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Research Article

Cytogenetic study of the genus *Cousinia* (Asteraceae, section *Serratuloideae*) in Iran

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Abstract

Meiotic studies of ploidy level, chromosome paring and chiasma frequency were performed on 11 *Cousinia* (Asteraceae) species of the section *Serratuloideae*. The diploid number of the species studied was 2n = 2x = 24 and 26 so these species possess two different basic numbers (x = 12 or 13), a phenomenon common to other sections of the genus. The chromosome numbers of 9 species are reported here for the first time. When the 2n = 24 and 2n = 26 species were subjected to cluster analysis based on relative meiotic characters two different clusters were formed indicating their distinctness. Our data support the results obtained from morphometry, anatomy, pollen morphology and molecular studies of the genus *Cousinia*.

Key words: Cousinia, chiasma frequency, cluster analysis, meiosis.

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Introduction

The genus *Cousinia* (Asteraceae) is of worldwide distribution and comprises approximately 672 species, of which about 235 occur in central, western, eastern and southeastern Iran. According to Boissier (1875) the *Serratuloideae* section contains 9 species while Rechinger (1970) in *Flora Iranica* considers 11 species for this section.

Earlier reports on Iranian *Cousinia* are have been confined mainly to taxonomic studies (Ghahreman *et al*., 1999; Attar and Ghahreman, 2000; 2002) and a few chromosome number reports (Ghaffari and Djavadi, 1998; Ghaffari *et al*., 2000). Our study reported in this paper was the first cytological analysis of 11 Iranian species of the section *Serratuloideae*. We also discuss the cytological changes which play a role in the species diversification of this section.

Materials and Methods

Cytological studies were performed on 11 *Cousinia* species (Table 1), vouchers specimens are being deposited in the Herbarium of Tehran University (HTU), Iran.

For cytological studies young flower buds of each species were collected from at least 10 randomly selected plants and fixed in acetic acid: ethanol (1:3 v/v) for 24 h after which they were washed and preserved in ethanol at 4 °C until used (Sheidai *et al*., 2002). For each species, squash slides were prepared and stained with 2% (w/v) aqueous aceto-orcein and the chromosome numbers and chiasma frequency determined from 50 pollen mother cells at diakinesis-metaphase I (Sheidai *et al*., 2002). Due to insufficient number of meiotic cells, chiasma frequency and distribution was not analyzed in *C. serratuloides*.

In order to determine any significant difference in chiasma frequency and distribution among the species with either chromosome number (n) = 13 or n = 12 we performed analysis of variance (ANOVA) by the least significant differences (LSD) method (Sokal and Rolf, 1995; Sheidai *et al*., 2002).

Grouping the n = 12 and n = 13 species was performed using various cluster analysis methods including single linkage, unweighted paired group with arithmetic mean (UPGMA) and the WARD method as well as ordination based on principal component analysis (PCA) in each group separately (Podani, 2000; Sheidai *et al*., 2002). For numerical analyses meiotic characteristics such as chiasma frequency and distribution (terminal and intercalary chiasmata) and chromosomes association (ring and rod bivalents) as well as univalents were used.

For grouping both the species having n = 12 and 13, cluster analysis and PCA ordination was performed using relative meiotic characters (*i.e.* dividing the mean values of chiasma frequency and bivalents by the chromosome num-

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Table 1 - List of *Cousinia* species studied. All samples were collected by Attar and Mahdigholi.

