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Genetic relationships among annual species of *Cicer* (Fabaceae) using isozyme variation

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Abstract In order to determine the pattern of genetic diversity within and among the species of *Cicer* and to estimate interspecific genetic relationships, allelic variation was assayed for 23 isozyme loci in 63 accessions of 11 species of *Cicer* using starch gel electrophoresis. The total allozymic variation observed in the genus (H_t) was equal to 0.60. When partitioned (G_{st}), 96% of this allelic diversity was found among rather than within species. The allelic diversity among species (D_{st}) and allelic diversity within species (H_s) were equal to 0.58 and 0.02, respectively. *Cicer reticulatum* and *C. pinnatifidum* had the highest proportion of polymorphic loci (17.39%) and the highest mean number of alleles per locus (1.22 and 1.17, respectively). UPGMA cluster analysis of Nei's unbiased genetic distance revealed four genetic groups. One includes *C. reticulatum*, *C. arietinum* and *C. echinospermum* where the first 2 species represent a putative derivative-progenitor pair. A second cluster contains *C. bijugum*, *C. pinnatifidum* and *C. judaicum*. *Cicer yamashitae*, *C. chorassanicum*, *C. anatolicum* and *C. songoricum* form a third group. Finally, *C. cuneatum*, which has a very distinct isozyme profile and peculiar morphological features, is the only member of a fourth species group. This species grouping agrees partially with those obtained from crossability and cytogenetic studies. The results suggest that the annual habit arose from perennial progenitors at least twice in the genus *Cicer*.

Key words Chickpea · Isozymes · Genetic diversity · Phenogram

Introduction

The genus *Cicer* L. belongs to the family Fabaceae, subfamily Papilionoideae. Interest in this genus arises from the fact that it contains the cultivated chickpea (*C. arietinum* L.). On a global basis, chickpea is the third most important pulse crop after dry beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.) (Saxena 1990). In addition to the cultivated species, the genus contains 42 wild species, 8 of which are annual, 33 perennial and one whose life-cycle has not been specified (van der Maesen 1987).

As a result of the economic importance of chickpea, the distribution of genetic diversity within and among the *Cicer* species and the genetic relationships among them warrant investigation. This information will help to give insight into species divergence and to exploit genetic resources for hybridization breeding. In addition, chickpea being an old world crop, long in cultivation, it is important to investigate how domestication has affected its genetic diversity. In the genus *Cicer*, most interspecific relationships have been determined among the annual species using karyotype and crossability data (Mercy and Kakar 1975; Ladizinsky and Adler 1976; Pundir and van der Maesen 1983; Jaiswal et al. 1984; Singh et al. 1984) as well as seed storage protein analysis (Ladizinsky and Adler 1975; Vairinhos and Murray 1983; Smirnoff et al. 1981). The 9 annual species have been classified into four groups. The first group includes *C. arietinum*, *C. reticulatum* Ladizinsky and *C. echinospermum* P. H. Davis. *Cicer reticulatum* has been classified as a subspecies of the cultivated chickpea and proposed as its putative progenitor (Ladizinsky 1975). *Cicer bijugum* K. H. Reshinger, *C. judaicum* Boissier, *C. pinnatifidum* Jaubert and Spach and *C. yamashitae* Kitamura comprise a second group. The remaining two annual species, *C. chorassanicum* (Bunge) M. G. Popov and *C. cuneatum* Hochst. ex Rich form additional groups. Recently, Kazan and Muehlbauer (1991) and Ahmad et al. (1992) concluded that the grouping of the annual *Cicer* species based on allozyme variation agrees with species grouping based on crossability and morphological similarities. Kazan and Muehlbauer (1991) also found some correlations between genetic distances and geographic distribution of the species. Finally, they

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concluded that all of the annual species are monophyletic based on an isozyme duplication event (for aldolase) shared by all annual species.

The study presented here is more comprehensive than that of Kazan and Muehlbauer (1991) and Ahmad et al. (1992). We include two perennial species in order to test better the hypothesis of the origins of annual species of *Cicer*, and in particular, the cultivated *C. arietinum*. Furthermore, our more comprehensive allozyme analysis gives better estimates of gene diversity among and within species and of genetic distances among species.

Materials and methods

Plant material

Plant material used in this study included 9 annual and 2 perennial *Cicer* species, which is all of the material available in living seed collections. Among the 9 annual species, 8 were wild and 1 was cultivated. For the cultivated species, *Cicer arietinum*, 25 accessions, obtained from the chickpea collection of the University of California, Riverside (UCR), were examined (Table 1). These represented the desi (small-seeded) and kabuli (large-seeded) types of chickpea and included genotypes with seed coat colors ranging from light cream to black and covered a wide geographical distribution. Among the 8 wild annual species, 36 accessions were assayed (Table 2). The seeds were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) in Allepo, Syria and from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. Also, 1 accession of each of 2 perennial species, *C. songoricum* and *C. anatolicum*, was analyzed (Table 2). Seed samples of these 2 species were provided by the United States Department of Agriculture, Western Regional Plant Introduction Station, Pullman, Washington. Voucher specimens of the wild accessions used are deposited in the Herbarium, Department of Botany and Plant Sciences, University of California, Riverside.

