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Linkage Maps in Pea

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Manuscript received May 20, 1991
Accepted for publication November 25, 1991

ABSTRACT

We have analyzed segregation patterns of markers among the late generation progeny of several crosses of pea. From the patterns of association of these markers we have deduced linkage orders. Salient features of these linkages are discussed, as is the relationship between the data presented here and previously published genetic and cytogenetic data.

THE construction of linkage maps of whole genomes has been a preoccupation in plant genetics laboratories since Punnett embarked on his linkage analysis of sweet pea in 1908 (PUNNET 1923, 1925, 1932). With the advent of molecular techniques, first of isozyme analysis and later of restriction fragment length polymorphism (RFLP) analysis (BOTSTEIN *et al.* 1980), such maps have become more common, and are now available for *Arabidopsis thaliana* (KOORNEEF *et al.* 1983; CHANG *et al.* 1988), lettuce (LANDRY *et al.* 1987), maize (HELENTJARIS 1987), potato (BONIERBALE, PLAISTED and TANKSLEY 1988), tomato (BERNATZKY and TANKSLEY 1986) and rice (MCCOUCH *et al.* 1988). Linkage maps have previously been presented for pea based on morphological, physiological and pigmentation characters (BLIXT 1974) on isozyme data (WEEDEN and MARX 1987) and the combination of morphological, isozyme and DNA markers (WEEDEN and WOLKO 1990).

We have undertaken a linkage analysis of several recombinant inbred populations of pea, and here present our results. For one cross we derive a linkage map comprising 151 markers, spanning approximately 1700 cM. The relationship between the different crosses is discussed, as is the relationship between our map and previously presented linkage maps of pea.

MATERIALS AND METHODS

Plant material: We have used lines with the following John Innes Pisum germplasm accession numbers JI 15 (=WBH 1458), JI 61 (=WBH 761), JI 117 (=Glano 0632 = WBH 19), JI 281 (*Pisum sativum* from Ethiopia), JI 399 (=cv Cennia), JI 813 (carries *yp*, from cv Vinco; MURFET 1967), JI 1156 (Minnesota Early Sweet), JI 1194 (MISOG-1 conventional) and JI 1201 (MISOG-1 *af,st,tl*). WBH accession numbers refer to the Pisum germplasm collection of the Weibullsholm Plant Breeding Institute, Sweden, MISOG-1 are a series of lines developed by the late G. A. MARX at Cornell (see MARX 1977, 1987a). The pedigree of JI 61 is relevant to later discussion: JI 61 = WBH 761, WBH 761 is derived from the cross L118 (Extra rapid) × L206 (Olympia) (M. AMBROSE, personal communication); L83 (=T3S-5L, LAMM 1951) is equivalent to JI 145 (Extra rapid) and was used by SIMPSON *et al.* (1990). Recombinant inbred lines derived from crossing JI 15 × JI 61, JI 15 × JI 1194, JI 281 × JI 399, and JI 813 × JI 1201 have been used in these analyses. The population sizes of these recombinant inbred populations are; 38, 50, 71, and 48, respectively. In addition, F_2 populations derived from the crosses JI 181 × JI 430 and JI 117 × JI 1156 are discussed; the data derived from the former cross have been presented previously (ELLIS *et al.* 1984, 1986; DOMONEY, ELLIS and DAVIES 1986). The cDNA clones corresponding to storage protein genes, lipoxygenase and gr-16 were derived from cv Birte. The rDNA clone cDB 1.07 was derived from cv Feltham First, and the repeated sequence probe *PDR1* was derived from cv Dark Skinned Perfection. Markers scored in various crosses are listed in Table 1.

Nucleic acid manipulation: Conditions for DNA preparation, restriction enzyme digestion, gel electrophoresis, Southern transfer, hybridization and autoradiography have been presented elsewhere (ELLIS *et al.* 1984), but sodium diethyldithiocarbamate is no longer used as a consequence of the release of carbon disulfide from aqueous solutions of this substance. Except for the case of the simultaneous analysis of the segregation of multiple loci (LEE *et al.* 1990), the details of the procedures we have employed are not critical for the results we have obtained. We have avoided the use of nylon membranes since discovering one batch of Hybond-N which did not bind DNA after any combination of baking or UV crosslinking; similar, but less-well characterized difficulties with Genescreen plus membranes convinced us that nitrocellulose membranes were more reliable, and in the long term, more efficient because we considered it unlikely that we would run out of DNA corresponding to a given genotype when using recombinant inbreds.

The bulk of the hybridization probes we have used were cDNA clones derived from a petal cDNA library, which is described elsewhere (HARKER, ELLIS and COEN 1990). Very few of the randomly selected cDNAs from this library have proved to be repetitions of previously chosen clones.

Data collection: The markers scored (Table 1) corresponded to pigmentation or morphological characters, or were RFLPs, with the exception of P7 and *Lg*-1 in the cross JI 281 × JI 399 which were scored from Coomassie blue-stained sodium dodecyl sulfate (SDS) gels of total seed protein. For RFLP analysis we have used mostly *Eco*RI

TABLE 1

Markers and probes scored in various crosses

Classical markers, rRNA and storage protein genes:

281 × 399: *a, F, i, le, rb*, cDB 1.07, 5S/1, 5S/2, 5S/3, *Cvc, Lg-1, Lg-J &/2, Vc-2, Vc-3, Vc-5*, pCD 72

15 × 1194: *a, gp, le, Pl, r*, cDB 1.07, *Lg-J, Lg-32, Vc-2, Vc-5*

15 × 61: *a, bt, F, gp, le, Pl, r, v*, cDB 1.07/1 &/2, 5S*Mbo*I, 5S*Taq*I, *Cvc, Lg-J, Lg-32, Vc-2, Vc-3, Vc-5*

1201 × 813: *a, af, st, tl*, cDB 1.07, 5S TaqI, *Vc-5*

Miscellaneous:

281 × 399: P7, pAt-T4/1* /2* /3* /4* /5 /6 /7* /8, pCHS 2, pPE 1036a, pPE, 923b/1 to /3, pCD 7/3kb & 7/5kb & 7/6kb, GST 10/1 to /4, pPSR 546/1 to /4, gr-16/1 to /9 (/4*), pST LL1, pST H, pST P, pST T, D15B/1, D15B/2, 0.9 MI/1 & /2, DR 1 to DR 26 (5** 15** 21** 22* 23* 24* 25**)

15 × 1194: pPSR 546/1 to /8, D15B, MI, cosmid 10a, DRP 1 to 15

15 × 61: pCD 7/3kb & 7/5kb, GS, pPSR 546, D15B, MI, cosmid 8g, cosmid 10a

1201 × 813: pPE 1036a, pPE 923b/1 & /2

cDNA clones:

281 × 399: 1, 2a, 23, 24, 34, 38a, 39, 40/1 to /8, 41, 44, 48a, 53, 56/1 & /2, 67a, 75, 76, 119, 125/1 /02 to /04 /08 /11 /20 & /180, 133, 136, 137, 148/1 to /6, 150/1& /2, 164, 186, 187, 189a, 194/1, 194/2, 200, 204/1 to /4, 206, 228, 231, 243, 260, 267, 280, 286, 289 & /2, 324, 331, 373

15 × 1194: 1, 2a, 23, 24, 40, 41, 53, 56, 99, 101a, 136, 137, 148/1 & /2, 150, 155a, 169, 188, 194, 204, 213, 231, 267, 271, 277, 278, 280, 286, 289

15 × 61: 1, 40, 41, 53, 56, 101a, 102, 118, 133 136, 137, 148/1 to /5, 164, 188, 194, 213, 231, 267, 280, 286, 289

1201 × 813: 44, 53, 200

Characters have been scored at the F_8 generation, and a few underlined at the F_6 only. For *r* the data for the crosses 15 × 61 and 15 × 1194 have been presented elsewhere (BHATTACHARYYA *et al.* 1990). Probes other than *PDR* (DR & DRP, LEE *et al.* 1990) which detect multiple loci are denoted "/#", where # is a number, although this need not correspond between crosses. Clone subfragments are indicated by a letter. Gene and probe symbols are as follows: pAt-T4 is an Arabidopsis telomere clone (gift of F. AUSUBEL) (RICHARDS and AUSUBEL 1988); CHS (cDNA 1, pCHS 2) is a chalcone synthase probe (HARKER, ELLIS and COEN 1990). The 5S rRNA genes are described in ELLIS *et al.* (1988); cDB 107 is a pea rDNA cosmid clone (gift of J. GATEHOUSE); GS(T) is glutamine synthase (a gift of B. MIFLIN); pCD 7 is an HSP70 related sequence (DOMONEY *et al.* 1991); pPE 923 and pPE 1036 are lipoxygenase sequences (EALING and CASEY 1988; 1989; DOMONEY *et al.* 1990); pPSR 546 is an ADH cDNA (gift of J. FINNEGAN) (LLEWELLYN *et al.* 1987); Cvc is a convicilin locus (ELLIS *et al.* 1986); *Lg-1* is a legumin locus (DAVIES 1980; MATTA and GATEHOUSE 1982; DOMONEY, ELLIS and DAVIES 1986); *Lg-J* is the legumin locus containing *leg J* [DOMONEY, ELLIS and DAVIES (1986), GATEHOUSE *et al.* (1988); it does not correspond to *Lg2* of MATTA and GATEHOUSE (1982)]. *Lg-J/2* is detected by a second probe from the *legJ* genomic clone. *Lg-32* is identified by pCD 32 [it is not *Lg-3* of MATTA and GATEHOUSE (1982)]. The pST probes were the gift of S. TURNER, and the gr-16 probe was the gift of P. MULLINEAUX. *Vc-2, Vc-3* and *Vc-5* are vicilin loci defined in ELLIS *et al.* (1986), *Vc-5* seems to be mistaken for *Vc-2* in WEEDEN and WOLKO (1990). The classical markers are all described in BLIXT (1972, 1974), and see WEEDEN and WOLKO (1990). Markers not assigned to a group *, ** segregation ratio suggests two markers.

digests; these have been done in bulk (*ca.* 200 μg DNA) and checked for completeness with the rDNA probe cDB 1.07; *Taq*I and *Mbo*I digests were used to score some 5S rRNA gene variants. These digests were then available for many gels and hybridization probes. Nitrocellulose filters have been reused by leaving the bound counts to decay before reprobing.

