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Genetic mapping of an ancient translocation in the genus *Lens*

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Summary. Segregation of 18 marker genes was monitored in selfed progeny of a *Lens culinaris* × *L. ervoides* hybrid; five linkage groups were mapped, one of which contained a reciprocal translocation break-point that differentiates between the parents. Four markers were found to be linked to the translocation break-point: *Aco-1* and *Pgm-2* on one side and *Gs* and *Got-2* on the other. The gene pairs on both sides of the translocation are not linked in *L. culinaris* or in *L. orientalis*. The *L. ervoides* gene order was also found in *L. odemensis* but with significantly reduced map distances. Analysis of monogenic segregations in a number of *Lens* interspecific crosses revealed some consistent patterns of deviations from the expected Mendelian ratios. The factors responsible for these unequal segregations, genotypic effects on recombination frequencies, negative interference, and the possible ancient origin of the translocation are discussed.

Key words: *Lens* – Isozymes – Translocation break-point – Linkage – Evolution

Introduction

Mapping of chromosomal translocations is aided by the ability to carry out cytological analysis and by the fact that the linkage order of marker genes may vary. Association between genetic markers and a translocation break-point was discovered in rye (Figueiras et al. 1984), maize (Kleese and Phillips 1972), *Glycine max* L. (Sacks and Sadanaga 1984) and *Capsicum* (Tanksley 1984), and now also in lentils.

Members of the genus *Lens* Miller are diploid ($2n = 14$) annuals that reproduce predominantly by self-pollination. The *Lens* species share a similar karyotype of four metacentric chromosomes, one of which carries a satellite, and three acrocentric chromosomes (Ladizinsky 1979a). The species of *Lens* form two crossability groups (Ladizinsky et al. 1984); the first includes *L. culinaris*, *L. orientalis*, and *L. odemensis* and the second includes *L. ervoides* and *L. nigricans*. The members of the different crossability groups are reproductively isolated from one another due to embryo break-down of their hybrids. Within the same group, crosses give rise to variable F_1 hybrids; however, because of the considerable chromosomal repatterning in the genus, the hybrids are often only partially fertile.

The genetics of eight electrophoretically detectable isozyme markers and a morphological marker was determined in crosses between *L. culinaris* and *L. orientalis* (Zamir and Ladizinsky 1984). The allozymic divergence was used to study the evolutionary relationships of the genus *Lens* (Pinkas et al. 1985). Two main branches were observed in the phylogeny: the first included *L. nigricans* and in the second branch *L. culinaris* and *L. orientalis* were clustered together, separated from *L. odemensis* and *L. ervoides*.

By means of embryo culture, Ladizinsky et al. (1985) obtained a hybrid between *L. culinaris* and *L. ervoides*. The hybrid was heterozygous for a single reciprocal translocation and 18 assayable genetic markers. In the present study we established the linkage relationships of this translocation break-point and determined the divergence of the mutation in the genus.

Materials and methods

Plant material

The six parental lines involved in the crosses, their origins and the number of analyzed plants are presented in Table 1.

All seeds germinated and were planted in a greenhouse at temperatures of 25/18 °C (day/night). Each F_2 seed from Cross I was cut in half; the cotyledon section was used for extraction of total seed proteins and the radicale section for sowing.

Table 1. Parental lines involved in crosses

Cross no.	Female parent	Origin	Male parent	Origin	Tested generation	No. of plants
I	*L. culinaris* 7	Ethiopia	*L. ervoides* 32	Israel	F_2	107
					F_3	[a]
II	*L. culinaris* 162	Spain	*L. odemensis* 37	Israel	F_2	56
III	*L. culinaris* 160	Canary Islands	*L. odemensis* 37	Israel	F_2	42
IV	L. culinaris 158	Portugal	*L. odemensis* 37	Israel	F_2	22

[a] A minimum of six selfed progeny from each heterozygous F_2 plant were analyzed for their isozymic genes. Eight different seeds were analyzed for seed proteins. For *Gs* a minimum of 16 selfed progeny were scored

Seeds from each F_2 plant were collected separately. Equal numbers of plants from each F_3 family were pooled to form the bulk F_3 (Table 1).

Cytology

Flower buds were fixed in a 3 : 1 solution of absolute ethyl alcohol and glacial acetic acid for 24 h, stored in 70% ethyl alcohol and later stained with aceto-carmine. Chromosome pairing was analyzed on a minimum of 5 pollen mother cells per plant at MI. Pollen viability was measured on 200 grains from each F_2 plant stained with aceto-carmine. Grains that were fully stained and regularly shaped were considered viable.

Morphological markers

The epicotyl color (*Gs*) and pod dehiscence (*Pi*) were scored according to Ladizinsky (1979b).