Table 1 *Cicer arietinum* L. accessions[a] assayed for putative allozyme variation and their origin

Accession	Origin
Aztec	Idaho
Blanco Cordobes	Mexico
Blanco Lechose	Spain
CP 13	California
CP 60532	Russia
Garnet	USDA, Washington
Gavillan	USDA, Washington
GR 351	Morocco
GR 386	Morocco
Hermosillo	Mexico
Lyons	Idaho
Macarena	Mexico
Mission	California
P.I. 439798	Iran
P.I. 439850	Iran
P.I. 458870	Iran
P.I. 462164	Iran
P.I. 704061	Chile
Portugal	Portugal
Sarah	USDA, Washington
Surutato	Mexico
UC 5	California
UC 15	California
White Spanish	California
X-24	California

[a] Seeds were obtained from the chickpea collection of the University of California, Riverside

Table 2 Wild *Cicer* species accessions assayed for putative allozyme variation and their sources

Species	Accession	Source
C. anatolicum Alef (Turkey)	ICCW 14 JM 2153	USDA
C. bijugum Rech.	ILWC # 227	ICARDA
(Turkey)	ILWC # 228	ICARDA
	7/5-3	ICARDA
	8-3	ICARDA
	ICCW 41 # 200	ICRISAT
	JM 2113	ICRISAT
C. chorassanicum (Bge.) Pop. (Afghanistan)	ICCW 26 JM 2230	ICRISAT
C. cuneatum Rich.	37/5-1	ICARDA
(Ethiopia)	37/7-5	ICARDA
	ILWC # 232	ICARDA
	ICCW 47 SL-157	ICRISAT
C. echinospermum Dav.	ILWC 239	ICARDA
(Turkey)	35/5-1	ICARDA
	ICCW 44 # 204	ICRISAT
C. judaicum Boiss.	3-1/2	ICARDA
(Lebanon)	4/1	ICARDA
	ILWC # 46	ICARDA
	ILWC # 50	ICARDA
	ICCW 7 # 33	ICRISAT
	ICCW 34 # 182	ICRISAT
C. pinnatifidum Jaub.	4-1	ICARDA
(Turkey)	7-5	ICARDA
	ILWC 22	ICARDA
	ILWC 49	ICARDA
	ICCW 37 # 188	ICRISAT
	ICCW 38 # 189	ICRISAT
C. reticulatum Lad.	21-3/2	ICARDA
(Turkey)	21-16	ICARDA
	ILWC 223	ICARDA
	ILWC 229	ICARDA
	ICCW/JM2100	ICRISAT
	ICCW/JM2105	ICRISAT
C. songoricum Steph. (Turkey)	–	USDA
C. yamashitae Kitam.	ILWC # 215	ICARDA
(Afghanistan)	4-3/2	ICARDA
	ICCW 1 JM 2021	ICRISAT
	ICCW 2 JM 2022	ICRISAT

Ten seeds from each accession of the various species were assayed. *Cicer* species are predominantly self-pollinated (van der Masen 1987), and this sample size should represent the level of variation found within populations. In selfers, a greater proportion of gene diversity within a species exists among rather than within its component populations (Crawford 1990). Interpopulational sampling, therefore, is more critical than intrapopulational sampling. In addition, individual *Cicer* species have restricted geographical distribution, and the accessions surveyed are assumed to represent different populations within the area of distribution of the species.

Enzyme systems

Starch gel electrophoresis was used to examine the following 12 enzyme systems : aconitase (ACO, E.C.4.2.1.3), alcohol dehydrogenase (ADH, E.C.1.1.1.1), aldolase (ALD, E.C.4.1.2.13), glutamate oxaloacetate transaminase (GOT, E.C.2.6.1.1), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), malate dehydrogenase (MDH, E.C.1.1.1.37), malic enzyme (ME, E.C.1.1.1.40), menadione reductase

(MR, E.C.1.6.99.2), phosphoglucoisomerase (PGI, E.C.5.3.1.9), phosphoglucomutase (PGM, E.C.2.7.5.1), 6-phosphogluconate dehydrogenase (6PGD E.C.1.1.1.44) and shikimate dehydrogenase (SKDH, E.C.1.1.1.25).

Procedure

Preliminary experiments revealed a better resolution and staining quality of bands obtained from seed rather than from leaf extracts. Consequently, seed extracts were used throughout the study. A total of 630 seeds were assayed. Two starch gel systems were used. The isozymes of ADH, MR, PGD, PGI, PGM and SKDH were resolved in the histidine citrate (HC) system. The gel consisted of 9.6% starch and 0.009 *M* L-histidine titrated to pH 6.0 with citric acid. The electrode buffer was 0.065 *M* L-histidine (Ellstrand 1984). The remaining isozymes, ACO, ALD, GOT, IDH, MDH and ME, were studied in the morpholine citrate (MC) system. The gel consisted of 9.6% starch and 0.0016 *M* citric acid, and the electrode buffer consisted of 0.04 *M* citric acid. both buffers were titrated to pH 7.0 by the addition of *N*-(3-aminopropyl)-morpholine (O'Malley et al. 1980). Methods of enzyme extraction, electrophoresis and enzyme staining generally followed those of Soltis et al. (1983) and Garvin et al. (1989).

The different sets of bands observed for a given enzyme were numbered sequentially with the most anodally migrating set designated as 1. Each band within a set was presumed to be specified by an individual coding locus. Electrophoretic variants within a region were considered putative "allozymes" and were assigned letters, with the fastest band designated "a", the second fastest "b" and so forth.

Analysis

Electrophoretic data were analyzed using BIOSYS-1, the computer software package of Swofford and Selander (1981). Data were entered as single-individual phenotypes. In addition, a hierarchial arrangement of the populations was established at the species level so that the data could be analyzed within and among species and summarized per species. It was assumed in this study that different populations in each of the species represented the genetic variability present in a taxon.

Genetic variability was assessed using the number of putative alleles per locus, the percent polymorphic loci per species and the observed and expected heterozygosity. Observed mean heterozygosity was determined by the direct count method, and the expected mean heterozygosity was calculated by the unbiased estimate method of Nei (1978), which adjusts for small sample sizes.