Data analysis: Alleles were scored as "+," "−," or "0;" "+" and "−" correspond to one of the parental types, assigned by a convention within a given cross, "0" corresponds to missing data, a failure to score, or the heterozygous condition. The data presented here are mainly from the F_8 generation (Table 1) where heterozygosity is minimal, but some of the markers were scored in the F_6 generation; several have been scored in both generations. It should be noted that there are a small number of cases of disagreement between F_6 and F_8 data which are a consequence of heterozygosity at either the F_6 or F_5; the F_6 plants from which DNA was prepared were not ancestral to the F_8 (*i.e.*, they were killed), but the F_5 plant was the common parent for both lineages. This difference may increase slightly the estimated map distance between markers scored in the different generations and consequently reduce the likelihood that linkage is detected. The few remaining heterozygotes are unlikely to occur preferentially in one combination of flanking alleles, so ignoring heterozygotes is unlikely to generate a systematic error in the estimation of linkage distance.

All the data have been analysed by simple BASIC programs run on a VAX computer; copies of these programs are available on request. The data are condensed to a matrix of "+"s, "−"s and zeros, with rows representing markers and columns representing the particular recombinant inbred of a given cross. A list of marker names refers to a list of gel numbers, blots and probings which is the primary data set. This matrix of scores can be represented by $E_{m,r}$, with E being an entry, m being a marker and r being a recombinant inbred line. A test for linkage between the markers 1 and 2 counts the number of instances where $E_{1,r}$ and $E_{2,r}$ are both "+," both "−" and the two instances where they are different; if either is represented by "0," then no count is made. These four values are considered as a 2 × 2 contingency table, and a chi-square test performed. If the chi-square value is above 8 (approximately $P < 0.05$ by chance alone, for $n = 3$), then it is recorded for the given pair together with the fraction of lines where the parental alleles have been recombined. If this fraction is greater than 0.5 this is noted; if the fraction is below 0.5, the recombination frequency for a single meiosis is calculated according to HALDANE and WADDINGTON (1931), and a map distance derived assuming HALDANE's (1919) mapping function. The error in this recombination frequency is $\sqrt{pq/n}$, and from this the corresponding limits to the map distance calculated.

A LOD score (EDWARDS 1972) was also calculated; chi-square values and LOD scores were well correlated. For the data set derived from JI 281 × JI 399 a chi-square value of 8 corresponds roughly to a LOD score of 1.5, while a LOD score of 3 represents a chi square of almost 14.

Multipoint linkage analysis has been carried out as suggested by LANDER and GREEN (1987), the scored data for groups of up to ten markers which are candidates for a linkage group being abstracted from the data set. From these abstracted data, the number of recombinant lines, in all possible pairwise combinations, is calculated and the orders of markers which give the 20 lowest values for the sum of the corresponding interval distances are recorded together with the associated sum. The order with the lowest sum is the simplest linkage hypothesis for that group; however, this does not imply that the markers are a genuine linkage group. We have used several criteria to deduce whether or not a particular set of markers corresponds to a linkage group. First, all the intervals in the "most likely" order should correspond to two point tests detected as linked markers. Second the position of a given marker should be conserved when different sets of markers are chosen along a linkage group. An additional criterion which we have used where possible is that a given group of markers exhibit the same linkage in other crosses; however this criterion is not always valid because of chromosomal rearrangements, or the presence of a given sequence at multiple loci.

RESULTS

A linkage map derived from the cross JI 281 × JI 399: In Figure 1A we present a linkage map which summarises the data obtained from the analysis of the cross JI 281 × JI 399, and Figure 1B shows all pairwise associations of markers detected on the basis of a $\chi^2 >$ 11. Several of these associations involve different linkage groups, which is as expected since about 1% of all pairwise associations found should be a consequence of chance alone (*q.v.*), which is in close agreement with the frequency of postulated spurious linkages shown in Figure 1B.

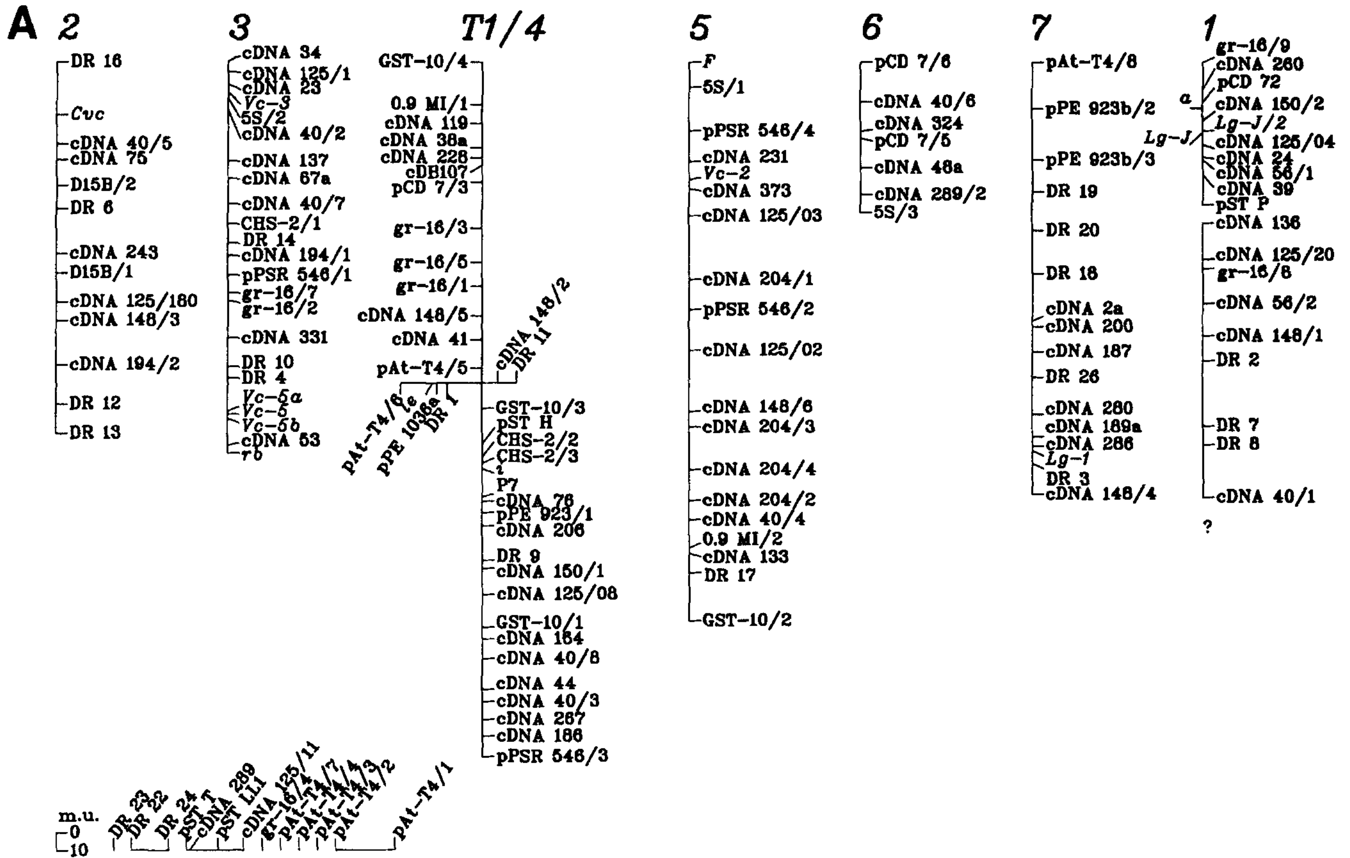
The recombination frequencies observed within the linkage groups shown in Figure 1 are presented in Table 2 (2.1–2.8). The linkage groups have been numbered *1* to *7* relating a drawn linkage group to the appropriate part of Table 2. The correspondence (or lack of it) between these designated linkage groups, and those presented by other authors is discussed below.

