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Cryptic speciation in *Lens culinaris*

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Summary

Three crossability groups have been identified in the wild progenitor of lentil, *Lens culinaris* ssp. *orientalis*. The common one which is predominant in this taxon and in the cultigen, the unique one, which at present is known only from three populations in southern Turkey and northern Syria and the intermediate one, known from four populations in that general region. Crosses between members of the common and unique groups yield aborted seeds which can be rescued by embryo culture. Members of the intermediate group are cross-compatible with both other groups. Crossability potential seems to be controlled by a few major genes and minor genes of quantitative nature. One population of the unique group is characterized by novel karyotype and chromosome rearrangements and is reproductively isolated from any other population of *Lens culinaris*. The implication of the three crossability groups and the novel chromosome rearrangement for lentil taxonomy are briefly discussed.

Introduction

Lens culinaris Medik. ssp. *orientalis* (Boiss.) Ponert, which treated as *L. orientalis* (Boiss.) Schmalh. in botanical literature, is the wild progenitor of the cultivated lentil, *Lens culinaris* ssp. *culinaris*. The two are interfertile, share the same morphological characteristics and their differences result from evolution under domestication of the cultigen (Ladizinsky, 1979). The wild progenitor has a wide distributional range from Turkey to Tadjikistan and from Iran to the Crimean peninsula. It is restricted to relatively dry habitats with shallow, stony soil. The populations are usually small and comprise a small number of plants. Chromosome number of ssp. *orientalis* is $2n = 14$, as in other *Lens* species, and the karyotype possesses three pairs of submetacentric chromosomes, three pairs of acrocentrics and one pair of metacentrics with a secondary constriction very close to the centromere (Fig. 1). While this karyotype appears quite stable in ssp. *orientalis* and in the cultigen, chromosomal rearrangement is indicated by multivalent formation at meiosis in intraspecific hybrids (Ladizinsky et al., 1984). The standard chromosome arrangement, which is also seen in the cultigen, is common throughout the distribution range, i.e. Turkey, Cyprus, Syria, Iran, Turkmenia, Uzbekistan and Tadjikistan but not in Israel. The Israeli populations differ by a single translocation between metacentric and acrocentric chromosomes (Ladizinsky et al., 1984). Different translocations between meta-centric and acrocentric chromosomes have been detected in a few Turkish and Syrian populations. In another Turkish population a paracentric inversion was detected and translocation between two metacentric chromosomes was observed in a population from Iran (Ladizinsky et al., 1984).

In this paper we report a new karyotype in ssp. *orientalis* and emerging crossability barriers within