Cousinia species	Collection site	Altitude (meters)	Accession number
C. elbursensis (Mahdigholi, Attar, Sheidai and Ghahreman)	Mazandaran	2400	32192
C. pinarocephala (Boiss)	Mazandaran, Rudbarak, Alam-kooh	2350	2869
C. pterocaulos (C.A. Mey)	Gilan, Talesh, Aghvelar village	1600	32200
C. crispa (Jaub and Spach.)	Tehran, Kandowan	2700	27825
C. adenostegia (Resch)	Khorasan, Neishabur, Barfriz village	1800	32203
C. concolor (Bunge)	Khorasan, Neishabur, Pivehgen village	2100	27647
C. hypoleuca (Boiss)	Tehran, Kandowan	2500	27824
C. irritans (Resch)	Semnan, Shahrood road to Azad shahr	1600	21893
C. sheidaei (Attar, Ghahreman and Mahdigholi)	Markazi	2000	21817
C. serratuloides (Boiss)	Tehran, Lasem village	2100	32205
C. discolor (Bunge)	Khorasan, Neishbur, Disband	2200	25396

ber to obtain meiotic values per chromosome) unaffected by the chromosome number (Sheidai *et al*., 2002). Euclidean distance was used for cluster analysis.

Results and Discussion

Variation in prophase sub-stages of meiosis-I

The meiotic analysis of the *Cousinia* species studied showed variation in the prophase sub-stages of meiosis-I (Figure 1, A-D). In the first sub-stage instead of the leptotene and zygotene stages a synezetic knot occurred consisting of thin chromatin strands surrounding the nucleolus and eventually totally covering it (Figure 1, A). Later, paired chromosomes unraveled from the knot and appeared as thick strands, ushering in the pachytene stage (Figure 1, B) of which end-to-end attachment of chromosomes is a feature reported in taxa showing a synezetic knot stage (John *et al*., 1989; Sheidai and Inamdar, 1991).

After pachytene, decompaction of the chromosomes occurs, commencing with the diffuse stage (Figure 1, C-D), which has been reported in several plant species (Sybenga, 1992), which may be complete with whole chromosomes undergoing decompaction or partial in which only part of the genome becomes decompacted. Our study showed an almost complete diffuse stage in the *Cousinia* species examined. Various reasons have been suggested for the occurrence of the diffuse stage, including high synthetic activity (*i.e.* the lampbrush stage in amphibian oocytes), shedding of the lateral elements of the synaptonemal complex, post pachytene elimination or modification of histone proteins and meiotic arrest to withstand adverse environmental conditions (John *et al*., 1989; Sheidai *et al*., 2003). We were not able to determine the exact reason for the occurrence of a diffuse stage in the *Cousinia* species studied by us but since these species grow in regions of Iran where the environmental conditions are harsh it may be an adaptation to these conditions. Unusual meiosis occurs in *Cousinia* species with different chromosome numbers, as has been reported in diploid and polyploid species of other plant groups (Sheidai and Inamdar, 1991) and the genomic control of the diffuse stage is not affected by ploidy level.

Chromosome number, chiasma frequency and distribution

Of the *Cousinia* species studied by us, *elbursensis*, *pinarocephala*, *pterocaulos* and *crispa* were n = 12 while *adenostegia*, *concolor*, *hypoleuca irritans*, *sheidaei*, *dis-*