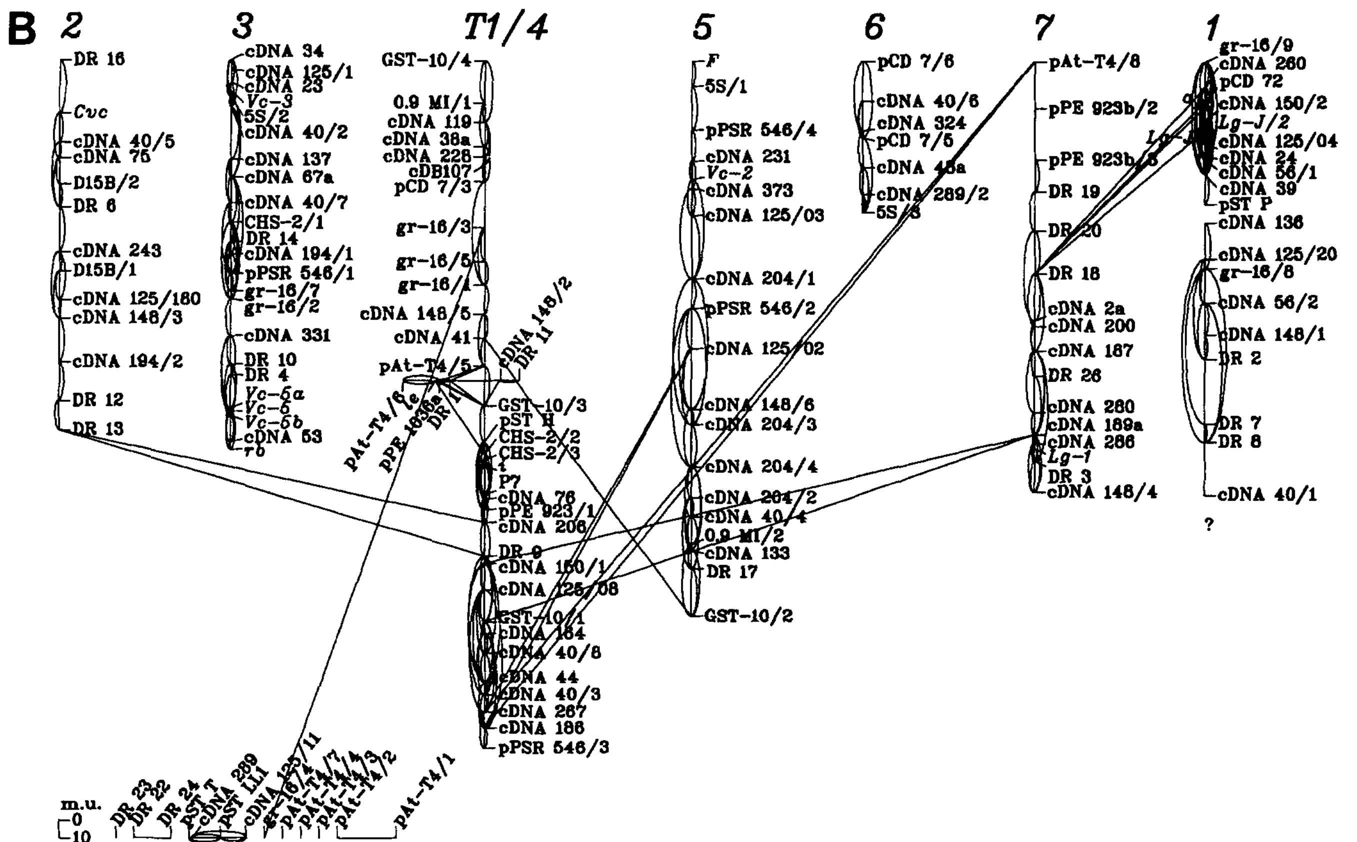
Table 2 gives numerical data corresponding to the linkage map; however, some of the associations we have detected are not given in Table 2; those for a χ^2 > 11 are shown in Figure 1B. Some other difficulties with the linkage orders we have presented can be seen in Figure 2. For example in the second group (Table 2.2) we have given the order: . . . *DR 6–cDNA 243–D15B/1–cDNA 125/10–cDNA 148/3–cDNA 194/2* . . . yet *cDNA 243* maps closer to *cDNA 148/3* than *D15B/1*; the reason for choosing this order is that *DR 6* is not detectably linked to *D15B/1*, but does show linkage to *cDNA 243*. Furthermore *cDNA 148/3* shows linkage to *cDNA 194/2* while *cDNA 125/10* and *D15B/1* do not. Other such difficulties can be found in the tables, with varying degrees of complexity or severity. These difficulties are unquestionably deficiencies in the data where we have attempted to produce a linear order in our map given the restrictions outlined in methods. In other words, a map with a greater frequency of (double) recombinants may be more accurate; additional markers in these areas will doubtless clarify the issue.

Linkage group *4* of Figure 1 (marked *T1/4*, see later) presents at least two problems. The *DR 9–pPSR 546/3* group could be associated with either group *2*, or group *4* as drawn in Figure 1. The data are ambiguous, but in principle we can expect to resolve this problem with additional data. The pattern of linkage in the region of *GST-10/3* is more problematic; the difficulty of attempting to establish a unique linear order can be seen graphically in Figure 2 which compares linear and nonlinear maps of this region. The cruciform map suggests there may be a reciprocal translocation involving markers from this region in the cross JI 281 × JI 399. Presumably one standard chromosome is represented by a group containing the marker detected by cDB 1.07 and *Le* (see below) (POLANS, WEEDEN and THOMPSON 1986; WEEDEN and WOLKO 1990), while the other represents a second chromosome, probably involving linkage group *I*. Informative markers on this second chromosome are *i* [assigned to linkage group *I* by BLIXT (1974) and WEEDEN and WOLKO (1990)], and *pPE923/1*, which shows linkage to *af* in our cross JI 1201 × JI 813; *i* and *af* are linked (MARX 1969; SNOAD 1971; KIELPINSKI 1982; WEEDEN and WOLKO 1990). Our data do not easily support linkage between the *i–af* segment and any of our group *1* markers which show linkage to *a* (Figure 1A). A more complex map could be drawn tying the linkage group around *a* to *i* and *pPE923/1*, but this would also involve our linkage group *7* (see Figure 1B). If the cruciform map is accepted, then our linkage map represents nine major linkage groups, to be distributed among the seven chromosomes of the pea complement.

The map presented in Figure 1 is, nevertheless, the best current representation of our linkage data. A few markers (indicated * in Table 1) which we have scored have not been assigned a map position because they have either a deficiency in their data set, or an aberrant segregation ratio. An aberrant segregation ratio can arise if a restriction fragment of a given size is present in one parental line at two loci which segregate independently. Some telomere related markers also remain unassigned to linkage groups; these are discussed later.

Some probes detect multiple markers in linear sequence within a linkage group: In our linkage group *3* of the cross JI 281 × JI 399, three closely linked markers are resolved by the vicilin gene probe, pJC 2-7, which identifies the *Vc-5* locus (LEE *et al.* 1988). We have designated these *Vc-5*, *Vc-5a* and *Vc-*


A
2
DR 16
Cvc
cDNA 40/5
cDNA 75
D15B/2
DR 6
cDNA 243
D15B/1
cDNA 125/180
cDNA 148/3
cDNA 194/2
DR 12
DR 13
3
cDNA 34
cDNA 125/1
cDNA 23
Vc-3
5S/2
cDNA 40/2
cDNA 137
cDNA 67a
cDNA 40/7
CHS-2/1
DR 14
cDNA 194/1
pPSR 546/1
gr-16/7
gr-16/2
cDNA 331
DR 10
DR 4
Vc-5a
Vc-5
Vc-5b
cDNA 53
rb
T1/4
GST-10/4
0.9 MI/1
cDNA 119
cDNA 38a
cDNA 228
cDB107
pCD 7/3
gr-16/3
gr-16/5
gr-16/1
cDNA 148/5
cDNA 41
pAt-T4/5
cDNA 148/2
DR 11
pAt-T4/6
le
pPE 1036a
DR 1
GST-10/3
pST H
CHS-2/2
CHS-2/3
i
P7
cDNA 76
pPE 923/1
cDNA 206
DR 9
cDNA 150/1
cDNA 125/08
GST-10/1
cDNA 184
cDNA 40/8
cDNA 44
cDNA 40/3
cDNA 287
cDNA 186
pPSR 546/3
5
F
5S/1
pPSR 546/4
cDNA 231
Vc-2
cDNA 373
cDNA 125/03
cDNA 204/1
pPSR 546/2
cDNA 125/02
cDNA 148/6
cDNA 204/3
cDNA 204/4
cDNA 204/2
cDNA 40/4
0.9 MI/2
cDNA 133
DR 17
GST-10/2
6
pCD 7/6
cDNA 40/6
cDNA 324
pCD 7/5
cDNA 48a
cDNA 289/2
5S/3
7
pAt-T4/8
pPE 923b/2
pPE 923b/3
DR 19
DR 20
DR 18
cDNA 2a
cDNA 200
cDNA 187
DR 26
cDNA 280
cDNA 189a
cDNA 286
Lg-1
DR 3
cDNA 148/4
1
gr-16/9
cDNA 260
pCD 72
a
cDNA 150/2
Lg-J/2
Lg-J
cDNA 125/04
cDNA 24
cDNA 56/1
cDNA 39
pST P
cDNA 136
cDNA 125/20
gr-16/8
cDNA 56/2
cDNA 148/1
DR 2
DR 7
DR 8
cDNA 40/1
?
m.u.
0
10
DR 23
DR 22
DR 24
pST T
cDNA 289
pST LI1
cDNA 125/11
gr-16/4
pAt-T4/7
pAt-T4/4
pAt-T4/3
pAt-T4/2
pAt-T4/1


