Extensive Conservation of Linkage Relationships Between Pea and Lentil Genetic Maps

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A 560-cM linkage map consisting of 64 morphological, isozyme, and DNA markers, has been developed from an interspecific cross (*Lens ervoides* × *L. culinaris*). In addition, nine markers were scored that assorted independently of any of the multilocus linkage groups. Comparison of this map with that established previously for *Pisum sativum* reveals eight regions in which linkages among marker loci appear to have been conserved since the divergence of the two genera. These conserved linkage groups constitute at least 250 cM, or approximately 40% of the known linkage map for *Lens*. The two genera represent disparate lineages within the legume tribe Viceae, indicating that all members of this tribe may possess linkage groups similar to those identified in *Lens* and *Pisum*. Instances where the *Pisum* and *Lens* maps differed included the regions surrounding the 45S ribosomal tandem repeats and the position and distribution of the genes encoding the small subunit of ribulose bisphosphate carboxylase. We also found a highly repeated sequence unique to *Lens* that maps within a linkage group shared between the two genera and a cDNA sequence that displays significant variation in copy number within the genus *Lens*.

Chromosomal linkage maps have long served as a convenient method for summarizing much of the genetic information known about a species. Until about 1980, only a few of the most intensively studied species such as tomato (*Lycopersicon esculentum*), maize (*Zea mays*), and garden pea (*Pisum sativum*) had relatively complete linkage maps. However, with the discovery of allozyme and, more recently, DNA polymorphisms, the number of segregating markers that can be conveniently scored in a progeny has increased tremendously, permitting detailed linkage maps to be established for a considerable number of plant species (Bernatzky and Tanksley 1986; Chang et al. 1988; Gebhardt et al. 1989; Helentjaris et al. 1986; Landry et al. 1987; McCouch et al. 1988; Slocum et al. 1990). Despite this great increase in efficiency, the generation of a linkage map de novo still requires considerable effort and expense. Additional approaches or techniques that might facilitate the mapping operation continue to be sought and investigated.

One such approach, successfully applied in several instances, takes advantage of the conservation of linkage relationships in closely related genera. Isozyme loci initially shown to be syntenic in *Triticum aestivum* (Hart and Langston 1977) are also syntenic in many relatives of wheat (Hart 1979). DNA markers have demonstrated a nearly perfect retention of linkage alignment in tomato and potato (Bonierbale et al. 1988), and linkage conservation has been found among sorghum and its relatives (Hulbert et al. 1990). Comparisons of more divergent taxa, such as tomato and pepper (Tanksley et al. 1988), have detected few conserved linkages.

We have been using both isozyme and DNA markers to develop linkage maps for two legume species: garden pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.). Both are members of the Viceae, a tribe of north-temperate legumes that also includes faba bean (*Vicia faba*) and sweet pea (*Lathyrus sativus*). The genus *Vicia* is currently believed to be basal to the tribe, with *Lens* representing one evolutionary lineage and *Lathyrus* and *Pisum* another (Radzhi 1971). All diploid species of *Pisum, Lathyrus,* and *Lens* possess a somatic chromosome number of 14. The species of *Vicia* show more variability but generally have $2n = 14$, although *V. faba* has $2n = 12$. Thus, linkage groups conserved between *Pisum* and *Lens* have a high probability of being present in all members of the Viceae.

At present, *P. sativum* is the only Viceae species with a detailed linkage map that

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consists of morphological and physiological mutants, allozyme and other protein variants, RFLPs, and structural genes identified from DNA clones (Blixt 1974; Weeden and Wolko 1990). Several partial linkage groups also have been identified in lentil, again consisting of morphological, protein, and DNA markers (Havey and Muehlbauer 1989; Muehlbauer et al. 1989; Tadmor et al. 1987). Although homologous relationships between loci conditioning morphological traits are notoriously difficult to demonstrate, those between isozyme loci, cloned DNA sequences coding specific genes, or low-copy-number random DNA sequences are much more readily determined. Initial studies indicated that at least a few linkages appeared to be conserved between garden pea and lentil (Weeden et al. 1988). In order to estimate the degree to which linkages have been maintained with the Viceae, we have extended the linkage map of lentil to include many loci homologous with those mapped in pea and have compared the relative arrangement of the loci.

Materials and Methods

Plant Material

The parents used for the interspecific cross in *Lens* were *L. ervoides* (Ladizinsky #32) and *L. culinaris* (Ladizinsky #7). The two lines are known to differ by a reciprocal translocation, but otherwise they generate relatively fertile F_2 progeny (Ladizinsky et al. 1985). Dr. D. Zamir generously donated seed from approximately 100 individual F_2 plants, the F_2 already having been scored for 18 morphological and isozyme marker loci (Tadmor et al. 1987). Ten seeds from each F_2 plant were germinated, and we used 60 of these F_3 families for our analyses. For most segregating markers, data were obtained on at least 40 F_3 families, although in several cases this number was reduced due to poorly resolved phenotypes. We determined morphological and allozyme phenotypes on five F_3 plants. The morphological markers epicotyl color (*Gs*), pod indehiscence (*Pi*), and seed coat spotting (*Scp*) were scored as described by Havey and Muehlbauer (1989). DNA was extracted from pooled leaf tissue of all F_3 plants from a particular F_2. In this way the genotype of the F_2 could be determined both for isozyme loci and RFLPs.

