Genetical aspects of hybrid embryo abortion in the genus *Lens* L.

S. ABBO* & G. LADIZINSKY

Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot 76100, Israel

Hybrid embryo abortion is an effective crossing barrier in the genus *Lens* L. It divides the genus into two crossing groups: the Culinaris and the Nigricans groups; but it is also evolving within the Culinaris group. The abortion of the F₁ embryo is strongly affected by dominant gene action. In the segregating generations embryo abortion is of a quantitative nature and has both components, dominant and additive. These were expressed differentially in the *Lens ervoides* × *L. culinaris* and *L. orientalis* × *L. culinaris* cross combinations. The genetic control of embryo abortion was indicated further by the association between marker loci and embryo abortion rates in segregating generations. Embryo abortion was not associated with chromosomal aberrations. The establishment of an embryo abortion reproductive barrier by a single somatic mutation in a locus affecting seed development is proposed as a first step in a build-up of genetic isolation in the genus *Lens*.

Keywords: crossing barrier, genetics, hybrid embryo abortion, *Lens* L., quantitative control.

Introduction

Wild as well as cultivated lentil are usually free of any embryo abortion following self-pollination. Hybrid embryo abortion after interspecific hybridization in lentil was first described by Ladizinsky *et al.* (1984). They classified the five taxonomic species of the genus *Lens* L. into two crossing groups: the Culinaris group, which includes the cultivated lentil *Lens culinaris* and the wild taxa *L. orientalis* and *L. odemensis* and the Nigricans group, which contains *L. nigricans* and *L. ervoides*. Crossing within each group is usually successful and the resulting F₁ plants are fully or partially fertile. Crosses between the groups fail due to embryo abortion within 14 days after pollination (Ladizinsky *et al.*, 1984). Interestingly, embryo abortion is also typical to a few cross combinations within the Culinaris group (Brown, 1985). In a recent paper (Abbo & Ladizinsky, 1991) the authors described the anatomical aspects of hybrid embryo abortion in lentil.

Ladizinsky *et al.* (1985) obtained an interspecific hybrid between *L. ervoides* and *L. culinaris* using embryo rescue techniques. This F₁ plant produced normal and aborted embryos at a ratio of 1:1. Among

the F₂ progeny, however, pod abortion ranged from 10 per cent to 90 per cent, thus implying quantitative control of embryo abortion (Ladizinsky *et al.*, 1985).

As *Lens* species are self-pollinated, interspecific embryo abortion is apparently caused by a heterozygous condition, an epistatic interaction or both. Given the fact that continuous variation of embryo abortion was recorded on plants of early segregating generations derived from intra- and interspecific hybrids (Brown, 1985; Ladizinsky *et al.*, 1985), it seems plausible that embryo abortion is a result of multiple gene action.

The genus *Lens* therefore provides a unique opportunity to study the genetics of crossing barriers between species through hybrid embryo abortion. One possible approach is to look for linkage between marker loci and quantitative trait loci (QTL) affecting embryo abortion (Tanksley *et al.*, 1982). Secondly, narrow sense heritability could provide a valuable estimate for the additive component of genetic variance of embryo abortion in lentil. Thirdly, as parental lines may differ in chromosomal linear order, backcrosses may help to assess the role of chromosomal aberrations, if any, in hybrid embryo abortion. We present a genetic analysis of hybrid embryo abortion in two segregating populations derived from intra- and interspecific cross combinations of lentil.

*Correspondence: The Field Crops Department, Faculty of Agriculture, Rehovot 76100, Israel.

Materials and methods

Plant material

L. ervoides #32 × *L. culinaris* #7. An F₁ plant was obtained with the aid of embryo culture (Ladizinsky *et al.*, 1985). Sixty-five F₅ plants originating from 65 individual F₂ plants were obtained by the single seed descent procedure. Fifty-four F₆ families were grown in a randomized design (about seven plants per F₆ family) in the greenhouse during Winter–Spring 1990. Thirty-seven F₇ families were grown in the greenhouse during Winter–Spring 1991.

L. orientalis #133 × *L. culinaris* #2. Two F₁ seeds were obtained after approximately 400 hand pollinations (Brown, 1985). Eighty-six F₂ plants were grown in Autumn 1987. A random sample of 44 F₃ families (about 12 plants per family, progeny of 44 individual F₂ plants) were grown as described above during Winter–Spring 1989. Forty F₅ families, bulked progeny of F₄ families were grown as described above during Winter–Spring 1991.

