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Chloroplast DNA phylogeny of *Lens* (Leguminosae): origin and diversity of the cultivated lentil

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Abstract. A restriction-site analysis of chloroplast DNA (cpDNA) variation in *Lens* was conducted to: (1) assess the levels of variation in *Lens culinaris* ssp. *culinaris* (the domesticated lentil), (2) identify the wild progenitor of the domesticated lentil, and (3) construct a cpDNA phylogeny of the genus. We analyzed 399 restriction sites in 114 cultivated accessions and 11 wild accessions. All but three accessions of the cultivar had identical cpDNAs. Two accessions exhibited a single shared restriction-site loss, and a small insertion was observed in the cpDNA of a third accession. We detected 19 restriction-site mutations and two length mutations among accessions of the wild taxa. Three of the four accessions of *L. culinaris* ssp. *orientalis* were identical to the cultivars at every restriction site, clearly identifying ssp. *orientalis* as the progenitor of the cultivated lentil. Because of its limited cpDNA diversity, we conclude that either the cultivated lentil has passed through a genetic bottleneck during domestication and lost most of its cytoplasmic variability or else was domesticated from an ancestor that was naturally depauperate in cpDNA restriction-site variation. However, because we had access to only a small number of populations of the wild taxa, the levels of variation present in ssp. *orientalis* can only be estimated, and the extent of such a domestication bottleneck, if applicable, cannot be evaluated. The cpDNA-based phylogeny portrays *Lens* as quite distinct from its putative closest relative, *Vicia montbretii*. *L. culinaris* ssp. *odemensis* is the sister of *L. nigricans*; *L. culinaris* is therefore paraphyletic given the current taxonomic placement of ssp. *odemensis*. *Lens nigricans* ssp. *nigricans* is by far the most divergent taxon of the genus, exhibiting ten autapomorphic restriction-site mutations.

Key words: *Lens culinaris* – Lentil – Restriction site analysis – cpDNA – Cytoplasmic bottleneck

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Introduction

The genus *Lens* Miller (Leguminosae) includes the cultivated lentil and its wild relatives native to western Asia, northern Africa and the Mediterranean region. The plants are diploid (2n = 14) and annual, and grow in sites with shallow, stony soil. The lentil is one of the oldest grain crops of the Middle East and, despite its considerable economic importance, it has been the subject of modern breeding programs and systematic studies only during the past 10 years. In fact, all the lentil cultivars grown currently in the U.S.A. are descended from only a few plant introductions (Havey and Muehlbauer 1989).

Using cytology and the interfertility of *Lens* taxa, Ladizinsky et al. (1984) revised the taxonomy of the genus. As currently recognized, *Lens* comprises two species: *L. culinaris* Medikus and *L. nigricans* Godr. The former includes ssp. *culinaris* (the cultivated lentil), ssp. *orientalis* (Hand.-Mazz.) Williams, and ssp. *odemensis* Ladizinsky; *L. nigricans* includes ssp. *nigricans* and ssp. *ervoides* (Grande) Ladizinsky. Hereafter in this paper, these taxa will be referred to by their subspecific epithets. Subspecies *orientalis* ranges from Turkey south to Israel and east to Afghanistan; *odemensis* ranges from Turkey south to Israel; *nigricans* is distributed along the northern and eastern coast of the Mediterranean, from Turkey to Italy, with disjunct

distributions in southern Spain and Ethiopia, and *ervoides* occurs from Turkey west to Spain and in northern Morocco and Algeria. All taxa are sympatric from Turkey south to Israel.

Morphological distinctions among the taxa are few and subtle, consisting mainly of the shape and orientation of stipules and the length of calyx teeth (Ladizinsky et al. 1984). Moreover, the transferral (Ladizinsky et al. 1984) of ssp. *odemensis* (with semi-hastate stipules) from *L. nigricans* (with semi-hastate stipules) to *L. orientalis* (with lanceolate stipules) weakens the strength of stipule shape in differentiating between the species. Several recent studies have employed enzyme electrophoresis (Hoffman et al. 1986) or molecular techniques (Pinkas et al. 1985; Havey and Muehlbauer 1989; Muench et al. 1991) to test Ladizinsky's classification, but no study to date has used a cladistic approach to reconstruct the phylogeny of the group.

