Pl. Syst. Evol. 175: 11–21 (1991)

—Plant—
Systematics
and
Evolution

Allozyme variation and phylogeny in annual species of *Cicer* (*Leguminosae*)

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Received March 23, 1990

Key words: Angiosperms, *Leguminosae*, *Cicer*. – Allozymes, isozyme gene duplication, phylogenetic relations, polymorphism.

Abstract: Allozymic variation at 30 isozyme loci was examined electrophoretically in nine annual and one perennial species of *Cicer*. While most of the accessions examined were monomorphic, species can be differentiated on the basis of their enzyme phenotypes. Several groups of species were identified based upon genetic distance values. For example, *C. arietinum*, *C. reticulatum*, and *C. echinospermum* shared the same alleles for most of the loci exmained. Perennial *C. anatolicum* is also closely related to this group. Similarly, *C. judaicum*, *C. bijugum*, and *C. pinnatifidum* formed another group. Two annual species, *C. chorassanicum* and *C. yamashitae* clustered together, whereas *C. cuneatum* was the most distantly related species. Correlations were found between genetic distances and geographic distribution. Results from enzyme electrophoresis tend to support the previously reported taxonomic treatments based upon crossability and morphological similarity. However, *C. yamashitae*, which has been classified in the second crossability group, is quite distinct genetically and morphologically from the remaining species of the group. An isozyme gene duplication observed in the genus suggested the monophyletic origin of the species examined in the present study.

The genus *Cicer* (*Cicereae* ALEF.) includes nine annual and 31 perennial species. Almost all the species are self-pollinating and have $2n = 2x = 16$ chromosomes. Four sections have been recognized in the genus on the basis of morphological characteristics, life cycle (perennial vs annual), and geographical distribution (reviewed by VAN DER MAESEN 1987). Section *Monocicer* comprises eight annual species (*C. reticulatum*, *C. echinospermum*, *C. pinnatifidum*, *C. judaicum*, *C. bijugum*, *C. yamashitae*, and *C. cuneatum*) including the cultivated species (*C. arietinum*). Section *Chamaecicer* includes one annual (*C. chorassanicum* M. POP.) and one perennial species [*C. incisum* (WILLD.) K. MALY.]. 23 perennial and seven woody perennial species were grouped in sectt. *Polycicer* and *Acanthocicer*, respectively. Annual species of *Cicer* have also been subjected to numerous taxonomic studies (KUPICHA 1977; VAN DER MAESEN 1987; LADIZINSKY & ADLER 1975, 1976 a, b; NOZZOLILLO 1985). LADIZINSKY & ADLER (1976 a, b) studied phylogenetic relationships by means of crossability and fertility of hybrids in interspecific crosses. Based upon these studies, nine annual species of *Cicer* were divided into four

crossability groups (reviewed by LADIZINSKY & al. 1988). The first group includes three species, *C. arietinum, C. reticulatum*, and *C. echinospermum*. Among these, *C. reticulatum* has been recognized as a subspecies (MORENO & CUBERO 1978) and proposed as the putative progenitor of the cultivated *C. arietinum* (KABIR & SINGH 1988, LADIZINSKY 1975). *Cicer bijugum, C. pinnatifidum*, and *C. judaicum*, were grouped in the second crossability group. Later, *C. yamashitae* was also included in this group. The remaining two species, *C. chorassanicum* and *C. cuneatum*, which cannot be crossed with any other annual species or with each other, form the third and fourth groups.

The barriers to interspecific hybridization within and between crossability groups were examined by AHMAD & al. (1988). In their study, normal pollination and fertilization were observed in many interspecific combinations, but factors resulting from the genetic disharmony between the maternal and paternal genomes were believed to be the cause of sterility.

Using enzyme electrophoresis it is possible to estimate divergence of particular genes in related species and to clarify systematic relations among the related taxa. In the present study, several collections and germplasm accessions belonging to nine annual species and one perennial species of *Cicer* were examined electrophoretically to assess the allozymic variability for 19 enzyme systems representing 30 putative gene loci. *Cicer* spp. mentioned above represent the three sections *Monocicer, Chamaecicer*, and *Polycicer* (perennial *C. anatolicum*). Currently, collections from other perennial and woody perennial species are not available. We describe the evolutionary relationships among these *Cicer* spp. based on allozyme data and compare these results to the previously reported taxonomy of the genus.