Scoring of aborted embryos

Aborted embryos were shrunken and smaller (1–2 mm in diameter) than viable seeds. All pods from each plant of the analysed populations were removed before dehiscence. Viable and aborted seeds were counted. For each plant the ratio of aborted seeds, AR, was calculated as follows: AR = No. of aborted seeds / (No. of aborted + viable seeds).

Heritability estimates

Heritability estimates of transformed AR values were obtained by the correlation coefficient from parent × offspring correlation after Cahaner & Hillel (1980). The genetic expectation of heritability estimate is determined by genotype frequencies in the analysed populations. Therefore, marker loci were used to detect mean heterozygosity in the segregating populations of both cross combinations. The basic formulae given by Cahaner & Hillel (1990) were amended according to the actual decrease in heterozygosity found in both cross combinations.

Chromosome analyses

Karyotypes were characterized by mitotic root tip chromosomes in Feulgen-stained squash preparations. Meiotic chromosomal behaviour was studied during diakinesis, metaphase I and anaphase I–II in pollen mother cells (PMC) using aceto-carmine preparations.

Isozyme electrophoresis

Starch gel electrophoresis, isozyme activity staining and genotype scores at the *Skdh* and *Gal-8.5* loci were as described by Tadmor *et al.* (1987) and Wendel & Weeden (1989), respectively.

DNA polymorphism analyses

Restriction length markers (RFLP) and random amplified markers (RAPD) were scored with bulked DNA preparations made from more than six plants per family in each of the analysed populations. In this way every analysed family served as a progeny test to detect heterozygosity within the parental population.

RFLP. The RFLP markers were developed by M. Havey (Havey & Muehlbauer, 1989). DNA preparation, digestion with restriction enzymes, agarose electrophoresis, blotting onto nylon membranes, as well as radioactive probe labelling, autoradiogram development and genotype scores, were as described by Havey & Muehlbauer (1989).

RAPD. Polymerase chain reaction conditions, primer sequences, electrophoresis of amplified products and genotype scores were described by Abbo *et al.* (1992).

Statistical analyses

Comparison of genotypic groups (within each marker locus), analyses of variance and correlation analyses were carried out using SAS software (SAS, 1982).

Linkage analyses

Two point linkage tests were done using the LINKAGE-1 program (Suiter *et al.*, 1983). Because the current lentil genetic map (Weeden *et al.*, 1992) was derived from the F₂ of the very same *L. ervoides* #32 × *L. culinaris* #7 cross described herein, marker loci on that map were used as anchors in the two point tests to localize RAPD and morphological markers into established linkage groups. In the same way, markers segregating in the *L. orientalis* #133 × *L. culinaris* #2 were localized to the linkage groups described by Havey & Muehlbauer (1989) for a *L. culinaris* × *L. orientalis* mapping population.

Results

The F₁ plant of the cross *L. ervoides* #32 × *L. culinaris* #7 (Le #32 × Lc #7) yielded aborted and viable seeds in a 1:1 ratio (see Ladizinsky *et al.*, 1985). So did

those from the *L. orientalis* #133 × *L. culinaris* #2 (Lo #133 × Lc #2) which yielded 46:57 and 71:71, aborted:viable seeds, respectively. These ratios may suggest the involvement of one major locus, determining embryo abortion in the heterozygous condition. Under this assumption all heterozygous individuals would be eliminated from the population (as aborted F_2 seeds). The viable F_2 individuals would then represent the two parental homozygous groups, free of subsequent embryo abortion. However, continuous variation in ratios of aborted embryos as found on plants among segregating generations of the Le #32 × Lc #7 cross, up to F_7 , rules out monogenic control.

The partially fertile interspecific hybrids and the quantitative nature of embryo abortion allowed us to use the following three methods for studying the genetics of hybrid embryo abortion: (1) heritability estimation of embryo abortion ratio, to differentiate between environmental effects, and genetic variation; (2) detection of factors affecting embryo abortion with the aid of marker loci; and (3) backcrosses (BC) to parental lines, to assess crossability relations between the families of segregating generations and the parental lines, and secondly to assess the effect of chromosomal rearrangements on embryo abortion.