Although most work has pointed to ssp. *orientalis* as the progenitor of the domesticated lentil (Barulina 1930; Zohary 1972; Zohary and Hopf 1973; Williams et al. 1974; Ladizinsky 1979; Ladizinsky et al. 1984; Muench et al. 1991), other studies have reached equivocal or conflicting conclusions (Renfrew 1969; Hoffman et al. 1986). Many of the aforementioned studies also assessed the genetic diversity within the cultivated and wild accessions of *Lens* in an attempt to identify genetic markers useful in breeding programs. However, only a relatively small number of cultivated accessions have been included in these surveys, and only a minor amount of variation was revealed.

The purpose of the present study was to employ restriction-site analysis of chloroplast DNA (cpDNA) in order to: (1) assess the cpDNA variation among all available accessions of the cultivated lentil, (2) identify the progenitor of the cultivated lentil, and (3) elucidate the cpDNA phylogeny of the genus *Lens*. In the past 10 years, molecular-based methods, such as restriction-site analysis of cpDNA, have produced significant contributions to our understanding of crop evolution. Because many crop plants have undergone extensive morphological divergence from their wild relatives, traditional methods of analysis are often unsuccessful in clarifying phylogenetic relationships (Doebley 1992). Molecular approaches can provide numerous conservative markers that are easily interpreted and valuable in identifying progenitors of crop plants, reconstructing phylogenies, revealing genetic bottlenecks, and exposing possible cases of introgression where nuclear and cytoplasmic phylogenies differ (see review in Doebley 1992). Restriction-site analysis of cpDNA has been valuable in phylogenetic studies of a number of crop species, including barley (Clegg et al. 1984; Neale et al. 1988), cotton (Wendel 1989; Wendel and Albert 1992), maize (Timothy et al. 1979; Doebley et al. 1987; Doebley 1990), mustard and the cole crops (Erickson et al. 1983; Palmer et al. 1983), pea (Palmer et al. 1985), potato (Hosaka 1986; Hosaka and Hanneman 1988 a, b), sorghum (Duvall and Doebley 1990), squash and pumpkin (Doebley 1992), sunflower (Rieseberg 1990), and wheat (Ogihara and Tsunewaki 1988).

By using 20 restriction enzymes and all available accessions of the cultivated lentil, we attempted to detect genetic variation that was not accessible by previous studies and to reconstruct the phylogeny of *Lens* using cladistic methods. Although Muench et al. (1991) used cpDNA variation to address relationships in *Lens*, that study employed only four restriction enzymes, analyzed restriction fragments rather than sites, and interpreted the data phenetically. Therefore, our larger study, with a stronger emphasis on the genetic interpretation of pattern differences and phylogenetic analysis, will provide a new perspective on the phylogeny of *Lens*.

Materials and methods

Plant material was provided by the Grain Legume Genetics and Physiology Research Unit, U.S.D.A. Agricultural Research Service. The accessions included (Fig. 1) represent the Research Unit's total inventory that was available for analysis. Total DNA was isolated from 114 accessions of ssp. *culinaris* and 11 accessions representing the four wild taxa (Table 1) using a modification of the hot CTAB miniprep procedure of Saghai-Maroof et al. (1984) and Doyle and Doyle (1987). Each DNA isolate was digested with the following 20 restriction endonucleases (resulting fragments in parenthesis): *Apa*I (6), *Bam*HI (28), *Ban*I (20), *Bgl*I (2), *Bgl*II (24), *Bst*EII (8), *Bst*NI (29), *Cfo*I (40), *Dra*I (32), *Eco*RI (36), *Eco*RV (22), *Hae*II (16), *Hin*dIII (14), *Hpa*II (42), *Nci*I (33), *Pst*I (8), *Sac*I (10), *Sal*I (5) *Xba*I (20), and *Xho*I (8). *Bst*NI and *Nci*I recognize a 5-bp sequence, and *Cfo*I and *Hpa*II recognize 4-bp sequences; the remaining enzymes have 6-bp recognition sequences. The resulting fragments were separated by electrophoresis through 1% agarose gels, denatured, and transferred to nylon membranes (Zetabind, Cuno Laboratory Products, Meriden, Conn.). The filter-bound DNA was probed through hybridization with ^{32}P-labeled, cloned fragments of the chloroplast genomes of lettuce (Jansen and Palmer 1987) and petunia (Palmer 1983). The lettuce and petunia clones were provided by Bob Jansen and Jeff Palmer.