At each polymorphic locus, the total allelic diversity is represented by H_t, which is partitioned into the mean allelic diversity within populations, H_s, and the allelic diversity among populations, D_{st}. These quantities are related by the expression $H_t = H_s + D_{st}$. The proportion of total allelic diversity found among populations, G_{st}, is calculated as the ratio D_{st}/H_t. The unbiased genetic distances of Nei (1978) were calculated for all possible pairwise comparisons among the 63 populations. The unweighted pair group method with arithmetic averaging (UPGMA) was then performed on the 63 × 63 distance matrix. Finally, the genetic distances were averaged among populations within a species, and UPGMA was employed to generate a phenogram illustrating species clustering.

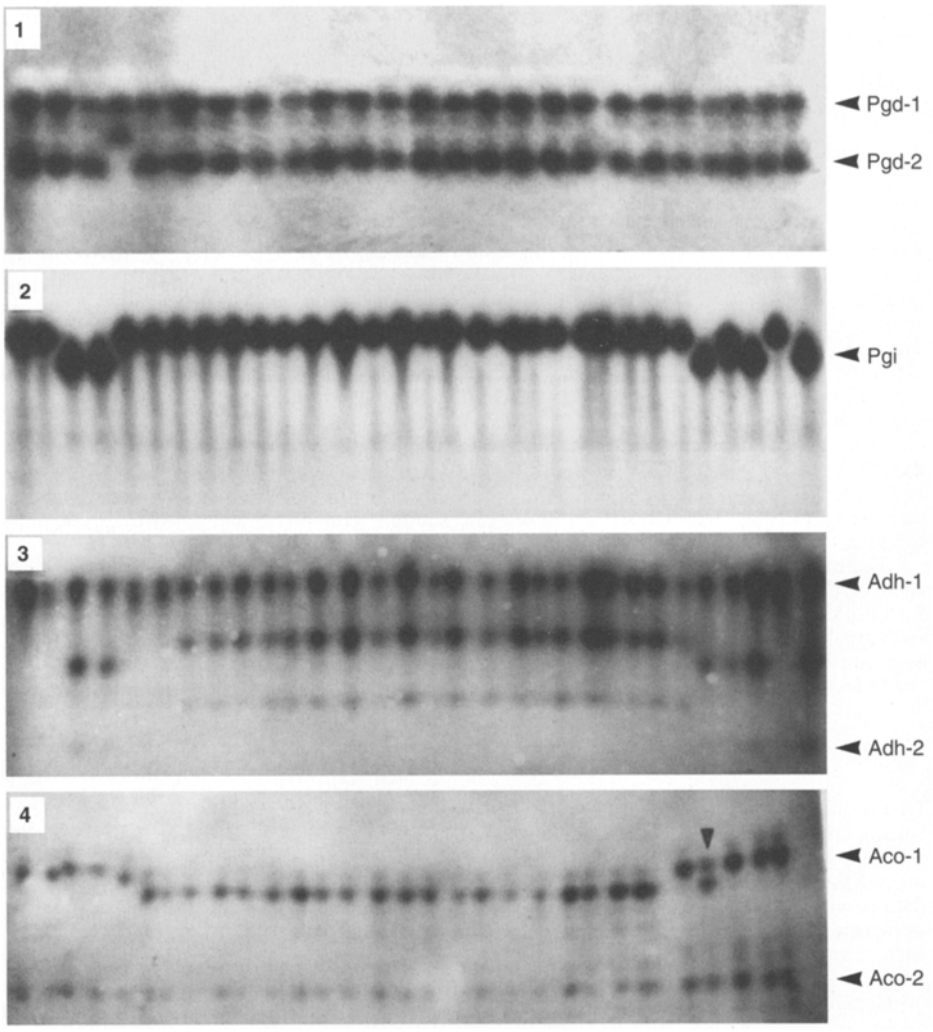
Results

Of the 12 enzyme systems analyzed, 1, menadione reductase (MR), produced unresolved and non-scorable bands and thus was excluded from the analysis. The remaining 11 systems revealed 23 scorable and polymorphic loci, presumed products of individual coding loci. These were *Aco-1*, *Aco-2*, *Adh-1*, *Adh-2*, *Ald1*, *Ald2*, *Got-1*, *Got-2*, *Got-3*, *Idh-1*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Mdh-4*, *Me-1*, *Me-2*, *Pgd-1*, *Pgd-2*, *Pgi*, *Pgm-1*, *Skdh-1*, *Skdh-2* and *Skdh-3* (Figs. 1–4). All of the enzyme bands migrated anodally, The monogenic inheritance of allozymes of these systems was reported by Gaur and Slinkard (1990 a,b). Their results were based on F_2 segregation in interspecific crosses of *C. arietinum* with *C. reticulatum* and *C. echinospermum* and intraspecific crosses in *C. reticulatum*.

In the present study, ADH produced intergenic hybrid bands as it does in many diploid species (Garvin et al. 1989) (Fig. 3). The results for the MDH system were not very clear since the inheritance and genetics of this enzyme are not documented in *Cicer*. However, MDH shows intergenic heterodimers in many plant species. *Cicer arietinum*, *C. reticulatum*, *C. echinospermum*, *C. bijugum*, *C. yamashitae*, *C. chorassanicum*, *C. songoricum* and *C. anatolicum* displayed four-banded phenotypes. *Cicer pinnatifidum* and *C. judaicum* had four- to five-banded phenotypes, whereas *C. cuneatum* showed an eight-banded phenotype. However, for the purpose of this study, four common bands were considered over all species and were assumed to represent 4 different loci. Aldolase exhibited two zones of activity. In each zone, a single band that displayed varying electrophoretic mobility in various species was observed (Table 3). Kazan et al. (1991) reported the presence of duplicate genes coding the plastid-specific aldolase and described a multiple-banded electrophoretic pattern for this enzyme. Since seed extracts were used for the isozyme assays in the present study, it is highly probable that it was the cytosolic form of the aldolase enzyme that was active rather than the plastid form. This could account for the discrepancies in the banding patterns observed in the two studies. There is strong evidence indicating that the plastid and cytosolic isozymes of aldolase are specified by distinct genes (Weeden 1983).