Heritability estimation

Le #32 × Lc #7. Means, ranges and standard deviations of the resultant AR values are given in Table 1. A slight reduction in AR values is evident among the F_6 families compared with the F_5 population. The distribution range narrowed and shifted towards the lower values. Nevertheless, the AR distribution among the F_7 families was still wide and close to that described for the F_2 generation (10–90 per cent) by Ladizinsky *et al.* (1985).

By definition, AR values may range from 0.0 to 1.0 and are therefore not normally distributed. To enable

analyses of variance and correlation analyses AR values were normalized as follows: Transformed AR (TAR) = arcsine(square root (AR)). The distribution of TAR values was tested using SAS' Varcomp procedure and was found normal.

The genetic expectation of heritability estimates is determined by genotype frequencies. As deviations from the expected Mendelian segregation were reported for this cross combination (Tadmor *et al.*, 1987), an estimate for genotype frequencies was obtained by six non-linked markers (according to Weeden *et al.*, 1992). Segregation of an F_6 family in any of the marker loci was interpreted as an indication of heterozygosity in the F_5 plant. About one-quarter of the F_5 plants were found heterozygous, a greater proportion than the expected 1/16 after four generations of selfing (Table 2). As heterozygous frequency in the F_2 population was 0.5 (Tadmor *et al.*, 1987) and 0.25 among the F_5 , a reduction of 0.2, instead of 0.5, in heterozygous frequency after each generation of selfing apparently took place. Therefore, heritability expectations were accordingly amended based on the basic formulae given by Cahaner & Hillel (1980) (Table 3).

As a considerable fraction of heterozygosity was detected in the F_5 generation the heritability expectations are not free of the V_D component (Table 3). The heritability estimate according to the parent × offspring correlation was $r = 0.47 \pm 0.09$.

Lo #133 × Lc #2. This population was derived from two F_1 seeds obtained after 400 hand pollinations (Brown, 1985). Seed set is usually over 50 per cent in crosses within the Culinaris group. Eighty-six F_2 progeny were scored for aborted embryos during Autumn 1987. The following morphological markers were also scored: epicotyl coloration (*Gs*), pod pubescence (*Hp*), pod dehiscence (*Pd*), growth habit (*Gh*), hard seed coat (*Ger*), cotyledon colour (*Cot*) and seed coat pattern (*Sc*) (Table 4). A random sampling of

Table 1 Descriptive statistics of AR in the segregating populations of the analysed cross combinations

Cross combination	Generation	Mean	Range	Standard deviation
Lc #32 × Lc #7	F_5	0.32	0.00–0.68	0.189
	F_6	0.22	0.01–0.47	0.127
	F_7	0.29	0.00–0.89	0.230
Lo #133 × Lc #2	F_2	0.30	0.06–0.60	0.143
	F_3	0.16	0.02–0.34	0.064
	F_5	0.08	0.00–0.25	0.060

44 F_3 families was grown and scored for aborted embryos and morphological markers during Winter–Spring 1989. RFLP and RAPD analyses were performed with bulked DNA preparations of F_4 progeny (to represent the genetic status of the F_3 family). AR and TAR were calculated as described for the previous cross combinations. Descriptive statistics of AR in the F_2 , F_3 and F_5 generations are given in Table 1. A marked reduction in AR values is evident in F_3 compared with F_2 . A further reduction of AR values was evident among the F_5 families.

The F_3 families were used to detect heterozygosity in the F_2 generations by the above morphological markers. The number of segregating F_3 families for each of those markers is given in Table 2. In all marker loci but the *Ger* locus, no deviations from the expected Mendelian segregation were found. Therefore, the heritability expectations for TAR (Table 3) were as developed by Cahaner & Hillel (1980). The heritability estimate of TAR obtained by the correlation coefficient was $r = 0.54 \pm 0.1$.