Restriction-site data were analyzed by "Phylogenetic Analysis Using Parsimony" (PAUP version 3.0s: Swofford 1991) to reconstruct the phylogeny of *Lens*. A heuristic search with stepwise simple addition and TBR branch swapping was performed, followed by a search employing the "branch-and-bound" algorithm (Hendy and Penny 1982), a more time-consuming approach guaranteed to identify all optimal trees. *Vicia montbretii* was used as the outgroup because this species was formerly classified within *Lens* but is now considered distinct from the genus and a likely close relative (Ladizinsky and Sakar 1982). Those mutations that were observed among accessions of *Lens*, but could not be polarized, were coded as missing data for *Vicia*. Estimates of DNA sequence divergence were calculated from restriction-site data for each pair of *Lens* accessions using equations 8 and 10 of Nei and Li (1979).

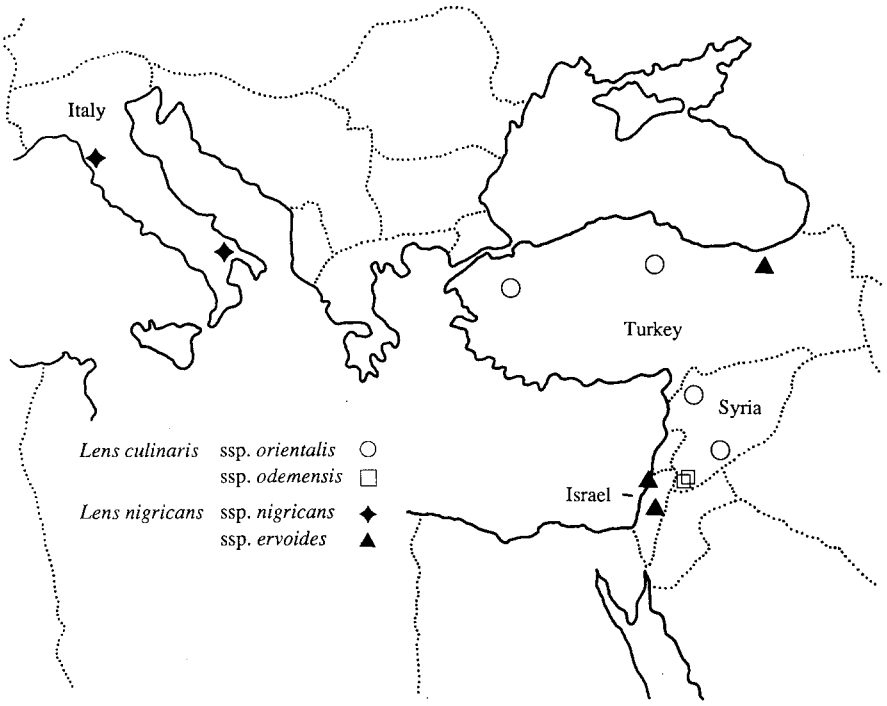

Fig. 1. Locations of the wild accessions analyzed in this study

Table 1. Accessions of wild and cultivated *Lens* analyzed, listed by numbers and names of the cataloging system of the Grain Legume Genetics and Physiology Research Unit, U.S.D.A./A.R.S.

Lens culinaris				
ssp. *culinaris*				
LC660789	LC660324	LC660342	WA8649084	LC760254
LC660798	WA8649090	WA8649085	LC760377	WA9849044
LC760394	LC660194	LC660206	LC760393	Emerald
LC6601096	LC6601165	LC6601042	Benewah	LC560189
LC660952	LC660980	Brewer	LC660999	WA8649014
Chilean 78	Palouse	LC760218	LC760235	WA8649041
LC860053	LC860209	LC760630	LC760154	Rose
LC860210	LC860137	LC960090	LC960105	LC960134
LC960024	LC960158	LC960149	LC960098	LC960156
LC960148	LC960159	LC960007	LC960142	LC960008
LC960099	LC960093	LC960092	LC960144	LC960102
LC960013	LC960104	LC960010	LC960009	LC960015
LC900002	LC960154	LC900001	LC960115	LC960096
LC960003	LC960114	LC960256	LC960253	LC960257
LC960232	LC960182	LC960179	LC960198	LC960255
LC960180	LC960224	LC960174	LC960166	LC960189
LC960178	LC960245	LC960247	LC960203	LC960206
LC960209	LC960214	LC960202	LC960254	LC960249
LC960223	LC460007	LC860175	LC860208	LC860047
Redchief	LC860049	LC760273	Crimson	LC764099
LC860213	LC660819	LC860174	LC860048	LC860200
LC860168	LC760809	LC760819	LC860188	LC860063
LC760414	LC860186	LC860129	LC860152	
ssp. *odemensis*				
	Ld163	Ld164		
ssp. *orientalis*				
	Lo77	Lo156	Lo157	Lo108
Lens nigricans				
ssp. *nigricans*				
	Ln13	Ln21		
ssp. *ervoides*				
	Le52	Le53	Le85	

