Distribution of Unlinked Transpositions of a *Ds* Element From a T-DNA Locus on Tomato Chromosome *4*

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ABSTRACT

In maize, receptor sites for unlinked transpositions of *Activator (Ac)* elements are not distributed randomly. To test whether the same is true in tomato, the receptor sites for a *Dissociation (Ds)* element derived from *Ac*, were mapped for 26 transpositions unlinked to a donor T-DNA locus on chromosome *4*. Four independent transposed *Ds*s mapped to sites on chromosome *4* genetically unlinked to the donor T-DNA, consistent with a preference for transposition to unlinked sites on the same chromosome as opposed to sites on other chromosomes. There was little preference among the nondonor chromosomes, except perhaps for chromosome *2*, which carried seven transposed *Ds*s, but these could not be proven to be independent. However, these data, when combined with those from other studies in tomato examining the distribution of transposed *Ac*s or *Ds*s among nondonor chromosomes, suggest there may be absolute preferences for transposition irrespective of the chromosomal location of the donor site. If true, transposition to nondonor chromosomes in tomato would differ from that in maize, where the preference seems to be determined by the spatial arrangement of chromosomes in the interphase nucleus. The tomato lines carrying *Ds* elements at known locations are available for targeted transposon tagging experiments.

TRANSPOSABLE genetic elements were first described by McCLINTOCK (1947, 1951). When such elements insert into genes, mutant alleles are induced and the corresponding gene can be isolated using the transposable element as a probe. Transposable elements of maize (SCHWARZ-SOMMER *et al.* 1985) and *Antirrhinum majus* (COEN *et al.* 1989) are well characterized and have been used for the isolation of genes mutated by transposon insertion (FEDOROFF *et al.* 1984; MARTIN *et al.* 1985; O'REILLY *et al.* 1985; THERES *et al.* 1987; HAKE *et al.* 1989; COEN *et al.* 1989).

In many plants, endogenous transposable elements have not been sufficiently well characterized for use in gene isolation. To establish gene isolation systems based on tagging, transposons such as the maize *Activator (Ac)* or *Dissociation (Ds)* elements have been introduced into heterologous plant species and shown to transpose (BAKER *et al.* 1986; VANSLUYS *et al.* 1987; KNAPP *et al.* 1988; YODER *et al.* 1988; ZHOU and ATHERLY 1990; ZHANG *et al.* 1991; DEAN *et al.* 1992) and mutagenise plant genes (CHUCK *et al.* 1993; JONES *et al.* 1994; WHITHAM *et al.* 1994). For several species, transposon systems have been established in which the transposable element and transposase gene are provided as separate components (HEHL and BAKER 1989; LASSNER *et al.* 1989; SWINBURNE *et al.* 1992). In maize (GREENBLATT 1984; DOONER and BELACHEW 1989) and other species (JONES *et al.* 1990; DOONER *et al.* 1991; BANCROFT and DEAN 1993; KELLER *et al.* 1993; CARROLL *et al.* 1995), *Ac* and *Ds* transpose preferentially to linked receptor sites.

DOONER *et al.* (1994) studied the distribution of unlinked receptor sites after transposition of *Ac* elements from the maize *bronze* (*bz*) gene on chromosome *9* and found it to be nonrandom with an apparent preference for sites on the same chromosome as the donor site. It was suggested that the nonrandom pattern of transpositions to the remaining chromosomes (preferentially to chromosomes *5* and *7*) might reflect higher order spatial organization of the chromosomes in the interphase nucleus. Thus, unlinked receptor site distribution could provide an interesting insight into nuclear organization and raises the issue of whether this nonrandom distribution might also prevail for transposition from other maize loci and in other species.

We set out to test if there is also a nonrandom pattern to the unlinked transpositions of *Ds* in tomato, and we report here on the distribution of unlinked transposed *Ds*s (tr*Ds*s) from the tomato chromosome *4* T-DNA locus designated 1561E. Of 103 families analyzed that harbored germinally tr*Ds* elements, 36 carried tr*Ds*s genetically unlinked to the T-DNA donor site. Using inverse PCR (TRIGLIA *et al.* 1988) we have amplified tomato DNA sequences adjacent to unlinked tr*Ds*s for 33 families, and we have determined the RFLP map