Table 2 Number of segregating families of the two analysed cross combinations at morphological and RFLP markers

<i>L. ervoides</i> × <i>L. culinaris</i>		<i>L. orientalis</i> × <i>L. culinaris</i>	
Locus	No. of segregating F_6 families	Locus	No. of segregating F_3 families
<i>Gs</i>	14/54	<i>Gs</i>	25/44
<i>Pd</i>	19/54	<i>Pd</i>	21/44
<i>Ger</i>	11/54	<i>Ger</i>	17/44
<i>St</i>	14/54	<i>Gh</i>	21/44
<i>P68</i>	10/54	<i>Cot</i>	28/44
<i>P110d</i>	14/54	<i>Sc</i>	20/44
Mean	1/4		1/2
Expected heterozygous frequency	1/16		1/2

Table 3 Estimating procedures and genetic expectations of heritability estimates of TAR for the two analysed cross combinations

Combination	h^2 estimation procedure	h^2 genetic expectation
Le # 32 × Lc # 7	$r = \frac{\text{Cov } F_6 F_5}{\sqrt{V_{F_6}} * \sqrt{V_{F_5}}}$	$\frac{1.5 V_A + 0.6 V_D}{\sqrt{(1.6 V_A + 0.6 V_D + 0.14 V_E)} * \sqrt{(1.5 V_A + 0.76 V_D + V_E)}}$
Lo # 133 × Lc # 2	$r = \frac{\text{Cov } F_3 F_2}{\sqrt{V_{F_3}} * \sqrt{V_{F_2}}}$	$\frac{V_A + 0.5 V_D}{\sqrt{(V_A + 0.25 V_D + 0.08 V_E)} * \sqrt{(V_A + V_D + V_E)}}$

Genetic association between abortion factors and marker loci

To pursue further the genetic nature of the factors affecting hybrid embryo abortion we attempted to find an association between this quantitative characteristic and known genetic markers.

Le # 32 × *Lc* # 7. Within the F_6 generation of this cross combination the following markers were scored: morphological markers *Gs*, *Ger*, *Pd*, stipule shape *St*, RFLP markers *E14*, *P68*, *P110a*, *P110b*, *P110c*, *P110d* and 36 RAPD polymorphic bands. Within the F_7 population, *Gs*, *Pd*, *St*, the isozymic markers *Skdh* and *Gal-8.5* and the RFLP markers *C81*, *E5*, *P111a* and *P111d* were scored. The above genetic markers cover approximately 80 per cent of the lentil genome as estimated by means of chiasmata per cell by Ladizinsky (1979) and according to the proposed map of Weeden *et al.* (1992).

Among the 38 markers scored in the F_6 generation two morphological markers, *Pd*, *Ger* and eight RAPD markers were found in association with embryo abor-

Table 4 Monogenic segregation of marker loci as scored in the F_3 generation of the cross *L. orientalis* # 133 × *L. culinaris* # 2

Marker locus	Genotypic groups			Total	d.f.	Expected ratio	χ^2
	<i>cul</i>	<i>het</i>	<i>ori</i>				
<i>Ger</i>	21	17	4	42	2	1:2:1	15.28
<i>Pd</i>	6	21	13	40	2	1:2:1	2.55
<i>Gh</i>	15	21	7	43	2	1:2:1	3.0
<i>Gs</i>	11	25	7	43	2	1:2:1	1.88
<i>Hp</i> ^a	33	33	11	44	1	1:3	0.0
<i>Cot</i>	8	28	7	43	2	1:2:1	1.37
<i>Sc</i>	12	20	11	43	2	1:2:1	0.26

^aScored in F_2 only.

Table 5 Probability of *F* value obtained by the one-way ANOVA for the effect of the marker loci on TAR in the *F*₆ *L. ervoides* # 32 × *L. culinaris* # 7 population

Marker locus	<i>P</i> (<i>F</i>)
<i>Pd</i>	0.067
<i>Ger</i>	0.006
<i>P3f</i>	0.03
<i>P7c</i>	0.06
<i>P7d2</i>	0.03
<i>P8e2</i>	0.009
<i>P8e7</i>	0.006
<i>P11d</i>	0.04
<i>P11e</i>	0.007
<i>P11g</i>	0.02

tion (Table 5). Only *P7c* of the eight unmapped RAPD markers was tightly linked to a pair of known markers, *P110b* and *Pd*, and was therefore localized in linkage group 3 according to Weeden *et al.*'s (1992) map.