Results

The approximate size of the chloroplast genome of *Lens* is 120 kb, smaller than the chloroplast genomes of most land plants, but comparable to those of related legumes (Palmer et al. 1985). This small size is due to the loss of a 25-kb inverted repeat sequence in the common ancestor of most of the papilionoid legumes (Lavin et al. 1990). However, our calculation of the lentil chloroplast genome size is also smaller than the value of 125 kb derived by Muench et al. (1991), who employed ^{35}S end-labelling of restriction fragments to simultaneously visualize all cpDNA fragments generated by a single restriction endonuclease. The discrepancy in the two estimates may be due to our difficulty in detecting fragments smaller than 400 bp. We constructed restriction site maps for three enzymes, *Hin*dIII, *Eco*RV, and *Hae*II, using ssp. *culinaris* accession #LC6601096 (Fig. 2).

An analysis of 399 restriction sites (approximately 2274 bp or 1.9% of the genome) in the 114 lentil cultivars revealed a single restriction-site loss (#16, Table 2) shared by accessions LC6601165 and LC660189 (hereafter referred to as 1165 and 0189) and a single distinct insertion (LM 1, Table 2) in accession LC860168. Poor digestion of DNA samples, or failure of probe hybridization, resulted in missing data points for accessions LC660789, LC660194, LC860049, LC960092, and LC960009. Nineteen restriction-site mutations and two length mutations were detected among the 11 wild accessions (Table 2). Only eight of these restriction-site mutations could be polarized

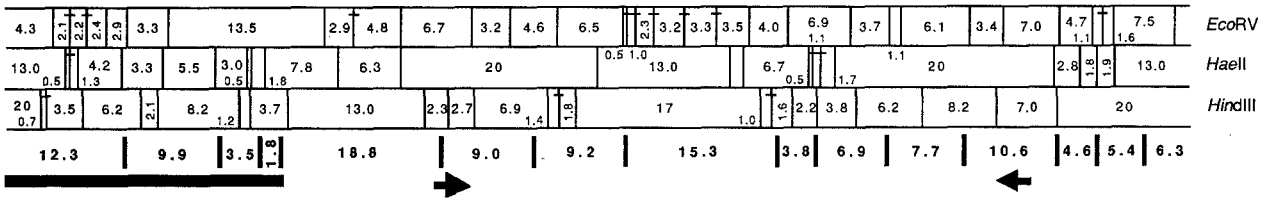

Fig. 2. Restriction-site map of the chloroplast genome of the cultivated lentil. Approximate fragment sizes (kb) are noted within the blocks; a *horizontal bar* overlapping blocks denotes an arbitrary ordering of those blocks. The approximate positions of the lettuce and petunia probes in the genome are noted below the map; a *dark band* underlies the section of the genome that is repeated in most land plants, and *arrows* mark the endpoints of the 50-kb inversion common to all legumes

Table 2. Restriction-site mutations and length mutations (LM) detected in *Lens*, fragment size in kb; parentheses denote primitive character state, mutations without parentheses could not be polarized. The cloned *Lactuca Sac*I and *Petunia Pst*I fragments used to detect each mutation are noted. Accessions that bear a particular mutation can be identified in Fig. 3