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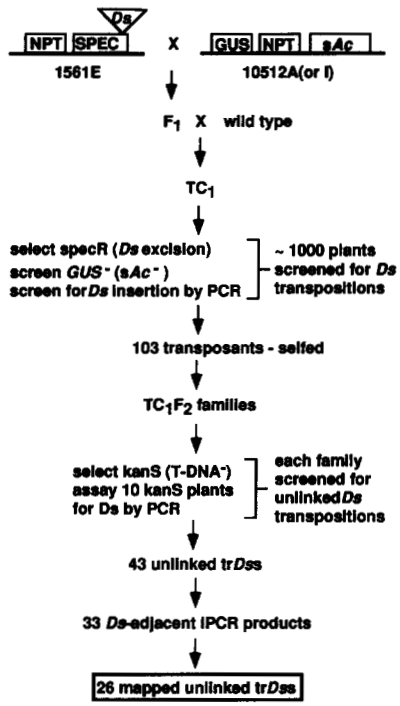

FIGURE 1.—Experimental strategy for the generation, recovery and mapping of transposed *Ds* elements unlinked to the donor T-DNA 1561E located on chromosome *4* of tomato.

locations of 26 tr*Ds*s. Our results, like those for maize (DOONER *et al.* 1994) are consistent with preferential transposition to unlinked sites on the same chromosome as the donor locus *vs.* sites on other chromosomes. However, unlike the results for maize, our results suggest little preference among the other chromosomes, with the possible exception of chromosome *2*.

MATERIALS AND METHODS

Isolation of tr*Ds*s unlinked to the donor T-DNA: All of the DNA constructs and transgenic tomato lines used in this study were generated and described previously (CARROLL *et al.* 1995). The donor T-DNA 1561E (derived from construct SLJ1561), located on chromosome *4*, contains a 35S:*NPT* transformation marker conferring kanamycin resistance, a *nos:SPEC* excision marker conferring spectinomycin resistance and a *Ds* element in the 5′ untranslated leader of the *nos:SPEC* gene (Figure 1). Transposition of the *Ds* was induced by either 10512A or 10512I T-DNAs (derived from construct SLJ10512) carrying stabilized *Ac* (s*Ac*); this provided a source of transposase and a 2′:*GUS* marker conferring β-glucuronidase activity that was used as a histochemical marker for the presence of s*Ac* (Figure 1).

The crossing and screening strategy for the generation and recovery of tr*Ds*s unlinked to the donor T-DNA is outlined in Figure 1. Individuals carrying s*Ac* and 1561E were crossed either individually, as seed parents, or bulked, as pollen parents, to wild-type plants to generate several TC_1 populations. TC_1 seed was germinated on spectinomycin-containing medium, and individuals carrying a tr*Ds* and lacking s*Ac* were identified by carrying out PCR (to detect the *Ds*) on spectinomycin resistant individuals that lacked a *GUS* gene as determined by staining plant tissue for β-glucuronidase activity. Each TC_1 plant of this phenotype was allowed to self-pollinate to generate TC_1F_2 seed. To assay for the presence of the donor T-DNA, seed of each family was germinated on MS medium containing 300 μg ml^{-1} kanamycin. To distinguish unlinked and linked tr*Ds* elements, 10 kanamycin sensitive (kanS) seedlings of each family (*i.e.*, 20 gametes) were assayed for the presence of the *Ds* by PCR. PCR analyses were carried out as described by KLIMYUK *et al.* (1993) and four oligonucleotide primers were added to each PCR. Two (D60 and D75, Table 1) amplified a 334-bp fragment composed of the 3′ end of *Ds* and the other two (2995AR and 2995AL) (see KLIMYUK *et al.* 1993) amplified a 141-bp fragment from tomato chromosome *11* as a positive control.

Upon transposition of the *Ds* to a site closely linked to the T-DNA, little or no recombination will occur between the T-DNA and the tr*Ds*, so the majority or all of the kanS TC_1F_2 individuals should lack *Ds*. In contrast, upon transposition of the *Ds* to an unlinked site, the T-DNA and the tr*Ds* should assort independently, and 75% of the kanS plants will inherit the *Ds* element. The proportion of kanS plants lacking a tr*Ds* was used to identify families likely to carry a tr*Ds* unlinked to the T-DNA. Families with five or more kanS individuals car-

TABLE 1

Oligonucleotide sequences of primers used for PCR and IPCR analysis of transposed *Ds*s