B
2
DR 16
Cvc
cDNA 40/5
cDNA 75
D15B/2
DR 6
cDNA 243
D15B/1
cDNA 125/180
cDNA 148/3
cDNA 194/2
DR 12
DR 13
3
cDNA 34
cDNA 125/1
cDNA 23
Vc-3
5S/2
cDNA 40/2
cDNA 137
cDNA 67a
cDNA 40/7
CHS-2/1
DR 14
cDNA 194/1
pPSR 546/1
gr-16/7
gr-16/2
cDNA 331
DR 10
DR 4
Vc-5a
Vc-5
Vc-5b
cDNA 53
rb
T1/4
GST-10/4
0.9 MI/1
cDNA 119
cDNA 38a
cDNA 228
cDB107
pCD 7/3
gr-16/3
gr-16/5
gr-16/1
cDNA 148/5
cDNA 41
pAt-T4/5
cDNA 148/2
DR 11
pAt-T4/6
le
pPE 1036a
DR 1
GST-10/3
pST H
CHS-2/2
CHS-2/3
i
P7
cDNA 76
pPE 923/1
cDNA 206
DR 9
cDNA 150/1
cDNA 125/08
GST-10/1
cDNA 184
cDNA 40/8
cDNA 44
cDNA 40/3
cDNA 287
cDNA 186
pPSR 546/3
5
F
5S/1
pPSR 546/4
cDNA 231
Vc-2
cDNA 373
cDNA 125/03
cDNA 204/1
pPSR 546/2
cDNA 125/02
cDNA 148/6
cDNA 204/3
cDNA 204/4
cDNA 204/2
cDNA 40/4
0.9 MI/2
cDNA 133
DR 17
GST-10/2
6
pCD 7/6
cDNA 40/6
cDNA 324
pCD 7/5
cDNA 48a
cDNA 289/2
5S/3
7
pAt-T4/8
pPE 923b/2
pPE 923b/3
DR 19
DR 20
DR 18
cDNA 2a
cDNA 200
cDNA 187
DR 26
cDNA 280
cDNA 189a
cDNA 286
Lg-1
DR 3
cDNA 148/4
1
gr-16/9
cDNA 260
pCD 72
cDNA 150/2
Lg-J/2
cDNA 125/04
cDNA 24
cDNA 56/1
cDNA 39
pST P
cDNA 136
cDNA 125/20
gr-16/8
cDNA 56/2
cDNA 148/1
DR 2
DR 7
DR 8
cDNA 40/1
?
m.u.
0
10
DR 23
DR 22
DR 24
pST T
cDNA 289
pST LI1
cDNA 125/11
gr-16/4
pAt-T4/7
pAt-T4/4
pAt-T4/3
pAt-T4/2
pAt-T4/1

5B and the relevant parental and recombinant types are shown in Figure 3. It seems likely that these arise as a consequence of crossing over between different members of this tightly linked gene cluster; confirmation of this suggestion must, however, await the construction of a physical map in this region.

There are other cases of an array of related markers which probably arise in a different manner. One such array occurs in the cross JI 15 × JI 1194 where three ADH related sequences span 16 map units adjacent to the single copy chalcone synthase gene detected by cDNA 1 at high stringency (HARKER, ELLIS and COEN 1990). This sequence is found at a locus defined by the RFLP designated CHS 2/1 on the map derived from the cross JI 281 × JI 399 (linkage group *3*). These three contiguous loci containing ADH related sequences in the cross JI 15 × JI 1194 should perhaps be represented by a single locus: there is a single ADH locus adjacent to the corresponding chalcone synthase locus in the maps derived from both JI 281 × JI 399 and JI 15 × JI 61. This situation could arise if the parents differed, not only as a consequence of fragment length, but also as a consequence of DNA methylation.

Another grouping occurs in group *5* of the map presented in Figure 1, where three markers detected by the probe cDNA 204 are present in a contiguous group. The region covers some 40 map units, and does not appear to distort the surrounding linkages (Table 2.3) so is probably a genuine representation of the organization of these sequences in the genome.

Relationship between linkage groups in different crosses: Although most of the data presented above derive from the analysis of the progeny of a single cross, the comparison between data from different crosses has been informative.

Alignment of linkage orders: Table 1 shows that many of the markers scored in JI 281 × JI 399 have been scored in other crosses. There is, in general, colinearity between linkage orders, although map distances are not necessarily in a fixed proportion; exceptions to colinearity are discussed below.

Our map has made extensive use of multiple marker probes, which necessarily generate some ambiguity when making comparisons between crosses. However, even "single locus" markers can suffer such ambiguity; Table 1 shows several examples of cDNA clones which identify different numbers of genetic loci in different crosses, for example ADH (as detected by pPsR 546), cDNA 40 and cDNA 148. In the case of markers detected by the probes cDNA 40 and gr-16 (the latter has been scored only in the cross JI 281 × JI 399), there is clearly a copy number difference in DNA sequences detected by these probes, between the parental lines (Figure 4). It is thus difficult to tell, for example, which locus detected by cDNA 40 (in JI 281 × JI 399) corresponds to the single locus scored in two other crosses. It follows then that the two single *cDNA 40* loci (scored in JI 15 × JI 61 and JI 15 × JI 1194 respectively) may be different. Linkage data for these single loci detected by cDNA 40 suggests that the locus in JI 15 × JI 1194 corresponds to *cDNA 40/2* of JI 281 × JI 399, while for JI 15 × JI 61 this seems unlikely, but further data are required to be certain.

There may be copy number variation for a given probe, and it may detect different subsets of a variety of possible loci in different lines. Thus we cannot be sure, *a priori*, that a single locus identified by a given probe in different crosses is identical in all instances. In classical genetic terms this is genetic heterogeneity.

Identification of a translocation: There is a major departure from colinearity between markers, which can be attributed to a translocation in JI 61. The region of the genetic map involved in this translocation is shown for the crosses JI 15 × JI 61 compared to JI 281 × JI 399 in Figure 5. The line JI 61 is related to, and has the same karyotype as JI 145 = LAMM's line L-83, (they are both isolates from the cultivar Extra Rapid, D. FOLKESON personal communication; P. R. SIMPSON personal communication; see "plant material"). This karyotype has been designated a chromosome *3*/chromosome *5* translocation (FOLKESON 1990), and one chromosome, T 3S-5L, in the JI 145 complement (which is equivalent to JI 61), has an additional site for *in situ* hybridization with a 5S rRNA gene probe (SIMPSON *et al.* 1990).

In the cross JI 1201 × JI 813 (data not shown) we have detected linkage between *st* and a polymorphic *Taq*I site in a 5S RNA gene array (21 map units). According to BLIXT (1974) *st* is classically a linkage group *III* marker; this *Taq*I variant of the 5S rRNA gene repeat is therefore a linkage group *III* marker. We have identified a polymorphic *Taq*I site in the 5S rRNA gene repeat which segregates in the crosses JI 15 × JI 61 and JI 281 × JI 399; by inference, these markers, and those linked to them, are assigned to linkage group *III*.

BLIXT (1974) assigns *gp* to linkage group *V*, yet this is clearly linked to *5STaq* in the cross JI 15 × JI 61. The linkage group containing *5STaq* and *gp* in JI 15 × JI 61 thus appears to represent a 3/5 translocation on the basis of classical cytogenetics (FOLKESON 1990), *in situ* hybridization (SIMPSON *et al.* 1990) and linkage analysis.

FIGURE 1.—Linkage groups for the cross JI 281 × JI 399. A, Scale drawing of linkage groups. B, As (A), but connections are drawn corresponding to all pairwise linkages which gave a χ^2 value greater than 11. Curved lines show within linkage group linked pairs, and straight diagonal lines are between group pairs. Note that as the map has been constructed from primary two point data where $\chi^2 > 8$, some associations in the linear maps do not correspond to linkages at $\chi^2 > 11$, *e.g.*, pPE923b/2 and pPE923b/3 (see top of group 7).