Polymorphism at some isozyme loci was detected within 4 species, *C. arietinum*, *C. reticulatum*, *C. judaicum* and *C. pinnatifidum* (Table 3). In *C. arietinum*, polymorphism was observed among the accessions at the following loci: *Adh-2* (where CP13, Garnet and Sarah had a null allele as compared to the rest of the accessions), *Pgm-1* (where Garnet had a unique phenotype) and *Pgd-2* (where CP605 was different from the rest of the accessions). In *C. reticulatum*, *Aco-1*, *Adh-2* and *Pgi* were polymorphic within the 2 accessions ICCW JM 2100 and ICCW JM 2105, and *Pgm-1* was polymorphic among the accessions. *Aco-1*, *Aco-2* and *Pgm-1* loci were polymorphic among the accessions of *C. judaicum*. Finally, *C. pinnatifidum* accessions were polymorphic at *Aco-1*, *Aco-2*, *Idh-1* and *Mdh-4* loci. The 7 remaining species were monomorphic for all of the loci assayed.

By analyzing genetic variability (Table 4), we found the percent of polymorphic loci to be the highest in *Cicer reticulatum* and *C. pinnatifidum* (17.39). Genetic variability measurements and the mean heterozygosity of these 2 species are represented in Table 5. *Cicer arietinum* and *C. judaicum* had a lower value of 13.04. The remaining species, *C. echinospermum*, *C. bijugum*, *C. cuneatum*, *C.*



Figs. 1–4 Zymograms illustrating polymorphic putative isozyme loci in *Cicer* species. Variation in banding patterns of: PGD among 25 accessions of *C. arietinum* (**Fig. 1**) and PGI within 6 accessions of *C. reticulatum* (**Fig. 2**). In **Fig. 2** every 5 samples represent one accession. Note variation within the first and last accessions. **Fig. 3** Electrophoretic pattern of putative *Adh-1* and *Adh-2* loci in 6 accessions of *C. reticulatum*. Every 5 samples represent one accession. **Fig. 4** Zymogram showing polymorphism in *Aco-1* in 6 accessions of *C. reticulatum*. Every 5 samples represent 1 accession. *Arrow* indicates the heterozygous individual in the last accession

yamashitae, *C. chorassanicum*, *C. anatolicum* and *C. songoricum* showed no enzyme polymorphism. The mean number of alleles per locus was highest in *C. reticulatum* (1.22) followed by *C. pinnatifidum* (1.17). *Cicer arietinum* and *C. judaicum* had 1.13 alleles per locus, while the other species had 1 allele per locus.

The total allozymic variation (H_t) observed in these *Cicer* species was 0.60. When partitioned, 96% of this allelic diversity was found among rather than within species (G_{st}) $= D_{st}/H_t = 0.96$) with D_{st} and H_s values equal to 0.58 and 0.02, respectively.

The unbiased genetic distance coefficients of Nei (1978) were calculated for all possible pairwise comparisons among the 63 populations. These were subjected to UPGMA clutering (Fig. 5). Two interesting features of the population cluster analysis are worth mentioning. First, 2 populations of *C. reticulatum* (JM2100 and JM2105) clustered closer to *C. arietinum* than to the remaining 4 populations of *C. reticulatum*. Second, populations of *C. judaicum* and *C. pinnatifidum* did not cluster according to species boundaries. Rather, populations of *C. pinnatifidum* were nested within the populations of *C. judaicum*.

The genetic distance coefficients were averaged among populations within a species (Table 6). Small genetic distance values (D) were observed between *C. arietinum*, *C. reticulatum* and *C. echinospermum* (these species are referred to as group 1). It was observed that *C. reticulatum* ($D = 0.18$) is closer to the cultivated species than is *C. echinospermum* ($D = 0.24$). Similarly, *C. judaicum*, *C. bijugum* and *C. pinnatifidum* were closely related, as demonstrated by their low D values (these species are referred to as group 2). However, when compared with group 1, the species of group 2 had the lowest genetic distance with *C. echinospermum*. In general, *C. cuneatum* was quite distant from all other species, as can be concluded from the high D values obtained. When compared with other annual species, *C. yamashitae* and *C. chorassanicum* showed relatively high genetic distance values. However, they had a low genetic distance ($D = 0.57$) from each other. Both perennial species, *C. anatolicum* and *C. songoricum*, were more

Table 3 Allelic variation for 23 isozyme loci in 11 *Cicer* species[a]. Numbers in parentheses indicate the relative percentages of the different alleles, respectively