Materials and methods

Plant material. Collections of nine annual and one perennial species obtained from the United States Department of Agriculture Western Regional Plant Introduction Station, Pullman, WA, and 57 desi (small seeded with pigmented flowers) and 38 kabuli (large seeded with nonpigmented flowers) type germplasm accessions of *C. arietinum* obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, were used in the experiment (Table 1). Ten plants were assayed for each accession/collection.

Enzyme assays and electrophoresis. Enzyme extracts were obtained by grinding two or three leaflets of young seedlings in a 100 µl potassium phosphate, pH 7, extraction buffer according to SOLTIS & al. (1983). For alcohol dehydrogenase (ADH, EC 1.1.1.1.), seeds were soaked 18 hours in water prior to enzyme extraction. A pH 6.1 histidine gel and electrode buffer (CARDY & al. 1980) was used to resolve alcohol dehydrogenase, aldolase (ALD, EC 4.1.2.13), diaphorase (DIA, EC 1.8.1.4), glyceraldehyde-3-phosphate dehydrogenase (GPD, EC 1.2.1.12), NADP+ specific isocitrate dehydrogenase (IDH, EC 1.1.1.42), malic enzyme (ME, EC 1.1.1.40), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), phosphoglucomutase (PGM, EC 2.7.5.1.), phosphoglucoisomerase (PGI, EC 5.3.1.9), and shikimic dehydrogenase (SKDH, EC 1.1.1.25). A tris-citrate/lithium borate, pH 8.1, gel system (SELANDER & al. 1971) was used to resolve aspartate aminotransferase (AAT, EC 2.6.1.1), aconitate hydratase (ACO, EC 4.2.13), amylase (AMY, EC 3.2.1...), leucine aminopeptidase (LAP, 3.4.11.1), and triosephosphate isomerase (TPI, EC 5.3.1.1). A citrate/N-(3-aminopropyl)-morpholine gel, pH 6.1 (CLAYTON & TRETIAK 1972), was used to resolve acid phosphatase (ACP, EC 3.1.3.2), and β-D-galactosidase (GAL, EC 3.2.1. pH 8.5). Electrophoresis was carried out using a 12% starch gel for all the gel systems. The gel was

Table 1. *Cicer* spp. used in the experiment and their origins

Species	Accession no.	Origin
C. arietinum L.	38 kabuli 57 desli	U.S.S.R., Spain, India, Pakistan, Syria, Turkey
C. reticulatum LAD.	PI 489777 PI 489778 PI 510655	Turkey Turkey Turkey
C. echinospermum DAV.	PI 489776	Turkey
C. bijugum RECH.	PI 458550 PI 458551 PI 458552	Turkey Turkey Turkey
C. judaicum BOISS.	PI 458558 PI 458559 PI 504291	Lebanon Israel Jordan
C. pinnatifidum JAUB.	PI 458555 PI 458556 PI 510663	Turkey Turkey Turkey
C. cuneatum RICH.	PI 458554	Ethiopia
C. chorassanicum (BGE.) POP.	PI 458553	Afghanistan
C. yamashitae KITAM.	PI 504550 PI 510664	Afghanistan Afghanistan
C. anatolicum ALEF.	PI 383626	Turkey

run for 3 h at a constant voltage of 310 for the assays conducted on histidine gel system. For the other assays, the gel was run at 45 mA for 4 h in a refrigerated cabinet. Assays for ACP, ALD, AAT, GPD, IDH, LAP, ME, PGI, PGM, PGD, SKDH, and TPI were described by SOLTIS & al. (1983). The GAL assay was identical to that described by MUEHLBAUER & al. (1989). ADH was assayed according to VALLEJOS (1983). Amylase bands were detected in gel slices assayed for aconitase that were kept overnight in the refrigerator. The ACO, DIA, and SOD assays were described by CARDY & BEVERSDORF (1984).

Subcellular compartmentation. Subcellular location of aldolase isozymes was determined by comparing the electrophoretic patterns of pollen leachate, leaf extract, and chloroplast preparations according to the method of WEEDEN & GOTTLIEB (1980).