Starch gel electrophoresis

Crude extracts were obtained by macerating 12 leaflets in buffer A (for systems 1 and 2) and buffer B (system 3) (Zamir and Ladizinsky 1984). Three gel and electrode buffer systems were used to resolve the enzyme bands: 1) Tris citrate/boric acid mixture pH 7.7 (Tanksley 1979), for the assaying of acid phosphatase (APS), aconitase (ACO), leucine aminopeptidase (LAP) and peptidase (PEP); 2) Tris citrate/boric acid mixture pH 8.1, for the assaying of diaphorase (DIA), esterase (EST), glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM) and peroxidase (PRX); 3) histidine/Tris citrate mixture (Zamir and Ladizinsky 1984), for the assaying of malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGD) and shikimic dehydrogenase (SKDH). Staining for enzyme activity was done according to Vallejos (1983).

SDS-PAGE

SDS polyacrylamide gel electrophoresis (PAGE) for the separation of total seed proteins was carried out using 15% bisacrylamide gels. Extraction of the proteins, preparation of the gels, running conditions, staining and destaining were according to Galili and Feldman (1983).

Statistical analysis

Chi square tests were used to assess the goodness of fit to the expected Mendelian ratios for single loci, the independence segregation of pairs of loci and the homogeneity of monogenic and digenic segregations.

Recombination frequencies between loci were estimated by the maximum likelihood equations given by Allard (1956). Linkage between the translocation break-point and codominant markers was calculated using the following maximum likelihood formula:

$$0 = (1-2p) \times \{(-2)\,(a+e)/(1-2p\,(1-p)) + (b+c+f)/p\,(1-p) - d/(1-2p\,(1-p))\}$$

with the standard error:

$$\text{s.e.} = \sqrt{p\,(1-p)\,(1-2p\,(1-p))/2n\,((1-2p)^2)}\,,$$

where p = recombination frequency; a = the observed number of plants homozygous for the parental chromosome arrangement and the *L. culinaris* allele of the tested marker; b = the observed number of plants heterozygous for the reciprocal translocation and homozygous for the *L. culinaris* allele; c = the observed number of plants homozygous for the parental chromosome arrangement and heterozygous for the tested locus; d = the observed number of plants heterozygous for the reciprocal translocation and heterozygous for the tested locus; e = the observed number of plants homozygous for the parental chromosome arrangement and for the *L. ervoides* allele; f = the observed number of plants heterozygous for the reciprocal translocation and homozygous for the *L. ervoides* allele; and n = sample size.

The maximum likelihood formula for estimating recombination frequency between a codominant marker and a translocation break-point in an F2 population was calculated as follows: consider two parental lines, P_1 and P_2, that differ in alleles of a codominant marker (A) and a reciprocal translocation (T). The two homozygotes TT and tt are fertile (F) while the heterozygotes for parental chromosomal arrangement Tt are semifertile (SF).

Genotype of P_1 = AATT

Genotype of P_2 = aatt

Genotype of F_1 = AaTt

Assuming a recombination frequency p between the two loci:

Gametes produced by the F_1	Expected frequency
AT	p/2
at	p/2
At	(1 − p)/2
aT	(1 − p)/2

The four types of gametes unite randomly in 16 combinations that form the following six phenotpyes in an F_2 (for each phenotype the observed number (a–e) is scored in the population):

	AA		Aa		aa	
	exp.	obs.	exp.	obs.	exp.	obs.
SF	$p(1-p)/2$	b	$(1-2p(1-p))/2$	d	$p(1-p)/2$	f
F	$(1-2p(1-p))/4$	a	$p(1-p)$	c	$(1-2p(1-p))/4$	e

According to the maximum likelihood formula the parameter H will achieve its maximum value when the maximizing p value is used:

$$H = n!/a!\,b!\,c!\,d!\,e!\,f! \times ((1-2p(1-p))/4)^a \times (p(1-p)/2)^b \times (p(1-p))^c \times ((1-2p(1-p))/2)^d \times ((1-2p(1-p))/4)^e \times (p(1-p)/2)^f,$$

L, the logarithm of H, will also have its maximum value when the same p is substituted:

$$L = \log(n!/a!\,b!\,c!\,d!\,e!\,f!) + a\log((1-2p(1-p))/4) + b\log(p(1-p)/2) + c\log(p(1-p)) + d\log((1-2p(1-p))/2) + e\log((1-2p(1-p))/4) + f\log(p(1-p)/2).$$

After differentiating L by p the estimation equation takes the following form that is equal to zero when the most likely p value is substituted:

$$dL/dp = (1-2p) \times \{(-2)(a+e)/(1-2p(1-p)) + (b+c+f)/p(1-p) - d/(1-2p(1-p))\}$$

with a standard error of

$$\text{s.e.}(p) = \sqrt{p(1-p)(1-2p(1-p))/2n((1-2p)^2)}.$$

Results

Segregations in cross I (L. culinaris × L. ervoides)

Cytological analysis. Cytogenic analysis of F_1 and F_2 pollen mother cells revealed that metacentric and acrocentric chromosomes were involved in the translocation (Fig. 1A). The metacentric chromosome which carries the nucleolus is not involved in the quadrivalent (Fig. 1B).