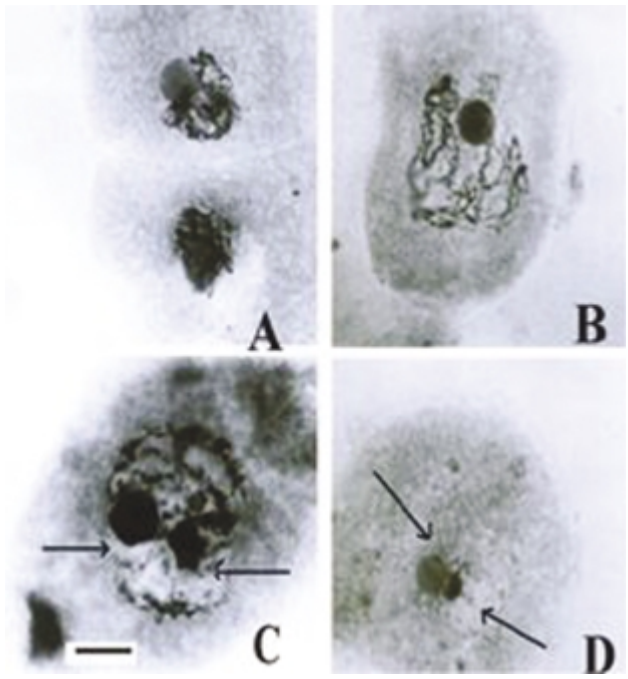


Figure 1 - Representative pollen mother cells of *Cousinia* species showing prophase-I sub-stages of meiosis. A & B = synezetic knot and pachytene stage in *C. hypoleuca*, C = Start of diffuse stage (arrows) in *C. irritans*, D = complete diffuse stage (arrows) in *C. concolor*. Scale bar = 10 μm.

color and *serratuloides* were n = 13 (Table 2, Figure 2). Except for *C. crispa* and *C. hypoleuca* this is the first time that chromosome numbers have been published for these species. In general, our study supports the chromosome number reported for *C. hypoleuca* by Afzal-Rafii (1980), although this author reported 2n = 26 for *C. crispa* as opposed to the n = 12 (2n = 24) which we recorded for this species. Our data shows that two different basic chromosome numbers (12 or 13) occur in the *Serratuloideae* section, a similar situation having been reported for other *Cousinia* sections including *Cousinia* and *Leiocaules* (both either n = 12 or 13) and *Eriocousinia* (n = 11 or 13, Susana *et al*., 2003). These data suggest aneuploidy, most probably by chromosome reduction during the evolution of the genus *Cousinia* (Susana *et al*., 2003).

Data on chiasma frequency and distribution as well as chromosome pairing is presented in Table 2. For the n = 12 species, *C. elbursensis* had the highest total number of chiasmata (17.00) and terminal chiasmata (16.90), while the highest number of intercalary chiasmata (0.70) occurred in *C. pinarocephala* and *C. crispa*. Among the n = 13 species the highest number of total and terminal chiasmata and ring bivalents occurred in *C. discolor* while the lowest values occurred in *C. hypoleuca* (Table 2). It is interesting to note that *C. adenostegia*, *C. irritans* and *C. discolor* did not form intercalary chiasmata but showed ter-

Table 2 - Meiotic characters of the *Cousinia* species studied. Due to insufficient number of meiotic cells, chiasma frequency and distribution was not analyzed in *C. serratuloides*.

Cousinia species	n	RB	ROD	I	TX	IX	TOX	RN	IN	RDN	IXN	TXN	TOXN
C. elbursensis	12	5.00	6.90	0.10	16.90	0.10	17.00	0.42	0.01	0.58	0.01	1.41	1.42
C. pinarocephala	12	5.00	6.80	0.20	14.90	0.70	15.60	0.42	0.02	0.57	0.06	1.24	1.30
C. pterocaulos	12	4.33	7.34	0.33	15.44	0.66	16.00	0.36	0.03	0.61	0.06	1.29	1.33
C. crispa	12	4.82	6.77	0.41	15.41	0.70	16.11	0.40	0.03	0.56	0.06	1.28	1.34
C. adenostegia	13	7.40	5.20	0.40	20.10	0.00	20.10	0.58	0.02	0.40	0.00	1.55	1.55
C. concolor	13	8.33	4.37	0.30	20.69	0.15	20.86	0.64	0.02	0.34	0.01	1.59	1.60
C. hypoleuca	13	6.47	5.30	1.23	18.17	0.12	18.29	0.50	0.09	0.41	0.01	1.40	1.41
C. irritans	13	7.22	5.34	0.44	19.77	0.001	9.77	0.56	0.03	0.41	0.00	1.52	1.52
C. sheidaei	13	7.29	5.67	0.14	19.85	0.29	20.14	0.56	0.01	0.44	0.02	1.53	1.55
C. discolor	13	9.50	3.00	0.50	21.00	0.00	21.00	0.73	0.04	0.23	0.00	1.62	1.62