Lo # 133 × *Lc* # 2. The seven morphological markers used for the estimation of heterozygosity in the *F*₂ and *F*₃ populations were tested for association with embryo abortion factors. In addition, all but the *Sc* locus were found to be linked to each other. These data do not agree with the map proposed by Havey & Muehlbauer (1989) in which four of the six linked morphological markers were placed onto different linkage groups. As the parental lines differed by at least three reciprocal translocations (Ladizinsky *et al.*, 1984) the 'morphological linkage group' is considered to consist of four chromosomes (out of a total of seven). All the linked markers were found to affect the AR. Most of the RFLP and RAPD markers (data not shown), scored using bulked DNA of *F*₄ plants per family, clustered onto the 'morphological linkage group', suggesting that this 'pseudo-linkage' group contains a considerable portion of the genome.

Weller (1987) proposed a method to estimate the effect of a chromosomal segment on a quantitative trait. According to Weller's method the effect of a chromosomal segment defined by two linked markers (*m* and *n*) is calculated from the means of the QTL value of the two double homozygous groups as follows: Chromosomal segment effect = $X_{m1n11} - X_{m2n22}$; where X_{m1n11} and X_{m2n22} are the means of the *F*₃ families doubly homozygous for the linked markers *m* and *n*. If the chromosomal segment contains only one QTL for the analysed trait, the accuracy of this approach increases with decreasing map distance between the markers because double recombinants of the two markers are less likely to occur. Surprisingly,

Table 6 Map distances between marker loci in the 'pseudo-linkage' group and the calculated effect of chromosomal segment on TAR as defined by pairs of marker loci (after Weller, 1987)

Marker pairs	<i>r</i> (cM)	Effect on embryo abortion (in TAR units)
<i>Ger-Gh</i>	22	0.20
<i>Gh-Gs</i>	32	0.21
<i>Gs-Pd</i>	19	0.26
<i>Ger-Pd</i>	73	0.31

the segment defined by the most remote markers *Ger-Pd* (Table 6) had the largest effect on the embryo abortion ratio. If embryo abortion were affected by only one locus in the analysed segment then estimating the effect of a larger segment should result in a reduced effect. In this case the opposite was true, i.e. analysis of a larger segment resulted in a much greater effect on embryo abortion.

We interpret this as an indication that embryo abortion is affected by number of loci, as indicated by the analysis of the first cross, where ten marker loci were found to associate with embryo abortion. It is not clear if all these markers represent distinct abortion factors. However, evidence was found for the involvement of several loci in affecting embryo abortion in both cross combinations.

Backcrosses

Le # 32 × *Lc* # 7. Thirty-seven *F*₇ families of the cross *Le* # 32 × *Lc* # 7 were crossed with the parental lines. Of these, 20 crossed freely with *Lc* # 7 and partially with *Le* # 32. Seven families were crossed successfully to both parental lines, although in some cases a small proportion of aborted seeds was found. Five families did not cross at all with *Le* # 32 and crossed partially with *Lc* # 7. For each family the TAR for two BC directions was calculated. The one-way ANOVA, used for detecting linkage between marker loci and TAR, was adopted for detection of linkage between marker loci and the AR after BC with each of the parental lines.

The *E5*, *P110b* and *P110c* marker loci were found to affect crossability with *Lc* # 7. In each of them, the high aborting groups carried the *L. ervoides* alleles. The *P110c*, *St* and *Gal-8.5* marker loci were found to affect crossability with *Le* # 32. In the first two loci, the high aborting groups carried the *L. culinaris* alleles. These results suggest that embryo abortion after BC resulted from heterozygous interaction (dominant gene action). It is important to note that none of the loci

which affected TAR in the BC did so after self-pollination on the F_6 and F_7 plants.

$Lo \# 133 \times Lc \# 2$. Five F_4 families of this cross were selected for backcrosses. Three of the families ($\# 13$, 93 , 96) were homozygous for the *culinaris* alleles throughout the 'pseudo-linkage groups'. The other two families ($\# 26$, 43) were homozygous to the *orientalis* alleles in all the analysed markers. $Lo \# 133$ and $Lc \# 2$ differ karyotypically from each other (Fig. 1a, b). $Lc \# 2$ has a large satellite on a metacentric chromosome whereas in $Lo \# 133$ most of the satellite is translocated and the satellited chromosome is very small, hence acrocentric (evidence for translocation in Fig. 1c). The first three families had the satellited chromosome of $Lc \# 2$ and the other two had the $Lo \# 133$ satellited chromosome. These latter families crossed freely with both parental lines. Two of the

three families with the *culinaris* satellited chromosome ($\# 13$, 93) crossed freely with $Lc \# 2$ but did not cross with $Lo \# 133$, the third one ($\# 96$) crossed with either parental line, although more freely with $Lc \# 2$.

