Further evidence that *Td* is located on pea linkage group III

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Lamprecht (3, 4) reported linkage between *Td,* a genetic locus that conditions leaflet dentation in pea, and three linkage group IV markers, *fa, z* and *n.* Marx (6), however, found no evidence of linkage between *Td* and either *z* or *was* (another group IV marker closely associated with *z*) upon performing an F_2 analysis of a different cross. Instead, he demonstrated linkage between *Td* and *b,* a linkage group III marker, noting that a loose association between *Td* and *b* had been indicated by Lamprecht's original data as well. More recently, Grajal-Martin and Muehlbauer (2) used two independent crosses to show that *Td* is linked with *Lap-1,* a linkage group III isozyme marker, establishing a composite *Td—Lap-1* recombination value of approximately 23%. Given that *Lap-1* and *b* are closely linked (9), this figure is similar to the approximately 15% (17.1% and 14.2%) recombination value Marx (6) determined for *Td* and *b.*

In the present study, I have obtained additional evidence supporting the assignment of *Td* to linkage group III. An evaluation of the F_2 progeny of two independent crosses, A73-91 x A778-26-6 and A1078-234 x 82-14n, reveals that *Td* is linked with *st* (the linkage group III gene for reduced stipules) in both crosses with recombination values of 20.2% and 22.0%, respectively (see Table 1), using the LINKAGE-1 computer program (8). The plant materials, all obtained originally from G.A. Marx and N.F. Weeden, have been characterized as either *Td* (A778-26-6 and 82-14n) or *Td* (A73-91 and A1078-234); A778-26-6 was one of the parental lines used in the Grajal-Martin and Muehlbauer study. Both crosses involved standard marker lines (A73-91 and A1078-234) and produced fully fertile hybrids.

The A1078-234 x 82-14n cross also segregated for three other linkage group III markers, *Pea2* (an RFLP marker corresponding to a light-regulated gene), *Vc-5* (an RFLP marker corresponding to a member of the vicilin multigene family) and *Lap-1* (7). Pairwise comparisons among these loci reveal that *Vc-5* and *Lap-1* are both linked with *Td* as well, displaying recombination values of 27.6% and 22.2%, respectively. *Pea2* showed linkage with *Vc-5* (22.6%) and a very loose, perhaps questionable, linkage with *st* (44.9%). The 22.2% recombination value for *Td* and *Lap-1* found in this cross is very close to the 23% figure reported by Grajal-Martin and Muehlbauer (2).

When 46 F_2 progeny from the A1078-234 x 82-14n cross were analysed using the MAPMAKER multipoint linkage package (5), the integrated map shown in Fig. 1 was generated based in part on the LOD scores summarised in Table 2. The LOD scores reveal five particularly strong linkage relationships which characterise Fig. 1 and which are generally consistent with the broad pairwise relationships established in Table 1. The data clearly indicate that *Td* is closely associated with *Lap-1* and *st.* They further link *st* with *Vc-5,* and *Vc-5* strongly with *Pea2.* The most reasonable conclusion to draw from these findings corroborates the suggestion by Marx (6) and Grajal-Martin and Muehlbauer (2) that *Td* is located on pea linkage group III.

Table 1.   Joint segregation analyses for linkage group III genetic marker loci.

Loci	Number of F$_2$ progeny in each genotypic class [a]									n	χ^2	P	Recomb. value (%) [f]
	11,11	11,12	11,22	12,11	12,12	12,22	22,11	22,12	22,22				
A1078-234 x 82-14n [e]													
Pea2, Vc-5 [b]	14	0	0	2	16	8	0	11	1	52	47.1	<<0.001	22.6±4.8
Pea2, st [c]	12		2	17		10	12		0	53	7.3	<0.05	44.9+8.3
Vc-5, st [c]	21		4	37		4	9		14	89	22.1	<<0.001	25.6±5.2
Vc-5, Td [c]	22		3	37		4	11		11	88	15.8	<0.001	27.6±5.5
Vc-5, Lap-1 [b]	5	11	8	17	24	0	1	8	14	88	32.6	<<0.001	38.2±4.9
st, Td [d]	62		6				11		12	91	20.4	<<0.001	22.0±9.9
Lap-1, st [c]	22		2	38		8	10		14	94	18.9	<<0.001	24.8+5.0
Lap-1, Td [c]	23		0	37		6	12		12	90	20.2	<<0.001	22.2±4.9
A73-91 x A778-26-6 [e]													
st, Td [d]	30		9				2		12	53	16.9	<<0.001	20.2±13.1

Genotypic designations: 11 = 82-14n or A778-26-6 homozygotes, 22 = A1078-234 or
A73-91 homozygotes, and 12 = heterozygotes (for *Vc-5,* 1 and 2 represent haplotypes;
for *st* and *Td,* (masked) heterozygotes are scored as 11).
[b] 1:2:1:2:4:2:1:2:1 expected ratio.
[c] 3:1:6:2:3:1 expected ratio.
[d] 9:3:3:1 expected ratio.
[e] Reciprocal cross.
[f] Calculated using the LINKAGE-1 computer program (8); pairwise comparisons with P >
   0.05 or a recombination value = 50% are not listed

Table 2. Pairwise LOD scores for linkage group III genetic marker loci calculated by applying the MAPMAKER computer program (5) to 46 F_2 progeny from the reciprocal cross A1078-234 x 82-14n.

Loci	LOD scores
Pea2, Vc-5	4.28*
Pea2, st	0.02
Pea2, Td	0.00
Pea2, Lap-1	0.00
Vc-5, st	2.15*
Vc-5, Td	0.56
Vc-5, Lap-1	0.60
st, Td	2.75*
st, Lap-1	3.01*
Td, Lap-1	2.83*

* Log of the odds ratios (LOD scores) between 2.00 and 5.00 indicate that the likelihood of linkage by a stated recombinant fraction is between 100 and 100,000 times greater than the likelihood of no linkage, respectively.
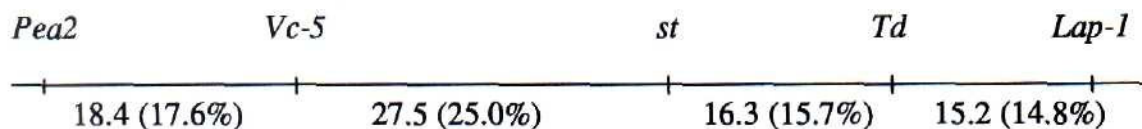

Fig. 1. Linkage associations for five linkage group III genetic loci. Recombination values (in parentheses) were determined using the MAPMAKER multipoint maximum likelihood algorithms (5). Map distances in cM were calculated using the Kosambi mapping function.

Grajal-Martin and Muehlbauer (2) offer two interesting explanations for the discrepancy between the original findings of Lamprecht (3, 4) and the findings of subsequent investigators. They reason that either 1) there are two *Td* genes, one located on linkage group III and the other located on linkage group IV, or 2) genomic rearrangements have resulted in a single *Td* gene being shifted between the two linkage groups of different pea lines. The first explanation would seem to be the less likely of the two given the precise characterisation of the *Td* phenotype by Marx (2, 6, and pers. comm.). Furthermore, the segregation and assortment ratios of a duplicate gene system which would accommodate all the data presented in these studies may require more elaborate and restrictive conditions than parsimony allows. By contrast, genomic rearrangements in pea appear to be fairly common, as evidenced most recently by the publication of a pea RFLP map (1) in which genetic markers were found to exist in several different linkage arrangements depending on the cross selected. Given the convincing presentations of all the *Td* data sets described in these studies, the latter explanation may provide the most appropriate hypothesis.

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