cross between diploid LL and a BC_1 triplo-8 segregant (as male parent). In the total of 61 BC_2's, 1 was triplo-8 *Wa*, 8 were 2n *Wa*, the remaining 52 were diploid+. The results demonstrated that S chromosome 8 can be transmitted as an extra by male gametes, and that genes on this chromosome can be transmitted to diploid progeny. Further data from this experiment and from other tests to be reported below revealed that other genes on this chromosome (*dl*^{s}, *Got-4*^{s}) were also transmitted to diploid progeny, but not always in concert. This breakdown in joint transmission proves that crossing over occurred between L and S chromosomes.

Our new research included testing segregations of *Wa* (derived from the diploid GH577) in progenies approaching purity for the LL background and testing linkage relations with selected markers of chromosome 8. Several segregations of the former type were observed as summarized in Table 3. The observed frequencies fit expected Mendelian ratios, suggesting the absence of phenomena engendered by the intergeneric hybridization that drastically interfere with meiosis or transmission. The linkage data summarized in Table 3 were derived from a test cross of a BC_3+/Wa segregant to a standard *l-bu-dl-ae* tester. The F_1 necessarily segregated for +/+ and +/*Wa*, hence the latter were selected for hybridization (as male parents) to the homozygous marker stock. We were surprised to find that all F_1+/Wa had free anthers, characteristic of *dl*, and the same phenotype was seen in all 299 testcross progeny, demonstrating that an allele (*dl*^{s}) linked with *Wa* had been transmitted in the segment of S chromosome 8 being investigated. Fortunately, for our purposes, *dl*^{s} does not manifest the trichome modifications associated with *dl* (Rick 1947), thus permitting discrimination between the two phenotypes. The five genes segregated in orderly 1:1 fashion. The array of estimated locus-to-locus distances together with the frequencies or absences of various multiple crossover classes summarized in Fig. 2 point incontrovertibly to a site on the long arm of chromosome 8 at approximately position 31, 2 cM distal to *dl*. This determination is in close

Table 3. Linkage segregation in BC_4 *l bu dl ae* × $\frac{+\ +\ dl^s\ Wa\ +}{l\ bu\ dl\ +\ ae}$

Crossovers	Phenotypic classes					F
Parental	+	+	*dl*^{s}	*Wa*	+	66
	l	*bu*	*dl*	+	*ae*	66
Single	+	*bu*	*dl*	+	*ae*	34
	l	+	*dl*^{s}	*Wa*	+	33
	+	+	*dl*	+	*ae*	13
	l	*bu*	*dl*^{s}	*Wa*	+	2
	+	+	*dl*^{s}	+	*ae*	3
	l	*bu*	*dl*	*Wa*	+	3
	+	+	*dl*^{s}	*Wa*	*ae*	32
	l	*bu*	*dl*	+	+	15
Double	+	*bu*	*dl*^{s}	*Wa*	+	2
	l	+	*dl*	+	*ae*	2
	+	*bu*	*dl*	*Wa*	+	0
	l	+	*dl*^{s}	+	*ae*	0
	+	*bu*	*dl*	+	+	5
	l	+	*dl*^{s}	*Wa*	*ae*	15
Double	+	+	*dl*	*Wa*	+	0
	l	*bu*	*dl*^{s}	+	*ae*	0
	+	+	*dl*	+	+	6
	l	*bu*	*dl*^{s}	*Wa*	*ae*	0
	+	+	*dl*^{s}	+	+	1
	l	*bu*	*dl*	*Wa*	*ae*	0
Triple	+	*bu*	*dl*^{s}	+	*ae*	0
	l	+	*dl*	*Wa*	+	0
	+	*bu*	*dl*^{s}	*Wa*	*ae*	1
	l	+	*dl*	+	+	0
	+	*bu*	*dl*	*Wa*	*ae*	0
	l	+	*dl*^{s}	+	+	0
	+	+	*dl*	*Wa*	*ae*	0
	l	*bu*	*dl*^{s}	+	+	0
Quadruple	+	*bu*	*dl*^{s}	+	+	0
	l	+	*dl*	*Wa*	*ae*	0

agreement with the aforementioned observation of tight linkage between *dl* and *Wa*. The data also provide the first good approximation of the *ae* locus, which heretofore had been known only to be situated toward the distal end of 8L.