Pollen viability was assayed in 104 plants from the F_2 population. The frequency distribution of pollen viability showed two peaks, one at 50% and the other at 90% (Fig. 2). In a random sample of five plants with viability higher than 85%, only cells with seven bivalents were observed. In 22 plants with less than 65% viability, only cells with five bivalents and a quadrivalent were observed. On the basis of these data we took the phenotype of all the plants with pollen viability higher than 85% to be homozygous for the parental chromosome arrangements, whereas plants with viability lower than 65% were assumed to be heterozygous for the reciprocal translocation. Cytogenetic determination was carried out for 14 plants whose pollen viability was between 65 and 85%. Sixty-three plants were found to be heterozygous for the reciprocal translocation and 41 were homozygous.

Monogenic segregations. In the F_2 of cross I we assayed 12 isozymic genes: *Got-2, Pgm-2* (Fig. 3), *Aco-1, Aps-1, Prx-3, Pep-1, 6-Pgd-1, Est-1, Skdh-2, Got-3, Lap-1* and *Prx-1,* four seed protein genes: *Sp-1, Sp-2, Sp-3* and *Sp-4* (Fig. 4), two morphological markers (*Gs, Pi*) and the translocation break-point, *Bp* (Table 2). The genotype of each F_2 plant was determined on the basis of seed and plant assays and was verified by progeny tests for all loci except *Pi* and the translocation break-point (*Bp*). Segregation of nine of the 19 loci deviated significantly ($P < 0.05$) from the expected Mendelian ratios. In the bulk F_3, eight codominant loci and eight

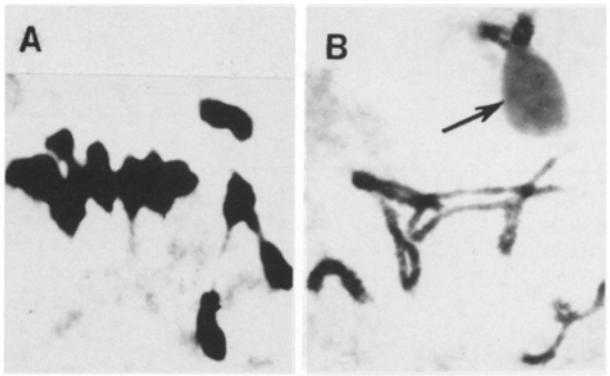

Fig. 1 A, B. Pollen mother cells stained with aceto-carmine at: **A** MI and **B** diakinesis; arrow indicates the nucleolus

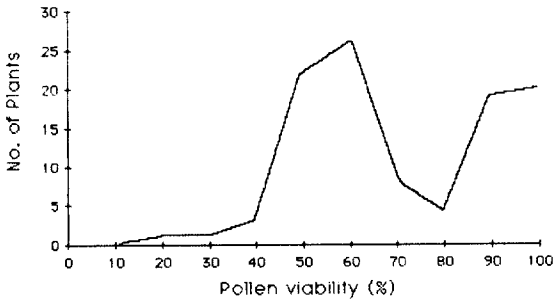

Fig. 2. Distribution of pollen viability among 104 F_2 plants of cross I

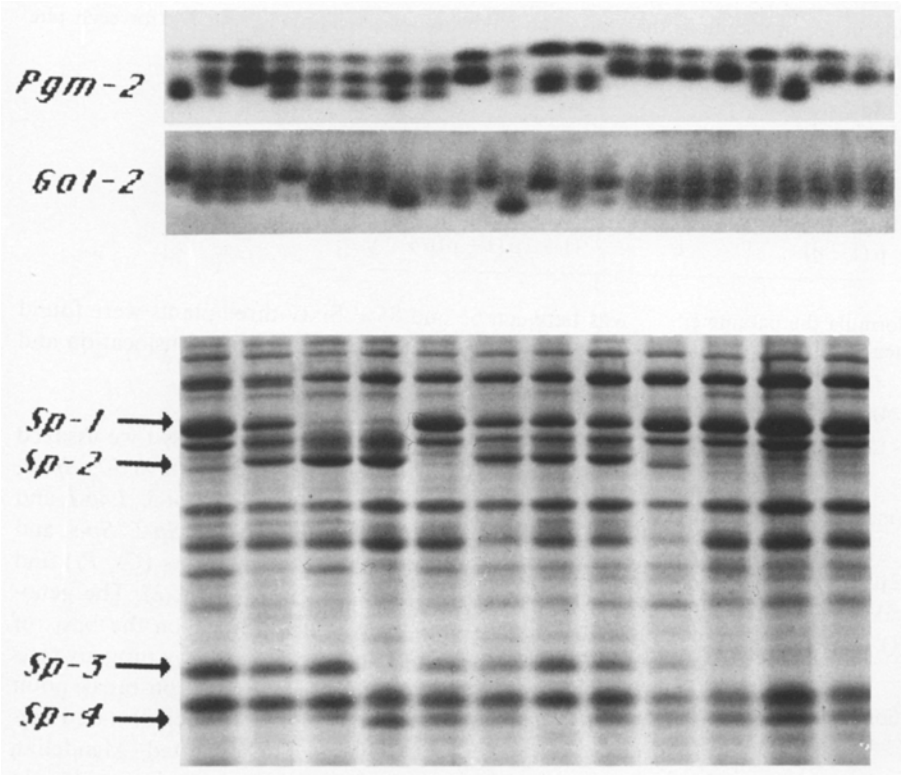

Fig. 3. Zymograms showing the allelic variation for the translocation break-point *Pgm-2* and *Got-2*

