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ORIGINAL PAPER

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Interspecific relationships of the genus *Cicer* L. (Fabaceae) based on *trn*T-F sequences

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Abstract The *trn*T-F region in chloroplasts was sequenced to elucidate interspecific phylogenetic relationships in the genus *Cicer*. Twenty-five species representing four sections and two outgroups were analyzed. A phylogenetic analysis revealed three major clades in the genus *Cicer*. Inferred phylogenetic relationships support multiple origins of annual species in the genus *Cicer*. Low variation within the most perennial species in the sequence regions suggests they may have originated during a period of rapid diversification after the genus arose. High levels of sequence divergence, biogeographical patterns and morphological traits between African and Eurasian groups of species suggest that *Cicer* may have independently diverged on each continent. Phylogenetic analysis of sequence data did not support the monophyly of the currently recognized sections and indicated the need for a revision of the infrageneric classification.

Introduction

Cicer L. (Fabaceae) consists of about 43 species which are distributed throughout the Northern Hemisphere. Thirty-nine *Cicer* species occur in central and western Asia and four species in isolated areas of north and northwest Africa and Europe (Van der Maesen 1987). The genus contains herbaceous and shrubby species. The annual chickpea, *C. arietinum* L., is a well known cultivated species and is the third most important legume crop. Other annual and perennial species are important sources for animal feed, traditional human usage and agents of soil fertility.

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The genus was traditionally classified into four sections, reflecting differences in annual and perennial habits. The first section, *Cicer* (=*Monocicer* M.G. Pop.), includes the cultivated species *C. arietinum* and seven annual species. The second section, *Chamaecicer*, contains two species, one annual and one perennial. These species grow in the mountainous regions of western Asia and Crete. The third section, *Polycicer*, is comprised of 25 perennial species. The members of this section grow widely throughout the range of the genus. The fourth section, *Acanthocicer*, contains seven perennial species which grow in mountainous regions of Persia, Afghanistan and Central Asia (Popov 1976; Van der Maesen 1987). The life cycle of the species *C. laetum* Rassulova et Sharipova has not been described (Van der Maesen 1987).

The cultivated species *C. arietinum* is conventionally divided into two types: 'Kabuli' and 'Desi.' 'Kabuli' (large, ram-shaped, with cream- or beige-colored seeds) is predominantly distributed throughout Mediterranean countries and the Near East. 'Desi' (small, angular, with dark-colored seeds) is prevalent in the eastern and southern parts of the distribution area of the crop (Van der Maesen 1972; Zohary and Hopf 1993).

Determination of the interspecific relationships of the genus *Cicer* is important for the breeding of new chickpea lines where germplasm from closely related *Cicer* species can be incorporated into the chickpea germplasm. Cytological (Ladizinsky and Adler 1976; Singh and Ocampo 1993) and seed storage protein analyses (Ladizinsky and Adler 1975; Ahmad and Slinkard 1992), isozyme variation (Kazan and Muehlbauer 1991; Ahmad et al. 1992; Ladbi et al. 1996; Tayyar and Waines 1996), random amplified polymorphic DNA (Ahmad 1999; Iruela et al. 2002; Sudupak et al. 2002; Javadi and Yamaguchi 2004), RFLP (Serret et al. 1997), AFLP (Sudupak et al. 2003) and microsatellite markers (Udupa et al. 1999; Choumane et al. 2000) have been used to investigate this area. The general consensus among these studies is that *C. reticulatum* is the most likely candidate for the wild progenitor of *C. arietinum*. However, the results of several of these studies have shown conflicting patterns in the species