Enzyme nomenclature. The locus specifying the most anodally migrating isozyme was designated as 1, the next 2 and so on. Similarly, the most anodal allozyme at a given locus was assigned with "a" whereas the subsequent letters were used to indicate the slower migrating allozymes.

Data analysis. Genetic distances were calculated according to unbiased estimates of NEI (1972, 1978). A cluster analysis was then performed to construct a dendrogram based on estimates of genetic distance using the computer software package of SWOFFORD & SELANDER (1981).

Results

Allozymic variation for the loci assayed in nine annual and one perennial species of *Cicer* is given in Table 2. Most of the accessions studied were monomorphic for the loci assayed. Although the number of polymorphic loci within each species is very low, almost all species can be differentiated. Polymorphisms were detected in *C. arietinum* only for *Aco-2, Gal-1, Pgd-1, Pgd-2*, and *Adh-2* and in *C. reticulatum* only for *Amy-1-, Ald-1, Adh-2*, and *Pgd-2*. Desi and kabuli type *C. arietinum* accessions had common alleles for most of the allozymes examined. A *Gal-1* (pH 8.5) polymorphism was detected only in kabuli type germplasm accessions, whereas an *Adh-2* polymorphism was unique to desi types. *Pgd-2* was the most polymorphic locus in both kabuli and desi type populations. A relatively high level of poly-

Table 2. Allelic variation for the isozyme loci in *Cicer* spp. * Most common allele (see Table 3 for details). ari *C. arietinum*, ret *C. reticulatum*, ech *C. echinospermum*, bij *C. bijugum*, jud *C. judaicum*, pin *C. pinnatifidum*, cun *C. cuneatum*, cho *C. chorassanicum*, yam *C. yamashitae*, ana *C. anatolicum*

Locus	*ari*	*ret*	*ech*	*bij*	*jud*	*pin*	*cun*	*cho*	*yam*	*ana*
Aat-3	d	d	c	c	c	c	c	b	a	d
Aco-1	a	a	a	b	c	c	d	c	c	c
Aco-2	d*/b	b	b	a	b	a	c	c	c	c
Acp-1	a	a	a	c	c	c	b	b	c	c
Adh-1	a	a	a	c	c	c	a	a	b	b
Adh-2	c*/b	c-b/n	c	c	c	c	a	d	b	c
Ald-1	a	a*/c	a	a	a	a	b	a	a	a
Ald-2	c	c	c	c	c	c	c	b	a	a
Amy-1	b	b*/a	a	b	a	a	c	a	a	b
Dia-1	a	a	a	a	a	a	b	c	c	c
Dia-2	a	a	a	a	a	a	a	b	a	a
Gal-1	a*/b	a	a	a	b	b	c	a	b	b
Gpd-1	b	b	b	b	b	b	a	b	b	b
Gpd-2	b	b	b	b	b	b	a	b	c	b
Gpd-3	b	b	b	b	b	b	a	b	c	b
Gpt-1	c	b	c	a	a	a	b	a	c	b
Gpt-2	c	b	b	a	a	b	b	a	a	b
Idh-1	c	c	c	b	c	a	c	c	c	c
Lap-3	d	d	d	d	d	d	c	a	b	a
Mel-1	b	b	b	c	c	a	b	d	b	b
Pgd-1	b*/a	b*/a	a	b	a	a	b	a	a	a
Pgd-2	c*/b	b*/c	b	d	d	d	a	a	a	b
Pgi-1	a	a	a	a	a	a	a	a	a	a
Pgi-2	b	a	b	b	b	b	a	d	c	c
Pgm-2	b	d	d	b	c	b	b	a	b	c
Skdh-1	a	a	a	a	a	a	b	c	c	a
Skdh-2	a	a	a	a	a	a	b	c	c	a
Sod-1	a	a	a	a	a	a	b	c	d	c
Tpi-1	b	b	b	a	a	a	b	c	c	b
Tpi-2	b	b	b	a	c	c	b	c	c	b