TABLE 2

Pairwise map distances within linkage groups as drawn in Figures 1 and 2

2.1. Group *1*

gr-16/9											
4.54	cDNA 260										
8.93	4.25	pCD 72									
8.76	4.25	3.17	*a*								
16.8	5.5	11.1	7.15	cDNA 150/2							
11.3	4.25	7.02	3.12	5.17	*Lg-J/2*						
13.5	5.56	10.6	5.36	6.53	0.9	*Lg-J*					
13.9	13.5	13.2	9.78	12.8	6.82	6.86	cDNA 125/04				
20.8	13.2	17	10.7	9.83	7.15	6.53	6.98	cDNA 24			
24.8	17.3	19.2	12.3	12.5	8.43	9.55	9.78	1.58	cDNA 56/1		
21.9	27.9	26.6	22.1	22.4	17.4	16.8	17.8	8.93	7.85	cDNA 39	
.....	.....	.....	.....	.....	.....	.....	.....	.....	27.6	16.8	pST P

2.2. Group *2*

DR 16												
28.6	*Cvc*											
.....	15.9	cDNA 40/5										
.....	15.1	8.35	cDNA 75									
.....	.....	28.9	14.3	D15B/2								
.....	.....	15.6	7.85	12.4	DR 6							
.....	.....	.....	.....	.....	24.5	cDNA 243						
.....	.....	.....	.....	.....	.....	10.6	D15B/1					
.....	.....	.....	.....	.....	.....	5.5	15.7	cDNA 125/10				
.....	.....	.....	.....	.....	.....	3.44	15.1	10	cDNA 148/3			
.....	.....	.....	.....	.....	.....	.....	.....	.....	24.1	cDNA 194/2		
.....	.....	.....	.....	.....	.....	29.3	.....	23.7	29.1	21.3	DR 12	
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	16	DR 13

2.3. Group *3*

cDNA 34																						
5.62	cDNA 125/187																					
8.45	6.67	cDNA 23																				
8.45	5.36	4.42	*Vc-3*																			
13.2	7.85	5.56	3.44	5S/2																		
15.1	11.3	8.93	5.17	5.36	cDNA 40/2																	
.....	.....	.....	.....	27.4	28.5	cDNA 137																
.....	.....	.....	.....	21.8	22.8	9.64	cDNA 67a															
.....	32.4	.....	.....	.....	.....	23.5	13.7	cDNA 40/7														
.....	.....	.....	.....	.....	.....	.....	13.2	10.9	CHS-2/1													
.....	.....	.....	.....	.....	.....	.....	18	27.3	10.6	DR 14												
.....	.....	.....	.....	.....	.....	.....	.....	.....	23.9	6.53	cDNA 194/1											
.....	.....	.....	.....	.....	.....	.....	26.9	25.8	13.4	5.26	10.7	pPSR 546/1										
.....	.....	.....	.....	.....	.....	.....	32.5	.....	17.9	14.3	20.2	9.64	gr-16/7									
.....	.....	.....	.....	.....	.....	.....	.....	.....	24.5	14.7	20.7	9.83	4.2	gr-16/2								
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	31.5	20.2	cDNA 331							
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	15.6	DR 10						
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	38.2	20.2	6.2	DR 4					
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	38.1	.....	21.2	15.6	18.3	*Vc-5a*				
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	21.2	14	16.6	1.49	*Vc-5*			
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	28.7	19.7	22.8	4.06	2.32	*Vc-5b*		
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	14.3	14.3	14.7	cDNA 53	
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	19.7	15.7	12.7	3.92	*rb*

2.4. Group *1* markers of the *4/1* translocation

```
DR 11
10.4  cDNA 148/2
..... 22.4  GST-10/3
..... ..... 19.6  pST H
..... ..... 32.5   6.13 CHS-2/2
..... ..... ..... 11.6  4     CHS-2/3
..... ..... ..... 10.1   2.29  3.12 i
..... ..... ..... 17.3  18.2  24.8  15.3  P7
..... ..... ..... 13.3  20.2  26.9  22.3   2.6  cDNA 76
..... ..... ..... 14.8  20.9  26.9  20.9   8.35  6.03 pPE 923/1
..... ..... ..... ..... ..... ..... ..... 19.2  17     7.33 cDNA 206
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 18.8  DR 9
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 18.2   4.42 cDNA 150/1
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 14.3  14.3  cDNA 125/08
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 21.8  19.7  18    GST-10/1
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 29.3  22.7   6.31 cDNA 164
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 30.4  25.8  22    10.2  10.7  cDNA 40/8
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 26.6  23    25.1  10.6   3.33 16.4  cDNA 44
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 30.3  24.8  27.6  15    10    21.3   6.8  cDNA 40/3
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 19.6  27.9  16.8  ..... 12.7   9.7  cDNA 267
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 25.5  ..... 27.9  ..... 21.3  21.8   9.11 cDNA 186
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 31.2  30.3  21.9  11.1  pPSR 546/3
```

2.4. Group *4* markers of the *4/1* translocation

```
GST-10/4
23     0.9MI/1
21.2  10.4  cDNA 119
..... 21.4  13.1  cDNA 38a
..... ..... 18.6   5.5  cDNA 228
..... ..... 23     8.71  4.2  cDB107
..... ..... ..... 16.8   9.55  9.32 pCD 7/3kb
..... ..... ..... ..... ..... ..... 25    gr-16/3
..... ..... ..... ..... ..... ..... 23.5  18.7  gr-16/5
..... ..... ..... ..... ..... ..... 13.8   9.32 12.8  gr-16/1
..... ..... ..... ..... ..... ..... ..... ..... ..... 16    cDNA 148/5
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 11.1  pAt-T4/5
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 26.7  DR 1
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 14.3   5.88 pPE 1036a
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 29.3  18.8   2.17 le
..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... ..... 17.1  17.8  pAt-T4/6
```

2.4. Markers around the proposed translocation point in a linear order (see Figure 2)

```
cDNA 148/5
13    cDNA 41
11.1  15.7  pAt-T4/5
..... ..... 27.4  GST-10/3
..... ..... 26.7  16.1  DR 1
..... ..... 14.3   5.72  5.88 pPE 1036a
..... ..... ..... 33.5  ..... 17.1  pAt-T4/6
..... ..... 29.3  13.4  18.8   2.17 17.8  le
..... ..... ..... 32.5  ..... ..... ..... 33.6  CHS-2/2
..... ..... ..... ..... ..... ..... ..... .....  2.29 i
..... ..... ..... ..... ..... ..... ..... .....  4     3.12 CHS-2/3
..... ..... ..... 19.6  ..... ..... ..... .....  6.13 10.1  11.6  pST H
..... ..... ..... ..... ..... ..... ..... ..... 18.2  15.3  24.8  17.3  P7
..... ..... ..... ..... ..... ..... ..... ..... 20.2  22.3  26.9  13.3   2.6  cDNA 76
```

TABLE 2—*Continued*

2.5 Group *5*																		
F																		
13.8	5S/1																	
.....	23.7	pRSR 546/4																
.....	26.4	16.8	cDNA 231															
.....	.....	29.1	10.2	*Vc-2*														
.....	28.2	30.3	8.91	4.67	cDNA 373													
.....	.....	.....	22.4	14.7	14.7	cDNA 125/03												
.....	.....	.....	33.2	26.3	18.7	34.6	cDNA 204.1											
.....	.....	.....	.....	.....	.....	.....	16.4	pRSR 546/2										
.....	.....	.....	.....	.....	.....	.....	40.9	22.2	cDNA 125/02									
.....	.....	.....	.....	.....	.....	.....	19.2	13.7	33.2	cDNA 148/6								
.....	.....	.....	.....	.....	.....	.....	16.1	5.88	20.2	8.71	cDNA 204/3							
.....	.....	.....	.....	.....	.....	.....	53.6	28.2	.....	41.5	23.2	cDNA 204/4						
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	30.1	.....	16.8	cDNA 204/2					
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	19.2	10.6	cDNA 40/4				
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	19.2	10.6	15.6	0.9	MI/2		
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	25.1	16.1	17.2	2.47	cDNA 133		
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	25.5	26.6	16.6	11.1	DR 17	
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	23.9	23.5	26.2	GST-10/2
2.6. Group *6*																		
pCD 7/6																		
21.7	cDNA 40/6																	
16.2	16.4	cDNA 324																
16.3	24	3.61	pCD 7/5															
.....	38.6	11.4	15.9	cDNA 48a														
.....	.....	.....	17.5	14.8	cDNA 289/2													
.....	.....	30.1	23.5	24.7	9.89	5S/3												
2.7. Group *7*																		
pAt-T4/8																		
25.5	pPE 923b/2																	
.....	27.9	pPE 923b/3																
.....	.....	17.4	DR 19															
.....	.....	20.2	21.3	DR 20														
.....	.....	.....	.....	23.5	DR 18													
.....	.....	.....	.....	30.7	25.9	cDNA 2a												
.....	.....	.....	.....	34.6	24.8	2.91	cDNA 200											
.....	.....	.....	.....	.....	20.8	14	13.7	cDNA 187										
.....	.....	.....	.....	.....	.....	16.4	.....	13.8	DR 26									
.....	.....	.....	.....	.....	.....	.....	.....	14	20.2	cDNA 280								
.....	.....	.....	.....	.....	.....	.....	.....	15.1	12.2	12.3	cDNA 189a							
.....	.....	.....	.....	.....	.....	.....	.....	30.4	.....	28.2	5.26	cDNA 286						
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7.7	2.65	*Lg-1*					
.....	.....	.....	.....	.....	.....	.....	.....	24.8	.....	.....	11.7	6.43	6.94	DR 3				
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	19.6	16.4	15.1	16.8	cDNA 148/4			
2.8. Unassigned groups																		
cDNA 136												pST T						
19.7	cDNA 125/20											1.66	cDNA 289					
22.4	5.26	gr-16/8										12.7	15.7	pST LL1				
.....	19.7	18.8	cDNA 56/2									24.6	23.7	13.9	cDNA 125/11			
.....	.....	.....	17.8	cDNA 148/1														
.....	.....	22.4	12.1	13.4	DR 2							DR 22			pAt-T4/1			
.....	.....	34.6	33.5	.....	35.8	DR 7						20.3	DR 23		32.4	pAt-T4/2		
.....	19.7	14.6	25.1	.....	.....	10.3	DR 8											
.....	.....	.....	.....	.....	.....	.....	28.9	cDNA 40/1										