Isozyme Analysis

The generation of extracts from lentil leaf, seed, or root tissue, as well as the gel systems used for the resolution of the various enzyme systems, was described in Muehlbauer et al. (1989), except that we resolved glutamine dehydrogenase and ribulose bisphosphate carboxylase (RUBISCO) on the tris-citrate/lithium borate system. Data for the segregation of seed proteins were used directly from sheets supplied with the seeds and were not confirmed in our laboratory. Enzyme assays were performed as described in Wendel and Weeden (1989).

RFLP Analysis

We obtained high molecular weight DNA from young leaves of 6- to 8-week old lentil seedlings using a CTAB extraction essentially as described in Polans et al. (1985). DNA from the parents was screened for RFLPs using the enzymes *Eco*RI, *Eco*RV, *Bam*HI, *Bgl*II, and *Hin*dIII. Restriction digests were performed for 6–8 h at 37°C, in buffers supplied with the enzymes. Electrophoresis was carried out in 0.9% agarose gels. After electrophoresis the gels were exposed to UV light (302 nm) for at least 2 min to generate nicks in the DNA molecules. The DNA was denatured by soaking in 0.4 N NaOH with 0.6 M NaCl for 20 min and transferred to Genescreen Plus (Dupont) nylon filters using the alkaline transfer procedure of Reed and Mann (1985).

We obtained plasmids containing lentil DNA inserts from libraries described by Havey and Muehlbauer (1989). Pea DNA probes included the 45S ribosomal DNA repeat (pHA2, Jorgensen et al. 1987), genes coding the plastid-specific, cytosolic, and nodule-specific glutamine synthase (pGS185, pGS299, pGS340, Tingey et al. 1987), the plastid-specific glyceraldehyde 3-phosphate dehydrogenase gene (pNcoI, Cerff et al. 1986), and the gene coding the chloroplast ribosomal protein-22 (rpl22, Gantt et al., 1991). A plasmid containing an alfalfa leghemoglobin sequence (pALb, Dunn et al. 1988) and one containing a soybean actin gene (pSAc3, Shah et al. 1982) also were used in the study. Except for pHA2, which was labeled in entirety, inserts were excised and separated from the vector before labeling with ^{32}P by the method of Feinberg and Vogelstein (1983). Hybridization was performed overnight at 65°C in 0.5 M $NaHPO_4$, pH 7.2, containing 10× Denhardt's solution, 1 mM EDTA, 0.1 mg/ml sonicated calf thymus DNA, 0.6% (w/v) SDS, and 2.5% (w/v) dextran sulfate. Filters were washed three times with 2× SSC containing 0.1% SDS at 65°C for 30 min. Autoradiography was performed with intensifying screens at −70°C.

Linkage Analysis

Linkage between morphological, isozyme, and RFLP markers was estimated from F_2 segregation data using the method of maximum likelihood as applied by the LINKAGE-1 program (Suiter et al. 1983). Linkage groups were constructed from estimates of recombination between pairs of markers and by inspection of primary data to minimize the number of recombinants within a linkage group. All RFLP data were obtained starting with the same initial set of 20 individuals and proceeding sequentially through the progeny. Thus, for those markers in which only partial data sets exist, the number of F_3 families compared when two such markers were analyzed for linkage was equal or nearly equal to the size of the smaller data set. Linkages greater than 30 cM or with a $P \geq .005$ were not reported.

Pisum Linkage Map and Markers

Linkages involving molecular markers in pea were summarized in Weeden and Wolko (1990) with appropriate primary references cited therein. The morphological characters discussed below are described in Blixt (1974).

Results

Sixty-six segregating markers were scored in the progeny (Table 1). These markers could be assembled into 11 multilocus linkage groups (Figures 1 and 2). Eleven of the markers displayed non-Mendelian segregation ratios, and six of these (*Sp-1, Aat-p, Gs, Rrn, Pgm-p,* and *Gdh*) were involved with the linkage groups containing the reciprocal translocation. The four loci close to the breakpoint (*Lghb, Rrn, Gs,* and *Pgm-p*) all displayed an excess of the heterozygous genotype (Table 1), although for *Lghb* this excess did not produce a significant deviation from the expected 1:2:1 ratio. Segregation at two other regions of the genome, the *Sp-3* end of linkage group 4 (three markers) and the region around *Lap-1* on linkage group 6 (two markers) gave significant deviations from expected ratios. In each of these cases the *L. culinaris* allele was predominant, the excess being particularly evident as one progressed toward *Sp-3* along linkage group 4.