relationship. For example, Ahmad (1999) examined nine annual species and grouped them into four clusters. The first cluster consisted of *C. arietinum*, *C. reticulatum* and *C. echinoseprmum*. The second included *C. yamashitae* and *C. chorassanicum*. The third comprised *C. pinnatifidum*, *C. judaicum* and *C. echinospermum*. *C. cuneatum* made the (far distant) fourth group. He found that the placement of two annuals, *C. chorassanicum* and *C. yamashitae*, adjacent to the cluster containing *C. arietinum*. However, Kazan and Muehlbauer (1991), Ahmad and Slinkard (1992) and Tayyar and Waines (1996) indicated that these two annuals are farther removed from *C. arietinum* when compared with other annual species. Sudupak et al. (2002) indicated the grouping of three annuals, *C. pinnatifidum*, *C. judaicum* and *C. bijugum*, with perennial species, while Iruela et al. (2002) did not find this clustering. Kazan and Muehlbauer (1991) and Choumane et al. (2000) examined nine annuals and one perennial (*C. anatolicum*) and reported that there was a close relationship among *C. anatolicum*, *C. reticulatum*, *C. echinospermum* and *C. arietinum*, while Tayyar and Waines (1996), Iruela et al. (2002), Sudupak et al. (2002, 2003) and Javadi and Yamaguchi (in press) studied more than one perennial species (2–7) and did not find this relationship. On the whole, insufficient taxon sampling, particularly among perennial species, hampers comparisons among studies.

DNA sequence data have been used as a source of phylogenetic markers; and these data provide more reliable information on evolutionary relationships at different taxonomic levels. The chloroplast genome is well suited for evolutionary and phylogenetic study, because chloroplast (cp)DNA is a relatively abundant component of plant total DNA, providing an extensive background of molecular information on the chloroplast genome. Owing to these advantages, the comparative analysis of cpDNA sequence data is a quickly expanding area (Clegg and Zurawski 1992). The cpDNA region from the *trn*T (UAA) 5′ exon to *trn*F (GAA) has been used extensively as a source of phylogenetic markers, particularly for the resolution of interspecific relationships (e.g., Gielly and Taberlet 1996; Ohsako and Ohnishi 2000; Mummenhoff et al. 2001). The region includes the 5′ *trn*L exon, the *trn*L intron, the 3′ *trn*L exon, the intergenic spacer and the *trn*F exon regions. The region between the *trn*T (UGU) and *trn*F (GAA) genes in the large single-copy region was particularly suitable for: (1) the succession of conserved *trn* genes and several hundred base pairs of non-coding regions, (2) the absence of gene rearrangements among tobacco, rice and *Marchantia* and (3) the higher rate of molecular evolution of the single-copy regions (Taberlet et al. 1991). With regards to previous research, no phylogenetic relationship within the genus *Cicer* using DNA sequencing has so far been performed.

The purposes of this paper were to use cpDNA sequences in order to determine the following: (1) the interspecific relationships of the genus *Cicer* and (2) the monophyly of annual and perennial species of the genus *Cicer*.

Materials and methods

Plant materials

Twenty-nine accessions from 25 species were studied, representing four sections of *Cicer*, with *Lens ervoides* (Brign.) Grande and *Pisum sativum* L. as outgroups (Table 1). The number of accessions analyzed for each species were: two for *C. reticulatum*, two for *C. echinospermum*, two for *C. arietinum*/ ‘Desi’, two for *C. arietinum*/ ‘Kabuli’ and one each for the remaining 22 species (Table 1). The voucher specimens are deposited in the Herbaria of Osaka Prefecture University and Kyoto University. The complete nucleotide sequences are deposited in DDBJ/EMBL/GenBank data bases.