	Enzyme	Mutation	Probe
1	*Bam*HI	4.2 + 0.4 − 4.6	*Sac*I 12.3/9.9/3.5/1.8
2	*Eco*RV	2.4 + 0.9 − 3.3	*Sac*I 12.3/9.9/3.5/1.8
3	*Xho*I	(15.7) − 10.5 + 5.2	*Sac*I 12.3/9.9/3.5/1.8
4	*Eco*RI	(1.6 + 0.1[a]) − 1.7	*Sac*I 18.8
5	*Eco*RV	(3.0 + 4.8) − 7.8	*Sac*I 18.8
6	*Hpa*II	(2.2) − 1.4 + 0.8	*Sac*I 18.8
7	*Pst*I	(5.7 + 2.8) − 8.5	*Sac*I 18.8
8	*Eco*RV	(6.7 + 3.8) − 10.5	*Sac*I 18.8/*Pst*I 9.0/9.2/15.8
9	*Bam*HI	(8.8) − 4.6 + 4.2	*Pst*I 9.0/9.2/15.8
10	*Eco*RI	4.3 + 2.0 − 6.3	*Pst*I 9.0/9.2/15.8
11	*Eco*RI	4.6 + 2.0 − 6.6	*Pst*I 9.0/9.2/15.8
12	*Eco*RI	3.5 + 1.1 − 4.6	*Pst*I 9.0/9.2/15.8
13	*Eco*RI	3.1 + 1.2 − 4.3	*Pst*I 9.0/9.2/15.8
14	*Eco*RV	5.6 + 1.2 − 6.8	*Pst*I 9.0/9.2/15.8
15	*Xba*I	4.8 + 1.7 − 6.5	*Pst*I 9.0/9.2/15.8
16	*Ban*I	(9.0 + 3.2) − 12.2	*Sac*I 3.8/6.9/7.7/10.6
17	*Eco*RV	5.0 + 2.4 − 7.4	*Sac*I 3.8/6.9/7.7/10.6
18	*Eco*RV	4.0 + 3.4 − 7.4	*Sac*I 3.8/6.9/7.7/10.6
19	*Sal*I	(14.7) − 12.2 + 2.5	*Sac*I 3.8/6.9/7.7/10.6
20	*Bgl*II	1.85 + 0.15[a] − 2.0	*Sac*I 4.6/5.4/6.3
LM1		+ 0.1	*Sac*I 18.8/*Pst*I 9.0/9.2/15.8
LM2		+ 0.1	*Pst*I 9.0/9.2/15.8
LM3		− 0.1	*Sac*I 3.8/6.9/7.7/10.6

[a] Fragment inferred, too small for detection

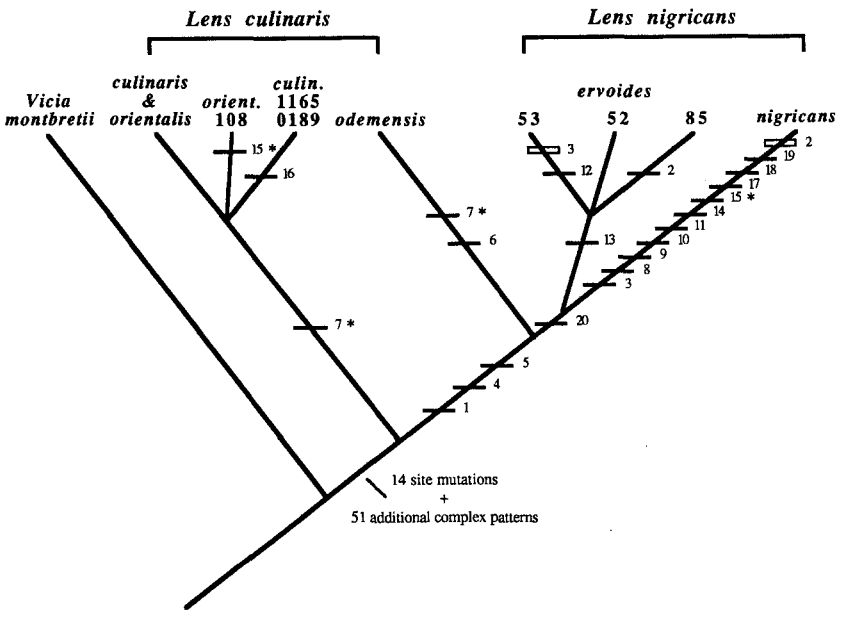

Fig. 3. Phylogeny of *Lens* taxa based on a cladistic analysis of cpDNA restriction-site data. The numbered *hatch marks* represent the restriction site mutations listed in Table 2; *asterisks* mark the two homoplasious site changes, and the *open boxes* represent the length mutations; LM 1 is not shown

unambiguously through comparison with patterns from *V. montbretii*, but both length mutations were polarizable through outgroup comparison and were interpreted as an insertion (LM 2) and a deletion (LM 3). No variation was detected among accessions of either *odemensis* or *nigricans*. In contrast, three autapomorphic mutations differentiated the three accessions of subspecies *ervoides* (#2, #12, LM 3), and a homoplasious site loss shared by *nigricans* and accession 108 of *orientalis* (#15) distinguished this population of *orientalis* from the other three populations analyzed. The chloroplast genome of *V. montbretii* is extremely divergent from all members of *Lens*: 14 interpretable restriction-site mutations and at least 51 other ambiguous restriction fragment patterns (data not shown) distinguish *V. montbretii* from *Lens*.