The linkage map developed for the cross by Tadmor et al. (1987) was further defined and extended. A marker, CMH52c, was located between *Sp-1* and *Sp-2* in linkage group 1, and another marker, CMH81, was mapped between *Sp-3* and *Sp-4* in linkage group 4. This latter group was com-

Table 1. Segregation of markers in the F_2 from the cross *Lens culinaris* × *L. ervoides*

Marker	*N*	No. individuals with designated phenotype[a] Lc	H	Le	Expected ratio	χ^2	Linkage group assignment[b] *Lens*	*Pisum*
Aat-c	60	17	31	12	1:2:1	0.90	11	3
Aat-mb	42	11	19	12	1:2:1	0.43	8	n.d. (1)
Aat-p	60	6	32	22	1:2:1	8.80*	1	1
Aco-1	60	11	31	18	1:2:1	1.70	1/2[c]	n.d. (1)
Act-1	44	9	29	6	1:2:1	5.86	3	3[d]
Act-2	43	9	22	12	1:2:1	0.44	11	n.d. (3)
Act-3	42	8	0	34	1:3	0.67	4	n.d. (3)
Act-4	40	35	0	5	3:1	3.33	n.d. (2)	n.d. (3)
Act-5	43	31	0	12	3:1	0.19	4	n.d. (3)
Aps-1	59	15	29	15	1:2:1	0.05	3	3[d]
CMH3	11	2	7	2	1:2:1	0.82	6	n.d. (1)
CMH33	27	8	11	8	1:2:1	0.93	7	n.d. (1)
CMH34	31	4	22	5	1:2:1	5.52	4	7
CMH41	24	6	12	6	1:2:1	0.00	3	4
CMH52a	24	5	10	9	1:2:1	2.00	10	n.d. (3)
CMH52b	23	4	14	5	1:2:1	1.17	n.d. (2)	—[d]
CMH52c	41	13	22	6	1:2:1	2.60	1	1[d]
CMH58	32	7	17	8	1:2:1	0.52	5	7
CMH65a	60	14	0	46	1:3	0.04	9	n.d. (3)
CMH65b	50	45	0	5	3:1	6.00*	6	n.d. (3)
CMH71	38	5	24	9	1:2:1	3.45	2	5
CMH81	47	21	22	4	1:2:1	12.48**	4	n.d. (2)
Dia-1	42	11	21	10	1:2:1	0.05	n.d. (2)	3
Dia-2	43	8	22	13	1:2:1	1.17	2	2[d]
EMH1	29	6	11	12	1:2:1	4.17	3	n.d. (3)
EMH5	48	13	29	6	1:2:1	4.12	6	n.d. (3)
EMH8	35	9	19	7	1:2:1	0.48	n.d. (2)	n.d. (3)
EMH14a	33	12	18	3	1:2:1	6.58*	4	n.d. (3)
EMH14b	33	11	18	4	1:2:1	4.34	9	n.d. (3)
Est	59	14	25	20	1:2:1	2.59	4	7[d]
Gal-1	40	10	24	6	1:2:1	2.40	3	3
Gdh	35	5	14	16	1:2:1	8.31**	2	n.d. (1)
Gs	60	6	41	13	1:2:1	9.69**	1	1[d]
Gsyn-c	33	20	0	13	3:1	3.74	6	3
Gsyn-n	27	7	15	5	1:2:1	0.63	4	7[d]
Lap-1	60	18	36	6	1:2:1	7.20*	6	3
Lap-2	54	9	26	19	1:2:1	3.78	n.d. (2)	3
Lghb-1	49	9	29	11	1:2:1	1.81	1	1
Lghb-2	51	41	0	10	3:1	0.79	n.d. (2)	n.d. (3)
Lghb-3	43	14	0	29	1:3	1.31	n.d. (2)	n.d. (3)
NcoI	37	11	15	11	1:2:1	1.32	3	n.d. (3)
Pep-1	60	16	24	20	1:2:1	2.93	5	7[d]
Pgd-p	60	13	30	17	1:2:1	0.53	5	7
Pgm-p	60	7	39	14	1:2:1	7.03*	1/2	2
Pi	60	16	0	44	1:3	0.02	3	3[d]
PMH68	41	10	24	7	1:2:1	1.68	2	n.d. (3)
PMH95	21	6	11	4	1:2:1	1.46	1	n.d. (3)
PMH110a	40	8	18	14	1:2:1	2.20	3	n.d. (3)
PMH110b	40	9	17	14	1:2:1	2.15	3	n.d. (3)
PMH110c	40	9	0	31	1:3	0.13	1/2	n.d. (3)
PMH111a	48	38	0	10	3:1	0.44	n.d. (2)	n.d. (3)
PMH111b	37	13	16	8	1:2:1	2.05	3	n.d. (3)
PMH111c	48	40	0	8	3:1	1.78	10	n.d. (3)
PMH111d	39	33	0	6	3:1	2.92	1/2	n.d. (3)
Px-1	24	5	16	3	1:2:1	3.00	2	5
Prx-1	60	19	31	10	1:2:1	2.76	n.d. (2)	6
Prx-3	60	14	37	9	1:2:1	4.10	3	6
RbcS	52	11	31	10	1:2:1	1.96	4	5
Rpl22	47	13	23	11	1:2:1	0.19	8	6
Rrn	55	7	37	11	1:2:1	7.14*	1	4/7
Scp	60	15	0	45	1:3	0.00	7	n.d. (3)
Skdh	58	10	34	14	1:2:1	2.27	4	7
Sp-1	60	27	25	8	1:2:1	13.70**	1	n.d. (3)
Sp-2	60	14	29	17	1:2:1	0.36	1	1[d]
Sp-3	60	33	21	6	1:2:1	29.70**	4	n.d. (3)
Sp-4	60	19	29	12	1:2:1	1.70	4	n.d. (3)

* $P \leq .05$.

** $P \leq .01$.

[a] Phenotypic designations: Lc = *Lens culinaris* parent; H = F_1 heterozygote; Le = *Lens ervoides* parent.

