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Genetic Relationships among the Annual Species of *Cicer* L.

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Summary. Genetic relationships between 7 annual species of the genus *Cicer*, including the cultivated chickpea, have been studied. These species were assigned to 3 crossability groups. In each group interspecific hybrids could be obtained but their fertility differed considerably in the various cross combinations. Crosses between members of different groups yielded no viable seeds. The possibility of gene transfer from the wild species to the cultivated chickpea *C. arietinum* was also assessed. Only two species could be considered for this purpose, *C. reticulatum*, which is the wild progenitor of the cultivated species, and *C. echinospermum*, which is in the secondary gene pool of *C. arietinum*. A unique postzygotic reproductive barrier mechanism was found between the members of Group II, *C. judaicum*, *C. pinnatifidum* and *C. bijugum*. It is based on a disharmony in the growth rate of the stigma and the anthers at the time of anthesis of the F_1 interspecific hybrid so that selfpollination is avoided. It is proposed that this kind of mechanism has been involved only when an effective spatial isolation between the three species had been obtained.

The genus *Cicer* L. is known by the cultivated species *C. arietinum* L. which is an important legume in many Old World countries. This genus has already been subjected to a comprehensive taxonomic treatment by Popov (1929), and later by Linczevski (1948), Davis (1970) and van der Maesen (1972). At present it includes 40 species, 9 of which are annual. Recently, valuable botanical information on the genus has been added and two new species, *C. echinospermum* Davis (Davis 1969) and *C. reticulatum* Ladizinsky (Ladizinsky 1975), described. So far, no information on species relationships among the annual chickpeas has been published, probably due to the inaccessibility of the wild species. During the course of the present study a field trip to Turkey was undertaken, in which valuable information on the distribution and ecology of the *Cicer* species growing there was obtained and seeds were collected.

The present study deals with cytogenetic relationships between the following 7 annual chickpeas, *C. arietinum*, *C. reticulatum*, *C. echinospermum*, *C. pinnatifidum* Jaub. and Spach, *C. judaicum* Boiss., *C. bijugum* Rech. and *C. cuneatum* Hochst. and Rich. Due to lack of seeds, the two Afghanian species *C. chorassanicum* (Bge.) M. Pop. and *C. yamashitae* Kitam. were not included in this study.

Material and Methods

Chromosome numbers of the various species were counted in mitosis and meiosis. Somatic chromosome number was counted in root tips. The seeds were germinated in petri dishes, the seed coat being scarified to hasten germination. Roots of 1-1.5 cm were cut and placed in cold water (about 0°C) for 20-24 hr., fixed in 3:1 ethanol absolute-acetic acid, hydrolysed in 1 N HCL for 10 min. at 60°C and stained with Feulgen. Interspecific crosses were made in green house and phytotron. The lines used in the various cross combinations are listed in Table 1. Buds were emasculated close to anthesis and pollinated a day later. For the meiotic study, buds at the appropriate stage were fixed in 3:1 and stored in 70 % ethanol. Individual anthers were smeared with acetocarmine. Pollen fertility was determined by staining mature pollen grains with acetocarmine. At least 500 pollen grains were scored in each case. Pollen grains were considered normal if they had regular shape and darkly stained cytoplasm. Seed set was determined by the proportion of flowers developed to seed-bearing pods.

Results and Discussion

The chromosome number of all the 7 species examined was 2n = 16. Fourteen out of the 21 possible cross combinations between the 7 species were attempted. In some combinations only a few crosses were made, in others more than a hundred (Table 2). The outcome of these crosses suggests a tentative assignment of these 7 species to the following three crossability groups: I. *C. arietinum*, *C. reticulatum*, *C. echinospermum*; II. *C. judaicum*, *C. pinnatifidum*, *C. bijugum*; III. *C. cuneatum*. Within each group, interspecific hybrids could be obtained but the fertility of the various hybrids differed considerably. Crosses between members of different groups failed or, at best, yielded small empty pods. The characterization of each of these three groups is as follows:

Table 1. Parental lines used in crosses

Species	Origin
C. arietinum	
No. IX	Ethiopia
58F	Ethiopia
77	Greece
127	India
187	Israel
C. reticulatum	Turkey about 9 km East of Savur, Mardin district
C. echinospermum	Turkey about 20 km East of Siverek, Urfa district
C. bijugum	Turkey about 15 km Northwest of Diyarbakir
C. pinnatifidum	Turkey about 5 km East of Gaziantep
C. judaicum	Israel, Jerusalem
C. cuneatum	Ethiopia, near Aksum

Table 2. The number of crosses performed between 7 annual *Cicer* species (Number of the seed bearing pods obtained in brackets)

	reticulatum	*echinospermum*	*judaicum*	*pinnatifidum*	*bijugum*	*cuneatum*
arietinum	88 (7)	54 (2)	337 (-)	61 (-)	67 (-)	187 (-)
reticulatum		230 (1)			12 (-)	
echinospermum					13 (-)	
judaicum				50 (4)	32 (1)	108 (-)
pinnatifidum					34 (3)	37 (-)

Group I

Economically this is the most important group among the annual chickpeas since it contains the cultivated species and its wild relatives (Ladizinsky and Adler, 1976). The seed coat structure is the main diagnostic trait differentiating between the wild and the cultivated species of the group. While the seed coat of *C. arietinum* can be smooth, granulate or rugous, that of *C. echinospermum* is covered by whitish echinate hairs and that of *C. reticulatum* has a reticulate surface. This latter species also has a prostrate growth habit. In its seed coat structure, the wild species *C. reticulatum* is closer to *C. arietinum* than *C. echinospermum*. They also share almost the same seed protein profile (Ladizinsky and Adler 1975). Crosses between 4 lines of the cultivated species and *C. reticulatum* were as successful as between lines of *C. arietinum*. The hybrids of this combination developed normally, had regular meiosis and were fully fertile (Ladizinsky and Adler 1976). The growth habit and the seed coat structure of the F_1 were intermediate to those of the parental species and segregation was encountered in F_2. While the difference between erect and semi-erect was not always clear, in the F_2, 13 out of 57 progeny had prostrate habit, indicating that this characteristic is governed by a single, partially dominant, gene (P=80). The genetics of the seed coat structure is apparently more complicated. None of the 37 F_2 progenies of the hybrid between a smooth seed coat *C. arietinum* variety and *C. reticulatum* had seed coat similar to that of the wild species, but 3 had a phenotype similar to that of the cultivated variety. The degree of seed coat reticulation in the F_2 varied considerably among the rest of the F_2 plants. While the examined F_2 population was obviously too small to determine the genetics of this characteristic, it seems that more than a single locus is involved here.

The rate of crossability between *C. arietinum* and *C. reticulatum*, the normal meiosis and fertility of their F_1 hybrid and the lack of breakdown in the F_2 tend to support the conclusion that *C. reticulatum* is the wild progenitor of the cultivated chickpea. The small morphological differences between them are governed by a few genes and probably result from evolution under domestication of the cultigen *C. arietinum*.