Got-4^s, another chromosome 8 marker, segregated in certain of the populations of these new experiments. This allele was lacking in GH577, thus demonstrating recombination in its origin. Since subsequent linkage tests were made with descendents of this individual, they afforded no opportunity for including *Got-4* in the linkage analysis.

Chromosome 9

The data for this chromosome were derived from the BC_2 issue of the cross between LL as pistillate parent × GH646 – one of a series of trisomic plants identified as triplo-9 in the progeny of LLS. Even though the trisomic was the staminate parent, an extra chromosome 9 was transmitted to 2 of the 84 progeny. Also segregating in this family were two dominant traits that were consistently observed in the triplo-9's in BC_1: *Fmb* – fimbriate leaf – and *Bif* – bifurcate inflorescence. In contrast to *Lac, Fmb* conditions greater leaf serration and margins are so ruffled that the lower surface is frequently displayed (Fig. 1 a). We do not pretend that we could distinguish both leaf types and *Frl* in cosegregating progeny, but the unique linkage relations discriminate between the three and leave no doubt that they are determined by alleles at three independent loci. The *Bif* inflorescence is distinguished from the normal by its subdivision into two equal branches with a resultant increase in floral display (Fig. 1 d). The cosegregations of these traits in diploid individuals are summarized in Table 4. Each of the traits appeared in each of the 2 trisomic individuals, *Bif* also in 7 diploids and *Fmb* in 13 diploids. Amongst diploids, the cosegregation of *Bif* and *Fmb* appears independent. These data demonstrate that recombination permitted the transmittal of each trait to diploid progeny, and that their respective loci are separated by a relatively large map distance.

Table 4. Segregation in BC_2 of $++ \times$ Triplo-9 $\frac{Bif\ Fmb}{+\ +}$ / $\frac{}{+\ +}$

	Phenotype				Total
	++	*Bif*+	+*Fmb*	*Bif Fmb*	
2n	65	4	10	3	82
2n+1	0	0	0	2	2

Chromosome 12

In BC_1, a single individual (GH617) was discovered that was diploid, but variant in its *Pgi-1*^{p}/*Pgi-1*^{s} genotype. Although no examination was made for *6Pgdh-2*, another marker of chromosome 12, it is evident that it must have carried the S allele because the corresponding allozyme segregated in a later generation. BC_2 segregated 17+/*Pgi-1*^{s} and 26+/*Pgi-1*^{p} (Table 5). Determination of *6Pgdh-2*^{s} in BC_3 was possible in most, but not all segregants. Of the 4+/*6Pgdh-2* individuals, three were also +/*Pgi-1*^{s}, the other three undetermined, but one individual heterozygous for the former locus was +/+ for *Pgi-1*. Thus, even though known to be linked at 12 cM

Table 5. Segregations for various monogenic traits

Gene	Ch'some	Generation	Mut/Mut	+/Mut	+/+	Total	χ^2 [a]	Recombination (%)
Bif	?	BC_2 ex 2n+1		7	75	82		8.5
Dls	6L	F_2 ex BC_2	25		12	37		
Got-4^s	8L	BC_2 ex 2n+1		1	59	60		1.6
Fmb	9	BC_2 ex 2n+1		13	69	82		16
Frl	7	BC_2 ex 2n+1		4	78	82		4.0
Lac	6L	BC_3 ex 2n+1		95	108	203		
		F_2 ex BC_3	9	28	11	48		
Pgi-1^s	12	BC_2 ex 2n		8	26	34	9.5***	
				9	52	61	30***	
		BC_3 ex 2n		6	22	28	8.0**	
Rug	6L	F_2 ex BC_2	38		10	48		
sp	6L	BC_3 ex 2n+1	113		90	203		
Wa	8L	BC_2 ex 2n+1		8	52	60		13
		F_2 ex BC_3	71		24	92		
		BC_2 ex 2n		49	50	99		
		BC_3 ex 2n		22	28	50		
		BC_4 ex 2n		14	18	32		
		BC_4 (linkage)		154	145	299		