[b] n.d. = not determined for one of the following reasons: (1) = no variation, (2) = assorting independently of all markers tested, or (3) = homology uncertain.

[c] 1/2 = located on the section of the genome involved in the translocation.

[d] The marker designation is different in *Lens* than in *Pisum*. The *Lens*/*Pisum* comparison is as follows: *Act-1* = *Act*; *Aps-1* = *Acp-3*; CMH52c = C52b; *Dia-2* = *Dia-3*; *Est* = *Est-2*; *Gs* = *D*; *Gsyn-n* = *Gs-n1*; *Pep-1* = *Pep-3*; *Pi* = *Dpo*; *Sp-2* = *Lg-J*.

bined with the *Skdh–Est* segment by the intermediate markers *RbcS* and EMH14a (Figure 2). Linkage groups 2, 3, and 6 were significantly extended, and four new linkage groups were identified. Except for the markers near the translocation breakpoint, each of the linkage groups was clearly defined, and markers within a group did not display linkage at $P \leq .05$ with markers in other groups.

Mapping the Translocation Chromosomes

A considerable number of markers showed nonrandom assortment with the breakpoint. However, the relative map distances among the markers did not lead to the generation of an unambiguous linear map. Inspection of the genotypes of each individual revealed that at least three linkage groups were interacting in this region (Figure 1). The sequence *Rrn–Gs–Lghb–Aat-p* was readily identified, as was the *Rrn–Pgm-p–Aco-1* linkage. However, *Rrn* also showed tight linkage to *Dia-2*, thereby including the ribosomal cluster in a linkage group containing *Px-1*, CMH71, PMH68, and *Gdh*. Finally, PMH95 was linked to *Rrn* (11 recombination units), but its segregation pattern did not fit into any of the three linkage groups mentioned above. The linkage arrangement that best fits our data is presented in Figure 1.

Characterization and Distribution of Several Multigene Families

The actin, leghemoglobin, and RUBISCO sequences were present as small families of genes. Lentil DNA restricted with *Hin*dIII displayed five RFLPs when hybridized with the soybean actin clone, and at least three additional fragments were invariant. Four of the five polymorphisms assorted independently, mapping to three different linkage groups (Figure 2). *Act-3* and *Act-5* displayed tight linkage and may represent a cluster of actin genes near *Skdh*. *Act-4* assorted independently of all other loci analyzed. The alfalfa leghemoglobin clone gave four *Eco*RI polymorphisms. Two of these gave identical segregation patterns and were treated as a single gene, *Lghb-1*, mapping near *Gs* on chromosome 1. The remaining two RFLPs assorted independently of other segregating loci.

One cDNA clone, CMH75, was determined to contain at least part of the sequence encoding the small subunit of RUBISCO. This was ascertained (1) by obtaining similar pea DNA restriction patterns with CMH75 as were obtained by Polans et al. (1985) using the pea small