Three minimum-length cladograms of 22 steps with a consistency index of 0.909, including autapomorphies, were generated by PAUP. The results from the heuristic search and branch-and-bound search were identical. All trees presented the *odemensis/ervoides/nigricans* clade (Fig. 3) as a monophyletic group within *Lens* and showed the same relationships among these subspecies. The tree presented was chosen to depict restriction-site mutation 7 as a homoplasious loss shared by the *culinaris/orientalis* clade and *odemensis*. The two trees not shown differ in the positions of *orientalis* 108 and the two *culinaris* cultivars (abbreviated *culin.* 1165 and 0189) relative to the *culinaris/orientalis* clade. One of these trees displays a tetrachotomy consisting of the *odemensis/ervoides/nigricans* clade, the *culinaris/orientalis* clade, *orientalis* 108, and *culinaris* 1165/0189. The other tree removes *orientalis* 108 from the tetrachotomy and places it as the sister to a trichotomy composed of the remaining three lineages. The analyses detected two homoplasious site changes (#7 and #15, Table 2). With the exception of the single autapomorphy (#16) in *culinaris* 1165 and 0189, the 114 cultivars of *culinaris* were identical to accessions 77, 156, and 157 of *orientalis* at every site; thus, most accessions of these two taxa share the same terminus on the tree. Despite being classified as a subspecies of *L. culinaris*, *odemensis* shares a closer common ancestor with members of *L. nigricans* than with *culinaris* or *orientalis*. *L. nigricans* ssp. *nigricans* and ssp. *ervoides* are sister taxa supported by mutation #20. Subspecies *nigricans* is extremely divergent from the remainder of the genus, forming a lineage supported by ten restriction-site mutations and one insertion.

Percent DNA sequence-divergence estimates between taxa in *Lens* range from 0.00 to 0.34 (Table 3). Subspecies *culinaris* (with the exception of 1165/0189) and *orientalis* (without 108) have identical cpDNAs and consequently a sequence-divergence estimate of 0.00; comparison of *culinaris/orientalis* and *nigricans* yielded the highest value.

Discussion

As in other members of the legume family, *Lens* displays a 50-kb inversion in the region equivalent to the large single-copy region of other angiosperms (Palmer et al. 1987). Naturally, this inversion precludes colinearity of the lentil chloroplast genome with those of lettuce or petunia, the sources of the cloned fragments for our restriction-site analysis. If two different

cloned fragments overlap the endpoints of this inversion, the same polymorphism could be scored as two independent mutational events. If this scenario did occur, we did not detect it, perhaps because the levels of polymorphism in *Lens* were quite low and mutations were easily interpretable.

Another characteristic of the lentil chloroplast genome is the absence of the large inverted repeated sequence common to almost all terrestrial plants (Lavin et al. 1990). Other legume chloroplast genomes that lack the inverted repeat have shown a higher frequency of inversional mutations than do chloroplast genomes possessing the inverted repeat. Palmer and his co-workers (Palmer and Thompson 1982; Palmer et al. 1987, 1988; Lavin et al. 1990) have suggested that the inverted repeat confers a stability to the chloroplast genome, and that sequence rearrangements are more common when it is absent. In the present study we did not identify any rearrangements unique to the lentil, but this may simply reflect the narrower taxonomic scope and objectives of our study and methods that were biased against revealing small rearrangements. It was evident, however, that the chloroplast genome of *Lens* is extremely divergent from that of its putative closest relative, *V. montbretii*, and the large number of ambiguous restriction-fragment patterns of *V. montbretii* (at least 51) could signify a considerable number of structural differences between the chloroplast genomes of *Vicia* and *Lens*. The distinctiveness of *V. montbretii* from *Lens* is also reflected in differences in chromosome number (n = 6 and 7, respectively) and an inability to hybridize (Ladizinsky and Sakar 1982).

Chloroplast DNA variation in Lens

Only a single restriction-site loss and a single insertion were observed in 114 accessions of the cultivated lentil. These results may indicate that the lentil was domesticated from an ancestor (apparently *orientalis*) that possessed little cpDNA diversity, or else that the lentil experienced a cytoplasmic "bottleneck" during domestication and lost much of its variation. The latter syndrome has occurred in the history of many cultivated plants (Doebley 1992), and it probably has affected the lentil as well. However, because only four accessions of *orientalis* were available for our study, the levels of cpDNA diversity observed in the putative progenitor of the cultivated lentil may underestimate the true genetic diversity of this taxon. On the other hand, these accessions of *orientalis* were collected from widely-distributed populations within the western portion of its range (see Fig. 1), offsetting, in part, the problem of small sample size and supporting the claim that *orientalis* is depauperate in cpDNA variation. The low amount of restriction-site and length variation present within the other subspecies of the genus indirectly supports the "depauperate" hypothesis. For example, the two accessions of *nigricans* are identical, the two accessions of *odemensis* are identical, and two of the three accessions of *ervoides* exhibit just one or two autapomorphies each (Fig. 3).