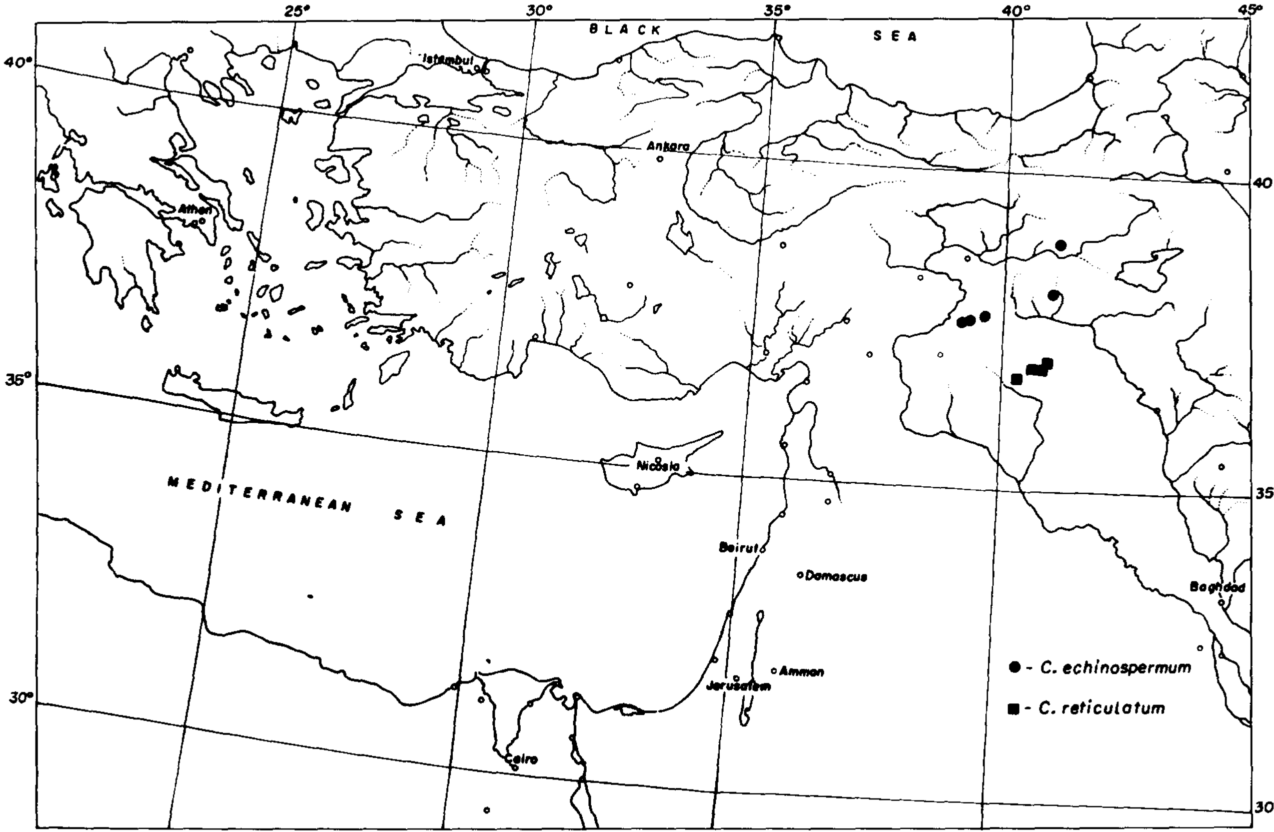

Fig. 1. The distribution of the wild species of Group I, representative locations

Hybrid seeds of *C. arietinum* × *C. echinospermum* are apparently much more difficult to obtain. Nevertheless, the hybrids developed normally, produced many branches and flowers, but were highly sterile. At meiosis, six bivalents and a quadrivalent were observed, instead of the eight bivalents of the parental species (Ladizinsky and Adler 1976). This quadrivalent indicates that the two species differ by a major reciprocal translocation, which by itself is insufficient to explain the sterility of this hybrid. It may also be due to cryptic structural hybridity which further reduces the proportion of balanced gametes in the F_1. The few seeds secured from these F_1 hybrids gave rise to normally developing plants which were, however, as sterile as the F_1. Back-crossing of these sterile F_2 plants to the cultivated species yielded some seeds.

The rate of crossability between the two wild species of Group I, *C. reticulatum* and *echinospermum* was even lower. The only hybrid obtained from this combination developed normally but was completely sterile. In this case too, six bivalents and a quadrivalent were observed in meiosis, which accords well with the meiotic behavior of hybrids of the two former combinations.