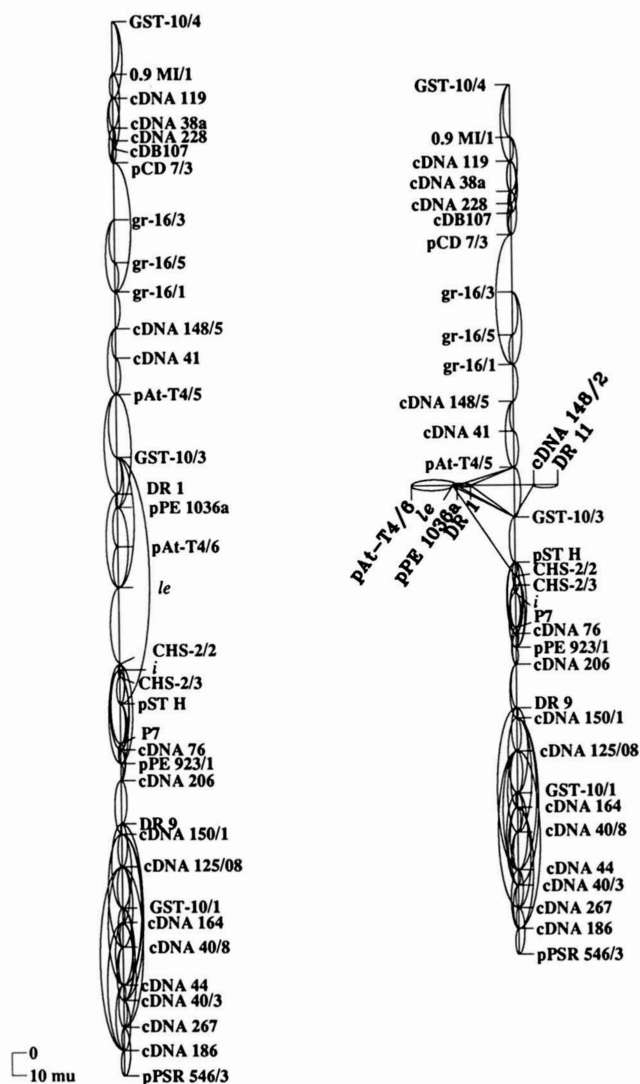


FIGURE 2.—Comparison of the linear and cruciform maps of group *T1/4*. All pairwise associations of markers are shown as arcs for χ^2 greater than 8.

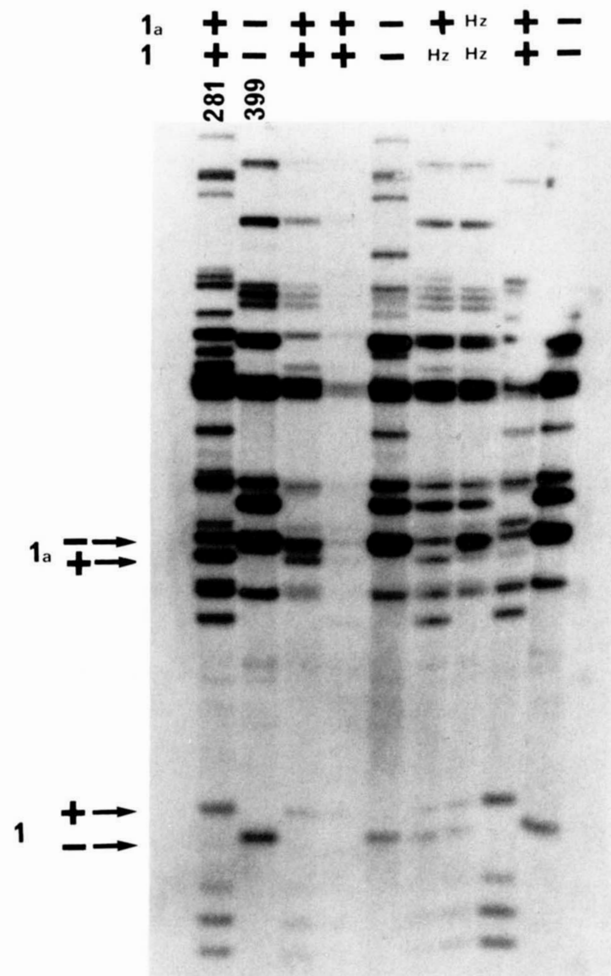


FIGURE 3.—Recombination within the vicilin multigene array at the *Vc-5* locus. The parental types JI 281 and JI 399 are indicated 281 and 399 respectively, a selection of seven DNA samples from the F_8 population is also shown. The allelic variants at the *Vc-5/1* and *Vc-5/1a* loci are indicated 1 and 1a above the tracks, and scored as "+," "−" or "Hz." The bands which, by their presence or absence, indicate the allelic condition at *Vc-5/1* or *Vc-5/1a* are arrowed and indicated 1 and 1a, respectively, and their allelic variants designated "+" and "−," respectively. In order to recognize the distinction between *Vc-5/1a* (and *Vc-5/1b*) the heterozygotes have been given the score corresponding to the allele different from *Vc-5/1* in both cases (*i.e.*, they were not scored as "0"; see MATERIALS AND METHODS). In effect this artificially doubles the actual value for the observed frequency of recombinants and thus exaggerates the map distance between the markers, but in both cases the map distance is smaller than its associated error. The filter was washed in 0.1 × SSC at 65°, 1 × SSC is 0.15 M NaCl, 0.015 M trisodium citrate.

If the translocation in JI 61 is reciprocal, we would expect to find two linkage groups in this cross exhibiting aberrant segregation behavior. We have been unable to assign such behavior to any other group in the cross JI 15 × JI 61, suggesting that the translocation may represent the relocation of a region of linkage group *III* to the end of linkage group *V* which remains otherwise undisrupted; this interpretation is consistent with the genetic maps provided in Figure 5.

This data set derived from the cross JI 15 × JI 61 serves several purposes; first, it allows us to correlate some of our linkage groups to those previously defined (BLIXT 1974; WEEDEN and WOLKO 1990). Second, we can be confident that the translocation identified by *in situ* hybridization is a 3/5 translocation chromosome, and third, we can see clearly how a translocation appears in our analysis: it has the predicted features of crossover suppression, the association of markers unlinked in other crosses, and ambiguity in the overall order of many of the markers.

We have been concerned from the outset of these experiments that the pea genome, riddled with dispersed repeated sequences (MURRAY, CUELLAR and THOMPSON 1978; MURRAY and THOMPSON 1982), in a species which has apparently recently adopted an inbreeding strategy, may be prone to homologous nonallelic recombination (SINGER 1988) and thus translocations may be very frequent. We have identi-

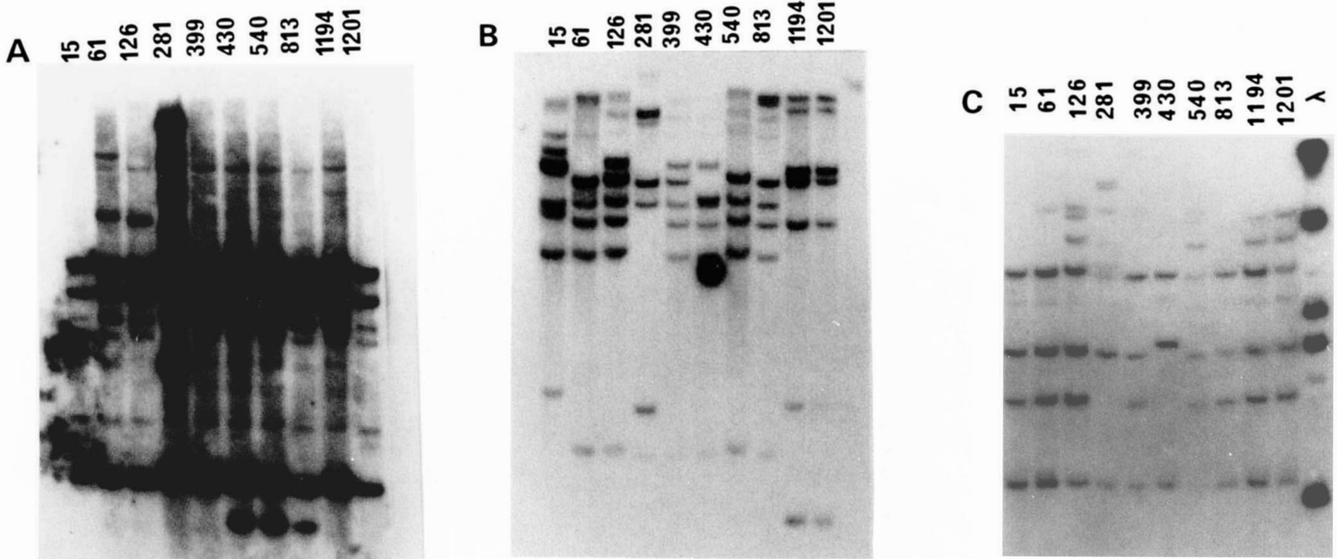


FIGURE 4.—Probes which show copy number variation. *Eco*RI-digested DNAs of the lines JI 15, JI 61, JI 126, JI 281, JI 399, JI 430, JI 540, JI 813, JI 1194 and JI 1201 (indicated by number) have been probed with cDNAs corresponding to gr-16, alcohol dehydrogenase (pPsR 546), and cDNA 40 (panels A, B and C, respectively) are shown. The variability in the number of bands in different lines should be noted. Filters were washed as for Figure 3, panels A and B are nitrocellulose filters (Schleicher and Schüll BA 85), panel C is the first probing of a Genescreen plus filter.