DNA extraction, marker amplification and DNA sequencing

Genomic DNA was extracted from freshly collected, silica gel-dried or herbarium specimen leaves, using a cetyltrimethyl ammonium bromide protocol with minor modification (Doyle and Doyle 1987). The cpDNA region *trn*T-F was used in this study and was amplified in two sections. The primers used to amplify and sequence this region are shown in Table 2. The primer pair trn-a and trn-b amplified the region between *trn*T and *trn*L. The primer pair trn-c and trn-f amplified the region between *trn*L and *trn*F. Since the intergenic spacer region between *trn*T (UGU) and *trn*L (UAA) was more than 900 bp long in the *Cicer* species and the CEQ 2000XL DNA sequencer (Beckman Coulter) could only read lengths about 600–700 bp long, four internal primers (trn-ab1F, trn-ab1R, trn-ab2F, trn-ab2R) were designed to read completely the intergenic spacer region (Table 2). The primers for sequence reactions of the *trn*L–*trn*F region were trn-c, trn-d, trn-e and trn-f. Polymerase chain reactions (PCR) were carried out in 25 μl reactions containing 13.9 μl of dH_2O, 2.5 μl of 10× reaction buffer, 2.5 μl of 25 mM $MgCl_2$, 2.5 μl of 1.25 mM dNTPs, 1.25 μl of each 10 μM primer, 0.1 μl Taq (5 units/μl) and 1 μl of 1.0 ng/μl genomic DNA. The PCR amplification began with initial denaturation for 2 min at 94 °C, followed by 35 cycles of denaturation (94 °C for 1 min), annealing (60 °C for 1 min) and extension (72 °C for 2 min), with a final extension at 72 °C for 5 min in a thermocycler (Gene Amp PCR System 2700, Applied Biosystems). The resulting PCR products were checked on a 2% agarose gel. The successful PCR reactions were purified using the QIAquick Spin PCR purification kit (QIAgen, Valencia, Calif.), following the manufacturer’s instructions.

The PCR amplification primers and internal primers were used as sequencing primers. The 20-μl cycle-sequencing reaction contained about 100 ng of template DNA, 4 μl of BigDye terminator RR mix (Applied Biosystems), 1 μl of primers (10 μM) and the appropriate amount of sterile water for a total volume of 20 μl. The cleaned cycle-sequencing products were analyzed on a CEQ 2000XL DNA sequencer (Beckman Coulter). Each sequencing was carried once for each accession. When there was an ambiguous sequencing, the cycle-sequencing was repeated.

Phylogenetic analyses

Sequences were aligned using the SeqEd program ver. 1.0.3 (Applied Biosystems) followed by manual corrections. Species of the genera *Vicia*, *Trifolium*, *Pisum* and *Lens* were initially selected as outgroups. However, the complete sequences of the *trn*T-L region of *Trifolium* and *Vicia* were unsuccessful.

Parsimony analyses of the data were performed using the heu- ris tic search options in PAUP* ver. 4.0b4a (Swofford 2000). The characters were equally weighted and the character states were specified as unordered. The gaps were treated as missing data and shared alignments gaps were scored as binary characters. The heuristic search strategies were performed according to the methods of Catalán et al. (1997) and Downie et al. (2000). Clade robustness was evaluated by the bootstrap method (Felsenstein 1985), using

Table 1 Taxa for sequencing of *trn* T-F region. ICC, International Crops Research Institute for the Semi-Arid Tropics (India); ILWC, Center for Agricultural Research in Dry Areas (Aleppo, Syria); PI, United States Department of Agriculture Plant Introduction Station (USA); TN, National Plant Gene bank of Iran (Iran); voucher, voucher specimen-extracted DNA