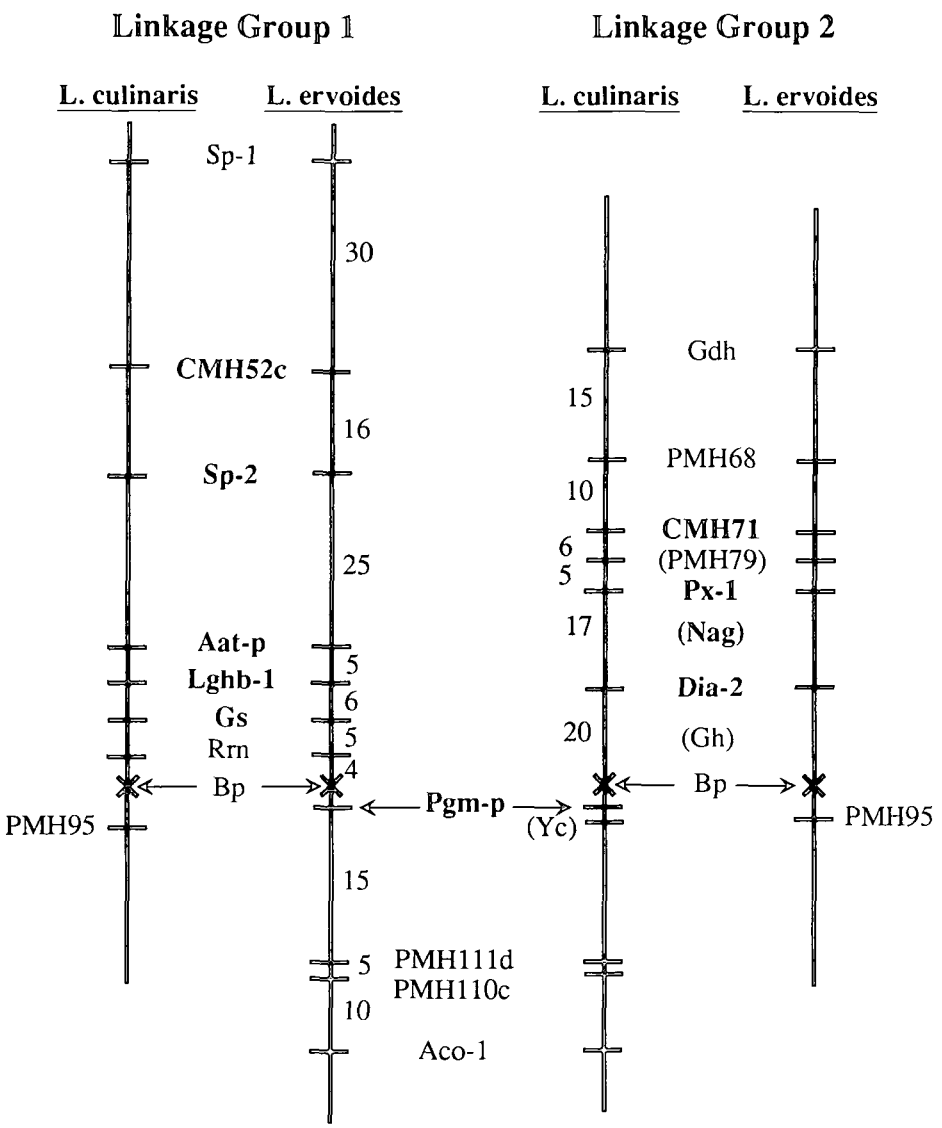

Figure 1. Linkage groups associated with the chromosomes involved in the reciprocal translocation existing between *Lens ervoides* #32 and *L. culinaris* #7. For each of the linkage groups the *L. culinaris* arrangement of genes is shown to the left and the *L. ervoides* arrangement to the right. The breakpoint is identified on each linkage group as 'BP.' Recombination values are given as the percentage of recombinants between adjacent loci. Shaded portions indicate regions of similarity with the garden pea linkage map. The shaded portion in linkage group 2 is broken into two sections because each appears to be homologous to a portion of a different chromosome in pea. Those loci with homologs actually mapped in garden pea are shown in bold type. Symbols in parentheses are loci that were not scored in the present cross but have been previously mapped to the respective linkage groups.

subunit clone, pSS15, and (2) by showing that the polymorphisms revealed in pea by CMH75 mapped to the same position as that known for the *rbcS* cluster (data not presented). Only one of the several RFLPs visualized by CMH75 was mapped, and this was linked to EMH14a and *Est* on linkage group 4. In addition, the RUBISCO holoenzyme showed polymorphism on starch gels and co-segregated with the CMH75 RFLP. Thus, the gene or genes on linkage group 4 are expressed in leaf tissue. The other RFLPs identified in this system appeared not to be linked to markers on linkage group 4.

Another cDNA clone, CMH65, gave a polymorphism of considerable interest. In *L. culinaris* it hybridized to two fragments (Figure 3), exhibiting a low-copy-number phenotype, as might be expected for a cDNA clone. In *L. ervoides*, however, the homologous sequence is present in 20 or more copies and the probe hybridized intensely to many *Eco*RV fragments (Figure 3). Two unlinked RFLPs were identified by CMH65, one of which (CMH65b) was a single 3.7-kb band present in *L. culinaris* but not in *L. ervoides*. The other CMH65 polymorphism involved nearly all the remaining fragments in the pattern and displayed loose linkage with EMH14b on linkage group 9 (Figure 2). A third segregating 6.6-kb polymorphism is evident in Figure 3. A band of similar size was present in CMH65a "plus" phenotypes and interfered with the scoring of this third polymorphism. We were not able to score a sufficient number of CMH65a "minus" individuals to locate this third polymorphism on the linkage map. In pea the probe hybridized to a single, approximately 15-kb *Eco*RV fragment (unpublished data).

Comparison with Pea Map

Of the markers mapped in *Lens*, 36 had homologous or possibly homologous counterparts mapped in pea. Only one (PMC119) of the random genomic clones (EMH- and PMH- designations) hybridized to pea DNA under relatively stringent conditions (two washes in 2 × SSC at 65°C). Thus, only the random cDNA clones and clones containing known genes were particularly useful for the examination of linkage conservation. Isozyme loci also were helpful, although in complex systems such as diaphorase, homology between specific pea and lentil isozymes was difficult or impossible to determine.

When the arrangement of markers on the lentil map is compared to that known for pea (Figure 4), eight regions can be identified where loci syntenic in pea are also syntenic in lentil. The sequence of markers from *Gs* to CMH52c on linkage group 1 is paralleled in pea by the sequence *D* through CMH52b, although at least one inversion, reversing the relative positions of *Aat-p* and the locus coding the seed protein (*Sp-2* or *Lg-J*), is required for the best match. *Pgm-p* is linked to a diaphorase locus in both pea (*Dia-3*) and lentil (*Dia-2*), although the homologous nature of the diaphorase loci has not yet been demonstrated. Group 2 also contained the CMH71-*Nag* region, which has its counterpart on chromosome 5 of pea. In pea, the *Pgm-p–Dia-3* segment is on a different chromosome than the CMH71-*Nag* segment. For this reason we have shaded these regions separately in lentil (Figure 1), despite their being on the same chromosome. Much of linkage group 3 in lentil appears to correspond to part of chromosome 3 in pea, and the lower portion of linkage group 4 is very similar to part of chromosome 7 of pea. Linkage groups 5 and 6 find parallels on chromosomes 7 and 3, respectively, of pea. Four of the five short linkage groups do not have corresponding regions in pea; however, many