Primer	Sequence (5′–3′)
Bar1	TTCTGGCAGCTGGACTTCAGCCT
Bar2	TCACCGAGATCTGATGACCCG
B34	ACGGTCGGTACGGGATTTTCCCAT
B35A	TATCGTATAACCGATTTTGTTAGTT
B38	GATATACCGGTAACGAAAACGAACGG
B39A	GTTTTCGTTTCCGTCCCGCAA
B49A	GAACATGAATTGATATGCAGGGAG
D60	GTGATCCAGATGTGAGCAAG
D71	CCGTTACCGACCGTTTTCATCCCTA
D72	GAATCTGTGAACTAACACGGCTGG
D73	TTTCCCATCCTACTTTCATCCCTG
D74	GTTAGTTTTATCCCGATCGATTTC
D75	ACGAACGGGATAAATACGGTAATC
D103	GTGATAAGTCTTGGGCTCTTGGCT
D104	ACATAAGAAGCCATATAAGTCTAC

TABLE 2

Primer combinations used to amplify IPCR products

Ds	Digest	Amplification	Primer combination
5′ end	All except *Dra*I	Primary	B35A/B34
		Nested	D74/D73
3′ end	*Bst*YI or *Bst*YI + *Bcl*I	Primary	B39A/Bar1
		Nested	D71/Bar2
	*Taq*I	Primary	B39A/B38
		Nested	D71/D75
	*Alu*I	Primary	B39A/B49A
		Nested	D71/D72
	*Dra*I	Primary	B39A/D103
		Nested	D71/D104

Amplifications occurred after cleavage with either *Bst*YI, *Bst*YI + *Bcl*I, *Taq*I, *Alu*I or *Dra*I and recircularization of DNA from plants carrying transposed *Ds*s. Primer sequences are shown in Table 1.

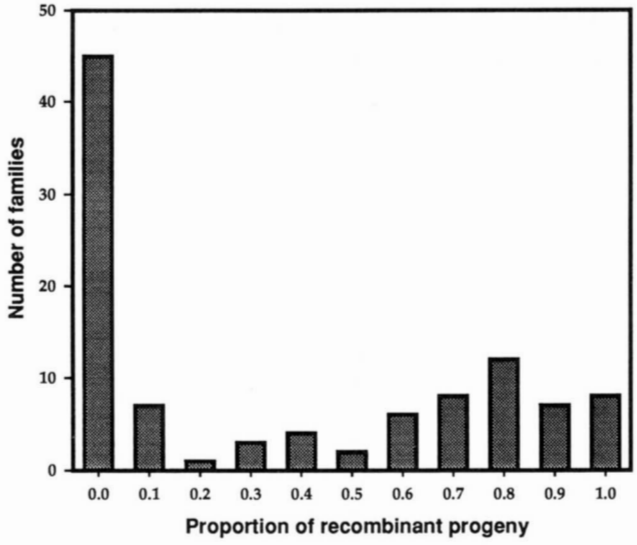

FIGURE 2.—Proportion of progeny recombinant between the donor T-DNA locus and a transposed *Ds* in 103 TC_1F_2 families carrying transposed *Ds*s. Progeny were tested for recombination between the T-DNA (kanamycin resistance) and the transposed *Ds* by screening kanamycin sensitive progeny for the presence of *Ds* by PCR.

rying *Ds* were presumed to carry an unlinked tr*Ds* (for five Ds^+:five Ds^- progeny $P = 0.08$ for a fit to a 3:1 ratio), whereas those with four or fewer kanS individuals carrying *Ds* were presumed to carry a linked tr*Ds* (for four Ds^+:six Ds^- progeny $P = 0.02$ for a fit to a 3:1 ratio).

Fifteen of the remaining kanR plants in each family carrying a presumed unlinked tr*Ds* were used to determine *Ds* copy number. Plants were bulked to make DNA that was digested with *Bsh*NI, Southern blotted and probed with the 334-bp 3′ end of *Ds*, which contains no *Bsh*NI site. In families harbouring more than one tr*Ds*, TC_1F_2 seedlings were grown in the greenhouse and sprayed at the two-to three-leaf stage with kanamycin solution (Weide *et al.* 1989). A week later kanS plants could be distinguished and seven to eight of these were subjected to Southern analysis as above, to identify individuals carrying a single unlinked tr*Ds*.