Scientific name/cultivar group	Section or tribe	Country	Accession number or voucher
Cicer arietinum L./Desi	*Cicer*	India	PI 315796
		Turkey	PI 595983
C. arietinum L./Kabuli	*Cicer*	Afghanistan	ICC 11234
		Turkey	ICC 10350
C. bijugum K.H. Rech.	*Cicer*	Iran	NPGBI
C. cuneatum Hochst. ex Rich	*Cicer*	Ethiopia	ILWC 37
C. echinospermum P.H. Davis	*Cicer*	Syria	PI 599067
		Turkey	PI 527930
C. judaicum Boiss.	*Cicer*	Lebanon	PI 599104
C. pinnatifidum Jaub. et Spach	*Cicer*	Turkey	PI 599109
C. reticulatum Ladiz.	*Cicer*	Turkey	PI 572537
		Turkey	PI 599042
C. yamashitae Kitamura	*Cicer*	Afghanistan	PI 510664
C. chorassanicum (Bge.) M.Pop.	*Chamaecicer*	Iran	TN 41-5274
C. anatolicum Alef.	*Polycicer*	Iran	Fathi 1637
C. canariense Santos Guerra et Lewis	*Polycicer*	Spain	PI 557453
C. flexusoum Lipsky	*Polycicer*	Tajikistan	PI 599102
C. kermanense Bornm.	*Polycicer*	Iran	Hamze-Nezhad 474
C. microphyllum Benth.	*Polycicer*	India	PI 599093
C. montbretii Jaub. et Spach	*Polycicer*	Turkey	PI 599090
C. multijugum Van der Maesen	*Polycicer*	Uzbekistan	PI 599085
C. nuristanicum Kitamura	*Polycicer*	Pakistan	PI 604497
C. songaricum Steph. ex D.C.	*Polycicer*	Uzbekistan	PI 599053
C. spiroceras Jaub. et Spach	*Polycicer*	Iran	TN 41-5282
C. subaphyllum Boiss.	*Polycicer*	Iran	Hatami, Jalilian, Fathi 6,6,1996
C. macracanthum M.Pop.	*Acanthocicer*	Pakistan	PI 599080
C. pungens Boiss.	*Acanthocicer*	Afghanistan	PI W6 14190
C. stapfianum K.H. Rechinger	*Acanthocicer*	Iran	Hatami, Jalilian, Fathi 5,6,1996
C. tragacanthoides Jaub. et Spach	*Acanthocicer*	Iran	TN 41-5261
Lens ervoides (Brign.) Grande	Vicieae	Turkey	PI 572314
Pisum sativum L.	Vicieae	Japan	

Table 2 Amplification and sequencing primers used in this study

Primer name	Primer sequence (5′ to 3′)	Use	Source
Forward			
trn-a	CATTACAAATGCGATGCTCT	Amplification, sequencing	Taberlet et al. (1991)
trn-ab1F	GAGGGATATTTT(T/C)GATTTAT	Sequencing	Designed in this study
trn-ab2F	GGTCTGTATCTATTCGCTCG	Sequencing	Designed in this study
trn-c	CGAAATCGGTAGACGCTACG	Amplification, sequencing	Taberlet et al. (1991)
trn-e	GGTTCAAGTCCCTCTATCCC	Sequencing	Taberlet et al. (1991)
Reverse			
trn-ab2R	ATAAATC(A/G)AAAATATCCCTC	Sequencing	Designed in this study
trn-ab1R	CGAGCGAATAGATACAGACC	Sequencing	Designed in this study
trn-d	GGGGATAGAGGGACTTGAAC	Sequencing	Taberlet et al. (1991)
trn-f	ATTTGAACTGGTGACACGAG	Amplification, sequencing	Taberlet et al. (1991)
trn-b	TCTACCGATTTCGCCATATC	Amplification, sequencing	Taberlet et al. (1991)

1,000 replicate, simple addition sequences and TBR branch-swapping.

Results

Sequence characteristics

The *trn*T-L sequences ranged from 727 bp (*C. cuneatum*, with a large deletion of 75 bp) to 1,047 bp (*C. microphyllum*). The final length of the *trn*T-L alignment was 1,253 bp. A total of 192 characters were parsimony-informative. The *trn*L-F region ranged from 661 to 784 bp in length. After alignment, including alignment of the outgroups, the total region consisted of 963 bp, in which 74 sites were phylogenetically informative. The combined data of *trn*T-L and *trn*L-F sequence length ranged over 1,388–1,795 bp. The combined aligned sequence was 2,216 bp, of which 266 characters were parsimony-informative. Adding the five gaps (one gap in *trn*T-L, four in *trn*L-F region) made a total of 271 characters.