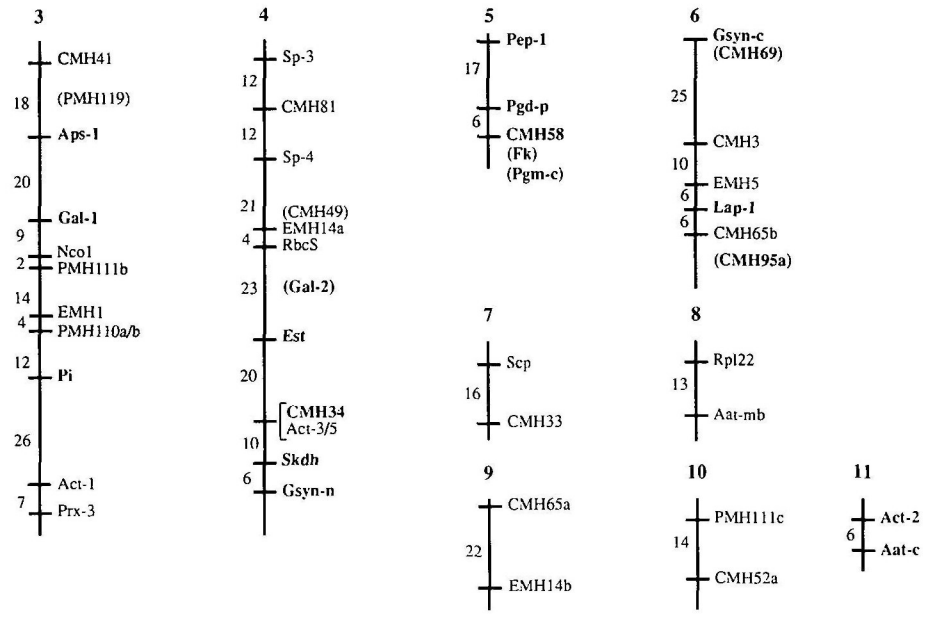

Figure 2. Additional multilocus linkage groups identified in the *Lens ervoides* #32 × *L. culinaris* #7 progeny. Numbers along the linkage groups reflect the recombinant fraction obtained between adjacent loci. Shaded portions indicate regions of similarity with the garden pea linkage map. Those loci within these shaded regions that have homologs mapped in pea are shown in bold type. Symbols in parentheses are loci that were not scored in the present cross but have been previously mapped to the respective linkage groups.

of the markers on these groups have yet to be located on the pea map.

Discussion

The linkage data generated for F_2 and F_3 populations from the cross *L. culinaris* × *L. ervoides* confirmed much of the genetic map previously established from studies on *L. culinaris* intraspecific crosses or *L. culinaris* × *L. orientalis* progenies (Havey and Muehlbauer 1989; Muehlbauer et al. 1989; Vaillancourt 1989). Except for the translocation region, the arrangement of the RFLP markers appeared to be co-linear with those mapped by Havey and Muehlbauer (1989). In addition, Havey detected linkage between CMH49 and CMH81 at an intensity of 33 cM and between *Aat-p* and a CMH52 fragment at an intensity of 36 cM in the progenies he studied (Havey MJ, personal communication). Our own data on CMH3 are very limited (Table 1), but its linkage with *Lap-1* and CMH69 was well documented by Havey and Muehlbauer (1989).

The *L. ervoides* × *L. culinaris* progeny had far more polymorphisms segregating (66) than any of the previous crosses used to develop the linkage map. Nearly all of the cloned genomic and cDNA sequences examined displayed polymorphism in this interspecific cross, about double the frequency at which RFLPs were found in crosses between more closely related taxa (Havey and Muehlbauer 1989). A number of morphological and isozyme loci that were not segregating in the *L. culinaris* × *L. ervoides* progeny or that did not exhibit linkage to other markers have been mapped by others (Havey and Muehlbauer 1989; Tadmor et al. 1987; Vaillancourt 1989). Where possible, we have included these in parentheses at their approximate location on the linkage map.

The primary goal of this study was to compare the pea and lentil genetic maps for conserved linkages. For such a comparison the precise distance between adjacent markers was not as important as the assignment of each marker to its correct linkage group. It also was important to have as much as possible of the lentil genome marked. Hence, we used a relatively small population and did not attempt to complete the data set for markers such as CMH3 that had been either clearly mapped in previous studies or mapped close to other markers for which we already had more extensive data. Although our linkage map for *Lens* is not complete, if we allot 10 cM for each terminus of a linkage group and 20 cM for each of the unmapped markers the coverage approaches the 10-Morgan anticipated minimum length for the lentil genome (Havey and Muehlbauer 1989). Each of the mapped markers displays linkage with only one region of the map, and the probability of the linkage being due to chance is less than .005. Thus, we are confident that each of the 11 linkage groups reported reflects a syntenic relationship of the markers. The smaller linkage groups undoubtedly represent chromosomal fragments that need to be connected by additional markers. We acknowledge that map distances given are associated with relatively high standard error values and