DNA isolation and Southern hybridization analysis: The procedure for isolation of *Lycopersicon esculentum* DNA was essentially as described previously (CARROLL *et al.* 1995). For isolation of DNA from *L. pennellii* and *L. esculentum* × *L. pennellii* F_2 plants, the method of TAI and TANKSLEY (1991) was used. To perform Southern analyses, 15 μg of each DNA were digested with the appropriate restriction enzyme, then resolved in 1% agarose gels containing Tris-borate/EDTA buffer (SAMBROOK *et al.* 1989) and blotted onto Hybond-N membranes as described by the manufacturer (Amersham). Thereafter, hybridization was conducted essentially as described by CHURCH and GILBERT (1984) and the blots were autoradiographed using a Bio-Imaging Analyzer BAS 1000 (Fuji Photo Film, Japan).

Mapping of tr*Ds*s: Inverse PCR (IPCR), to amplify tomato DNA flanking tr*Ds*s, was carried out essentially as described by THOMAS *et al* (1994), using the restriction endonuclease and primer combinations shown in Table 2. Primer sequences are shown in Table 1. IPCR products obtained from nested primer reactions were purified by agarose gel electrophoresis, electroeluted from gel slices and labeled to high specific activity with α^{32}P dCTP (3000 Ci/mmol) using a random priming kit (Pharmacia LKB). Labeled IPCR fragments were hybridized to Southern blots of *L. esculentum* and *L. pennellii* DNA

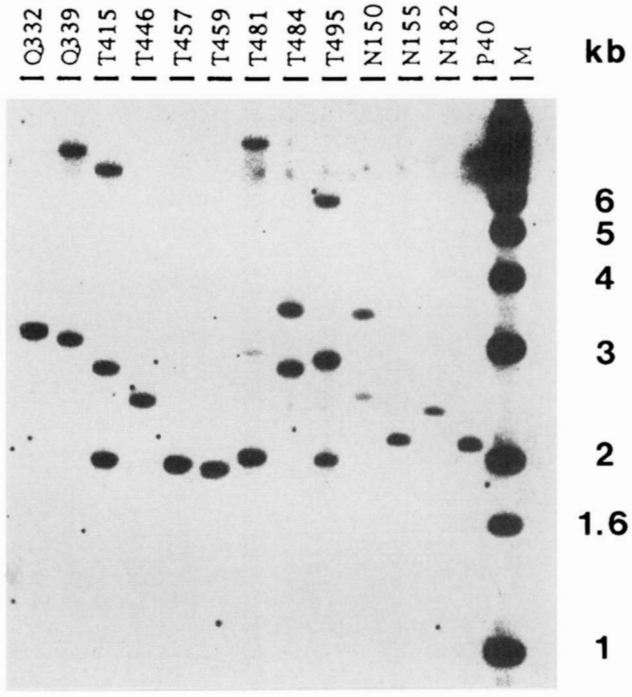

FIGURE 3.—Southern hybridization analysis of TC_1F_2 families carrying a transposed *Ds*. DNA from a pool of 15 kanamycin resistant plants was used to estimate the copy number of tr*Ds*s in each family. DNA was digested with *Bsh*NI, electrophoresed, blotted and probed with the 334-bp 3′ end of *Ds*, which contains no *Bsh*NI site. The figure shows Southern analysis of 13 different families. The origin of each family is listed in Table 3, except for P40, T415, T481 and T484, which were derived from TC_1 population 1. Lane M, marker DNA (1-kb ladder).

digested with *Eco*RI, *Eco*RV, *Dra*I or *Hin*dIII to identify restriction fragment length polymorphisms (RFLPs). The probes were then hybridized to blots of DNA from a mapping population of *L. esculentum* × *L. pennellii* F_2 plants (TANKSLEY *et al.* 1992) digested with the appropriate restriction enzyme. The position of each tr*Ds* element was then determined using the program MAPMAKER (LANDER *et al.* 1987) and segregation data for RFLPs covering the 12 tomato chromosomes (TANKSLEY *et al.* 1992).