During Winter-Spring 1991, BC and F_5 plants of these five families were compared for embryo abortion ratio. Chromosome pairing at meiosis in PMC was studied to verify the hybrid nature of the BC plants and to assess the effect of chromosomal aberrations on the AR. A one-way ANOVA within each of the families was used for the comparison of TAR values of the two BC groups and the F_5 group.

In family $\# 13$ TAR values were higher in the selfed progeny than in plants from BC with $Lc \# 2$ (Table 7). In families $\# 43$ and 93 the selfed progeny did not differ from the progeny of BC with $Lc \# 2$. Families $\# 26$ and 96 behaved differently. There, seeds from BC with both parents were obtained. The selfed

Fig. 1 Karyotypic differences between *L. culinaris* $\# 2$ and *L. orientalis* $\# 133$ and meiotic behaviour in backcrosses with their F_4 derivatives. (a) Karyotype of $Lo \# 133$, the satellited chromosomes are arrowed. (b) Karyotype of $Lc \# 2$, the satellited chromosomes are arrowed. (c) Anaphase I in a backcross between an F_4 plant possessing an $Lo \# 133$ karyotype and $Lc \# 2$. Note the asymmetric segregation, evidence for a quadrivalent separated into a trivalent and a univalent. (d) Normal pairing at diakinesis of an F_4 plant possessing an $Lo \# 133$ karyotype. Note the small acrocentric bivalent associated with the nucleolus. (e) Bridges at anaphase II in a backcross plant between an $Lo \# 133$ and $Lc \# 2$. Evidence for a paracentric inversion. (f) Anaphase I bridges in a backcross plant between an $Lc \# 2$ type and $Lo \# 133$. Note the bridges connect two independent pairs of chromosomes.

Table 7 Probability of F values obtained while comparing the three groups in each family and mean TAR in each of the groups

F_5 family	$P(F)$	Family TAR values		
		Selfed progeny	BC direction	
			LC # 2	Lo # 133
13	0.003	0.26 ^a	0.16 ^a	—
93	1.12	0.25 ^a	0.28 ^a	—
96	0.075	0.23 ^a	0.20 ^a	0.35 ^b
26	0.003	0.01 ^a	0.21 ^b	0.04 ^a
43	0.074	0.09 ^a	0.17 ^a	0.05 ^a

TAR values with the same letter did not differ statistically within families.

progeny did not differ in TAR values from the parental line of the same karyotypic arrangement: progeny of #96 × Lo #133, #26 × Lc #2 had higher abortion rates than in the selfed progeny of the respective families.

No chromosomal aberrations were found within families (Fig. 1d). Chromosomal aberrations were not found also in BC plants of Lc #2 with families #13, 93 or 96 nor in BC plants of families #26 or 43 with Lo #133. On the other hand the BC plants of families #26 and 43 with Lc #2 were heterozygous for three translocations and two inversions, similar to findings in Lc #2 × Lo #133 hybrids (Fig. 1c, e). The same was true in the BC plants of family #96 × Lo #133 (Fig. 1f). This indicates that chromosomal linear order of parties involved in the crosses has no effect on embryo abortion.

Discussion

A wide range of reproductive barriers has been described in the plant and animal kingdoms. However, understanding the genetic nature of these barriers is difficult because perfect genetic study material is hardly available. Genetic analyses are usually based on progeny tests but reproductive barriers prevent the formation of such progeny. The few existing reports on the genetic aspects of reproductive barriers, at best, present proportions of viable and nonviable progeny in the F_2 of interspecific crosses (Gou *et al.*, 1989; Hollingshead, 1930; Parker & Michaels, 1986; Rabakoarihanta *et al.*, 1979; Shii *et al.*, 1982).

The results of this study indicate that the abortion of F_1 hybrids is strongly affected by dominant or epistatic gene interaction. In the segregating genera-

tions embryo abortion is of a quantitative nature. The genetic nature of embryo abortion is indicated further by the association between abortion rate and several marker loci in the segregating populations.