	ari.	ret.	ech.	jud.	pin.	bij.	cun.	yam.	cho.	ana.	son.
Aco-1	b	b/c (31.7/68.3)	b	c/d (67/33)	c/d (53/47)	c	n	a	b	c	a
Aco-2	b	b	b	a/n (67/33)	c/d (73/27)	n	a	b	b	b	a
Adh-1	b	b	b	d	d	d	a	a	d	c	c
Adh-2	d/n (87.5/12.5)	c/d/n (67/20/13)	c	d	d	n	n	b	n	b	a
Ald-1	b	b	b	d	d	d	a	a	d	b	b
Ald-2	a	a	a	b	b	b	a	a	b	b	b
Got-1	d	d	c	c	c	c	a	b	c	c	c
Got-2	a	a	a	c	c	c	b	c	c	c	d
Got-3	a	a	a	c	c	c	b	c	c	c	c
Idh-1	c	c	c	b	b/c (47/53)	a	c	c	c	c	d
Mdh-1	a	a	a	a	a	a	a	b	b	c	b
Mdh-2	a	a	a	a	a	a	b	c	d	d	c
Mdh-3	a	a	a	a	a	a	b	c	c	d	c
Mdh-4	b	b	b	b	a/b (63/37)	b	c	c	c	d	d
Me-1	a	a	a	a	a	a	d	b	b	c	b
Me-2	a	a	a	a	a	a	c	b	b	d	b
Pgd-1	c	a	a	a	a	b	b	a	a	a	a
Pgd-2	b/c (4/96)	b	b	d	d	d	a	a	a	b	a
Pgi	b	b/c (83/17)	b	b	b	b	a	c	d	c	c
Pgm-1	b/c (4/96)	b/c (33/67)	b (33/67)	a/b	b	a	b	b	a	b	c
Skdh-1	b	b	b	b	b	b	n	a	c	d	d
Skdh-2	a	a	a	a	a	a	c	d	d	a	b
Skdh-3	a	a	a	a	a	a	c	d	d	a	b

[a] ari., *C. arietinum*; ret., *C. reticulatum*; ech., *C. echinospermum*; jud., *C. judaicum*; pin., *C. pinnatifidum*; bij., *C. bigugum*; cun., *C. cuneatum*; yam., *C. yamashitae*; cho., *C. chorassanicum*; ana., *C. anatolicum*; son., *C. songoricum*

Table 4 Genetic variability of 23 loci in 11 *Cicer* species (standard errors in parantheses)

Species	Mean sample size/locus	Mean # of allele/locus	Percent[a] of polymorphic loci
C. anatolicum	10 (0.0)	1.0	0
C. arietinum	250 (0.07)	1.13	13.04
C. bijugum	60 (0.0)	1.0	0
C. chorassanicum	10 (0.0)	1.0	0
C. cuneatum	40 (0.0)	1.0	0
C. echinospermum	30 (0.0)	1.00	0
C. judaicum	60 (0.07)	1.13	13.04
C. pinnatifidum	60 (0.08)	1.17	17.39
C. reticulatum	60 (0.11)	1.22	17.39
C. songoricum	10 (0.0)	1.0	0
C. yamashitae	40 (0.0)	1.0	0

[a] A locus is considered to be polymorphic if the frequency of the most common allele does not exceed 0.99

related to *C. yamashitae* than to any other annual species (Table 6). On the basis of Nei's (1978) unbiased genetic distance values, UPGMA was employed to generate a phenogram illustrating species clustering (Fig. 6). Four clusters were obtained. The first cluster included *C. arietinum*, C. reticulatum and *C. echinospermum*; The second cluster comprised *C. judaicum*, *C. pinnatifidum* and *C. bijugum*. *Cicer yamashitae*, *C. chorassanicum*, *C. anatolicum* and *C. songoricum* formed the third cluster, whereas *C. cuneatum* had its own branch.

Discussion

Little isozyme variation was found within the *Cicer* species examined. Five annual species, *C. bijugum*, *C. echinospermum*, *C. cuneatum*, *C. yamashitae* and *C. chorassanicum*, and the 2 perennial species, *C. anatolicum* and *C. songoricum*, were monomorphic over the 23 isozyme loci assayed. In self-pollinating plants, there is often minimal intra- and interpopulational variation (Crawford 1990), and populations may be monomorphic at almost all loci over their geographic ranges. However, in *C. arietinum*, the cultivated chickpea, 3 loci were polymorphic among the various cultivars sur-

Table 5 Genetic variability and mean heterozygosity in polymorphic populations of *Cicer reticulatum* and *C. pinnatifidum*

Species	Average sample size/locus	Average number of allele/locus	Percent of[a] polymorphic loci	Mean heterozygosity	
				Direct count	Hardy- Weinberg expected
C. reticulatum					
ICCW/JM2100	10	1.1	8.7	0.00	0.46
ICCW/JM2105	10	1.1	13.0	0.009	0.047
C. pinnatifidum					
7-5	10	1.0	4.3	0.00	0.23
ILWC22	10	1.1	13.0	0.00	0.046
ILWC49	10	1.0	4.3	0.00	0.023

[a] A locus is considered to be polymorphic if the frequency of the most common allele does not exceed 0.99