RESULTS

Isolation of tr*Ds*s unlinked to the donor T-DNA: Of 103 families assayed, 45 harbored a tr*Ds* closely linked to the donor T-DNA, with 0 recombinants in 20 gametes (Figure 2). Fifteen families showed an intermediate recombination frequency, with between one and four kanS progeny carrying a *Ds*. Forty-three families were classified as carrying tr*Ds* elements unlinked to the T-DNA and were used for mapping of tr*Ds*s (Figure 1). Eighteen of these 43 families (42%) were found to carry two or more tr*Ds*s (examples are shown in Figure 3). Individuals carrying a single unlinked tr*Ds* were isolated from 11 of these 18 families so that unlinked tr*Ds*s were isolated from 36 families in total. Seven of the 18 families containing multiple tr*Ds*s were found to have one copy linked to the donor T-DNA. Taking these families into

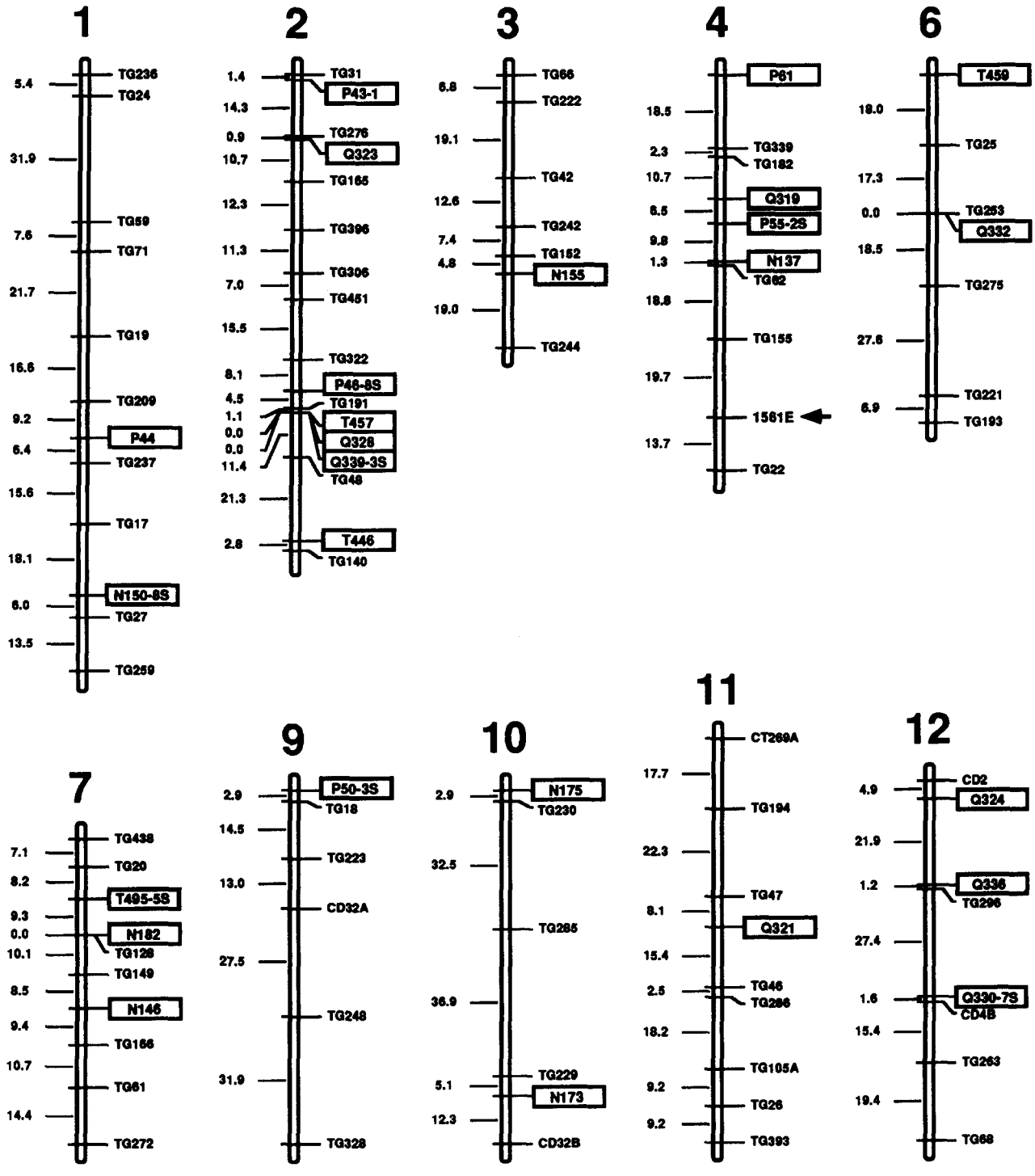

FIGURE 4.—RFLP map locations of tr*Ds*s unlinked to the donor locus 1561E on chromosome *4*. Only chromosomes carrying reinserted *Ds*s are included. Distances between succesive markers are given in centimorgans. Transposed *Ds* elements are shown in boxes and the map location of the donor T-DNA is indicated by an arrow.

account 67 (65%) of the families studied carried a *Ds* element transposed to sites linked to the donor T-DNA.