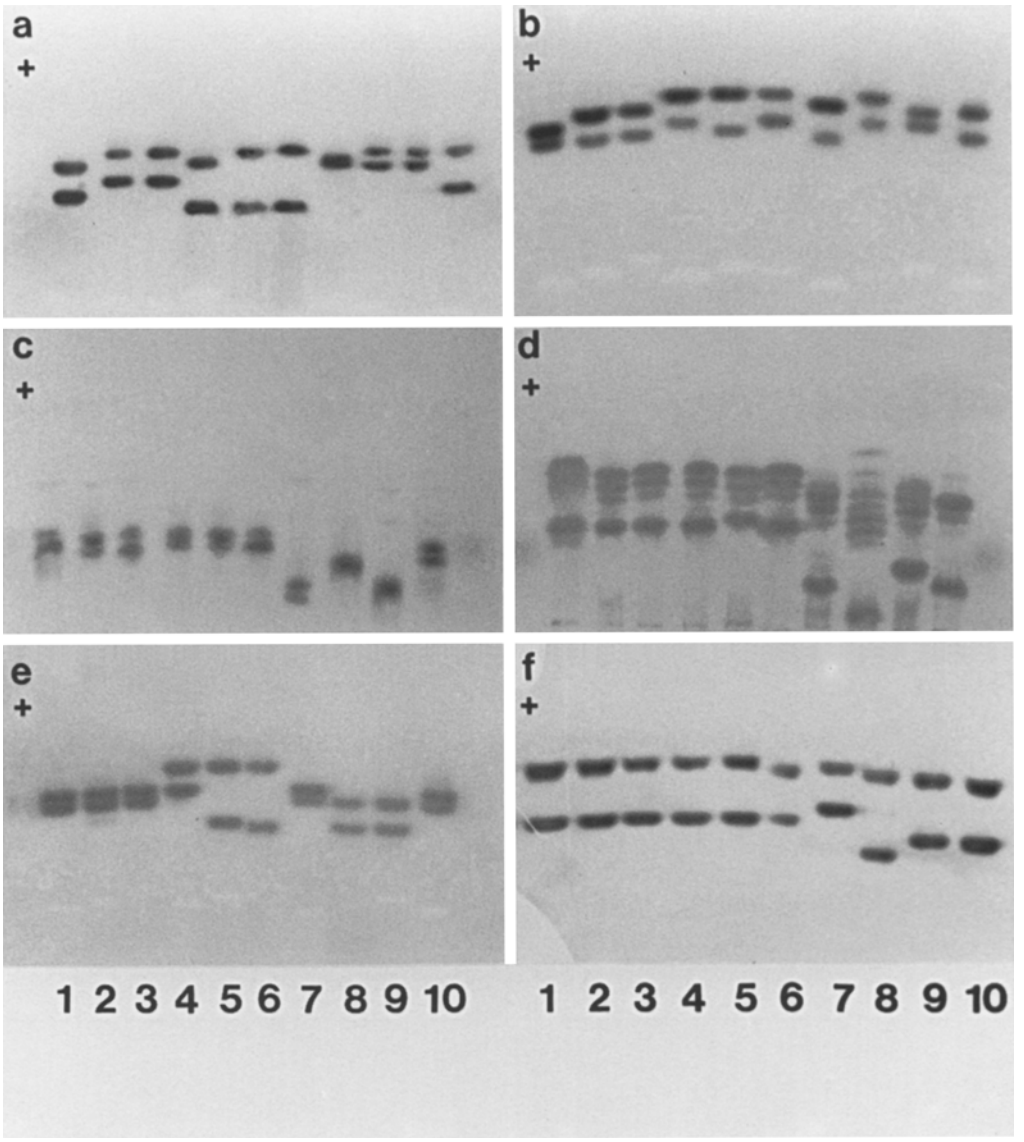

Fig. 1. Electrophoretic patterns of six enzyme systems (*a* PGD, *b* GPT, *c* SKDH, *d* MDH, *e* TPI, *f* PGI) in 10 species of *Cicer* (*1 C. arietinum, 2 C. reticulatum, 3 C. echinospermum, 4 C. bijugum, 5 C. pinnatifidum, 6 C. judaicum, 7 C. cuneatum, 8 C. chorassanicum, 9 C. yamashitae, 10 C. anatolicum*)

morphism found within *C. arietinum* can be partly explained by the large number (95) of populations examined in our study. All polymorphism reported for the species was due to the variation between populations.