Fig. 4. SDS-PAGE of the total seed proteins in the F_2 of cross I. *Sp-1* (40 Kilodalton (Kdal)) and *Sp-3* (19 Kdal) originate from *L. culinaris* while *Sp-2* (37 Kdal) and *Sp-4* (15 Kdal) originate from *L. ervoides*

Table 2. Monogenic segregations of 19 genetic markers in the F_2 of cross I

Loci[a]	1	2	3	χ^2
Sp-1	47	35	10	35.05***
Sp-2	25	44	34	3.75
Gs	18	44	44	15.81***
Got-2	21	63	23	3.45
Bp	41	63	–	4.24*
Pgm-2	20	61	26	2.77
Aco-1	35	53	17	6.18*
Aps-1	30	44	31	10.34**
Pi	30	—— 77 ——		0.37
Prx-3	22	66	17	7.42*
Pep-1	31	43	28	2.69
6-Pgd-1	20	62	25	3.17
Est-1	34	37	36	10.25**
Skdh-2	20	61	25	2.88
Sp-3	51	40	12	34.67***
Sp-4	29	50	24	0.57
Got-3	29	51	27	0.31
Lap-1	40	50	17	10.35**
Prx-1	35	45	22	4.73

[a] In all loci except *Pi* and *Bp*: 1 = homozygous for the *L. culinaris* allele; 2 = heterozygous; 3 = homozygous for the *L. ervoides* allele. In *Bp*, parental types share a similar phenotype and *Pi* was scored as a dominant/recessive locus
*, ** and *** significant χ^2 at levels of 0.05, 0.01 and 0.001, respectively

Table 3. Monogenic segregation of 16 genetic markers in the F_3 of cross I

Loci[c]	1	2	3	χ^2	χ^2 homo.[a]
Sp-1[b]	—— 279 ——		53	13.98*	1.48
Sp-2[b]	63	—— 209 ——		0.40	0.05
Gs[b]	102	—— 596 ——		40.00***	0.41
Got-2	74	232	92	12.52***	0.15
Pgm-2	36	154	75	18.45***	1.79
Aco-1	76	164	57	5.67	2.39
Aps-1[b]	86	—— 205 ——		4.82*	0.04
Prx-3	78	152	34	20.70***	3.00
6-Pgd-1	71	160	69	1.36	1.19
Est-1[b]	41	—— 138 ——		0.63	3.93*
Skdh-2	41	116	43	5.16	0.23
Sp-3[b]	—— 259 ——		61	5.70*	3.00
Sp-4[b]	117	—— 283 ——		3.63	0.05
Got-3	52	1167	48	16.9***	6.96**
Lap-1	113	164	66	13.550***	9.99
Prx-1[b]	120	—— 151 ——		59.50***	3.03

*, ** and *** significant χ^2 at levels of 0.05, 0.01 and 0.001, respectively
[a] χ^2 for homogeneity with F_2 segregations. In cases where the marker was scored as a dominant/recessive in bulk F_3, homogeneity was tested according to the two genotypic groups
[b] Scored as a dominant/recessive locus
1, 2, and 3 are as in Table 2

Table 4. Digenic segregations indicating linkage in the F_2 of cross I

Tested loci[d]		1/1	3/3	1/3	3/1	2/2	1/2	3/2	2/3	2/1	χ^2[a]	Distance (cM)
1st	2nd											
Linkage group I												
Sp-1	*Sp-2*	19	6	10	1	21	18	3	12	2	18.0**	36.7±4.7
Sp-2	*Gs*	10	19	6	2	23	9	12	17	4	18.1**	32.7±4.2
Sp-2	*Got-2*	10	11	3	3	30	12	20	7	7	12.5*	34.4±4.3
Sp-2	*Pgm-2*	10	12	45	2	29	11	20	9	6	14.2**	34.1±4.3
Gs	*Got-2*	15	17	1	1	35	2	26	4	5	66.8***	21.6±3.3
Gs	*Bp*[b]	16	18	–	–	37	1	25	6	–	33.3***	25.3±4.8
Gs	*Pgm-2*	17	20	0	1	37	1	23	5	2	66.8***	16.9±2.9
Gs	*Aco-1*	13	14	0	13	32	5	16	3	9	30.5***	34.8±4.3
Got-2	*Bp*[b]	17	18	–	–	56	3	4	6	–	56.9***	9.4±2.7
Got-2	*Pgm-2*	15	18	0	0	50	6	5	8	5	89.1***	12.0±2.4
Got-2	*Aco-1*	14	10	0	4	38	7	8	7	17	28.6***	26.5±3.7
Bp[b]	*Pgm-2*	17	22	–	–	57	2	–	4	2	74.2***	5.6±1.7
Bp[b]	*Aco-1*	14	11	–	–	39	14	–	5	19	9.7**	35.9±8.7
Pgm-2	*Aco-1*	12	11	0	3	35	7	11	6	20	26.5***	24.6±3.4
Linkage group II												
Aps-1	*Pi*[c]	19	28	11	3	–	–	–	37	7	27.2***	23.3±5.1
Pi[c]	*Prx-3*	10	17	0	12	–	19	47	–	–	10.0**	31.5±5.5
Linkage group III												
Pep-1	*6Pgd-1*	16	20	1	1	36	14	7	4	3	69.7***	17.1±2.9
Linkage group IV												
Est-1	*Skdh-2*	9	20	2	5	28	22	11	3	6	32.7***	30.7±4.0
Linkage group V												
Sp-3	*Sp-4*	24	11	1	7	30	4	6	0	20	52.6***	25.3±3.6