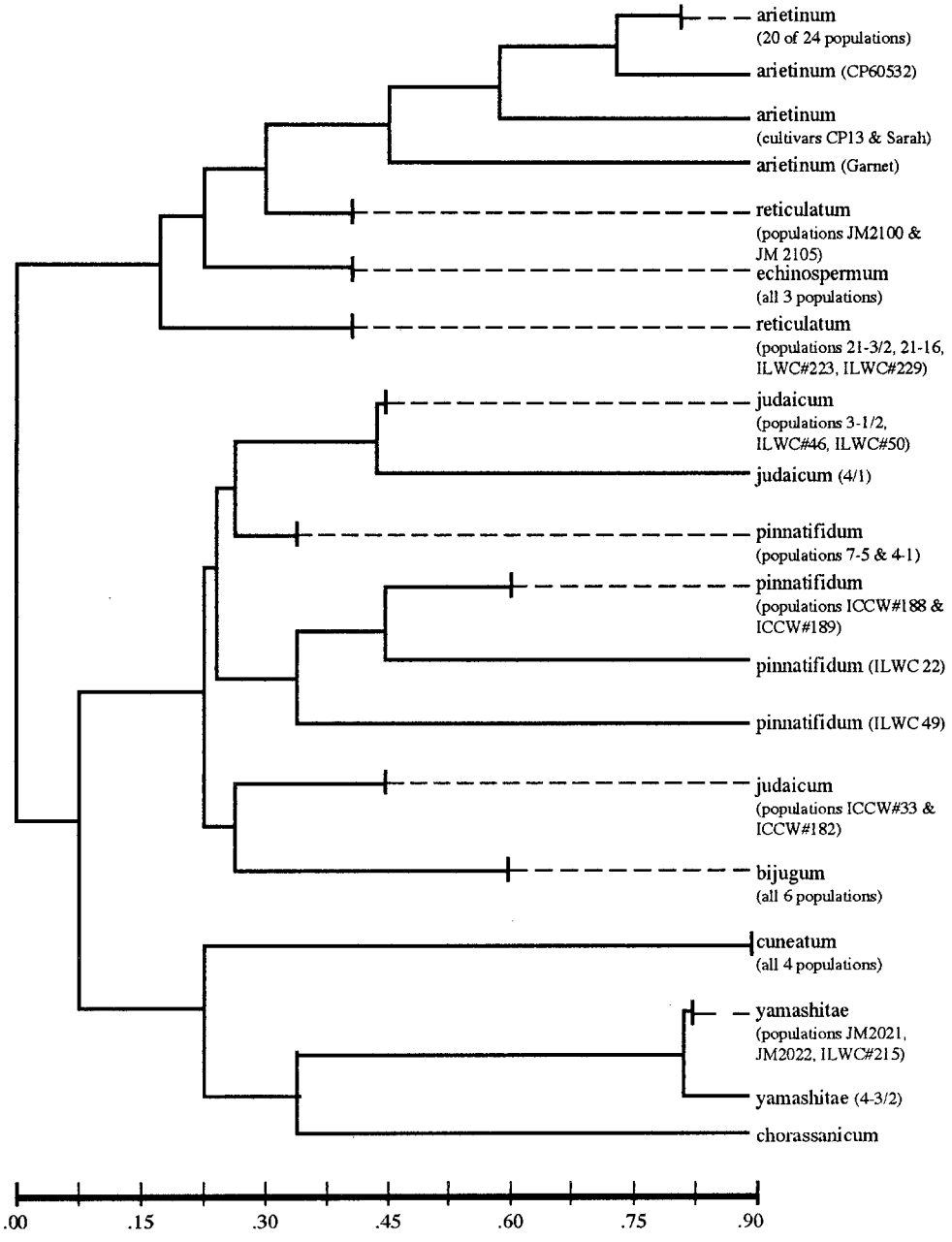


Fig. 5 Clustering of 63 populations of 11 *Cicer* species using Nei's genetic distances from isozyme data

Table 6 Nei's (1978) unbiased genetic distance values for 11 *Cicer* species. The range of values for population pairs in every species is given in parentheses. For abbreviations, see Table 3

	ari.	ret.	ech.	jud.	pin.	bij.	cun.	yam.	ccho.	ana.	son.
ari.	0.02										
	(0.00 – 0.14)										
ret.	0.18	0.07									
	(.11 – .24)	(.00 – .14)									
ech.	0.24	0.12	0.00								
	(.19 – .24)	(.09 – .14)	(.00 – .00)								
jud.	0.75	0.67	0.60	0.08							
	(.74 – .83)	(0.60 – .74)	(.57 – .65)	(0.00 – .14)							
pin.	0.75	0.67	0.57	0.13	0.09						
	(.71 – .88)	(.58 – .74)	(.55 – .61)	(.03 – .20)	(0.00 – .19)						
bij.	0.82	0.76	0.74	0.23	0.30	0.00					
	(.74 – .83)	(.94 – .84)	(.74 – .74)	(.19 – .24)	(.23 – .36)	(0.00 – 0.00)					
cun.	1.99	1.90	1.75	2.40	2.21	2.04	0.00				
	(1.53 – 2.04)	(1.59 – 2.04)	(1.75 – 1.75)	(2.04 – 3.14)	(2.04 – 2.44)	(2.04 – 2.04)	(0.00 – 0.00)				
yam.	2.43	1.90	1.75	1.60	1.42	2.04	2.04	0.00			
	(2.04 – 2.44)	(1.59 – 2.04)	(1.75 – 1.75)	(1.53 – 1.75)	(1.34 – 1.53)	(2.04 – 2.04)	(2.04 – 2.04)	(0.00 – 0.00)			
cho.	2.00	1.91	1.53	1.15	1.11	1.06	1.75	0.57	–[a]		
	(1.75 – 2.04)	(1.59 – 2.04)	(1.53 – 1.53)	(1.06 – 1.19)	(1.06 – 1.19)	(1.06 – 1.06)	(1.75 – 1.75)	.57 – .57)			
ana.	1.58	1.06	.97	1.19	1.08	1.40	2.43	0.78	1.12	–	
	(1.40 – 1.59)	(.98 – 1.09)	(.97 – .97)	(1.09 – 1.40)	(1.03 – 1.09)	(1.40 – 1.40)	(2.43 – 2.43)	(.78 – .78)	(1.12 – 1.12)		
son.	2.47	2.09	2.04	1.40	1.52	1.75	2.44	0.50	0.83	0.92	–
	(2.44 – 3.13)	(2.04 – 2.24)	(2.04 – 2.04)	(1.34 – 1.53)	(1.50 – 1.53)	(1.75 – 1.75)	(2.44 – 2.44)	(.50 – .50)	(.83 – .83)	(.92 – .92)	