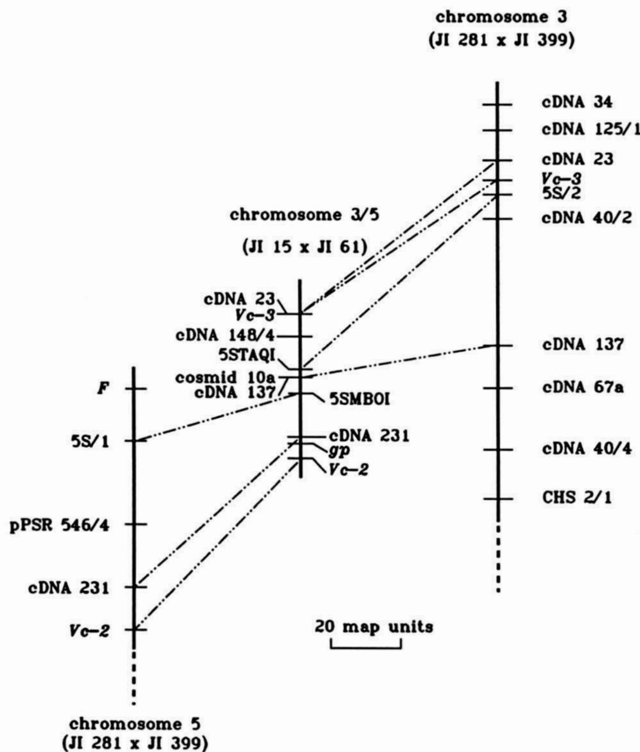


FIGURE 5.—A *3/5* translocation. Alignment of genetic maps derived from the cross JI 15 × JI 61 and JI 281 × JI 399. Corresponding markers in the different crosses are connected by diagonal lines.

fied translocations segregating in these experiments (see below), one translocation is well characterized, but the others are unexpected. We have been able to derive linear linkage maps corresponding to several regions of the pea nuclear genome, and ascribe instances of nonlinearity to differences between our parental stocks. This is of some importance for further genetic or breeding studies; however translocations do not arise at such a frequency as to make it impossible to deduce a linear order for most of the genome.

The linkage relationships around gp differ between crosses: A comparison between the linkage data derived from JI 281 × JI 399 and JI 15 × JI 1194 shows a further difference in the region around *gp* (Figure 6). The JI 15 × JI 1194 cross does not behave in the same way as JI 15 × JI 61; the group *3* markers which are involved in the 3/5 translocation in JI 61 and which have been scored in JI 15 × JI 1194 can be drawn in a map colinear with the one derived from JI 281 × JI 399 (not shown). In the (incomplete) linkage map derived from JI 15 × JI 1194, there is an association between group *5* and *7* markers, as defined in JI 281 × JI 399. However, there is no obvious crossover suppression, so possibly both lines in each of the pairs JI 15:JI 1194 and JI 281:JI 399 may have matching karyotypes as regards this region. The group 7 markers which have a novel linkage order in the cross JI 15 × JI 1194 represent a small, isolated, linkage group in the cross JI 15 × JI 61 in which the markers *r* and *gp* are not linked to one another, perhaps as a consequence of the 3/5 translocation in JI 61. The markers linked to *r* and *gp* in JI 15 × JI 1194 are on separate linkage groups in the cross JI 281 × JI 399 (neither *r* nor *gp* segregate in the cross JI 281 × JI 399), but the F_2 data derived from JI 430 × JI 181 indicate that these genes (or at least those linked to them) are again on the same linkage group.

There seems to be disruption of the *r–gp* linkage in two of these crosses, one as a consequence of a 3/5

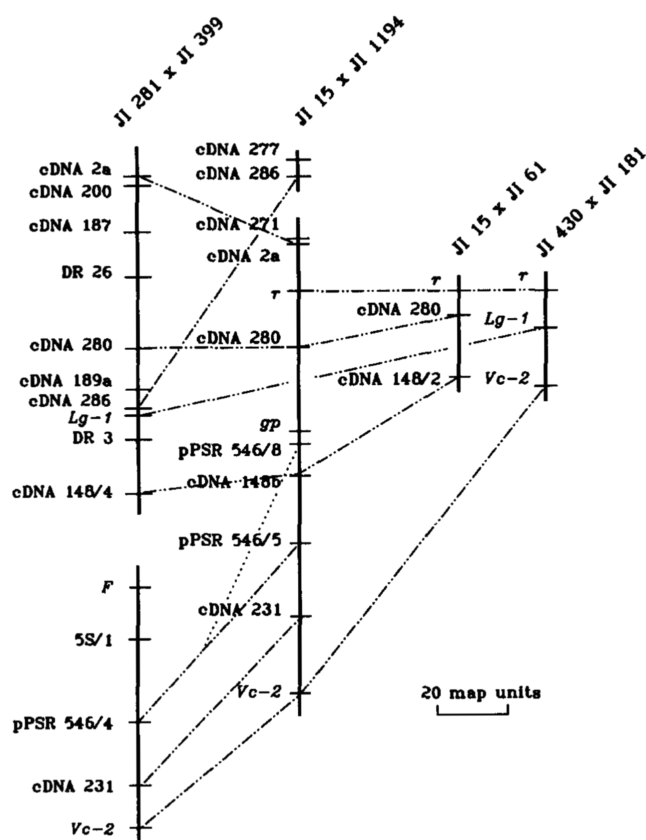


FIGURE 6.—A *5/7* translocation. Alignment of portions of linkage group *5* and group *7* of our map of JI 281 × JI 399 with corresponding regions of other crosses.

translocation and the other involves the classical linkage groups *V* and *VII*. There has been discussion of the possibility of linkage between the *gp* and *r* markers (LAMM 1951; LAMM and MIRAVALLE 1959; FOLKESON 1984, 1990; WEEDEN and WOLKO 1990; POLANS, WEEDEN and THOMPSON 1986; ELLIS and CLEARY 1988). Unfortunately our data do not resolve this issue because it is not clear whether the *gp* locus lies on the same group as *Lg-1* or as *Vc-2* in the cross JI 281 × JI 399; *gp* does not segregate in this cross, so we must await the outcome of the analysis of a further cross.

The *5S/1* proximal end of linkage group *5* appears to have three patterns of association in the crosses we have analysed here (Figures 5 and 6). The reason for this is not clear, but in two cases *Vc-2* is at or near the end of a linkage group while in the third it is subterminal. It may be that there is a site prone to breakage near to *Vc-2*. Breakage may result in the formation of an acentric fragment which is rescued as a telomere fusion.

DISCUSSION

We have presented a linkage map of pea, based largely on RFLP analysis of recombinant inbreds. The map is not universal; from the analysis of three recombinant inbred populations we have been able to show that at least one region of the genome can exist in several different linkage arrangements. These altered patterns of association of markers may have a simple relationship, involving the movement of a chromosome segment from one location to another. A much finer map is needed to verify this. We have shown that the different orders around the *r–gp* region are not necessarily simply related to each other: for example, the probe cDNA 286 detects a marker within this region in the cross JI 281 × JI 399 (Figure 1, group 7), but the marker it detects in the cross JI 15 × JI 1194 does not map to this region (Figure 6). This observation supports the view of FOLKESON (1984) that rearrangements of linkage groups may be "more complex than a simple reciprocal translocation." The map we present cannot be assumed to be precisely correct for all crosses. Nevertheless it would seem that the probes we have used can detect linkage to most regions of the pea genome.

We have exploited a variety of repeated and unique sequence probes. The repeated sequence probes map throughout the genome, and can thus be used to aid in the assay of introgression of a marker to a new background, or for the rapid assignment of the context of a new marker.

Relationship between this map and previously published maps: The maps we have presented here highlight the danger of attempting to compile a single linkage map of pea from the analysis of several crosses. This has been the only possible procedure for classical markers, so perhaps it is now less surprising that inconsistencies in the classical map (BLIXT 1974) have begun to emerge from the analysis of isozyme data (WEEDEN and MARX 1987; WEEDEN and WOLKO 1990), cytogenetics (FOLKESON 1984, 1990), and the use of molecular probes (ELLIS and CLEARY 1988; WEEDEN and WOLKO 1990).

We have given numbers to the linkage groups presented in Figure 1; the justification for these assignments is given below, but it must be appreciated that we do not necessarily expect a simple correspondence between these groups and BLIXT's (1974) map or the related map presented by WEEDEN and WOLKO (1990). For this reason, and to help in comparison between these maps, we refer to the previously described linkage groups in roman numerals while deduced linkage orders from this work are in arabic numerals.

Group *1*: The marker *a* (MENDEL 1866; DEHAAN 1932; HARKER, ELLIS and COEN 1990) segregated in the cross JI 281 × JI 399; *a/a* plants completely lack anthocyanin pigmentation and have been scored on the basis of white rather than coloured flowers. This is a group *I* marker according to BLIXT (1974) and we have used it to assign our linkage group *1*. In this

cross, *i* also segregates as does the linked marker *af* (MARX 1977, 1987a; YOUNG 1983; WEEDEN and WOLKO 1990) in another cross. These two markers have been assigned to linkage group *I* (PELLEW 1940; BLIXT 1974; WEEDEN and WOLKO 1990), but our data do not support their being on the same linkage group as *a*; we have assigned these two markers to a complex linkage group containing a translocation pair which includes group *4* (Figures 1 and 2). The group of markers around *a* are much more dense than at any other part of our maps (Figure 1), which suggests that some crossover suppression may occur in this region in the cross JI 281 × JI 399. This in turn raises the possibility that there is some karyotypic rearrangement which distorts the segregation pattern. The association of many of these markers with *DR 18* is very striking. We have no simple explanation for this pattern, but it could involve both a translocation and inversion. The evidence for such a speculation is lacking, and in the absence of cytogenetic data we prefer to leave the issue unresolved.