No variation among four accessions of the two major types of *C. arietinum* (‘Kabuli’ and ‘Desi’) was found in the *trn*T-L and *trn*L-F regions. Only one sequence of this

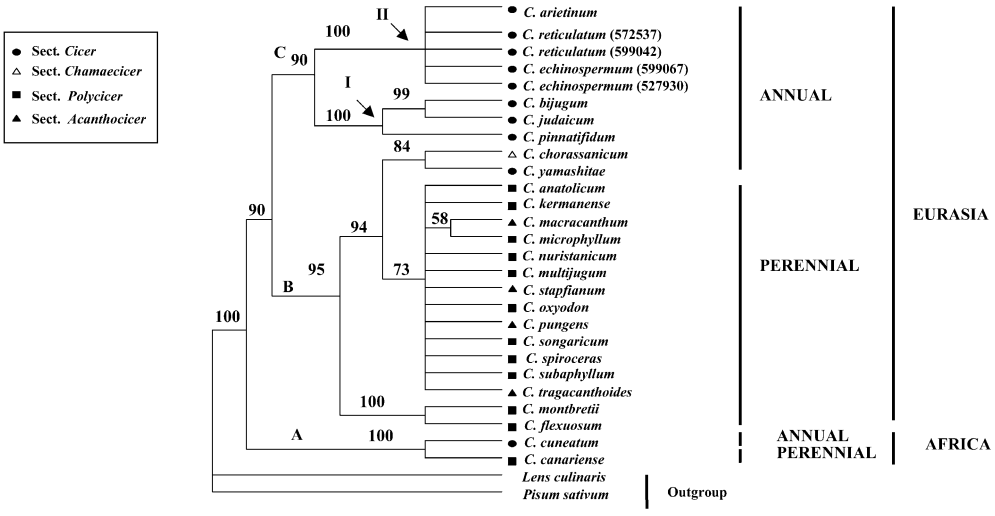

Fig. 1 Strict consensus tree of the 10,000 parsimonious trees resulting from phylogenetic analysis of the data for *trn*T-F. Consistency index =0.833, retention index =0.804. Numbers above branches indicate bootstrap values (%)

taxon was included in the data set for phylogenetic analysis. In the other species, *C. reticulatum* and *C. echinospermum*, base differences were observed and all sequences were therefore included in the analysis.

Phylogenetic reconstruction

The combined analysis recovered the same topology found by the separate analyses of *trn*T-L and *trn*L-F. The bootstrap value changed and increased in most clades and subclades, when compared with individual analyses. Therefore, we constructed a phylogenetic tree of the combined data.

An equal-weight maximum parsimony analysis of the combined molecular data set recovered from 27 taxa gave more than 10,000 most parsimonious trees of 860 steps (consistency index =0.833, retention index =0.804). The strict consensus tree with its bootstrap values is shown in Fig. 1. The *trn*T-F tree exhibited three major clades (A, B, C in Fig. 1). Clade A, with 100% bootstrap support, consisted of *C. cuneatum* (an annual) from north Africa and *C. canariense* (a perennial) from northwest Africa, which shared 17 substitutions. Clade B contained most of the perennial species of *Acanthocicer* and *Polycicer* and was supported by 95% bootstrap. Two perennial species in the section *Polycicer*, *C. flexuosum* and *C. montbretii*, with a high bootstrap value (100%), appeared as a basal group within clade B. *C. chorassanicum* (an annual species of section *Chamaecicer*) and *C. yamashitae* of section *Cicer* were sister taxa in this major clade B. Clade C contained subclades I and II (Fig. 1). Subclade I consisted of *C. pinnatifidum*, *C. bijugum* and *C. judaicum*, with a high bootstrap value (100%). Subclade II contained the cultivated species *C. arietinum*, two haplotypes of *C. reticulatum* and two haplotypes of *C. echinospermum*, with 100% bootstrap support. The members of these two subclades shared a 7-bp insertion (TGTATTT, located in the *trn*L-F region), with 90% bootstrap support.

Discussion

Interspecific phylogenetic relationships

The utility of this cpDNA in resolving the phylogenetic relationships in *Cicer* appears to be useful and suggests the monophyly of the examined species of the genus *Cicer* (100%; Fig. 1). Although neither of the cpDNA regions seems to be sufficiently fast to provide a completely resolved phylogeny of *Cicer*, the topology of the tree shows a clear, well supported phylogenetic pattern. Because the combined phylogeny has high resolution and bootstrap support, it provides information to discuss the interspecific relationship.