[a] Nothing with which to compare

veyed. This result is in agreement with previous reports that indicated that chickpea has limited variability at isozyme loci (Oram et al. 1987; Tuwafe et al. 1988; Kazan and Muehlbauer 1991). Furthermore, Ahmad et al. (1992) found no polymorphism at isozyme loci for the 25 accessions of *C. arietinum* that they surveyed. The 3 polymorphic isozyme markers could be helpful in breeding studies since very few markers have been previously identified. Similarly, 3 loci were found to be polymorphic among the accessions of *C. judaicum*. For the 2 remaining species, *C. reticulatum* and *C. pinnatifidum*, polymorphism was found only for a few loci among as well as within accessions (Table 5). In addition, *C. reticulatum* and *C. pinnatifidum* had the highest proportion of polymorphic loci (17.39%) and relatively the highest mean number of alleles per locus (1.22 and 1.17, respectively). There is not yet any satisfactory explanation for the higher rate of polymorphism observed among and within the accessions of these 2 species. Similarly, in their survey of the annual *Cicer* species, Ahmad et al. (1992) reported that *C. reticulatum* and *C. pinnatifidum* had the highest proportions of polymorphic loci. They attributed the variability observed to some association between polymorphism and geographical distribution or ecological factors, and to rare and spontaneous introgression among the various species.

The proportions of polymorphic loci observed for the species studied are within the range of values observed for self-pollinating species (Gottlieb 1981). *Cicer re-*

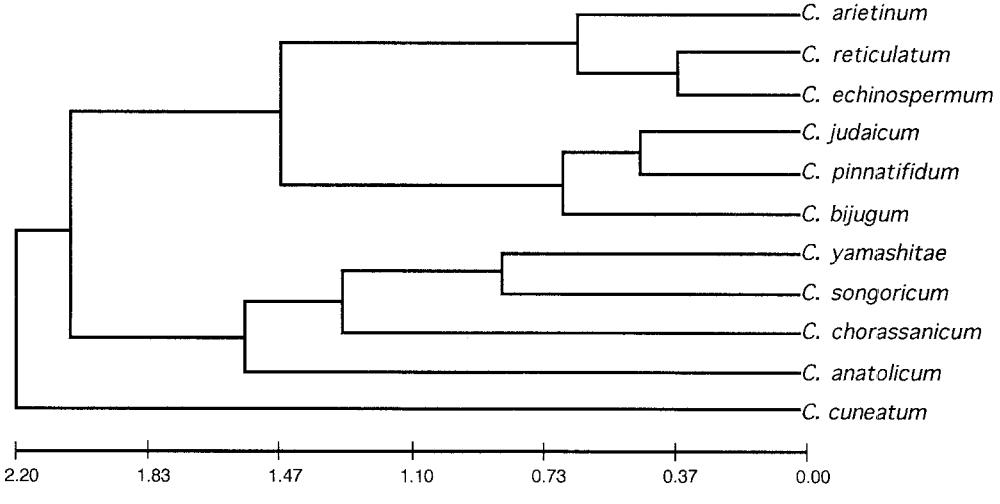


Fig. 6 Phenogram showing relationships among *Cicer* species, based on Nei's genetic distances from isozyme data

ticulatum and *C. pinnatifidum* had a proportion of polymorphic loci very close to the average value calculated for selfers (0.18) (Gottlieb 1981). However, the mean number of alleles at the polymorphic loci is lower than the values recorded for other selfing species. This might be due to the relatively low number of accessions surveyed (which was basically dependent on the availability of seeds at the International Centers). In contrast, Ahmad et al. (1992) reported that the proportions of polymorphic loci in the latter 2 species are higher than the average value calculated for selfers. This might be difficult to interpret considering the highly self-pollinating nature of the species (van der Maesen 1987).

The cause of the low genetic variability observed in *Cicer* could be mainly explained by the highly self-pollinated nature of the species. This has been well-documented for the cultivated species, *C. arietinum*, for which the rate of self-pollination was 98–100% (Eshel 1968; Gowda 1981; Niknejad and Khosh-Khui 1972). van der Maesen (1987) reported that, as far as is known, all *Cicer* species are almost exclusively self-pollinating. This statement also helps to explain the low level of heterozygosity observed; only 1 heterozygous individual was detected in *C. reticulatum*, for the *Aco-1* locus (Fig. 4). Another reason for the observation of low allozymic variation in *Cicer* could be the limited number of accessions of the wild species available for research purposes at both ICRISAT and ICARDA. In addition, the seeds obtained were the result of seed increase at the institutes, and they may not represent all of the genetic variability present in the original wild populations. Furthermore, the species were not equally represented in this study, which could have biased the results. Thus, *C. arietinum* was represented by 25 accessions whereas some species, such as *C. chorassanicum*, were represented by only 1 accession, which may not be representative of the species.

Hamrick and Godt (1990) reported that selfing species are characterized by a relatively high H_t value (0.33), a low H_s value (0.15) and a high G_{st} value (0.51). This indicates that while these species maintain high genetic diversity at their polymorphic loci, most of this variation is found among populations. Similarly, it might be extrapolated that in a genus like *Cicer*, which includes mostly selfing species, a large proportion of the total genetic diversity resides between rather than within species, as is indicated by the high G_{st} value obtained in this study (0.96). Recently, Ahmad et al. (1992) reported that allozyme variation in the annual species of *Cicer* was prevalent between species ($D_{st} = 0.51$) but not within species ($H_s = 0.05$). These values are similar to those found in the present study.