According to the present state of knowledge, *C. reticulatum* and *C. echinospermum* are restricted to different parts of Turkey (Fig. 1) and occupy different ecological niches, which prevent contact between them. Even if they should form a mixed population, gene flow between them would be extremely limited due to the sterility of their hybrid.

The morphological and cytogenetical information on this group also shed some light on the evolution of the cultivated species. As has already been noted, its wild progenitor, *C. reticulatum*, is apparently endemic to South East Turkey, which is also the central part of the traditional Fertile-Crescent. In that general area, wheat, barley, pea, and probably also lentil, were domesticated. While the wild species of the latter two legumes are widespread throughout the Crescent and could be domesticated simultaneously in several locations, the distribution of *C. reticulatum* is much more limited and apparently so is its place of domestication. It is also no-

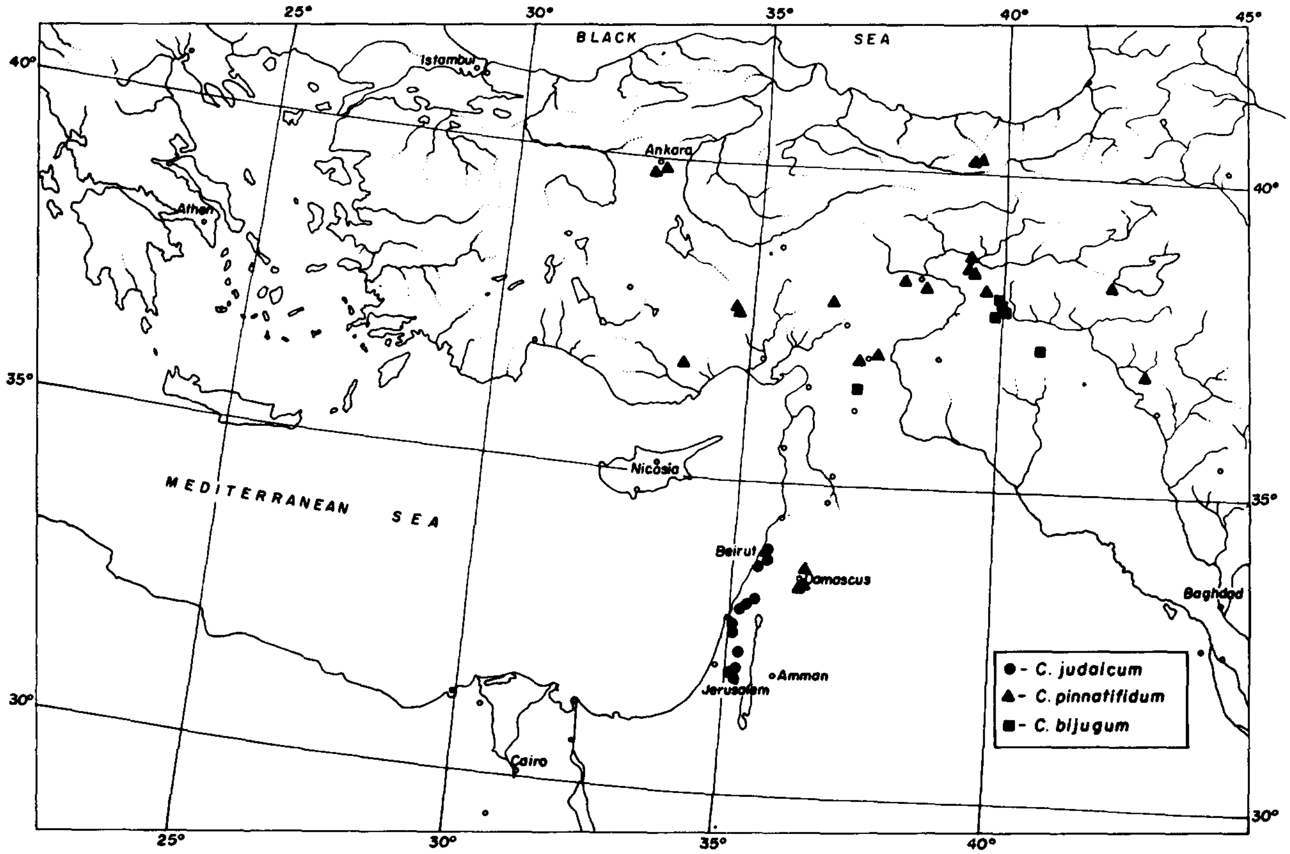

Fig. 2. The distribution of Group II, representative locations

teworthy that comparison of the seed dispersal mechanism of the wild peas and lentil with that of *C. reticulatum* suggests much better suitability of the latter for cultivation. While seeds of wild pea and lentil shatter immediately when ripe, the dry mature pods of *C. reticulatum* stay for a relatively long time on the plant before they fall intact and burst on the ground. It is likely that this mode of seed dispersal attracted the food gatherers during prehistoric time and resulted in a much greater proportion of chickpea among the pulses consumed by man. Also, when man commenced sowing wild plants, chickpea was probably favoured due to its lower loss of yield by shattering.