Despite the assumption that small numbers of genes affect the abortion of the hybrid embryos and those produced by the F_1 hybrids we could not determine their number. However, that number is assumed to be rather small, as indicated by the proportion of the F_4 families of the Lo #133 × Lc #2 and F_7 Le #32 × Lc #7 families which freely intercrossed with both parental lines. The association between embryo abortion and several of the marker loci is another genetic property of the abortion. Associations of this kind have been proposed as a tool for mapping QTL (Tanksley *et al.*, 1982; Weller, 1987). In the examined material, some of the factors affecting embryo abortion were mapped to a linkage group which includes the *Pd*, *P110b* and *P111b* loci.

The progress towards homozygosity was much slower in the Le #32 × Lc #7 cross combination. Despite increasing homozygosity the abortion rate remained virtually the same from F_2 to F_7 . This high value cannot be solely related to heterozygosity because with a mean heterozygosity of 0.5 in the F_2 of Lo #133 × Lc #2, a mean AR of 0.3 was recorded whereas at 0.25 mean heterozygosity in the F_5 of the Le #32 × Lc #7, a mean AR of 0.32 was found. The fixed AR value in advanced segregating generations of Le #32 × Lc #7 may be explained by interactions between different homozygous genes in the recombinant lines.

The TAR heritability estimates indicate a considerable additive effect. However, the fact that the abortion rate declined sharply in advanced generations of the Lo #133 × Lc #2 cross suggested that dominant effects are more pronounced in this cross combination.

The genetic attributes of hybrid embryo abortion in inter- and intraspecific crosses in the genus *Lens* shed some light on the evolution of the reproductive barrier. Morphologically Lo #133 is indistinguishable from other accessions of *L. orientalis* and its isozyme profile is similar to most of the examined *L. orientalis*-*L. culinaris* group (Pinkas *et al.*, 1985). However, Lo #133 is unique and differs from the main cytogenetic stock of *L. orientalis*-*L. culinaris* by at least five chromosomal rearrangements and its karyotype (Fig. 1). These rearrangements have no effect on abortion because some F_5 families with the chromosomal arrangement of Lo #133 freely intercrossed with both parental lines. Furthermore, another accession of *L. orientalis* (#124) which also caused hybrid embryo abortion following crosses with the *L. orientalis*-*L. culinaris* group (data not shown), is similar to the *main*

stock of that group. Yet, Lo # 133 and Lo # 124 freely intercross with one another and their F_1 hybrids are free of embryo abortion despite heterozygosity for two reciprocal translocations (data not shown). It seems, however, that in Lo # 124 the mechanism causing abortion with the main stock represented by Lc # 2 is more effective. A few hybrid seeds can be obtained in the cross Lo # 133 $\times$ Lc # 2 when the plants are exposed to relatively high temperatures; in the cross Lo # 124 $\times$ Lc # 2 not only were hybrid seeds never obtained but we could not rescue the hybrid embryos by transplanting them into embryo culture.

The factors governing hybrid embryo abortion in Lo # 133 $\times$ Lc # 2 are apparently different from those affecting abortion in the *L. ervoides* $\times$ Lc # 2 crosses and result in embryo abortion in crosses between Lo # 124, 133 and all *L. ervoides* tested. It is thus important to note that embryo abortion, which has similar anatomical characteristics in different crosses (Abbo & Ladizinsky, 1991), may be governed by different genetic systems.

The establishment of a reproductive barrier is a prerequisite for the formation of a new species. The evolution of these barriers is quite clear when it is based on chromosomal rearrangements but is much more obscure when it is an outcome of physiological and anatomical processes. In the genus *Lens*, which is predominantly a selfer, the evolution of a genetic mechanism which causes hybrid embryo abortion is not likely to have resulted from selection for achieving isolation from other members of the species or from other species. It could have occurred as a random process or as a by-product of selection for another trait. In any case, a mutation which affects embryo abortion is not likely to occur in the gametes because the heterozygous embryos would abort and the mutation would be lost. On the other hand, mutation in the shoot meristem would produce heterozygous inflorescences. Under self-pollination the heterozygous embryos would abort and the developing homozygous embryos would give rise to two reproductively isolated lines. This hypothetical course of evolution is a rather rapid one and results in speciation after a single step. Accumulation of additional mutations in minor genes could increase the genetic isolation between those lines.