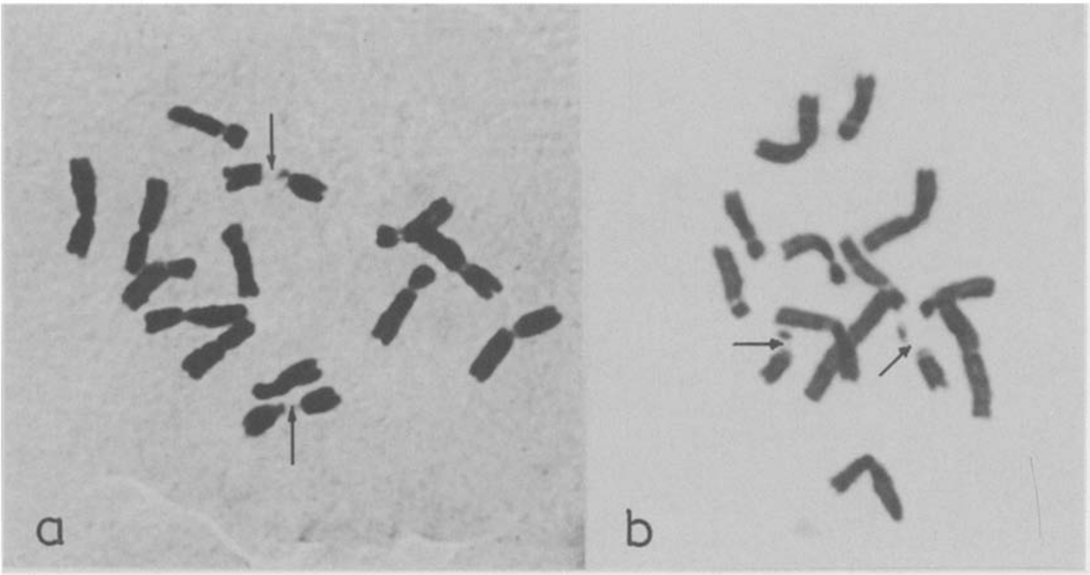

Fig. 1. a. Standard karyotype of *L. culinaris*, (× 2400). b. Rearranged karyotype of accession No. 133, (× 2400). Note the short satellite, arrows pointing to the secondary constrictions.

this taxon, indicating the imminent formation of a new species.

Material and methods

The lentil accessions employed in this study are as follows: two cultivated lines, Lc2 from Israel and Lc7 from Ethiopia, and six accessions of ssp, *orientalis* from northern Syria and southern Turkey. In addition a large number of accessions of this taxon were tested for their crossability potential but are not mentioned here because they all were members of the common crossability group (see below).

Karyotype examinations were made in root tips of germinating seeds. The root tips were placed in ice water for 24 h, fixed in 1:3 acetic acid glacial-95% ethanol, stored in 70% ethanol and stained with Feulgen after hydrolysis in 1N HCl for 10 min at 60°C. Chromosome pairing at meiosis was studied in pollen mother cells. Buds of appropriate size were fixed in 1:3 (as above) and stained with 2% acetocarmine. Pollen fertility was determined by their stainability in 2% acetocarmine. Regularly shaped mature pollen grains with darkly stained cytoplasm were considered normal and fertile.

Embryo culture. In a number cross combinations where hybrid embryos aborted embryo rescue was attempted by placing the immature, 14 days old, embryo on culture medium. The procedure and the medium were according to Cohen et al. (1984).

Results and Discussion

Chromosome reorganization

Accession No. 133 of ssp. *orientalis* was collected near Yukai Konak, about 50 km SE of Diyarbakir on the road to Mardin, Turkey. The ecology and size of the population were typical of the region but the plants were unique in that they had pubescent pods, a characteristic which is very rare in ssp. *orientalis*. This also appeared karyotypically divergent from all other accessions tested so far by us. Most of the satellite had been removed forming an additional acrocentric pair of chromosomes, and one of the submetacentrics had become larger (Fig. 1). The magnitude of the rearrangement was, however, much greater as indicated by the pattern of chromosome association at meiosis in hybrids involving No. 133 and the cultivated line Lc2 representing the standard chromosome arrangement (Table 1). These hybrids were obtained with the aid of embryo culture as indicated below. Trivalents and quadrivalents were common in metaphase I cells and a single cell with two trivalents and a quadrivalent indicated that the two accessions differed by three translocations. In addition, two bridges connecting different chromosomes and two fragments were observed in a number of anaphase cells, indicating that these hybrids were heterozygous for two paracentric inversions. As a result of these chromosomal aberrations pollen fertility was very low, 2-8%, and of the 2565 flowers counted on these hybrids, only 178 initiated pod development.

Karyotype and chromosome arrangement were also checked in five neighboring populations of ssp. *orientalis*. All had the standard arrangement, implying that the massive chromosome rearrangement in No. 133 had apparently occurred by a macromutation in a single step.

Similar chromosome reorganization was found also in *L. nigricans* (M.B.) Godr. where two populations diverged from other populations of the species by four rearrangements but which caused no karyotypic changes (Ladizinsky et al., 1984).

Crossability barriers

Accession No. 133 was cross-incompatible with most accessions of ssp. *orientalis* and with the cultivated lentil, regardless of the cross direction. Such crosses produced hybrid embryos which aborted 10-14 days after fertilization, sometimes after formation of shoot and root primordia (Braun, 1985). Using embryo culture, hybrid embryos involving No. 133 were rescued and reared to maturity. When crosses were made with this accession at high temperature, above 28°C, a few hybrid seeds were formed. Accession No. 124 which was collected 10 km E of Sakcagoz, Adana-Gaziantep road, in Turkey, and No. 76 collected NW of Aleppo on the road to Kalat Suman, in Syria, were cross-compatible with No. 133 but not with the common crossability group. Both had the standard karyotype and glabrous pods indicating a lack of association between cross-incompatibility and chromosome rearrangement. Nevertheless, because of irregular chromosome association at meiosis, hybrids involving No. 133 and these other two accessions were highly sterile.