Group *2*: No group *II* morphological marker segregated in any of our crosses; however, a convicilin gene has been mapped to linkage group *II* (MATTA and GATEHOUSE 1982), and the probe pCD75 (a cDNA corresponding to a convicilin message) detects a single convicilin locus (ELLIS *et al.* 1986). We have assigned linkage group *2* on the basis of the presence of the *Cvc* locus.

The *DR13–pPSR 546/3* segment of our linkage group has an ambiguous location. An association of this segment with group *2* could be made, but is dependent on the single linkage *DR 13–DR 12* (Table 2.2); the alternative position distal to the group *4* marker detected by cDNA 206 is preferred.

The restriction fragment which allows us to follow the segregation of *DR 13* has a bias in favor of one parental class, suggesting that the segregation of two loci could be responsible for the observed pattern of this restriction fragment among these progeny. The allele of this restriction fragment has not been found; it is likely to be a null (LEE *et al.* 1990). Thus the ambiguity over the assignment of the *DR 13–pPSR 546/3* segment may be a consequence of ambiguity as to whether *DR 13* alone represents one or two loci.

Groups *3* and *5*: Consideration of the behavior of a translocation segregating in one cross has enabled the correlation between two of our linkage groups and groups *III* and *V* of BLIXT (1974). This assignment is on the basis of cytogenetic data (SIMPSON *et al.* 1990) and by virtue of linkage to the markers *st* (PELLEW and SVERDRUP 1923; MARX 1977, 1987a) and *gp* (PELLEW 1940). WEEDEN and WOLKO (1990) have assigned an ADH locus to group *III* a little more than 20 cM from *st*; we have shown linkage (at 21 mu) of a 5S gene array to *st* in JI 1201 × JI 813 (not shown), and we have mapped one of the ADH related markers (of which there are many) to the middle of our linkage group *3*, which can be taken as weak supportive evidence.

The situation with regard to the marker *F* is unhelpful, but interesting. We are not clear whether the marker involved in this cross is *F*, a linkage group *III* marker, or *Fs* a linkage group *V* marker (BLIXT 1974), but we have assigned the marker to linkage group *5* of JI 281 × JI 399. *F* and *Fs* have been regarded as "polymeric genes" (LAMPRECHT 1942, 1953, 1961) and LAMPRECHT (1953) has suggested that polymeric genes arise as a consequence of duplications of sections of the pea genome.

An association of *rb* with linkage group *III* markers has been tentatively made by BLIXT (1974) and others (GRITTON 1971; MARX 1987b), but we have been unable to confirm this in an F_2 population segregating for *M* and *F* (JI 117 × JI 1156 data not shown). In the linkage map derived from JI 281 × JI 399 *rb* is at the end of our linkage group *3*. This terminal location for a marker reduces the chance that linkage to another marker can be detected, and may account for the previous difficulties in assigning a map location for *rb*.

Group *4*: The *Le* locus has been assigned to group *IV* (BLIXT 1974); on this basis we assign our linkage group carrying *le* to group *4*. POLANS, WEEDEN and THOMPSON (1986) have assigned one ribosomal gene cluster *Rrn1* to linkage group *IV*, and we find one ribosomal RNA gene cluster maps to this region, suggesting it identifies *Rrn1*. The presence of *I* and *Le* on the same linkage group is contrary to previous assignments; however, from our data this conclusion can be reached on the basis of several associations (Table 2.4).

A telomere related marker, *pAt-T4/6*, maps to this region and disrupts the tight linkage between *pPE 1036a* and *Le* in the linear map (Figure 2). This telomere related sequence may represent what was once a genuine telomere of linkage group *IV*, and perhaps represents the end of linkage group *IV* in other stocks. Thus the *I-Le* association in our linear map (Figure 2) represents a chromosomal rearrangement in either or both of the lines JI 281 and JI 399.

The alternative cruciform map (Figures 1 and 2) of this group also suggests that there is a chromosomal rearrangement in this region, but that it is segregating among the progeny of JI 281 × JI 399. The cruciform map places *pAT-T4/6* at the end of one arm, but leaves *pAt-T4/5* at an interstitial site.

Interstitial sites for telomere related sequences have been described for many vertebrates (MEYNE *et al.* 1990). The telomere related sequences mapped in these experiments do not exhibit *Bal*31 hypersensitiv-

ity (not shown); consequently even those telomere related sequences mapping to the end of a linkage group probably represent subtelomeric repeats.

Group 6: Our linkage group *6* is short, and it seems likely to be incomplete; however it may correspond to group *VI* on the basis of the 5S rRNA gene marker *5S/3*. The three 5S rRNA gene clusters have been assigned to chromosome *3*, *5* and *1* or *6* on cytogenetic grounds (SIMPSON *et al.* 1990). We have already identified the chromosome *3* and *5* loci (*q.v.*), and have assigned linkage group *I*, leaving the assignment of the *5S/3* containing group as *VI*. However it remains a possibility that our linkage groups *6* and *1* reside on the same chromosome, but that we have been unable to detect spanning linkages; linkage group *I* is fragmented on our map.

The only marker from BLIXT's linkage group *VI*, which segregates in the crosses we have analysed, is the black hilum character *Pl*. This does not show linkage to any other marker in JI 15 × JI 61, but linkage of *Pl* to *pCD 7/5* in the cross JI 15 × JI 1194 has been detected, suggesting some correspondence between our group *6* and linkage group *VI* of BLIXT (1974). However, our sixth linkage group (carrying *5S/3*) does not necessarily correspond to his linkage group *VI*.

Group 7: Our linkage group 7 carries no classical markers, but the storage protein gene locus *Lg-1* has been mapped to linkage group *VII* (DAVIES 1980; MATTA and GATEHOUSE 1982; DOMONEY, ELLIS and DAVIES 1986). The location of this legumin gene locus is the basis of our assignment of markers to linkage group *VII* of BLIXT. The location of *Lg-1* toward one end of our linkage group 7 is at variance with BLIXT's (1974) map, and also with WEEDEN and WOLKO (1990); however *in situ* hybridization results (SIMPSON, NEWMAN and DAVIES 1988) would lead us to expect this location in a normal karyotype.

The confusion of the existing literature (LAMM 1951; LAMM and MIRAVALLE 1959; FOLKESON 1984, 1990; WEEDEN and WOLKO 1990; POLANS, WEEDEN and THOMPSON 1986; ELLIS and CLEARY 1988) in relation to linkage groups *V* and *VII* has been discussed in connection with linkage relationships around *gp* above. In Figure 6 we show examples of linkage between markers near to *gp* and *r*, together with a disruption of this region.

Groups not assigned: The largest unassigned group spans *cDNA 136* and *cDNA 40/1*. This group shows some loose associations with other markers, but the pattern of association is problematic and involves several gr-16 related markers. A duplication could account for some of these features. The group *pST T–cDNA 125/11*, which contains *Rrn2* (POLANS, WEEDEN and THOMPSON 1974), is associated with the end of linkage group 7, but not with *cDNA 148/4*. Recently the second rDNA locus, *Rrn2*, has been identified on the basis of a minor restriction site variant, and its location near to *pST T* has been confirmed, but a spanning linkage to group 7 was not detected.

The telomere related markers *pAt-T4/1*, */2*, */3*, */4* and */7* do not show linkage to any other marker. Even though these markers do not show the pattern of *Bal*31 sensitivity expected of the actual telomere, they may be very close to the genuine telomeres in which case we would expect this behavior. Recombination has been suggested to be involved in yeast telomere replication (PLUTA and ZAKIAN 1989); if this mechanism is also employed in pea then the telomeric region may show a high frequency of recombination with respect to its physically closest marker. The lack of detected linkage with these markers may thus be a consequence of the biological function of telomeric sequences.

Conclusion: The data presented here are not a static map of the pea genome; the recombinant inbred lines represent multiply marked stocks segregating many characters of interest which are available for further investigation. Recombinant inbred analysis is a cumulative form of genetic mapping, and as these lines are investigated in more detail it is anticipated that some of the difficulties associated with this map will be resolved, and resolution of the map will become increasingly finer. The probes which we have exploited are a resource which can be exploited in further genetic analysis or breeding. It is further to be hoped that the correlation between good *in situ* probes and this developing map will continue and aid in the vexed issues of the relationship between linkage groups and chromosomes in pea.

We have presented our reservations concerning the simple transfer of this map to a different cross, but our map does direct the choice of markers which can be used for investigations in any cross of pea, and should be regarded as a general note of caution in dealing with the linkage maps which are being derived for many organisms.

This work has been funded by the Agricultural Genetics Company. The John Innes Institute is supported by a grant in aid from the Agricultural and Food Research Council.

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Communicating editor: M. R. HANSON