Sequence-divergence values summarizing cpDNA variation among accessions of *Lens* (Table 3) are low compared to estimates from genera such as *Clarkia* (Sytsma et al. 1990), *Sorghum* (Duvall and Doebley 1990), *Microseris* (Wallace and Jansen 1990), *Fuchsia* (Sytsma et al. 1991), *Gossypium* (Wendel and Albert 1992), and *Krigia* (Kim et al. 1992), but comparable to values estimated for taxa within *Lycopersicon* (Palmer and Zamir 1982), *Lisianthius* (Sytsma and Schaal 1985), *Gutierrezia* (Suh and Simpson 1990), and *Helianthus* (Rieseberg et al. 1991). This latter group of genera comprises taxa considered to have undergone relatively-recent and/or rapid radiation, a scenario that may apply to *Lens* as well. Perhaps the best study with which to compare our findings is an analysis of *Pisum* (Palmer et al. 1985), a close relative of *Lens* that comprises a similar number of species, and that also lacks

Table 3. Percent sequence-divergence estimates for pairs of *Lens* accessions based on restriction-site data (Nei and Li 1979), below diagonal; number of restriction sites differentiating pairs of accessions, above diagonal. Accessions 1165 and 0189 were not included in comparisons involving *culinaris*, therefore the divergence of *culinaris* and *orientalis* (without 108) is 0.00

	culinaris and *orientalis*	*orientalis* 108	*odemensis*	*nigricans*	*ervoides* 52	*ervoides* 53	*ervoides* 85
culinaris and *orientalis*	X	1	4	15	6	7	7
orientalis 108	0.02	X	5	14	7	8	8
odemensis	0.11	0.11	X	13	4	5	5
nigricans	0.34	0.32	0.30	X	11	12	12
ervoides 52	0.14	0.16	0.09	0.25	X	1	1
ervoides 53	0.16	0.18	0.11	0.27	0.02	X	2
ervoides 85	0.16	0.18	0.11	0.27	0.02	0.04	X

the inverted repeat (Palmer and Thompson 1981; overview in Lavin et al. 1990). Palmer et al. (1985) surveyed less than half the number of restriction sites we did and found 11 restriction-site mutations and 17 length mutations within and among species of *Pisum*. Estimates of sequence divergence between species of *Pisum* were calculated using the algorithm of Brown et al. (1979) and range from 0.10 to 0.81% (Palmer et al. 1985), higher than the values between species of *Lens* (range 0.09–0.34%), which were calculated using the algorithm of Nei and Li (1979). However, the algorithm of Brown et al. (1979) may overestimate levels of sequence divergence (Nei and Li 1979) and, in fact, does yield estimates of divergence in *Lens* that are approximately twice the magnitude (viz., 0.18–0.68%) of those calculated using the approach of Nei and Li (1979). Consequently, interspecific sequence divergence in *Lens* and *Pisum* is quite similar when only estimates derived using the Brown et al. (1979) approach are compared (ranges are 0.18–0.68% and 0.10–0.81%, respectively). The ratio of length mutations to restriction-site mutations in *Pisum* (17:11) is much higher than our value for *Lens* (3:20). However, 15 of the 17 length mutations observed in *Pisum* are due to two hotspot regions of frequent insertions and/or deletions (regions 1 and 3, Palmer et al. 1985), and if these regions are disregarded, *Pisum* and *Lens* are more comparable in the number of length mutations (2 vs 3, respectively). Furthermore, *Pisum* comprises four species whereas *Lens* comprises two, a difference that may also account for the greater cpDNA diversity in *Pisum*.

Origin of the cultivated lentil

The identical cpDNA restriction-site patterns of 112 accessions (of 114) of *culinaris* and three of four accessions of *orientalis* leave little doubt that the lentil was domesticated from *orientalis* ancestors. This progenitor/derivative relationship has been suggested by studies employing a variety of techniques: morphology (Barulina 1930), ecology and archeology (Zohary 1972; Zohary and Hopf 1973), numerical taxonomy and pollen morphology (Williams et al. 1974), cytology and hybrid fertility (Ladizinsky 1979; Ladizinsky et al. 1984), and analysis of cpDNA restriction fragment length polymorphisms (Muench et al. 1991). In contrast, Renfrew (1969) proposed *L. nigricans* as the progenitor on the basis of archeological evidence, and the most extensive allozyme analysis to-date (Hoffman et al. 1986) was not able to distinguish between *orientalis* and *odemensis* as the progenitor of *culinaris*. The chief difference between our investigation and the aforementioned studies lies in our cladistic method of inferring phylogenetic relationships rather than in a phenetic measure of overall similarity between taxa.