Annuals

The tree shows that the annual species of *Cicer* fall into three distinct clades (A, B, C in Fig. 1). The first clade (A) includes only the African *C. cuneatum* with the perennial *C. canariense*. The second annual clade (B) comprises *C. chorassanicum* and *C. yamashitae*, which is a sister to Eurasian perennial species. The third annual clade (C) consists of six annual species, the cultivated species *C. arietinum*, *C. echinospermum*, *C. reticulatum*, *C. bijugum*, *C. pinnatifidum* and *C. judaicum* (Fig. 1). These relationships, the three clades of annuals, are consistent with the crossability groups of Ladizinsky and Adler (1976) and previous results (Kazan and Muehlbauer 1991; Ladbi et al. 1996; Iruela et al. 2002). Members of each of the three clades have specific traits or share several morphological characters. Among the annual species, *C. cuneatum* is basically diverged from the others (Fig. 1). Unlike any other annuals, *C. cuneatum* has a climbing habit, a typical elliptic-obtuse pod, globular seed and occurs in North Africa. The present results, previous reports (Choumane et al. 2000, Iruela et al. 2002) and its morphological traits and geographical pattern provide some evidence for the possibility of a different ancestor and a divergent pathway separating this taxon from other annuals.

C. yamashitae was closer to *C. chorassanicum* than to the members of its section (*Cicer*) and it made a clear subclade (Fig. 1). Morphologically, *C. yamashitae* has five to seven leaflets and a long arista, while *C. chorassanicum* has a trifoliate leaf and small flowers. However, both have a similar range of distribution (Van der Maesen 1972). The grouping of the two annuals can be referred back to the point of view of Kazan and Muehlbauer (1991), who considered that the close relationship between them might be associated with geographical distribution. An alternative explanation for the close relationship between these two sympatric species is a chloroplast capture event between *C. chorassanicum* and *C. yamashitae*. Plastid *trn*T-F sequence data were incongruent with the result of Ahmad (1999). Furthermore, the placement of these two species in the large clade B suggests their different pathway of divergence from other annual species.

In the third annual clade C, three species of subclade I (*C. pinnatifidum*, *C. bijugum*, *C. judaicum*) share the character traits of imparipinnate leaf and weedy habit (Van der Maesen 1972, 1987; Fig. 1), although they are not synapomorphies because these characters are also found in species in other clades. Geographically, *C. pinnatifidum* and *C. bijugum* have a broad geographical distribution and their distribution overlaps that of *C. judaicum* (Van der Maesen 1972,1987). The data indicated that *C. bijugum* and *C. judaicum* are sister species with a high bootstrap value (99%). These results are in agreement with previous results (Ahmad 1999; Iruela et al. 2002). In subclade II, the cultivated species *C. arietinum* groups with *C. reticulatum* and *C. echinospermum*; and these are similar in many morphological traits, such as shape of leaflet, shape of calyx, number of flowers per peduncle, but their seed coat structure is a diagnostic morphological character (Van der Maesen 1972). The molecular data support the previous hypothesis (Ladizinsky and Adler 1975, 1976; Kazan and Muehlbauer 1991; Ahmad and Slinkard 1992; Ladbi et al. 1996; Tayyar and Waines 1996; Ahmad et al. 1999; Iruela et al. 2002; Sudupak et al. 2002, 2003) in which *C. reticulatum* and *C. echinospermum* were thought to be annual progenitors of *C. arietinum.*

The cpDNA sequence data indicate that three species (*C. pinnatifidum*, *C. judaicum*, *C. bijugum*) are closer to *C. reticulatum*, *C. ehinospermum* and *C. arietinum* than perennials, which is incongruent with the result of Sudupak et al. (2002, 2003). Moreover, the plant growth habit (erect to prostrate) is synapomorphic among them and they share a 7-bp insertion (TGTATTT). It is presumed that these six annual species may have a common ancestor. Furthermore, it is noteworthy that weedy species are closer to *C. arietinum* than to others, suggesting an important role of weedy species during the domestication process of *C. arietinum*.