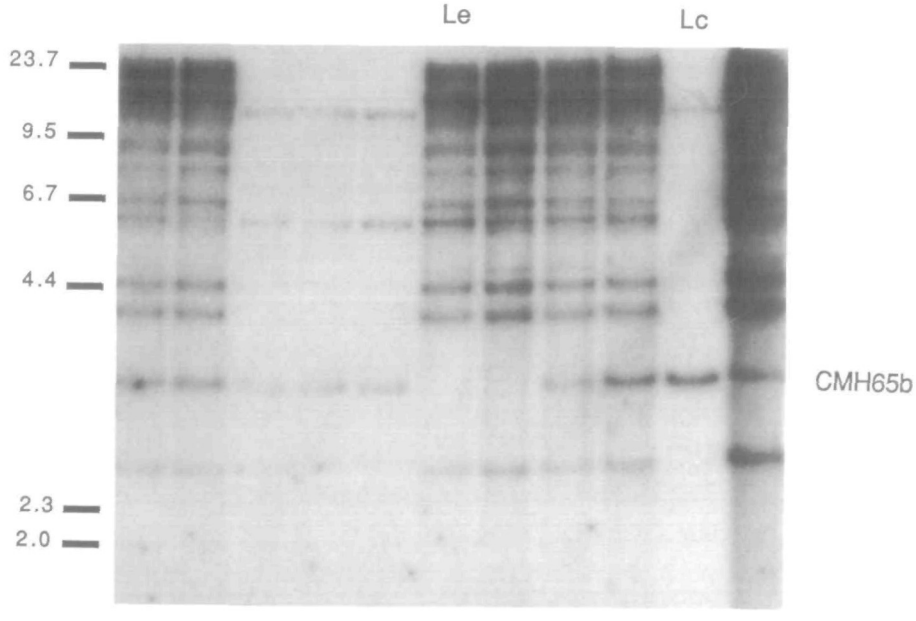

Figure 3. Restriction fragment polymorphism observed in 11 F_2 progeny using the cDNA clone, CMH65. The parental phenotypes are identified as Lc (*Lens culinaris*) and Le (*L. ervoides*). The cloned segment was originally isolated from a *L. culinaris* cDNA library and hybridizes to only two *Eco*RV fragments. One of these fragments (CMH65b) is not present in the *L. ervoides* phenotype. However, many additional fragments containing sequences homologous to CMH65 are present in the *L. ervoides* genome, most segregating as a single unit.

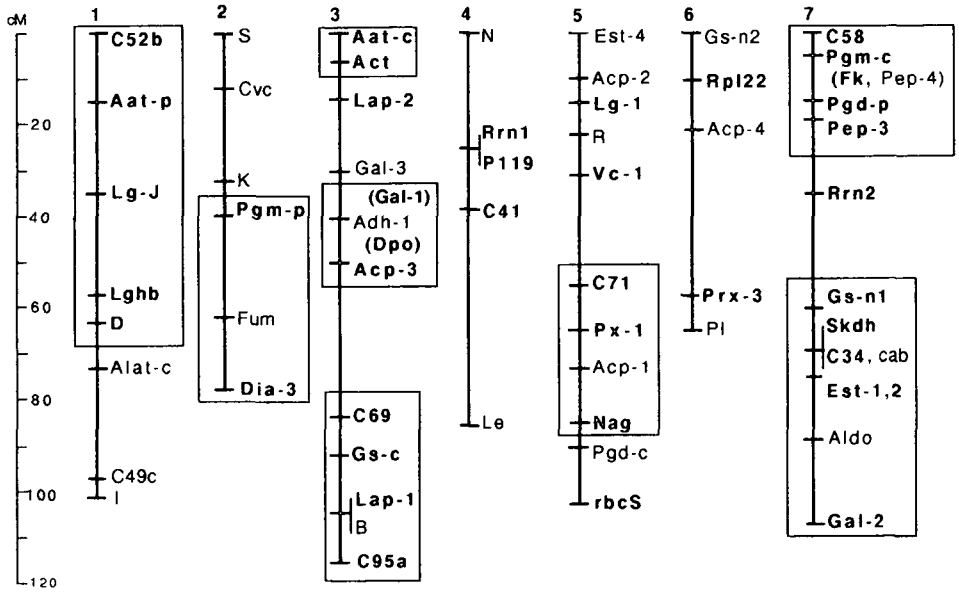

Figure 4. Outline of the linkage map for the garden pea showing regions of similarity with lentil (boxed). Those loci mapped in both pea and lentil are in bold type. Locus symbols C. . . and P. . . on the above map refer to the same clones that were designated CMH. . . and PMH. . . in lentil. A more complete description of the loci listed can be found in Weeden and Wolko (1990).

will require further analysis if precise estimates of recombination frequencies are desired.

The finding that a cDNA clone, CMH65, showed a vastly different copy number in *L. ervoides* than in *L. culinaris* was unexpected. Low-copy-number sequences isolated from tomato were also low-copy-number in pepper (Tanksley et al. 1988). Similarly, our results indicate a general tendency for copy number to be conserved between pea and lentil. The CMH65 insert appeared to be a major exception to this generalization, being low copy in pea and in the lentil genome from which it was isolated; yet it hybridized to many segments in the genome of *L. ervoides*, most of which appeared to be tightly linked. We do not know the function of this sequence, nor did we determine if the gene displays a higher level of expression in *L. ervoides*.