Lens phylogeny

Most contemporary phylogenetic studies use gene or organellar trees to estimate organismal trees, but the potential for incongruence between these phylogenies must be considered (Nei 1987; Avise 1989; Doyle 1992). Introgression and lineage sorting can cause a cpDNA-based phylogeny to suggest incorrect relationships among species (Rieseberg and Soltis 1991; Doyle 1992; Rieseberg and Brunsfeld 1992). A phylogeny of *Lens* taxa based on cpDNA restriction-site data offers no indication that either of these processes has influenced the topology of the tree. In fact, our interpretation of relationships within *Lens* differs from those based on allozymic data (Pinkas et al. 1985) or nuclear RFLP data (Muench et al. 1991) only because we used a cladistic method to analyze the restriction-site data; our estimates of sequence divergence, on the other hand, show patterns of overall similarity identical to the patterns revealed by these other studies. The cpDNA-based phylogeny of *Lens* taxa depicts relationships that generally support the current taxonomy of the genus. Subspecies *culinaris* and *orientalis* are closely related within *L. culinaris*, and sspp. *nigricans* and *ervoides* form the *L. nigricans* clade. The phylogenetic placement of *odemensis* as the sister taxon to *L. nigricans* is supported by three mutations. This result was unexpected, as it depicts *L. culinaris* as paraphyletic, i.e., in order for species *culinaris* to be monophyletic, it must now include *L. nigricans*.

These results reaffirm the utility of cpDNA variation in systematic studies at the inter- and even intraspecific levels (reviewed in Soltis et al. 1992) and demonstrate the advantage of cladistic methods over phenetic methods in phylogeny reconstruction. Previous phenetic analyses of isozymes (Pinkas et al. 1985; Hoffman et al. 1986) and both nuclear (Havey and Muehlbauer 1989) and cpDNA (Muench et al. 1991) restriction fragment length polymorphisms found both *odemensis* and *ervoides* more similar to *culinaris* and *orientalis* than to *nigricans*. Furthermore, most recent systematic studies have reported a high divergence of *nigricans* from all other taxa in *Lens* (e.g., cytology/crossability: Ladizinsky et al. 1984; allozyme analysis: Pinkas et al. 1985; Hoffman et al. 1986; nuclear DNA RFLP analysis: Havey and Muehlbauer 1989; cpDNA RFLP analysis: Muench et al. 1991). In the present study, cpDNA sequence divergence estimates (Nei and Li 1979) for each pair of accessions (Table 3) also depict the distinctness of *nigricans*, reflected in a higher divergence of both *ervoides* and *odemensis* from *nigricans* than from *orientalis* and *culinaris*. The corroboration of these data sets presents a strong case that *nigricans* has experienced an accelerated rate of evolution relative to the other members of *Lens*, and does not simply harbor a rapidly-evolving chloroplast genome.

It appears that the high degree of divergence of *nigricans* in both nuclear and cpDNA characteristics has caused analyses based on overall similarity in these genetic markers to group *culinaris, orientalis, odemensis,* and *ervoides* relatively closely, giving what we suggest may be an erroneous picture of evolutionary relationships within *Lens*. We therefore recommend using a cladistic approach both in the analysis of additional, independent data sets in *Lens* and in a reanalysis of previously-obtained data, where possible and appropriate. If our cpDNA-based phylogeny of *Lens* is accurate, it provides a basis for a classification that is fully consistent with stipule shape – one of only two morphological characteristics that have taxonomic utility in the genus. It is ironic that the semihastate stipule shape may represent a synapomorphic morphological character after all, and could be used to reunite ssp. *odemensis* with sspp. *ervoides* and *nigricans*, allowing monophyletic assemblages within both *L. nigricans* and *L. culinaris*.

Note added in proof

Ladizinsky (1993) has revised the taxonomy of *Lens*. This new interpretation is fully reconciled with the cpDNA phylogeny of the present paper. All subspecies have been elevated to species status with the exception of *culinaris* and *orientalis*, which are retained as subspecies under *L. culinaris*.

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