The allelic nature and the inheritance of the allozymes were determined for AAT, ACO, ACP, ADH, ALD, AMY, GPD, GPT, LAP, PGD, PGI, PGM, and GAL in intraspecific and interspecific crosses among *C. arietinum, C. reticulutum*, and *C. echinospermum*. A unique ADH-2 null allele was found in *C. reticulatum*.

The number of isozymes present for each enzyme system in *Cicer* (Fig. 1) is concordant with the number of isozymes observed in other diploid plants (GOTTLIEB

Table 3. Frequency of alleles at polymorphic loci in two *Cicer* spp.

Species	Locus	Allele	Frequency
C. arietinum	*Adh-2*	b	0.048
		c	0.952
	Gal-1	a	0.961
		b	0.039
	Pgd-1	a	0.011
		b	0.989
	Pgd-2	b	0.075
		c	0.925
	Aco-2	b	0.022
		d	0.978
C. reticulatum	*Amy-1*	b	0.769
		c	0.251
	Adh-2	b	0.090
		c	0.455
		null	0.455
	Ald-1	a	0.700
		c	0.300
	Pgd-1	b	0.943
		c	0.057
	Pgd-2	b	0.333
		c	0.777

Table 4. Genetic distance values (NEI 1978) among the *Cicer* spp. For abbreviations see Table 2

Species	*ari*	*ret*	*ech*	*bij*	*jud*	*pin*	*cun*	*cho*	*yam*	*ana*
ari	–									
ret	0.249									
ech	0.252	0.211								
bij	0.507	0.720	0.629							
jud	0.677	0.754	0.457	0.310						
pin	0.684	0.829	0.511	0.310	0.182					
cun	1.091	0.904	1.099	1.609	1.792	1.609				
cho	1.316	1.335	1.099	1.322	0.916	1.099	1.609			
yam	1.301	1.701	1.204	1.455	0.916	1.003	1.455	0.693		
ana	0.678	0.594	0.629	1.003	0.693	0.762	1.204	0.916	0.693	–

1982). However, some enzyme loci such as malate dehydrogenase (MDH, EC 1.1.1.37), esterases (EST, EC 3.1.1.2), anodal acid phosphatases (ACP, EC 3.1.3.2), and peroxidases (PRX, EC 1.11.1.7) were not included due to the complex allozyme patterns which make the interpretations relatively difficult. Some groups of species, however, can be resolved on the basis of allozymic patterns produced by these three loci. For example, all the species except *C. cuneatum*, which has an eight

banded pattern (Fig. 1 d), exhibited a four-banded pattern for MDH. *Cicer yamashitae* and *C. chorassanicum* shared the same MDH alleles for all the loci, whereas the remaining species formed another group based upon their similarity for MDH isozymes. Although we did not detect the allelic nature of MDH isozymes, we can assume that *Cicer* has four MDH isozymes encoded by separate genes. Four genes encoding MDH isozymes were also reported for most of the diploid plants so far examined (GOTTLIEB 1982). Therefore, the seven-banded pattern observed in *C. cuneatum* might be due to other reasons (i.e. postranslational modifications of the isozymes or duplication of certain loci).

The multiple-banded electrophoretic patterns observed for homozygous accessions suggested the presence of duplicate genes coding aldolase (KAZAN & al., unpubl.). This fixed-heterozygote phenotype did not segregate upon crossing but segregation was obtained in crosses between the species carrying different aldolase alleles. Subcellular localization studies also showed that both isozymes are expressed in the plastids and indicated that intergenic interaction occurs among the subunits of the polypeptides produced by the two duplicates.

Genetic distance values were calculated among the species. Several groups of species were identified based upon these values (Table 4). For example, *C. arietinum, C. reticulatum*, and *C. echinospermum* shared the same alleles for most of the loci examined. The perennial *C. anatolicum* also showed close similarity to this group. Three members of the second crossability group, *C. judaicum, C. bijugum*, and *C. pinnatifidum*, were closely related to each other. Two annual species, *C. chorassanicum*, and *C. yamashitae* formed another group, whereas *C. cuneatum* was the most distantly related to the other *Cicer* species.