[a] Reported only if significant

according to Bernatzky and Tanksley (1986), at least one recombinant was obtained. Furthermore, as noted above, segregation of *Pgi-1* deviated strongly from the expected 1:1 – a matter to be considered in the Discussion. Further study will be required to ascertain the nature of the aberrant segregations and to ascertain if any morphological traits are linked to the isozymic loci.

Unlocated S markers

Div (divergent anthers): For reasons to be discussed, *Div* is not allelic with dl^s, hence the application of a new symbol. It was first seen in a deviant diploid BC_1 individual (GH608). Although the separated anther trait of *L. pennellii* is slightly expressed in LP hybrids, this plant was exceptional in that all anthers of all its flowers were dialytic. Contrary to the expression of *dl*, but like that of dl^s, *Div* is not associated with the absence of marginal anther hairs that interlock to bind anthers together in LL (Rick 1947). This plant was also recombinant for the S allele of the chromosome 7 marker *Got-2*.

The question of relationship between *Div* and dl^s is readily resolved by considerations of dominance and linkage relations. With respect to the former, they differ in the dominance of *Div* and recessiveness of dl^s, and in the fact that the S parent, even if by chance heterozygous for a dominant and recessive allele at the same locus, could not have transmitted more than one allele, since all of our progenies trace to one LLS sesquidiploid.

With regard to the linkage argument, the experience with *Wa* established the order: *Got-4* – *dl* – *Wa* on the long arm of chromosome 8, *dl* being in close proximity to *Wa*. Now, if *Div* were indeed identical to dl^s, one might expect that either or both $Got\text{-}4^s$ and *Wa* be transmitted with the free anther trait, but GH608 was deviant for neither. Since transmission of dl^s without either of these markers would require a double crossover within a short chromosome segment, it is highly unlikely that this trait is determined by dl^s. The aforementioned presence of $Got\text{-}2^s$, suggesting, but not proving, that *Div* might be situated on chromosome 7, is also concordant with this conclusion.

By oversight, we failed to test GOT segregation in BC_2, but were able to do so in one small family of BC_3. Although in the latter, *Got-3* did not segregate, *Got-2* did for the P or S allele. For linkage detection, the source of the variant allele is immaterial, as the P allele would be expected to segregate in *trans* phase and the S allele in *cis*. The cosegregation was:

Got-2	+	+/s
+	5	4
+/*Div*	8	5

These data suggest independence, certainly not tight linkage. Thus, although available knowledge is not very enlightening, it is clear that *Div* is not allelic with *dl*, and if situated on chromosome 7, must be at a considerable distance from *Got-2*, thereby limiting it to a distal site on either the long or short arm.

Rug (rugose leaf): This trait was first observed in a single F_2 family derived from selfing a BC_2 derivative of GH588 (triplo-2). The family in question segregated distinctly into 38 rugose: 10 normal, and amongst the rugose individuals, degrees of intensity of leaf blistering could be discerned, suggesting a separation of +/*Rug* versus *Rug*/*Rug* (Fig. 1e). Leaves of rugose individuals were characterized by a dark green color, the upper laminar surface roughened by convex interveinal swellings even more extreme than in the familiar dwarf (*d*) phenotype. Their leaf segments appear to be somewhat broadened and their tips blunt, although these characters do not always appear to be associated. Since this trait was not detected in the grandparental BC_1 plant, we cannot be certain that *Rug* is affiliated with chromosome 2. Although this relationship is suggested, clearly more progenies need to be tested, and segregation with $Prx\text{-}2^s$ and other pertinent markers assayed.