*, ** and *** significant χ^2 at 0.05, 0.01 and 0.001 levels, respectively

[a] χ^2 for independence

[b] The two parental phenotypes were indistinguishable, therefore, 1/1 + 1/3, 3/1 + 3/3 and 2/3 + 2/1 were scored together

[c] *Pi* was scored as a dominant/recessive locus

1, 2 and 3 are as in the previous tables

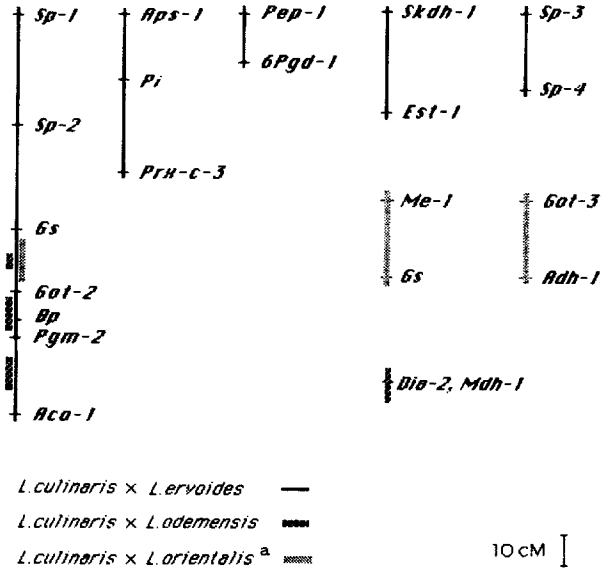

Fig. 5. Schematic linkage map of the genus *Lens*. [a] According to Zamir and Ladizinsky (1984)

dominant/recessive markers were analyzed (Table 3). Ten of the markers segregated unequally; seven of these loci deviated also in the F_2. Homogeneity between the segregations of the assayed genes in the F_2 and in the bulk F_3 was found in all cases except for *Est-1* and *Got-3* (Table 3).

Digenic segregations. Five linkage groups, covering a total map distance of 258 cM, were identified in the F_2 (Table 4, Fig. 5). Linkage group I included the translocation break-point and six markers, linkage group II had three markers and the rest of the linkage groups had two markers each. Linkages involving isozymes or seed proteins were verified in the bulk F_3; linkage group III was verified in selfed progeny of F_2 plant No. 35 (Table 5).

Mapping of the translocation break-point. In linkage group I the recombination frequency was 16.9 cM between *Gs* and *Pgm-2* and 21.6 cM between *Gs* and *Got-2*; however, we placed *Got-2* closer than *Pgm-2* to

Table 5. Digenic segregations indicating linkage in the F_3 of cross I

Tested loci[d]		1/1	3/3	1/3	3/1	2/2	1/2	3/2	2/3	2/1	χ^2[a]	Distance (cM)
1st	2nd											
Linkage group I												
Sp-1[b]	*Sp-2*[b]	38	22	73	3	–	–	–	–	–	3.8	30.8±7.7
Got-2	*Pgm-2*	29	51	2	0	109	15	15	19	8	193.6**	13.3±1.7
Got-2	*Aco-1*	23	18	3	2	70	13	28	15	15	52.6**	25.6±2.7
Pgm-2	*Aco-1*	19	21	2	2	72	9	28	14	18	55.8**	24.5±2.7
Linkage group IV												
Est-1[b]	*Skdh-2*	23	17	11	4	–	49	11	–	–	20.0**	27.3±5.1
Linkage group V												
Sp-4[b]	*Sp-3*[b]	74	44	118	4	–	–	–	–	–	14.6**	24.2±6.0

Digenic segregations indicating linkage in the F_3 of F_2 plant number 35

Tested loci[d]		1/1	3/3	1/3	3/1	2/2	1/2	3/2	2/3	2/1	χ^2[a]	Distance (cM)
1st	2nd											
Linkage group I												
Got-2	*Aco-1*	6	3	0	0	18	7	5	6	5	9.8*	27.5±5.5
Linkage group III												
Pep-1	*6Pgd-1*	11	3	0	0	20	5	5	2	4	23.5**	17.7±4.3

* and ** significant χ^2 at levels of 0.05 and 0.001, respectively
[a] χ^2 for independence
[b] Scored as a dominant/recessive locus
1, 2 and 3 are the as in the previous tables

Gs. This decision was taken in light of the findings of a previous study (Zamir and Ladizinsky 1984), in which *Gs* and *Got-2* were linked to each other whereas *Pgm-2* had segregated independently of this linkage group (Fig. 5). In the present study, the linkage between *Pgm-2* and *Got-2* occurred because of the translocation, and the break-point (*Bp*) was found to be located between them.