Discussion

The observation of low allozymic variation in *Cicer* is consistent with previous reports (WEEDEN & al. 1988, TUWAFE & al. 1988, ORAM & al. 1987). This could be due to: 1) the highly self-pollinated nature of the species, 2) localized distribution of the species and small population size, 3) genetic bottlenecks, 4) sampling bias made during collection, 5) relatively small number of plants examined in each accession. Currently, we do not know whether the genotypes in each accession represent a random sample of individuals in the population. Additionally, the base sample for the study is biased due to the fact that the cultivated species is represented by 95 samples while four other species are represented by a single sample. Therefore, we cannot exclude the possibility that additional variation can be found if more collections were available for study. Although analysis of a larger number of samples might have detected additional genetic variation, the number of plants assayed for each accession was limited due to the availability of seeds, especially of the wild species accessions. However, it is often recognized that sampling fewer individuals affects the variance of genetic distance to a lesser degree than decreasing the number of loci (NEI & ROYCHOUDHURY 1974, NEI 1978). Therefore, as many loci as possible were examined for each accession.

Some correlations exist between genetic distances and geographic distribution of the species. All annual species from Turkey or neighboring areas clustered together. Similarly, two species from Afghanistan were closer to each other than to the other species of the genus. *Cicer cuneatum* from Ethiopia was very distantly related to the rest of the species.

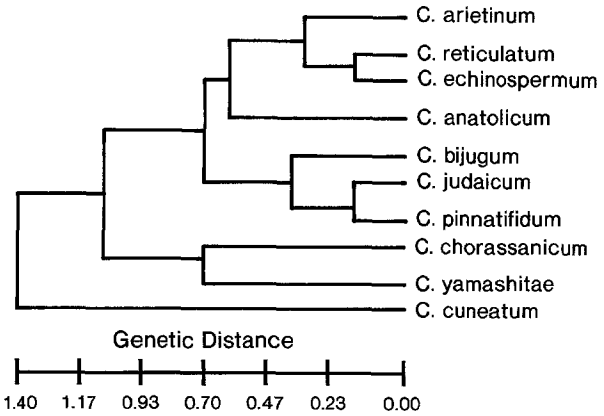

Fig. 2. Clustering of *Cicer* spp. by UPGMA analysis using NEI's (1978) unbiased genetic distance

The dendrogram obtained (Fig. 2) partially corroborates the previously reported taxonomy of the genus (LADIZINSKY & ADLER 1976 b). *Cicer reticulatum* and *C. echinospermum*, which occupy somewhat different ecological niches in southeastern Turkey (LADIZINSKY 1975) exhibited lower genetic distances. The close alloyzme similarity among the species of the first crossability group suggested that they have diverged recently. Based upon the data from enzyme electrophoresis, *C. arietinum* was derived either from *C. reticulatum* or from *C. echinospermum* since putative progenitor derivative species often exhibit high genetic identities (GOTTLIEB & PILZ 1976). It is likely that reproductive isolation has been achieved by chromosomal repatterning. The close geographic distribution and morphological similarity of these three species also supports the recent speciation hypothesis. This result is also in agreement with the previous studies concerning origin of the cultivated species (LADIZINSKY & ADLER 1975, 1976 a, b; KABIR & SINGH 1988). Interestingly, perennial *C. anatolicum* showed greater similarity to the species of first crossability group. These four species also have very similar karyotypes (AHMAD 1989), but crossability relations among them are not known. *Cicer anatolicum*, which also occurs in Turkey, might be the progenitor of all the annual species in the first crossability group.

Similarly, the first three members of the second crossability group, *C. bijugum, C. pinnatifidum*, and *C. judaicum*, are genetically closer. The close morphological similarity between the latter two species is also reflected in their allozyme genotypes. A genetic distance value of 0.182, which is closer to the mean value observed for subspecific species (HAMRICK 1983), was obtained between these two species. This is somewhat contradictory to the comparison of these species based on seed protein profiles which clearly separated the species (LADIZINSKY & ADLER 1975, KABIR & SINGH 1988). Although a morphological or natural reproductive barrier is reported for *C. pinnatifidum* and *C. judaicum* (LADIZINSKY & ADLER 1976 b), hybrids can be obtained by artificial pollination. Relatively regular meiosis was observed in the hybrids, indicating the genetic similarity of the species. Among these, *C. pinnatifidum* has a broader geographic range and its distribution overlaps that of *C. judaicum* in Lebanon, Israel, and Syria.