Mapping of tr*Ds*s: IPCR products were obtained for 33 out of the 36 tr*Ds*s recovered. Labeled IPCR fragments were hybridized to Southern blots of *L. esculentum* and *L. pennellii* DNA digested with *Eco*RI, *Eco*RV, *Dra*I or *Hin*dIII. The IPCR products from 19 tr*Ds*s hybridized to single copy sequences and were mapped. Seven hybridized to low (two to three) copy number sequences, and all of the copies mapped to the same location in each case. Seven could not be mapped because they hybridized to repeated sequences. The positions of the 26 mapped tr*Ds* elements on the tomato RFLP map are presented in Figure 4. Transpositions of

TABLE 3

Chromosomal locations of tranposed *Ds*s and the nature of the populations from which they were isolated

TC_1 population[a]	No. of F_1 plants used	Contribution to TC_1	No. of TC_1 seed	tr*Ds*	Chromosomal location
1	5	Bulked pollen	53,000	P44	*1*
				P43-1	*2*
				Q323	*2*
				P46-8S	*2*
				T457	*2*
				Q328	*2*
				Q339-3S	*2*
				T446	*2*
				Q319	*4*
				Q332	*6*
				P50-3S	*9*
				Q321	*11*
				Q324	*12*
				Q336	*12*
				Q330-7S	*12*
4	2	Bulked pollen	11,000	P61	*4*
5	4	Bulked pollen	56,000	P55-2S	*4*
				T459	*6*
				T495-5S	*7*
G248	1	Seed parent	<500	N150-8S	*1*
				N155	*3*
				N137	*4*
				N146	*7*
G249	1	Seed parent	<500	N175	*10*
				N173	*10*
G282	1	Seed parent	<500	N182	*7*

[a] As described in Tables 1 and 2 of CARROLL *et al.* (1995).

the *Ds* element from the 1561E locus on chromosome *4* occurred onto 10 out of 12 tomato chromosomes (chromosomes *5* and *8* did not receive a tr*Ds*). A statistical comparison of the observed and expected number of unlinked receptor sites based on the physical length of the chromosome (SHERMAN and STACK 1992) was performed for each chromosome (Table 3). The prediction for chromosome *4* takes into account the fact that much of it is linked to 1561E. The results suggest that the distribution of receptor sites for unlinked tr*Ds*s was not random because significantly more transpositions occurred onto chromosomes *2* ($P = 0.009$) and *4* ($P = 0.027$) than expected.

DISCUSSION

Transpositions of *Ac* tend to occur into genetically linked receptor sites in maize (DOONER and BELACHEW 1989), tobacco (JONES *et al.* 1990; DOONER *et al.* 1991) and Arabidopsis (KELLER *et al.* 1993). This tendency has also been reported previously for transposition of *Ds* elements originating from three different donor T-DNA loci in tomato, (CARROLL *et al.* 1995), but only 15 transposition events from each locus were examined. In the present study, 103 families harboring tr*Ds*s from one of these loci were analyzed, and 67 (*i.e.* 65%) of them were shown to carry a tr*Ds* linked to the T-DNA; 45 receptor sites were shown to cosegregate with the donor site. Other reports suggest that transpositions of *Ac* or *Ds* to sites linked to the donor T-DNA are much less frequent in tomato (OSBORNE *et al.* 1991; BELZILE and YODER 1992; HEALY *et al.* 1993). However, these authors studied somatic events and acknowledged that the real pattern could have been masked by secondary or even subsequent transpositions. They concluded that the distribution of unlinked tr*Ac*s in dispersed clusters was probably the result of primary transposition from the T-DNA to unlinked sites followed by linked transpositions around these new positions.