Discussion

Monogenic segregation

It is of interest to consider the segregation of the various monogenic traits described here. For this aspect, we are not concerned with segregations in BC_2 from alien addition trisomics because transmission of mutant gametes would be a function of chromosome substitution or recombination between S and L chromosomes. But in subsequent generations of a strictly diploid nature, it is worthwhile comparing observed versus expected BC ratios. In Table 5, nine relevant progenies are listed for the segregation of *Lac*, $Pgi\text{-}1^s$, *sp*, and *Wa*. Observed values do not deviate significantly from the expected, except for the segregation of $Pgi\text{-}1^s$ in BC_2 and BC_3, in which from 2–5 times as many normal homozygotes than heterozygotes were counted, the deviation being highly significant in each family. In the majority of the other populations, normal homozygotes also exceeded heterozygotes, although none of the deviations was significant. This type of deviation favoring alleles of the recurrent parent is common in backcross segregations of wide crosses, and many examples have been reported. Stephens (1939) first called attention to this phenomenon in his studies of interspecific hybrids of *Gossypium*; Rick (1963, 1969, 1971) reported numerous examples in *Lycopersicon*. The net effect of such deviations for the entire genotype would be a significantly more rapid than expected reconstitution of the recurrent parent genotype. We have indeed observed this trend in the segregation of quantita-

tive characters; for example, in both interspecific and intergeneric combinations, recovery of the large fruit size of the LL recurrent parent is so rapid that it is fully achieved in numerous segregants of BC_3. In general, this trend should expedite the goals of plant breeders and other investigators in their attempts to rapidly introgress one or a few wild alleles into the genetic background of the cultivated species.

Recombination or substitution?

The appearance of S traits in diploid progeny of sesquidiploid hybrids or alien addition trisomics could be explained theoretically by either genetic recombination between L and S chromosomes or by substitution of whole S for L chromosomes. The following arguments can be considered.

(a) Substitution is unlikely from cytological considerations. According to observed meiotic behavior in LLS and in alien addition types, the S chromosome is usually unpaired, and, if transmitted, it does so as an extra chromosome (Rick et al. 1986; DeVerna et al. 1987a). Even in the rarely observed trivalents, it is more likely that the two L chromosomes orient in opposite directions, so that each gamete would receive an L chromosome and an occasional extra S.

(b) Alien substitution heterozygotes should exhibit pairing disruption. Although we have not been able to examine the cytology of all derived diploid variants, our examinations of such individuals in BC_1 and later generations have not detected this phenomenon. For example, the three diploid BC_1 sources of *Pgi-1*^{s}, *Lac*, and *Wa* referred to above consistently displayed 12 bivalents in the diakinesis and metaphase of the first meiotic division. When examined, later derivatives of these and other S alleles show similar chromosome behavior.

(c) Alien substitution heterozygotes and homozygotes should be rather strongly modified in phenotype – stronger, in fact than alien addition races. We have not seen such extreme modification in the diploid variants.

(d) Unequivocal evidence of recombination has been detected genetically for nearly all genes studied in this survey. Thus, for transmission of genes from S chromosomes 6, 7, 8, 9, and 12, evidence of recombination was obtained from inheritance of traits from LLS hybrids, from alien addition types, or from diploid plants in BC_2 or later generations. Thus, for chromosome 6, recombination was detected between *Lac* and the *B* and *sp* markers; for chromosome 7, between *Frl*, *Bif*, and *Got-3*; for chromosome 8, between *Wa* and *Got-4* and four other standard markers; for chromosome 9, between *Bif* and *Fmb*; for chromosome 12 between *Pgi-1* and *6Pgdh-2*.

The total evidence therefore strongly supports recombination as the responsible phenomenon, although substitution cannot be ruled out in all instances.

Extent of recombination

Transmission of six monogenic S traits from BC_1 trisomics to BC_2 is summarized in Table 5. The frequencies of transmission amongst diploid progeny vary from 1.6% for *Got-4* to 16% for *Fmb*, the mean rate being 8.7%. In no instance did we fail to obtain desired recombinants, even in relatively small progenies. Thus, variable as these rates are, none are too low to block efforts to incorporate investigated genes from S into L, yet the rates appear to be low enough for us to achieve one of our goals – the development of a complete panel of S addition lines.