References

- ABBO, S. AND LADIZINSKY, G. 1991. Anatomical aspects of hybrid embryo abortion in the genus *Lens*. *Bot. Gaz.*, **152**, 316–320.
- ABBO, S., LADIZINSKY, G. AND WEEDEN, N. F. 1992. Genetic analysis and linkage study of seed weight in lentil. *Euphytica*, **58**, 259–266.
- BROWN, D. 1985. *Anatomical and genetic characterization of embryo breakdown in the genus Lens*. M.Sc Thesis, Faculty of Agriculture, The Hebrew University of Jerusalem (Hebrew).
- CAHANER, A. AND HILLEL, J. 1980. Estimating heritability and genetic correlation between traits from generations F_2 and F_3 of self-fertilizing species: a comparison of three methods. *Theor. Appl. Genet.*, **58**, 33–38.
- GOU, M., MOK, S. W. D. AND MOK, M. C. 1989. Isozyme banding pattern and embryo development in interspecific crosses of *Phaseolus*. *J. Hered.*, **80**, 29–32.
- HAVEY, M. AND MUEHLBAUER, F. J. 1989. Linkage between restriction fragment length, isozyme and morphological markers in lentil. *Theor. Appl. Genet.*, **77**, 395–401.
- HOLLINGSHEAD, L. 1930. A lethal factor in *Crepis* effective only in an interspecific hybrid. *Genetics*, **15**, 114–140.
- LADIZINSKY, G. 1979. The origin of lentil and its wild gene pool. *Euphytica*, **28**, 179–187.
- LADIZINSKY, G., BROWN, D., GOSHEN, D. AND MUEHLBAUER, F. J. 1984. The biological species of the genus *Lens* L. *Bot. Gaz.*, **145**, 253–261.
- LADIZINSKY, G., COHEN, D. AND MUEHLBAUER, F. J. 1985. Hybridization in the genus *Lens* by means of embryo culture. *Theor. Appl. Genet.*, **70**, 97–101.
- PARKER, J. P. AND MICHAELS, T. E. 1986. Simple genetic control of hybrid plant development in interspecific cross between *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray. *Plant Breeding*, **97**, 315–323.
- PINKAS, R., ZAMIR, D. AND LADIZINSKY, G. 1985. Allozyme divergence and evolution in the genus *Lens*. *Pl. Syst. Evol.*, **151**, 131–140.
- RABAKOARIHANTA, A., MOK, D. W. S. AND MOK, M. C. 1979. Fertilization and early seed development in reciprocal interspecific crosses of *Phaseolus*. *Theor. Appl. Genet.*, **54**, 55–59.
- SAS INSTITUTE 1982. *SAS User's Guide: Basics and Statistics*. SAS Institute, Cary, N. Carolina.
- SHII, C. T., RABAKOARIHANTA, A., MOK, M. C. AND MOK, D. W. S. 1982. Embryo development in reciprocal crosses of *Phaseolus vulgaris* L. and *P. coccineus* Lam. *Theor. Appl. Genet.*, **62**, 59–64.
- SUITER, K. A., WENDEL, J. F. AND CASE, J. S. 1983. LINKAGE-1: a PASCAL program for the detection and analysis of genetic linkage. *J. Hered.*, **74**, 203–204.
- TADMOR, Y., ZAMIR, D. AND LADIZINSKY, G. 1987. Genetic mapping of an ancient translocation in the genus *Lens*. *Theor. Appl. Genet.*, **73**, 883–892.
- TANKSLEY, S. D., MEDINA-FILHO, H. AND RICK, C. M. 1982. Use of naturally-occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. *Heredity*, **49**, 11–25.
- WEEDEN, N. F., MUEHLBAUER, F. J. AND LADIZINSKY, G. 1992. Extensive conservation of linkage relationship between pea and lentil genetic maps. *J. Hered.*, **83**, 123–129.
- WELLER, J. I. 1987. Mapping and analysis of quantitative trait in *Lycopersicon* (tomato) with the aid of genetic markers using approximate maximum likelihood methods. *Heredity*, **59**, 413–421.
- WENDEL, J. F. AND WEEDEN, N. F. 1989. Visualization and interpretation of plant isozymes. In: Soltis, D. and Soltis, P. (eds) *Isozymes in Plant Biology*, pp. 5–45. Discorides Press, Portland.