Abbreviations: n = haploid chromosome number; RB = Ring bivalents; ROD = Rod bivalents; I = Univalents; TX = Terminal chiasmata; IX = Intercalary chiasmata; TOX = Total chiasmata; RN = Ring bivalents/haploid chromosome number; IN = Intercalary chiasmata/haploid chromosome number; RDN = Rod bivalents/haploid chromosome number; IXN = Intercalary chiasmata/haploid chromosome number; TXN = Terminal chiasmata/haploid chromosome number; TOXN = Total chiasmata/ haploid chromosome number.

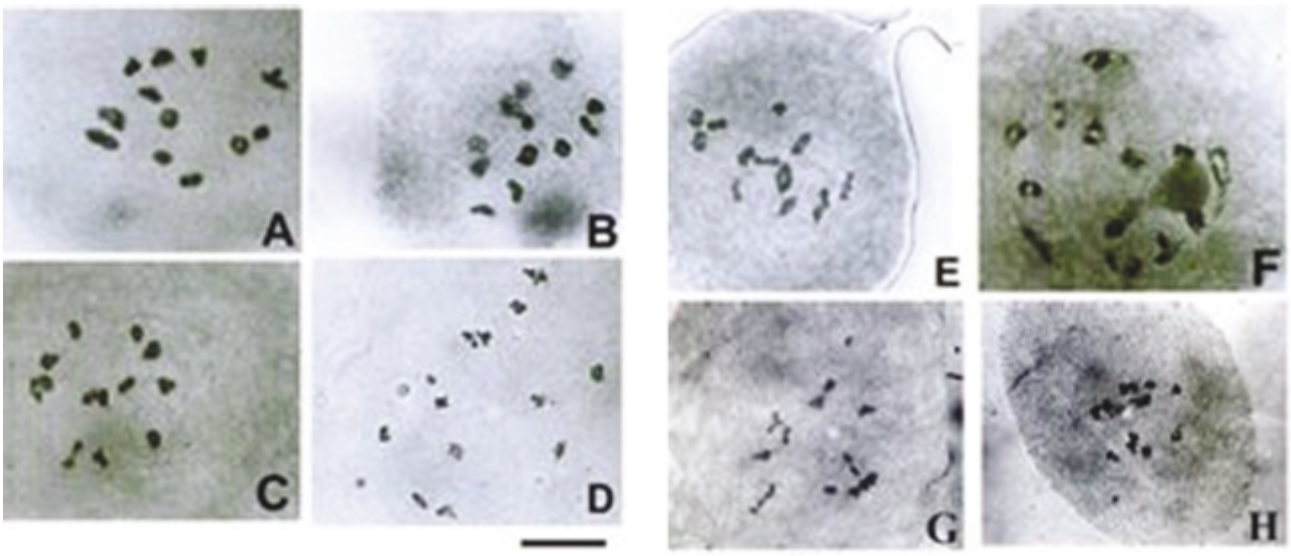


Figure 2 - Representative pollen mother cells of *Cousinia* species showing gametic chromosome number in diakinesis-metaphase I. A = *C. crispa* with n = 12; B = *C. elbursensis* with n = 12; C = *C. adenostegia* with n = 13; D = *C. discolor* with n = 13; E = *C. irritans* with n = 13; F = *C. sheidaei* with n = 13; G = *C. serratuloides* n = 13; H = *C. hypoleuca* n = 13. Scale bar = 10 μm.

minal chiasmata only (Table 2). Variation in chiasma frequency and localization is genetically controlled (Quick, 1993) and has been reported in several plant species as well as in crop plant varieties (Rees and Dale, 1974; Rees and Jones, 1977). Such variation between species and populations with the same chromosome number is considered to be a means for generating new forms of recombination which influences the variability within natural populations in an adaptive way (Rees and Dale, 1974).