In the domesticated species *C. arietinum*, no variation was observed in four accessions, including the 'Desi' and 'Kabuli' types, for the one *trn*T-F region. For this gene, it seems that the plastome evolves too slowly to expect any divergence over the time-frame of domestication.

Perennials

The genetic divergence among the perennial species did not appear to correlate with the pattern of phenotypic diversity. This result indicates that perennial species of major clade B showed high homoplasy in their morphological characters (Fig. 1). The perennial species *C. canariense* is significantly distant from other perennial species, as was reported by Iruela et al. (2002). A distant relationship between *C. canariense* and others might have accumulated autoapomorphic characters at both the morphological and molecular levels since divergence from its ancestor. Among perennials, there is a close relationship between *C. montbretii* and *C. flexuosum* (Fig. 1). Since the two species are found in different geographical regions (Europe–Anatolia vs Central Asia), different habitats (forests vs rubble) and show differences in a number of their morphological traits (e.g., erect vs shrubby; Van der Maesen 1987), these findings suggest rapid expansion and adaptation to habitats. The cpDNA analyses showed that *C. anatolicum* is part of a monophyletic group with other perennials and not with the annual species, *C. arietinum*, *C. reticulatum*, and *C. echinospermum*, as shown in the previous result of Tayyar and Waines (1996), although they used only two perennials.

Annuals and perennials

Chloroplast DNA phylogeny supports a close relationship between annual and perennial species. However, in evaluating obvious differences in morphology (Van der Maesen 1972) and molecular divergence among annual and perennial species, the data observed suggest a multiple origin of annuals, in contrast to the opinion of Kazan and Muehlbauer (1991). This evidence of the cpDNA *trn*T-F region can be used as a framework for the domestication process of the cultivated species *C. arietinum* and the evolutionary history of annual and perennial species in the genus *Cicer*.

Interestingly, the vetch-like (herbaceous) growing habit not typical of genus *Cicer* apparently occurs in *C. cuneatum* and *C. canariense* (Van der Maesen 1987). Both of these are distributed in the north and northwest parts of Africa and share more than 30 synapomorphic characters in the combined molecular data. It is therefore thought that the common ancestor of these two species could have been characterized by a vetch-like habit. The position of these two taxa seems in doubt in the genus *Cicer*.

Biogeography and evolutionary divergence within the genus *Cicer*

Within the present study, the two major groups, Eurasian and African, show various degrees of fidelity to biogeographic patterns. The Eurasian group represents most of the *Cicer* species. The African group includes the annual species *C. cuneatum* and the perennial *C. canariense*. Our

phylogenetic analysis suggests that *Cicer* may have independently diverged in each continent.

In conclusion, the molecular phylogeny presented herein has been useful in elucidating relationships within and among the taxa under study. Our molecular results may indicate the need for a revised intrageneric classification. A classification based largely on molecular data with the support of some morphological characters improves our understanding of *Cicer* phylogeny. The inclusion of additional taxa and sequences from other genes, such as a nuclear gene and a more rapidly evolving gene, should contribute to a more definitive resolution of the currently weakly supported clades. Of equal importance is the potential for the phylogenetic relationships presented here to serve as a framework to examine the gene pool of domesticated species and the evolution of morphological features.

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References

Ahmad F (1999) Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. Theor Appl Genet 98:657–663

Ahmad F, Slinkard AE (1992) Genetic relationships in the genus *Cicer* L. as revealed by polyacrylamide gel electrophoresis of seed storage proteins. Theor Appl Genet 84:688–692

Ahmad F, Gaur PM, Slinkard AE (1992) Isozyme polymorphism and phylogenetic interpretations in the genus *Cicer* L. Theor Appl Genet 83:620–627

Catalàn P, Kellogg EA, Olmstead RG (1997) Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndhF* gene sequences. Mol Phylogenet Evol 8:150–166