Table 1. Chromosome configurations at metaphase I in Lc2 × No. 133 hybrid (I = univalent, II = bivalent, III = trivalent, IV = quadrivalent)

Configuration	No. cells
5II, IV	5
I, 3II, III, IV	5
3II, 2IV	4
4II, 2III	4
2I, 4II, IV	3
I, 5II, III	3
6I, 4II	2
7II	2
2I, 6II	1
2II, 2III, IV	1
Total	30

All our attempts to obtain, via embryo culture, hybrids between No. 124 and representatives of the common crossability group were unsuccessful, apparently because of early abortion. Endosperm in these hybrid embryos was abnormal or totally lacking, and never reached the stage of producing shoot or root primordia (Abbo & Ladizinsky, 1991). No attempts were made to obtain hybrids with No. 76. Lentil pods typically contain two ovules, but one-seeded pods are quite common as a result of early natural ovule or embryo abortion. The proportion of one-seeded pods varies between lines and is also affected by environmental conditions. On F_1 hybrids from crosses involving No. 133 and the cultivated lentils Lc2 and Lc7, aborted ovules and seeds were distinguished according to the size of the aborted organ. The ratio between viable and aborted seeds was found to be close to 1:1, or 0.5 abortion rate, in 10 of the 14 examined hybrids (Table 2). Deviation from this ratio in four plants was probably due to environmental conditions. In segregating generations the mean abortion rate dropped from 0.3 in F_2 to 0.15 and 0.08 in F_3 and F_5 respectively, with an increasing number of virtually abortion free plants, as the parental lines (Abbot, 1991).

Five meiotically stable F_4 families of Lc2 × No. 133 were selected for backcrossing with the parental lines to test their crossability potential. In these families seven bivalents were regularly formed at metaphase I and the mean number of chiasmata per cell was in the range of that of the parental lines. Three of the families were morphologically and karyotypically similar to Lc2, and

Table 2. Viable and aborted seeds on L. *culinaris* × ssp. *orientalis* (No. 133) hybrids

Plant No.	Combination	Viable seeds	Aborted seeds	CH^2(1:1)
37	Lc7 × No. 133	56	52	0.14
38	Lc7 × No. 133	64	110	12.16*
83	Lc7 × No. 133	45	68	4.68*
219	Lc2 × No. 133	25	24	0.02
316	Lc2 × No. 133	41	25	3.87*
326	Lc2 × No. 133	48	62	0.16
328	Lc2 × No. 133	44	21	8.13*
334	Lc2 × No. 133	46	57	1.17
335	Lc2 × No. 133	71	71	0.00
340	Lc2 × No. 133	67	80	1.14
341	Lc2 × No. 133	46	29	3.84
343	Lc2 × No. 133	81	90	0.47
384	Lc2 × No. 133	52	70	2.65
1001	Lc2 × No. 133	72	55	2.27

*significant at the 5% level.

two to No. 133. The latter two families were highly male sterile and produced only a few pods upon selfing but many two-seeded pods were formed when pollinated with normal pollen. The five families were crossed to both parental lines to test their crossability potential. The proportions of viable and aborted seeds following selfing were estimated in 30 pods of the Lc2-like families and in each of the hybrid combinations, but only in 15 of the No. 133-like families (Table 3). Seed set was practically normal following crosses with the resembling parent. However, whereas seed abortion was common in crosses in the Lc2-like families with No. 133, it was relatively rare in crosses of the No. 133-like families with Lc2, indicating the No. 133-like families to be genetic recombinants.

In addition to these two crossability groups a third one was noted which was cross-compatible with both groups. Occasionally a few hybrid embryos aborted following crosses between members of the third group and those of either the first or the second group but usually hybrid seeds were easily obtained. At present four populations of this intermediate crossability group are known: No. 138, Mardin district, Turkey, and Nos S-74 and S-93, Aleppo district, and No. S-139 Damascus district, Syria. Chromosome arrangement in these populations is of the standard type, and pods are glabrous.