The phenogram obtained using Nei's (1978) unbiased genetic distances (Fig. 6) revealed four species clusters. This corroborates the grouping of the annual species obtained previously by Kazan and Muehlbauer (1991) and Ahmad et al. (1992). The first cluster includes *Cicer arietinum*, *C. reticulatum* and *C. echinospermum*. It corresponds to the first crossability group specified by Ladizinsky and Adler (1976). The low genetic distance value observed between *C. arietinum* and *C. reticulatum* ($D = 0.18$) is consistent with the previous hypothesis that the latter species is the progenitor of the cultivated chickpea (Ladizinsky and Adler 1975). Detailed reviews of electrophoretic data showed that high genetic identity is found between progenitor-derivative pair species (Gottlieb 1977; Crawford 1983, 1989). It appears that selection for certain features associated with domestication can cause rapid morphological divergence between progenitor and derivative taxa with little divergence at isozyme loci. In fact, in the present study, among the 26 alleles found at the 23 isozyme loci assayed in *C. arietinum*, 24 were shared with *C. reticulatum* (Table 3). The cultivated species had two unique alleles, one at locus *Pgd-1* and one at locus *Pgd-2*. This situation agrees with the models of species phylogeny, which state that most, if not all, of the alleles of the derivative should still be present in the progenitor, and the derivative should have very few or no unique alleles (Gottlieb 1981). This model is based on a model of speciation that proposes that reproductive isolation evolves abruptly in a rapid series of events frequently correlated with chromosomal repatterning (Lewis 1962, 1973; Gottlieb 1973).

Two populations of *C. reticulatum*, (JM2100 and JM2105) shared more alleles with the cultivated species than did the remaining 4 populations. For example, at the *Idh-2* locus, the individuals of JM2100 and JM2105 had either "d" or "n" alleles, which are both present in different cultivars, whereas, individuals of the remaining 4 accessions of *C. reticulatum* had a "c" allele that was also found in *C. echinospermum*. At the *Aco-1* locus, JM2100 and JM2105 individuals shared the "b" allele with the cultivated species and with *C. echinospermum*. Allele "c" was found in the rest of the populations of *Cicer reticulatum*. However, 1 individual of accession JM2105 was heterozygous and showed both the "b" and "c" alleles. Several interpretations of the above observations are possible. First, the 2 populations of *C. reticulatum* (JM2100 and JM2105) might represent the actual ancestral populations of the cultivated species or populations very close to them. Second, these 2 accessions of *C. reticulatum* may represent introgressive populations between the latter species and the cultivated chickpea. In this case, "the interspecific hybrids exhibit additive combinations of species-specific alleles at some loci" (Crawford 1983), as observed at the *Aco-1* locus. A third explanation could be that the separation between *C. reticulatum* populations is artificial and probably due to a sampling artifact. Accessions JM2100 and JM2105 may be represented by seed collected directly from wild populations, whereas the remaining 4 accessions may be represented by seed grown and maintained in germ plasm collections at institutes.

A relatively small genetic distance value is obtained between *C. arietinum* and *C. echinospermum*. This suggests that *C. echinospermum* may have played a role in the evolution of the cultivated species. Previous reports

have ruled out the possibility of *C. echinospermum* being the progenitor of the cultigen primarily because of the failure of the two species to hybridize (Ladizinsky and Adler 1976). However, the ability to interbreed should not be treated as the sole factor determining the degree of relatedness between species. Closely related species commonly lose the ability to interbreed and become genetically isolated due to chromosomal structural mutations.

A second cluster of the phenogram contains most of the species of the second crossability group (*C. bijugum*, *C. judaicum* and *C. pinnatifidum*) of Ladizinsky and Adler (1976). The latter 2 species are morphologically very close and in this study exhibited a very low genetic distance value ($D = 0.13$) typical of conspecific populations (Gottlieb 1977; Crawford 1983). In addition, their populations did not cluster according to species boundaries. This result disagrees with previous studies (Ladizinsky and Adler 1976; Kabir and Singh 1988) that concluded that *C. pinnatifidum* and *C. judaicum* were 2 separate and distinct species based on crossability data and seed protein analysis.

The 2 perennial species, *C. anatolicum* and *C. songoricum*, clustered in the phenogram with *C. yamashitae* and *C. chorassanicum*. This suggests that the annual habit may have arisen from perenniality at least twice in the genus *Cicer*. However, final conclusions should await more detailed studies that include as many perennial species as possible. The limited availability of seeds of perennial species makes it difficult to objectively and accurately trace the phylogeny and evolution of the genus *Cicer*. A previous report by Kazan and Muehlbaeur (1991) showed that the perennial species, *C. anatolicum*, clustered with the first crossability group as opposed to the results in the present study. The conflicting observations could have resulted from the small size of the sample surveyed in both studies in addition to variation in the source of seeds, in the enzyme systems examined and in the procedure of sample analysis. Moreover, it is apparent that *C. chorassanicum* classified in section *Chamaecicer* (van der Maesen 1972) may be better grouped with the other annual species in section *Cicer*. The striking morphological difference of the trifoliate leaf shape is probably caused by one to a few genes (Muehlbauer and Singh 1987). Also, the placement of *C. yamashitae* in the second crossability group based on the presence and activity of the seed storage proteins trypsin and chymotrypsin isoinhibitors (Smirnoff et al. 1981) should be reviewed. The morphology and isozyme profile of this species is quite different from that of the second crossability group.

Finally, the annual species *C. cuneatum* was quite distant from all other species. It has a very distinct isozyme profile and peculiar morphological features. It is the only annual species with a climbing growth habit. Its leaves mostly end in a branched tendril above leaflets fairly close and narrow-cuneate. Its pods are elliptic-obtuse as opposed to more ovate pods in other species. The seeds are round without a beak; the seed beak is quite characteristic of seeds of other annual *Cicer* species. No experimental hybridization has been reported between this species and any other annual species. In addition, *C. cuneatum* is the only *Cicer* species, annual or perennial, found in north-east Africa. This information clearly suggests an independent origin of the annual habit in this species. It is possible that this species should be separated into its own section.

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