Choumane W, Winter P, Weigand F, Kahl G (2000) Conservation and variability of sequence-tagged microsatellite sites (STMs) from chickpea (*Cicer arietinum* L.) within the genus *Cicer*. Theor Appl Genet 101:269–278

Clegg MT, Zurawski G (1992) Chloroplast DNA and the study of plant phylogeny. In: Soltis PS, Soltis DE, Doyle JJ (eds) Molecular systematics of plants. Chapman and Hall, London, pp 1–13

Downie SR, Katz-Downie DS, Watson MF (2000) A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoC1* intron sequences: towards a suprageneric classification of subfamily Apioideae. Am J Bot 87:273–292

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Gielly L, Taberlet P (1996) A phylogeny of the European gentians inferred from chloroplast *trn*L (UAA) intron sequences. Bot J Linn Soc 120:57–75

Iruela M, Rubio J, Cubero JI, Gil J, Millan T (2002) Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. Theor Appl Genet 104:643–651

Javadi F, Yamaguchi H (2004) RAPD and seed coat morphology variation in annual and perennial species of the genus *Cicer* L. Genet Res Crop Evol (in press)

Kazan K, Muehlbauer FJ (1991) Allozyme variation and phylogeny in annual species of *Cicer* (Leguminosae). Plant Syst Evol 175:11–21

Ladbi M, Robertson LD, Singh KB, Charrier A (1996) Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphism. Euphytica 88:181–188

Ladizinsky G, Adler A (1975) The origin of chickpea as indicated by seed protein electrophoresis. Isr J Bot 24:183–189

Ladizinsky G, Adler A (1976) Genetic relationships among the annual species of *Cicer* L. Theor Appl Genet 48:197–203

Mummenhoff K, Brüggemann H, Bowman JL (2001) Chloroplast DNA phylogeny and biogeography of *Lepidium* (Brassicaceae). Am J Bot 88 :2051–2063

Ohsako T, Ohnishi O (2000) Intra- and interspecific phylogeny of wild *Fagopyrum* (Polygonaceae) species based on nucleotide sequences of noncoding regions in chloroplast DNA. Am J Bot 87:573–582

Popov MG (1976) Genus *Cicer* and its species: on the problem of origin of mediterranean flora, and experiment in morphological and geographical monographing. Indian National Scientific Documentation Centre, New Delhi

Serret DM, Udupa SM, Weigand F (1997) Assessment of genetic diversity of cultivated chickpea using microsatelite-derived RFLP markers: implications for origin. Plant Breed 116:573–578

Singh KB, Ocampo B (1993) Interspecific hybridization in annual *Cicer* species. J Genet Breed 47:199–204

Sudupak MA, Akkaya MS, Kence A (2002) Analysis of genetic relationships among perennial and annual *Cicer* species growing in Turkey using RAPD markers. Theor Appl Genet 105:1220–1228

Sudupak MA, Akkaya MS, Kence A (2003) Genetic relationships among perennial and annual *Cicer* species growing in Turkey assessed by AFLP. Theor Appl Genet 10.1007/s00122-003-1505-8

Swofford DL (2000) PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b4a. Sinauer Associates, Sunderland, Mass.

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Tayyar RI, Waines JG (1996) Genetic relationships among annual species of *Cicer* (Fabaceae) using isozyme variation. Theor Appl Genet 92:245–254

Udupa SM, Robertson LD, Weigand F, Baum M, Kahl G (1999) Allelic variation at (TAA)n microsatellite loci in a world collection of chickpea (*Cicer arietinum* L.) germplasm. Mol Gen Genet 261:354–363

Van der Maesen LJG (1972) *Cicer* L.: a monograph of the genus, with especial reference to the chickpea (*Cicer arietinum* L.), its ecology and cultivation. Mededelingen Landbouwhogeschool Wageningen, Wageningen

Van der Maesen LJG (1987) Origin, history and taxonomy of chickpea. In: Saxena MC, Singh KB (eds) The chickpea. International Publishing, London, pp 11–34

Zohary D, Hopf M (1993) Domestication of plants in the old world. Oxford University Press, Oxford