Two annual species from Afghanistan, *C. yamashitae*, and *C. chorassanicum* formed another similarity group based upon genetic distance values. These two species are quite different morphologically but they have very similar geographic ranges. The position of *C. yamashitae* in the dendrogram does not agree with the previous classification based upon crossability. Apparently, this species is quite distinct from the remaining species of second crossability group.

Data from enzyme electrophoresis suggest that *C. chorassanicum*, which is the only annual species in sect. *Chamaecicer*, may be positioned better in sect. *Monocicer*; present data suggest that it is genetically similar to the other annual species. The morphology differences might be due to relatively few genes.

Finally, annual *C. cuneatum* (*Monocicer*), which occurs in Ethiopia and Sudan, was separated from all species studied by its unique isozyme phenotypes. It is also markedly different from other annuals in possessing a climbing habit and once-compound leaves.

We now have evidence indicating that all annual species of *Cicer* are monophyletic. The rationale for the common ancestor hypothesis is that an isozyme gene duplication event is shared by all annual species. Gene duplications as evidenced by enzyme electrophoresis are very rare events that could occur only once in the evolution of the species. It has been found that genera such as *Clarkia* with rapid chromosomal rearrangements are the most likely candidates for gene duplications because these duplications can occur via chromosomal translocations (GOTTLIEB 1982). Significantly, LADIZINSKY & ADLER (1976 a, b) have identified a translocation and a paracentric inversion in crosses between *C. arietinum* and *C. reticulatum*. Similarly, crosses of *C. echinospermum* with *C. arietinum* and *C. reticulatum* showed that *C. echinospermum* differs from each of two other species by a reciprocal translocation. Although cytogenetic evidence for translocations is not currently available for other species, available data suggest the possible role of chromosomal rearrangements in the evolution of the genus. Possibly such rearrangements gave rise to the duplicated segment before annual species diverged from a common ancestor which may or may not be extinct. This duplication might be shared by several other perennial species. At least one perennial species examined in the present study, *C. anatolicum*, showed the duplication. Thus, more perennial species should be examined as the seeds become available for genetic analyses. Present evidence also suggests that this duplication is unique to the tribe, *Cicereae*, since no duplications are known to occur in related but distant members such as *Pisum* and *Lens*.

The authors thank C. SPERLING for helpful comments on earlier version of this manuscript. Contribution no. 900-28, from the Department of Agronomy and Soils, College of Agriculture and USDA-ARS, Washington State University, Pullman, WA 99164, U.S.A.

References

AHMAD, F., 1989: The chromosomal architecture of *Cicer anatolicum* ALEF., a wild perennial relative of chickpea. – Cytologia **54**: 753–757.

– SLINKARD, A. E., SCOLES, G. J., 1988: Investigations into barrier(s) to interspecific hybridization between *Cicer arietinum* L. and eight other annual *Cicer* species. – Pl. Breed. **100**: 14–18.

CARDY, B. J., BEVERSDORF, W. D., 1984: A procedure for the starch gel elecrophoretic detection of isozymes of soybean (*Glycine max* [L.]. MERR.). – Tech. Bull. No. 119/8401, Dept. of Crop Sci., Univ. of Guelph, Ontario.

– STUBER, C. W., GOODMAN, M. M., 1980: Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). – Raleigh NC, NCSU Dept. Stat. Mimeo Series No. **1317**.

CLAYTON, J. W., TRETIAK, D. N., 1972: Amine-citrate buffers for pH control in starch gel electrophoresis. – J. Fish Res. Bd. Canad. **29**: 1169–1172.

GOTTLIEB, L. D., 1982: Conservation and duplication of isozymes in plants. – Science **216**: 373–380.

– PILZ, G., 1976: Genetic similarity between *Guara longiflora* and its obligately outcrossing derivative *G. demareei*. – Syst. Bot. **1**: 181–187.

HAMRICK, J. L., 1983: The distribution of genetic variation within and among natural plant populations. – In SCHONEWALD, L. M., CHAMBERS, S. M., MUCBIG, B., and THOMAS, L. (Eds.): Genetics and conservation, pp. 335–348. – MontePark, Cal.: Benjamin-Cummings.