In maize, transpositions of *Ac* tend to occur into genetically unlinked receptor sites on the donor chromosome in preference to sites on nondonor chromosomes (DOONER *et al.* 1994). This tendency has been observed previously in tomato for *Ac* or *Ds* elements originating from three different donor T-DNA loci, (OSBORNE *et al.* 1991; HEALY *et al.* 1993), but only a limited number of transpositions were examined for each locus. In the present study, four independent transpositions of *Ds* (Table 4) out of 26 tr*Ds*s genetically unlinked to the donor locus, were shown to be on the donor chromosome (Figure 4), confirming this preference in tomato. However, reports for other donor T-DNAs indicate no

TABLE 4

Testing the chromosomal distribution of 26 transposed *Ds* elements, for departure from randomness

Chromosome number	Observed no. of tr*Ds*s	Expected no. of tr*Ds*s	Probability[a]
1	2	3.50	0.30
2	7	2.47	0.009**
3	1	2.68	0.24
4	4	1.16[b]	0.027*
5	0	1.98	0.13
6	2	2.14	0.64
7	3	2.17	0.37
8	0	2.17	0.10
9	1	1.97	0.40
10	2	1.95	0.59
11	1	1.90	0.42
12	3	1.84	0.28

Ds elements were genetically unlinked to the donor T-DNA locus on chromosome *4*.

[a] Asterisks denote statistically significant (**$P < 0.01$; *$P < 0.05$) differences between the observed and expected numbers of tr*Ds*s.

[b] The number of tr*Ds*s expected for chromosome *4* is about half that for the whole chromosome because only sites unlinked to the donor T-DNA could serve as receptor sites for unlinked tr*Ds*s.

apparent preference for transpositions of *Ac* or *Ds* to genetically unlinked sites on the donor chromosome (OSBORNE *et al.* 1991; BELZILE and YODER 1992; ROMMENS 1992; ROMMENS *et al.* 1992; KNAPP *et al.* 1994; THOMAS *et al.* 1994). These differences may be consistent with the observation by DOONER *et al.* (1991) that different T-DNA loci can give rise to different patterns of *Ac* transposition in tobacco.

This study has also examined the distribution of tr*Ds*s among the nondonor chromosomes. The remaining 22 tr*Ds*s are distributed over nine of the 11 nondonor chromosomes, but chromosome *2* appears to carry significantly more tr*Ds*s than would be expected on the basis of a random distribution (Table 3). There are several possible explanations for this apparent preference for transposition to chromosome *2*.

First, chromosome *2* may comprise a site for preferential insertion of both T-DNAs and *Ds* or *Ac* elements. This might be consistent with the fact that many genes map to this chromosome, even though one arm consists primarily of the nucleolus organizing region (TANKSLEY *et al.* 1992). Chromosome *2* may be particularly active, carrying a higher proportion of "open" chromatin that might predispose the DNA to receive transpositions or T-DNA insertions. Chromosome *2* does appear to be a frequent site for T-DNA insertions (THOMAS *et al.* 1994).

Second, the presence of seven tr*Ds*s on chromosome *2* near RFLP marker TG191 (Figure 4) could have occurred because of an early transposition to chromosome *2* that gave rise to a large sector from which subsequent transpositions to linked sites occurred. This is possible because all seven of the transpositions to chromosome *2* arose from TC_1 population 1 (Table 3), so they are not necessarily independent. It seems unlikely that they are all secondary transpositions for several reasons. A large sector should, on average, have contributed to no more than $^1/_5$ of the TC_1 progeny, since pollen was bulked from 5 F_1 plants (Table 3), yet $^7/_{15}$ of these plants carry tr*Ds*s on chromosome *2*. The chance of seven or more tr*Ds*s arising from the same F_1 plant is 0.018. Some of the other populations described by CARROLL *et al.* (1995) did show evidence for large sectors of early transposition, but these populations were avoided in this analysis and there was no evidence for a common *Ds* hybridizing band among any of the individuals examined from population 1 (Figure 3). The currently accepted model for *Ac* or *Ds* transposition in maize is for transposition from replicated to either unreplicated or replicated DNA (CHEN *et al.* 1992). Assuming the mechanism of transposition in tomato is similar to that in maize, many of the individuals with a tr*Ds* on chromosome *2* should, if they were in fact secondary transpositions, have shown a common band comprising the primary transposed *Ds*. No common band was observed among the individuals carrying a tr*Ds* on chromosome *2* (Figure 3). Based on these arguments, it would seem unlikely that all the transpositions on chromosome *2* could be secondary transpositions. A mixture of primary and secondary transpositions seems more plausible. The four tr*Ds*s clustered in a 5.6-cM interval around RFLP marker TG191 are the most likely to have arisen by secondary transposition. The chance of four or more tr*Ds*s arising from the same F_1 plant is 0.35.