The map distances between *Aco-1* and *Pgm-2* (24.6 cM in the F_2 and 24.5 cM in the F_3), between *Aco-1* and *Got-2* (26.5 cM in the F_2 and 25.6 cM in the F_3) and between *Aco-1* and *Gs* (34.8 cM in the F_2) were also in accordance with the gene order proposed for linkage group I. Nevertheless, the map distances were not additive. The calculated map distance between *Aco-1* and *Got-2* (26.5 cM) was shorter than the sum of the distances between *Aco-1* and *Pgm-2* and between *Pgm-2* and *Got-2* (36.6 cM). A complete three-point test is not possible in this case. Three heterozygotic codominant genes can form eight (2^3) different gametes that can unite to form 27 different genotypes (Table 6). The identity of the gametes that form certain diploid genotypes cannot be determined: for example, an individual heterozygous at three loci (222 in Table 6) can result from four indistinguishable types of gamete combinations. However, there are still 20 genotypes whose parental gametes can be reliably identified. Table 7 presents the number of plants observed for each of these 20 genotypes in the F_2 and in a bulk of progeny of F_2 plants that were heterozygous in *Aco-1, Pgm-2* and *Got-2*.

On the basis of the map distances derived from an analysis of the sum of the homogeneous F_2 and F_3 data (Table 7), the expected rate of double crossing-over events was $0.144 \times 0.277 = 0.03989$. The observed frequency, $(7 + 6)/202 = 0.06436$, was significantly higher (obs./exp. $= 0.06436/0.03989 = 1.61344$; $P(Z) < 0.02$). Although this analysis is based on a restricted sample, we think it is nevertheless representative since the map distances calculated by this procedure are very similar to those presented in Tables 4 and 5.

Segregations in crosses II–IV (L. culinaris × L. odemensis)

Monogenic segregations. Three interspecific F_2 populations were assayed for five segregating isozymic genes and the epicotyl marker *Gs* (Table 8); six of 13 loci

Table 6. Expected genotypes in the selfed progeny of an individual heterozygous for three co-dominant genes

Female gamete	Male gamete							
	111	333	311	133	131	313	331	113
111	111*							
333	222[a?]	333*						
311	211*	322[b?]	311*					
133	122[c?]	233*	222[a?]	133*				
131	121*	232[e?]	221[d?]	132*	131*			
313	212[g?]	323*	312*	223[f?]	222[a?]	313*		
331	221[d?]	332*	321*	232[e?]	231*	322[b?]	331*	
113	112*	223[f?]	212[g?]	123*	122[c?]	213*	222[a?]	113*

1 = gamete with the allele of parent A or a plant homozygote for A allele; 2 = heterozygote; 3 = gamete with the allele of parent B or a plant homozygote for B allele; * = genotype resulting from only one combination of gametes; ? = genotype that can result from union of more than one combination of gametes. All combinations that give the same genotype are marked with the same letter (a–g)

Table 7. F_2 and bulk F_3 segregations of *Aco-1, Pgm-2* and *Got-2*

Genotypes			No. obs. in the F_2	No. obs. in the F_3	Crossing-over* in the gametes
Aco-1	*Pgm-2*	*Got-2*			
1	1	1	10	10	–
1	1	2	5	5	b
1	1	3	0	1	b
1	2	1	4	2	c
1	2	3	0	1	a+b
1	3	1	0	1	c+c
1	3	2	0	0	c+a
1	3	3	0	0	a+a
2	1	1	2	3	a
2	1	3	0	0	c+b
2	3	1	1	0	c+b
2	3	3	2	4	a
3	1	1	0	0	a+a
3	1	2	0	0	c+a
3	1	3	0	0	c+c
3	2	1	2	2	a+b
3	2	3	1	3	c
3	3	1	2	2	b+b
3	3	2	6	12	b
3	3	3	9	11	–
Total no. of known gametes:			44×2=88	57×2=114	
Total no. of parental gametes:			58	72	
Total no. of 'a' gametes:			6	10	
Total no. of 'b' gametes:			18	25	
Total no. of 'c' gametes:			6	7	

Recombination frequency between	in F_2	in F_3	in F_2+F_3
Got-2 and *Pgm-2*	(6+ 6)/88=0.136	(10+ 7)/114=0.149	29/202=0.144
Pgm-2 and *Aco-1*	(18+ 6)/88=0.273	(25+ 7)/114=0.281	56/202=0.277
Got-2 and *Aco-1*	(24+12)/88=0.409	(35+14)/114=0.430	85/202=0.421

* Parental type gamete, a = crossing-over between *Got-2* and *Pgm-2;* b = crossing-over between *Pgm-2* and *Aco-1;* c = double crossing-over

were found to deviate from the expected Mendelian ratios and in all cases the *L. culinaris* alleles were favored.