Group II

The members of this group are *C. bijugum*, *C. judaicum* and *C. pinnatifidum*, all with 2n = 16. The latter two species are sufficiently similar morphologically for some taxonomists to group them under the same species, for example in some regional floras (Post 1932; Zohary 1972). Following a more careful comparison, it has been decided to keep them as two different species (van der Maesen 1972). *Cicer judaicum* can be distinguished by its shorter petioles, less incised leaflet margins, obovate slightly incised stipules and smaller triangulate seed. These two species apparently also have allopatric distribution. While *C. judaicum* is common in the dwarf shrub formation in the hilly parts of Israel and Lebanon, *C. pinnatifidum* is found mainly in Turkey (Fig. 2). It grows there on calcareous bedrock and on the dry, metamorphic and igneous rubble slopes, where it is one of the very few annuals that manage to survive.

Morphologically the third species of Group II, *C. bijugum*, is characterized by much bigger leaflets, but only 5-7 per leaf, and by a subglobular seed covered with spiny hairs. The distribution of *C. bijugum* is much less known but it has been reported from N. Syria, N. Iraq and S. Turkey. In the Diyarbakir area of Turkey, this species is confined to a deep basaltic soil and is a common weed in cereal and legume fields including those of the cultivated chickpea.

Accomplishment of crosses between the three members of Group II is quite difficult because of the small

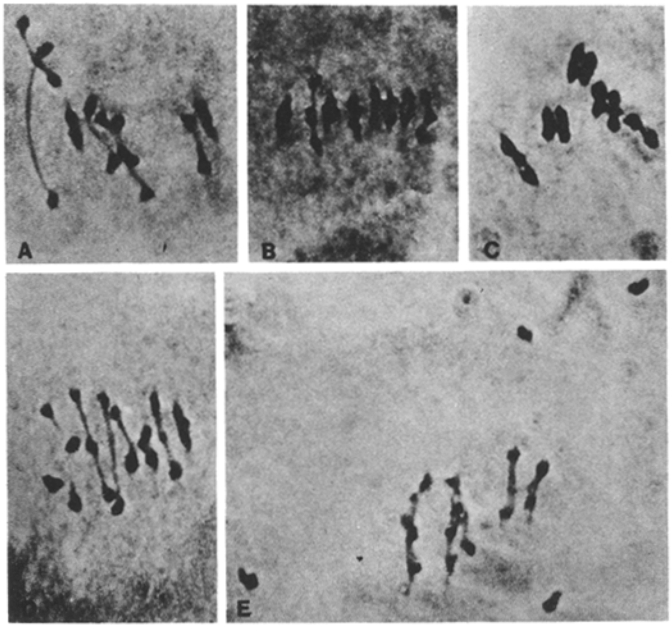

Fig. 3. Meiosis of species of Group II and their hybrids. 8II in MI of (A) *C. pinnatifidum*, (B) *C. judaicum*, (C) *C. bijugum*, (D) 7II + 2I in *C. pinnatifidum* × *C. bijugum* hybrid, (E) 6II + 4I in *C. judaicum* × *C. pinnatifidum* hybrid