The data include an anomalous situation for chromosome 8: the very low rate (1.6%) for *Got-4* versus the much higher rate (13.3%) for the linked *Wa* in the same progeny. The test clearly needs repeating. If indeed verified, it would point to greatly reduced pairing frequency in the region of *Got-4*. Since the latter must lie rather close to the centromere, such a relationship might mean that pairing and crossing-over are less likely to occur in pericentric regions – a phenomenon common in LP hybrids (Rick 1969, 1971).

Otherwise, the experience with these materials in attempted introgressions is straightforward. The plan is to backcross in consecutive generations. The first and/or second backcross from the sesquidiploid is made in order to transmit the S allele via recombination; as experienced, the rates are variable according to locus. After recombined, the allele should generally be inherited in normal Mendelian fashion, albeit with frequently reduced transmission of the wild allele. It is noteworthy that Clausen and Cameron (1957) reported essentially the same experience with introgression of traits into *Nicotiana tabacum* from other tobacco species. As they demonstrated, it is possible without cytological examination, but by segregation ratios and aneuploid phenotypes, to ascertain how traits are transmitted, first via the extra wild chromosome, later after recombination between wild and cultivated chromosomes, via normal segregation.

Limits of recombination

It is important to consider these results with respect to chromosomes and chromosomal regions from which recombinants have been derived. More concise mapping must be done before this matter can be satisfactorily evaluated. But perhaps it is worthwhile to evaluate the results using the present extent of our knowledge of the loci of the respective genes.

The best mapped loci are those of *dl*^{s} and *Wa*, which are fairly close to the centromere on 8L. Although mapped less precisely, *Dls* and *Lac* must be distally situated on 6L. *Got-2* lies in the proximal part of 7L, whilst *Got-3* is situated at the end of the rather poorly explored 7S. Although the map of chromosome 12 is poorly un-

derstood, the best version, developed by Bernatzky and Tanksley (1986) shows *Pgi-1* to be situated internally. The chromosomal location of other introgressed S genes of this study is not known; we shall be mapping them to the extent that our resources permit. Another shortcoming is that we have no way of knowing whether or not this small sample is typical of all characters that can be transferred by recombination. But a comparison of the exotic characters exhibited by alien addition types with those that we have been able to introgress suggests that recombination is not restricted to certain characters or regions of the genome. Thus, the introgressed alleles do not appear to be limited to distal regions, where recombination might seem easiest to achieve.

Another considerations is the chromosomal distribution of the located S alleles. Thus far, they have been limited to the shorter chromosomes (no. 6–12) of the complement; however, we suspect that this delimitation does not necessarily represent a nonrandomness of recombination, rather a restriction imposed on our tests by crossing barriers. At the time these crosses were made with the alien addition types, the widespread *Lycopersicon* unilateral barrier would not permit them to accept pollen of L sources. Also, pollen of addition lines with the longer chromosomes was too defective to permit their use as staminate parents. As our research proceeds, we anticipate that it might become possible to circumvent these barriers. Thus, none of the data suggests that introgression of S genes is limited in any marked fashion to specific parts of the complement.

Finally, we must conclude from this rather cursory survey that the prospects are good for transmission of a great wealth of variation from *S. lycopersicoides*. Although we have dealt with rather small progenies in the segregation of a relatively small number of traits of rather limited distribution in the genome, we have been able to introgress 13 monogenic traits from S into *L. esculentum* of various degrees of purity and thus far have not met with severe obstacles. In the case of leaf shape modification, *Fmb, Frl, Lac,* and *Rug* are genes of rather striking effect, even in heterozygotes, and in concert could conceivably account for most of the leaf shape differences between the two species. Thus, this experience certainly seems to bode well for breeding various traits, including those of economic importance, from this fascinating wildling.

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