Table 3. Percentage viable seeds in five meiotically stable F_4 Lc2 × No. 133 families and following crosses with the parental lines

F_4 families	selfing	× Lc2	× No. 133
Lc2-like			
13	75	88	0
93	72	94	0
96	83	74	2
No. 133-like			
26	86	63	89
43	100	71	98

To further pursue the genetic nature of cross-incompatibility in ssp. *orientalis* the proportions of viable and aborted seeds were observed in crosses involving members of the three crossability groups. To avoid gamete abortion due to irregular chromosome pairing at meiosis, No. 138 and Lc2 were crossed reciprocally and the hybrids were then crossed with No. 133 as male and female parent. The number of viable and aborted seeds obtained in three-way-crossing over three seasons are presented in Table 4. No clear pattern emerges from this Table, but viable seeds were common when No. 133 served as female parent, while aborted seeds were predominant when it was the pollen parent.

The occurrence of three crossability groups in ssp. *orientalis*, the pattern of abortion in crosses between the common and the unique groups, and the proportions of viable and aborted seeds on the F_1 hybrids and in segregating generations indicate that the genetics of crossability potential in this taxon is rather complex. The more or less 1:1 ratio (Table 2) between viable and aborted seeds on the Lc2 × No. 133 hybrids suggests the involvement of only a few major genes. To account for the occurrence of the three crossability groups at least two genes must be considered, Ea1 and Ea2. Tentatively the genotype ea1ea1,Ea2Ea2; Ea1Ea1,ea2ea2 and Ea1Ea1,Ea2Ea2 are assigned to Lc2, No. 133 and No. 138 respectively. Seed abortion would then occur when the two genes are heterozygous, Ea1ea1,Ea2ea2, or when one gene is

Table 4. Viable and aborted seeds involving members of the three crossability groups

Cross combination	Viable seeds	Aborted seeds
No. 133 × (No. 138 × Lc2)	49	25
No. 133 × (Lc2 × No. 138)	31	14
(No. 138 × Lc2) × No. 133	17	135
(Lc2 × No. 138) × No. 133	27	144

heterozygous and the other one is homozygous recessive, Ea1ea1,ea2ea2 or ea1ea1,Ea2ea2. This assumption is supported by the complete seed abortion in the Lc2 × No. 133 cross and the 1:1 ratio between viable and aborted seeds on the F_1 hybrids. The occurrence of No. 133-like F_4 families which are cross-compatible with both Lc2 and No. 133 is also consistent with the two gene model. On the other hand the proportion of viable and aborted seeds obtained in the three-way-crosses does not fit into the model.

The involvement of a few major genes however can not explain the continuous seed abortion observed in the segregating populations because the viable seeds are all expected to be homozygous dominant for at least one of the Ea genes. It seems that in addition to the two major genes, minor genes are also involved in the abortion but their action has become noticeable only after the removal of the major genes effect. The rapidly decreasing abortion rate from F_2 to F_5, in about half, each generation, implies a dominant effect of these genes.

Taxonomic implications

Progressive speciation in ssp. *orientalis* is occurring in southern Turkey and northern Syria, as indicated by emerging crossability barriers between populations and massive chromosome reorganization. The Yukari Konak population is the most divergent one and reproductively isolated from neighboring and most other populations of this taxon. The genetic isolation of this population is expressed by three complementary barriers: 1) hybrid seed abortion, 2) hybrids sterility due to irregular chromosome pairing at meiosis, and 3) abortion of about half the few embryos which may be produced on these hybrids. Furthermore, severe male sterility, despite regular chromosome pairing, may occur in segregating generations. These profound genetic changes, however, have not modified the ecology of that population, nor its isozyme profile (Pinkas et al., 1985). It also has not affected the main morphological features, aside from its unique pubescent pods.

The available information indicates that the Yukari Konak is a unique biological and phylogenetical entity which deserves a species ranking. However, before doing so, further study in the Mardin area is needed to find the geographic distribution of the modified karyotype, the hairy pod type and the association between them.

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