delicate flowers of *C. judaicum* and *C. pinnatifidum*. Nevertheless, the three possible hybrid combinations between them were reciprocally produced and analysed. The morphology of the hybrids in each of the combinations was intermediate to the parental species, particularly in the leaflets and stipule size and shape. Meiosis in the three hybrid combinations was characterized mainly by bivalent association and occasional univalent formation that hardly affected the number of chiasmata per cell (Table 3, Fig. 3). The lowest rate of univalent formation was observed in *C. pinnatifidum* × *C. bijugum* and was more frequent in the *C. judaicum* × *C. pinnatifidum* hybrid. In the latter combination a quadrivalent association was also observed. The meiotic behavior of the three hybrid combinations indicates close chromosome homology between them. However, some discrepancy between the morphological and the cytogenetical evidence is apparent. On morphological grounds, *C. judaicum* and *C. pinnatifidum* are so close that often they are treated as variants of the same species, while *C. bijugum* is distinct. On the other hand, in its chromosome architecture, *C. pinnatifidum* is closer to *C. bijugum* than to *C. judaicum*.

The pollen fertility was relatively high (30-50 %) but no seeds were produced in any of the F_1 hybrid combinations. The apparent sterility was caused by considerable elongation of the stigma at the time of anthesis. Very often it grew out of the keel and the wings, while the anthers remained inside (Fig. 4). The role of the spatial arrangement of the stigma and the anthers in that so-called sterility is shown by the relatively large number of seed produced in all the three hybrid combinations following hand self-pollination and back-crossing to the parental species (Table 3). The seeds obtained by this procedure germinated normally and the F_2 plants reached maturity. Great variation was encountered in the F_2, mainly in the leaf size, number of leaflets per leaf, leaflet shape and the extent of leaflet and stipule margin dentation. Restoration of self-fertility (hand pollinations were not used) was observed among the F_2 plants. Some of them were as fertile as the parental species, others were completely sterile. Usually there was a good correlation between restoration of fertility and morphological similarity to either parental species, but fertile progeny combining characteristics of the parental species were also detected.

The almost regular meiosis of the F_1 hybrids and their potentially high fertility, together with the lack of breakdown in F_2, confirm the close genetic rela-

Table 3. Meiosis and fertility of parental lines and hybrids of Group II

Parental lines and hybrids	No. cell	Univalents	Bivalents	Quadrivalents	No. chiasmata per cell	% pollen fertility	% seed set natural	% seed set hand-, self-pollination and back-cross
C. judaicum	36	-	8	-	9.66±1.13	94.8	63	
C. pinnatifidum	21	-	8	-	9.76±0.91	98.6	61	
C. bijugum	28	-	8	-	10.00±0.85	98.4	60	
C. judaicum × *C. pinnatifidum*	42	1 (0-4)	7.45 (6-8)	0.023 (0-1)	9.38±1.29	39.3	-	50
C. judaicum × *C. bijugum*	42	0.70 (0-4)	7.65 (6-8)	-	9.64±1.71	42.5	-	34
C. pinnatifidum × *C. bijugum*	30	0.20 (0-2)	7.90 (7-8)	-	9.33±0.97	69.7	-	70