KABIR, G., SINGH, R. M., 1988: Seed protein electrophoresis in six species and two F1s of *Cicer*. – Proc. Indian Acad. Sci. (Plant Sci.) **98**: 183–189.

KUPICHA, F. K., 1977: The delimitation of the tribe *Vicieae* ALEF. and the relationship of *Cicer* L. – Bot. J. Linn. Soc. **74**: 131–162.

LADIZINSKY, G., 1975: A new *Cicer* from Turkey. – Notes Bot. Gard. Edinburgh **34**: 201–202.

– ADLER, A., 1975: The origin of chickpea as indicated by seed protein electrophoresis. – Israel J. Bot. **24**: 183–189.

– – 1976a: The origin of chickpea, *Cicer arietinum* L. – Euphytica **25**: 211–217.

– – 1976b: Genetic relationships among annual species of *Cicer* L. – Theor. Appl. Genet. **48**: 197–203.

– PICKERSGILL, G. B., YAMAMOTO, K., 1988: Exploitation of wild relatives of the food legumes. – In SUMMERFIELD, R. J., (Ed.): World crops: cool season food legumes, pp. 967–978. – Dordrecht, The Netherlands: Kluwer Academic Publishers.

VAN DER MAESEN, L. J. G., 1987: Origin, history and taxonomy of chickpea. – In SAXENA, M. J., SINGH, K. B. (Eds.): The Chickpea, pp. 11–34. – Cambridge: CAB International.

MORENO, M.-T., CUBERO, J. I., 1978: Variation in *Cicer arietinum* L. – Euphytica **28**: 465–477.

MUEHLBAUER, F. J., WEEDEN, N. F., HOFFMAN, D. L., 1989: Inheritance and linkage relationships of morphological and isozyme loci in lentil (*Lens* MILLER). – J. Hered. **80**: 298–303.

NEI, M., 1972: Genetic distance between populations. – Amer. Naturalist **106**: 283–292.

– 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. – Genetics **89**: 583–590.

– ROYCHOUDHURY, A. K., 1974: Sampling variances of heterozygosity and genetic distance. – Genetics **76**: 379–390.

NOZZOLILLO, C., 1985: Seedling morphology and anatomy of eight *Cicer* species and their taxonomic value. – Canad. J. Bot. **63**: 1–6.

ORAM, R. N., SHAIKH, M. A. Q., ZAMAN, K. M. S., BROWN, A. H. D., 1987: Isozyme similarity and genetic differences in morphology between Hyprosola, a high yielding, high protein mutant of chickpea (*Cicer arietinum* L.) and its parental cultivar. – Envir. Exper. Bot. **27**: 455–462.

SELANDER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E., GENTRY, J. B., 1971: Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). – Univ. Texas Publ. **7103**: 49–90.

SOLTIS, D. E., HAUFLER, C. H., DARROW, D. C., GASTONY, G. J., 1983: Starch gel elec-

trophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. – Amer. Fern J. **73**: 9–27.

SWOFFORD, D. L., SELANDER, R. B., 1981: BIOYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. – J. Heredity **72**: 281–283.

TUWAFE, S., KAHLER, A. L., BOE, A., FERGUSON, M., 1988: Inheritance and geographical distribution of allozyme polymorphisms in chickpea (*Cicer arietinum* L.). – J. Heredity **79**: 170–174.

VALLEJOS, E., 1983: Enzyme activity staining. – In TANKSLEY, S. D., ORTON, T. J., (Eds.): Isozymes in plant genetics and breeding, A, pp. 469–516. – Amsterdam: Elsevier.

WEEDEN, N. F., GOTTLIEB, L. D., 1980: Isolation of cytoplasmic enzymes from pollen. – Pl. Physiol. **66**: 400–403.

– ZAMIR, D., TADMOR, Y., 1988: Applications of isozyme analysis in pulse crops. – In SUMMERFIELD, R. J., (Ed.): World crops: cool season food legumes, pp. 979–987. – Dordrecht, The Netherlands: Kluwer Academic Publishers.

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Accepted August 31, 1990 by B. L. TURNER