Although the map generated for the *L. ervoides* × *L. culinaris* progeny agreed with most previous reports of linkages in lentil, several linkages could not be verified. Muehlbauer et al. (1989) reported linkage between *Lap-1* and *Lap-2* and between *Aat-mb* and *Skdh*. Neither of these linkages was evident in the present study. *Lap-2* assorted independently of *Lap-1* and other loci on linkage group 6. *Aat-mb* was linked to *Rpl22*, but neither of these loci showed linkage to *Skdh* or other segregating loci in that linkage group. These differences indicate that chromosomal rearrangements may have occurred between *L. culinaris* #7 and the *L. culinaris* and *L. orientalis* lines used in the previous analyses.

Another inconsistency with previous results is the placement of the ribosomal gene cluster on chromosome 1. Tadmor et al. (1987) reported that the chromosome containing the nucleolar organizer region (and presumably the ribosomal DNA gene cluster) was not one of those involved in the translocation between *L. culinaris* #7 and *L. ervoides* #32. However, we found that the ribosomal DNA cluster mapped very near the breakpoint. An alternative explanation for the observed linkage would be the presence of genes in the region of the breakpoint that interacted with the nucleolar organizer region in such a way that recombinant phenotypes were strongly selected against. At present we have no reason to favor this alternative. Our order of markers on this linkage group differs slightly from that published by Tadmor et al. (1987). However, the gene order must be considered tentative until a more thorough study can be performed with parents that lack chromosomal rearrangements.

When this lentil linkage map is compared with the map recently published for the garden pea (Weeden and Wolko 1990), eight conserved linkages (indicated by shaded boxes in Figures 1 and 2) can be postulated. Very strong evidence for conserved linkage relationships exists for regions on linkage groups 1, 2, 4, 5, and 6. Inspection of the pea map indicates that most of the linkages on chromosomes 3 and 7 can be observed in lentil, although pea chromosome 3 appears to be divided into three linkage groups in lentil and chromosome 7 into two linkage groups. A total of about 250 cM, or about 40%, of the linkage map can be included within these regions. This estimate does not account for changes, such as inversions or deletions, within a linkage group. However, it does suggest that a significant proportion of the linkage map will be common to most members of the Vicieae. Thus, the Vicieae apparently represent an intermediate case of linkage conservation, with more divergence than that found between tomato and potato (Bonierbale et al. 1988) but considerably less divergence than that observed between tomato and pepper (Tanksley et al. 1988).

Two pairs of apparently homologous loci conditioning morphological characters were identified during our comparison. The genes *Gs* in lentil and *D* in pea both control the expression of anthocyanin pigment on the epicotyl and internodes (Ladizinsky 1979; Marx and Nozzolillo 1979). In pea, most alleles of *D* also cause anthocyanin synthesis in leaf axils and on stipules. The placement of both loci close to leghemoglobin genes on the respective genome maps and the finding that other linkage relationships have been conserved in this region provides further evidence that these two loci are homologous.

The second pair of morphological genes that confers similar polymorphisms and occupies similar positions in regions with conserved linkages are *Dpo* (in pea) and *Pi* (in lentil). Both genes in the homozygous recessive state inhibit the dehiscence of the pod when it ripens. Wild populations of both pea and lentil possess strongly dehiscent pods as a mechanism for seed dispersal. The recessive, indehiscent pod type has been selected in both crops during domestication in order to facilitate harvesting the seed. Apparently, in both crops the same locus may have been the primary focus of the selection.

Perhaps the most interesting and conspicuous of the linkages not conserved between pea and lentil is the region including the ribosomal genes. Two ribosomal gene clusters exist on the pea map (Jorgensen et al. 1987; Polans et al. 1985). One of these is linked to CMH41 and one of the fragments detected by PMH119; the other displays linkage with *Pgd-p* and *Pgm-c*. The *Pgd-p–Pgm-c* linkage is definitely conserved in lentil (linkage group 5, Figure 2) and the CMH41–PMH119 might be (linkage group 3). In lentil, the ribosomal clus-

ter is not associated with either of these linkage groups but shows relatively tight linkage to another series of markers (*Gs-Lghb-Aat-p*) that is also present in both pea and lentil.

The genes encoding the small subunit of RUBISCO also are in a different arrangement in lentil than in pea. In the former species there appear to be several unlinked clusters of *RbcS* genes. This distribution is similar to that found in tomato (Vallejos et al. 1986) but differs from the single cluster identified in pea (Polans et al. 1985). We can further conclude that the *RbcS* gene or genes actively transcribed and translated in leaf tissue appear to be in different linkage groups in pea and lentil. In pea the only cluster is linked to *Px-1-Nag-6pgd-c* on chromosome 5, whereas in lentil the gene or genes are linked to markers that in pea map to chromosomes 1 (CMH49) and 7 (*Gal-2, Est*).

Despite these rearrangements in gene order and copy number, as well as the presence of numerous inversions and translocations within both *Pisum* and *Lens*, it appears that significant portions of the lentil and pea genomes remain co-linear. Linkages conserved between lentil and pea are likely to be present in sweet pea, faba bean, and perhaps other closely related genera. Indeed, recent studies on the linkage relationships of isozyme loci in chickpea suggest that at least two of the linkage groups conserved between pea and lentil exist in this species (Gaur and Slinkard 1990a,b). Thus, the Viceae and the closely related Cicereae represent a pool of species in which genetic studies on one crop will be directly applicable to several others. The group also may provide an excellent system for the study of the evolution of homologous characters.

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