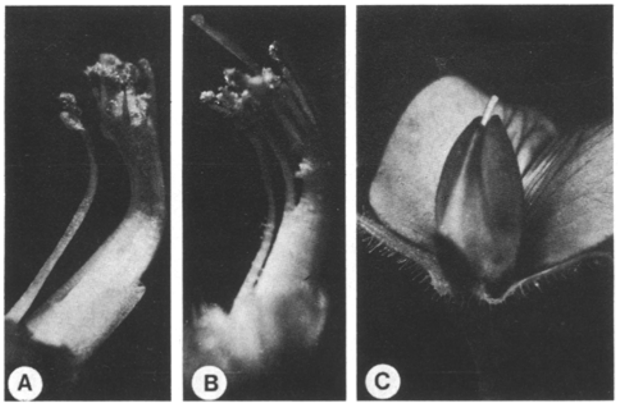

Fig. 4. Spatial arrangement of the stigma and anthers at the time of anthesis of species and hybrid of Group II. (A) The stigma and anthers are at the same level in *C. judaicum*, (B) The stigma is much higher than anthers in *C. judaicum* × *C. pinnatifidum* hybrid, (C) The outgrowing stigma of the same hybrid

tionships among the three species of this group. The reproductive barrier between them is rather weak, postzygotic in nature and does not result in genic disharmony and only partially in meiotic irregularities. The latter means of reproductive isolation could easily be evolved in this group of extremely cleistogamous plants even if they grew in nature side by side. Yet the main feature of the reproductive barrier between the three species of this group is an incompatibility in the growth rate of the stigma and the anthers of the F_1 interspecific hybrid at the time of anthesis. To the best of our knowledge such a mechanism has not previously been reported. Obviously this isolation barrier could have been evolved only after spatial isolation between these three species was completed. Otherwise, extensive gene flow between any two sympatric species of this group would be expected, due to the outpollination of the protruding interspecific hybrid stigma by pollen of both parental species, carried by insects.

While these three species of Group II have allopatric distribution and even occupy different ecological niches, the weedy species of the group, *C. bijugum*, very often grows side-by-side with the cultivated species as a weed in chickpea fields. Gene flow apparently does not take place in this case due to extreme cross-incompatibility.

Group III

The climbing chickpea *C. cuneatum* is the only member of this group. In addition to its unique growth habit, this species has been reported from Ethiopia and Sudan, far away from the main distribution area of the rest of the annual chickpeas. Numerous crosses were performed between this species and *C. arietinum* of Group I and *C. judaicum* and *C. pinnatifidum* of Group II (Table 2). In a few cases empty pods developed but not even a single hybrid seed was formed. This apparent cross-incompatibility further stresses the unique position of *C. cuneatum* among the annual chickpeas.

Within the distribution area of *C. cuneatum*, at least in Ethiopia, the cultivated species is extensively grown. Since *C. cuneatum* is mainly a weed of cultivation, some contact between these two species might be expected. The prezygotic reproductive barrier between them apparently prevents any gene flow in this case.

The assignment of the 7 annual chickpeas examined in the present study to three crossability groups also indicates the possible exploitation of these species for breeding purposes. Obviously, *C. reticulatum* is in the primary gene pool (Harlan and De Wet 1971) of the cultivated chickpea and gene exchange between these species should be as regular as between any other cultivated varieties. The second wild species of Group I, *C. echinospermum*, is, however, in the secondary gene pool. Gene exchange between this species and *C. arietinum* is impaired due to the high sterility of their F_1 hybrid. Back-crossing of this F_1 hybrid to the cultivated species might, however, increase fertility in advanced generations. Considering the possible significance of these two wild species for breeding of *C. arietinum* in the future, it is imperative to have additional material and further information on the variation and distribution of *C. reticulatum* and *C. echinospermum*.

The members of Groups II and III are apparently in the tertiary gene pool since crosses between them and the cultivated species yield no viable seeds. In view of the small number of crosses that have been made in several combinations, additional crosses are desirable to establish the crossability groups in the annual *Cicer* species.

On morphological grounds, one might suspect that the two annual chickpeas that were not included in this study, *C. chorassanicum* and *C. yamashitae*, can also be assigned to the tertiary gene pool of *C. arietinum*. By having trifoliate leaves, *C. chorassanicum* deviates considerably from the rest of the annual chickpeas. On the other hand, *C. yamashitae* is closer to *C. bijugum* of Group II. Nevertheless, it will be very interesting to test the crossability potential of these two species when their seeds become available.

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