Digenic segregations. Digenic segregations of the three *L. culinaris* × *L. odemensis* crosses are presented in Table 9. The data from these crosses were homogeneous ($P > 0.10$). The cosegregation data of the markers *Gs, Got-2, Pgm-2* and *Aco-1* gave rise to a linear order similar to that of the *L. culinaris* × *L. ervoides* cross (Table 9), with significantly reduced map distances ($P < 0.001$, Fig. 6). In addition, a linkage group involving *Mdh-1* and *Dia-2* was identified.

Table 8. Segregations of the markers in crosses II–IV

Loci	1	2	3	χ^2
L. culinaris 158 × *L. odemensis* 37				
Pgm-2	8	13	1	5.18*
Got-2	7	14	1	4.91*
Mdh-1	8	11	3	2.27
Dia-2	8	11	3	2.27
L. culinaris 160 × *L. odemensis* 37				
Gs[a]	11	— 31 —		0.03
Pgm-2	14	19	9	1.57
Aco-1	13	22	7	1.81
Mdh-1	13	27	2	9.19*
L. culinaris 162 × *L. odemensis* 37				
Gs[a]	20	— 36 —		3.43
Got-2	20	18	18	7.29*
Pgm-2	21	19	16	6.68*
Aco-1	14	25	17	0.96
Mdh-1	22	29	5	10.39**

1 = Homozygous for the *L. culinaris* allele; 2 = heterozygous; 3 = homozygous for the *L. odemensis* allele
* and ** significant χ^2 at levels of 0.05 and 0.01, respectively
[a] *Gs* was scored as a dominant/recessive locus

Discussion

The main findings of this study are: 1) deviations from the expected monogenic segregation ratios are determined by the activity of single genes; 2) map distances between genes vary with genetic backgrounds; 3) negative interference occurs for markers linked on both sides of the translocation break-point; and 4) the mapped translocation is probably of ancient origin.

Monogenic segregations

In a previous study (Zamir and Tadmor 1986) we determined the proportions of unequally segregating marker genes in intra- and interspecific crosses in the diploid genera *Lens, Capsicum* and *Lycopersicon.* In the pooled data the proportion of loci deviating from the expected monogenic segregation ratios was significantly higher for progeny of interspecific hybrids (61/114 genes, 54%) than for progeny of intraspecific hybrids (7/52 genes, 13%).

Table 9. Digenic segregations indicating linkage in crosses II–IV

Tested loci[d]		1/1	3/3	1/3	3/1	2/2	1/2	3/2	2/3	2/1	χ^2[a]	Distance (cM)
1st	2nd											
L. culinaris 162 × *L. odemensis* 37 (cross II)												
Gs[b]	*Got-2*	19	18	0	1	–	1	17	–	–	47.75***	3.4±3.3
Gs[b]	*Pgm-2*	19	16	0	2	–	1	18	–	–	43.99***	4.9±3.9
Gs[b]	*Aco-1*	13	17	0	1	–	7	18	–	–	30.00***	13.7±5.8
Got-2	*Pgm-2*	19	14	0	1	15	1	3	2	1	69.78***	8.2±2.7
Got-2	*Aco-1*	14	14	0	0	15	6	4	3	0	54.73***	12.1±3.3
Pgm-2	*Aco-1*	14	13	0	0	15	7	3	4	0	51.91***	13.0±3.4
L. culinaris 160 × *L. odemensis* 37 (cross III)												
Gs[b]	*Pgm-2*	11	9	0	3	–	0	19	–	–	29.81***	6.8±5.1
Gs[b]	*Aco-1*	8	7	0	5	–	3	19	–	–	12.68**	18.6±7.5
Pgm-2	*Aco-1*	11	6	0	0	16	3	3	1	2	39.78***	11.3±3.7
L. culinaris 158 × *L. odemensis* 37 (cross IV)												
Linkage group I												
Got-2	*Pgm-2*	6	0	0	0	11	1	1	1	2	11.10*	12.3±5.3
Linkage group II												
Dia-2	*Mdh-2*	8	3	0	0	11	0	0	0	0	44.00***	0.0

1 = Homozygous for the *L. culinaris* allele; 2 = heterozygous; 3 = homozygous for the *L. odemensis* allele
*, ** and *** significant χ^2 at levels of 0.05, 0.01 and 0.001, respectively
[a] χ^2 for independence
[b] *Gs* was scored as a dominant/recessive locus