Third, the excess of tr*Ds*s on chromosome *2* might be a consequence of close proximity between chromosome *2* and donor chromosome *4* at the time of transposition during interphase. The number of transpositions to genetically linked and unlinked sites on the same chromosome, strongly suggest that physical proximity increases the probability of a site receiving a transposition. In maize, a nonrandom distribution of unlinked tr*Ac*s was interpreted as possibly due to an ordering of the chromosomes in interphase nuclei (DOONER *et al.* 1994). These authors reported preferential *Ac* transpositions to chromosomes *5* and *7* and using the models of BENNETT (1984) for associations of chromosomes by arm length, predicted that in maize chromosomes *3* and *7* would be most frequently next to the donor *9S* and chromosome *5* to *9L*. A model of nuclear architecture that puts chromosomes with most similar arm lengths together indicates that tomato chromosome *4* is most likely to be near to tomato chromosomes *6* and *7*, not chromosome *2* (J. S. HESLOP-HARRISON, personal communication). However, it is not clear whether such packing models can be applied widely, particularly in species with smaller genome sizes and for which there

TABLE 5

Summary of the reported chromosomal distributions of transposed *Ac* or *Ds* elements genetically unlinked to their donor T-DNA loci

Chromosome												
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	Ref[a]
—	1	2	—	1	2	1	1	—	1	*	2	B
1	1	—	—	—	1	—	—	*	—	1	—	H
1	1	—	—	—	—	—	*	—	—	—	—	H
1	1	1	—	1	—	*	—	—	—	1	—	K
—	1	1	1	—	—	*	—	—	1	—	1	K
2	*	—	—	1	1	—	—	—	—	1	—	O
—	1	1	—	—	*	1 or 2	—	—	—	—	—	O
1	—	1	—	—	*	—	—	—	1	—	1	R
*[b]	1	—	—	—	—	—	—	—	—	—	—	R
*[b]	—	—	—	—	1	—	—	—	—	—	—	R
1	—	—	—	—	—	*	—	—	—	—	—	T
1	—	—	—	—	—	—	*	—	—	—	1	T
2	3 or 4	1	*	—	2	3	—	1	2	1	3	This work
10	10 or 11	7	1	3	7	5 or 6	1	1	5	4	8	Total

Donor chromosomes are indicated by asterisks. The number of single transpositions or clusters of transpositions presumed to have arisen by secondary transposition (*i.e.*, nonindependent transpositions clustered within an interval of 15 cM) are indicated.

[a] References: B, BELZILE and YODER (1992); H, HEALY *et al.* (1993); K, KNAPP *et al.* (1994); O, OSBORNE *et al.* (1991); R, ROMMENS (1992) and ROMMENS *et al.* (1992); T, THOMAS *et al.* (1994).

[b] The donor site was a site of primary transposition rather than a T-DNA.

is little independent evidence regarding the chromosomal organization of interphase nuclei. The spatial organization and relative positions of decondensed chromosomes within interphase nuclei can now be studied using *in situ* hybridization with chromosome painting probes and low copy probes in both animals (CREMER *et al.* 1993) and plants (HESLOP-HARRISON *et al.* 1993). It will be interesting to use this approach to study interphase chromosomal associations in tomato to establish whether there is a correlation with unlinked tr*Ds* receptor site distributions.

It will also be valuable to study the transposition patterns of more unlinked tr*Ds*s from additional T-DNA loci in tomato. A number of such studies already exist, but these are limited in extent and by lack of independence between transpositions. Nevertheless, a clear trend for recovery of dispersed clusters of transpositions, consistent with linked secondary transpositions arising from sites of primary transposition, has emerged. The distribution of presumptive primary transpositions among nondonor chromosomes is shown in Table 5 for each of these studies and for the present study, based on the assumption that each cluster observed represents a single primary transposition. In total, these studies suggest a preference for unlinked transpositions to some nondonor chromosomes *e.g.* chromosomes *1*, *2*, *6*, *7* and *12*, but not for others *e.g.*, chromosomes *4*, *8* and *9*, irrespective of donor chromosome. This raises the possibility that there are absolute site preferences for transposition among nondonor chromosomes in tomato and that transposition beyond the donor chromosome may depend more on chromosome composition than proximity. Any such preferences could be important for transposon tagging. Clearly, many independent nondonor chromosome transpositions need to be analysed for each of the 12 possible donor chromosomes of tomato to test this possibility rigorously.

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