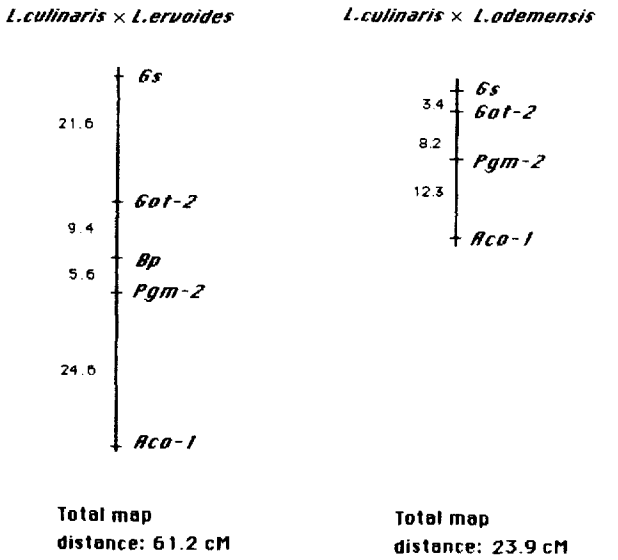

Fig. 6. Linkage group I based on F_2 of cross I and crosses II–IV

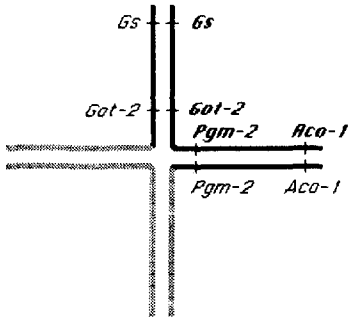

Fig. 7. Diagrammatic representation of the quadrivalent observed in heterozygotes for the reciprocal translocation that distinguishes *L. culinaris* and *L. orientalis* from *L. ervoides* and *L. odemensis*. *Got-2* and *Pgm-2* are linked in *L. ervoides* and *L. odemensis* while in the other two species they are located on different chromosomes

We interpreted these deviations as a product of linkage between the markers and genes that operate in the pre- and postzygotic phases of reproduction. Allelic variation in these loci within a species is low, whereas in interspecific hybrids many of these loci are heterozygous and lead to unbalanced reproduction. Our interpretation of the independent monogenic nature of the deviation causing factors is supported by the homogeneous aberrant segregations of markers in F_2 and F_3 of Cross I (Table 3). If the phenomenon results from the effect of a whole chromosome one would expect homogeneous monogenic segregations for linked markers. The heterogeneous segregations of *Est-1* and *Skdh-2* in linkage group IV ($P < 0.01$) and for *Sp-3* and *Sp-4* in linkage group V ($P < 0.01$) indicate that the deviations were not due to a whole chromosome effect.

In the crosses between *L. culinaris* and *L. odemensis* (II–IV) the deviations in all loci favored the *L. culinaris* alleles. In a similar interspecific F_2 population unidirectional deviations were previously detected at the chromosomal level (Goshen et al. 1982); the two species differed with respect to three reciprocal translocations. An excess of meiotically stable F2 plants were observed; most of them had the *L. culinaris* chromosome arrangements.

Map distances

A reduction of map distances in linkage group I of the *L. culinaris* × *L. odemensis* crosses compared to those of *L. culinaris* × *L. ervoides* crosses (Fig. 6) could be caused by one or more of the following: 1) a disproportionately large number of *L. culinaris* alleles, in the effective gametes population, would shorten the map distances in the *L. culinaris* × *L. odemensis* crosses (Mather 1951); 2) differences in specific genes that regulate synapsis and crossing-over (Kaul and Murthy 1985); and 3) greater homology between the chromosome of *L. ervoides* and *L. culinaris* than between those of *L. odemensis* and *L. culinaris* and hence a higher rate of crossing-over (Stern and Hotta 1978). Indeed isozyme phylogeny of the genus places *L. ervoides* closer to *L. culinaris* than is *L. odemensis* (Pinkas et al. 1985).

Negative interference

The high rate of double crossing-over between markers on both sides of the translocation break-point can be attributed to negative chromatoid interference due to the physical arrangement of the chromatids in the vicinity of the break-point (Sybenga 1975, 1980). Negative interference was found by cytogenetic analysis in *Melanoplus* (Hearne and Huskins 1935), *Trillium* (Huskins and Newcombe 1941) and *Lillium* (Kayano 1959); in *Disporum*, however, no such interference was found (Kayano 1960).

Evolution of the translocation

In 17 crosses involving 10 parental lines of *L. nigricans* we detected allozymic variability only for *Got-2*, and were therefore unable to determine the gene order in linkage group I for this species. In a previous study we demonstrated that in a number of *L. culinaris* × *L. orientalis* F_2 populations, *Gs* and *Got-2* belong to the same linkage group (14 cM apart) while *Pgm-2* is completely independent of this group (Zamir and Ladizinsky 1984). The results presented here indicate that in *L. ervoides* and *L. odemensis* the markers *Gs*, *Got-2* and *Pgm-2* are linked (Fig. 7). Bearing in mind the limits of

resolution of the mapping analysis, we suggest that a single translocation event may be responsible for the cosegregation of *Pgm-2* and *Got-2*. In such a case, it would follow that the translocation is of ancient origin in the evolution of *Lens*.

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