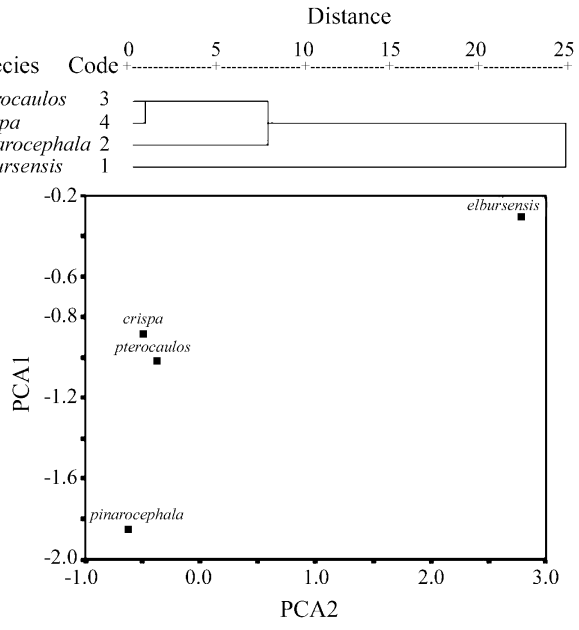
When ANOVA was performed on the cytogenetic data for the n = 13 species no significant differences were found between species, indicating that these species are homogeneous for such characters. However, a significant difference in the number of ring and rod bivalents was observed among the n = 12 species and since the frequency and distribution of chiasmata was not significantly different in these species the significant difference observed in the number of bivalents may be due to fast terminalisation of chiasmata only. A joint analysis was performed for the n = 12 and n = 13 species using relative meiotic data but no significant difference was seen.

The UPGMA and WARD cluster analysis and principal component analysis (PCA) of the n = 12 and n = 13 (performed separately) species produced similar results, the UPGMA Euclidean distance clustering dendrogram and PCA ordination for the n = 12 species being presented in Figures 3 and 4. Two species (*C. pterocaulos* and *C. crispa*) appear to be very similar and are placed very close to each other in a single cluster, while two other species (*C. pinarocephala* and *C. elbursensis*) appear to be more distant.

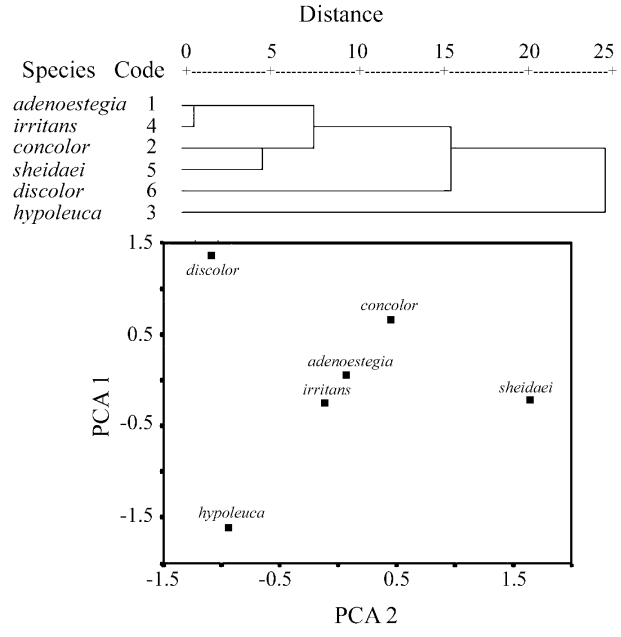
Principal component analysis of the meiotic data for the n = 12 species revealed that the first two PCA factors accounted for about 99% of the total variance. The first factor accounted for about 81% of the total variance, with the mean number of ring bivalents, mean number of univalents, and total and terminal chiasmata having the highest positive correlation (>0.86). The second factor accounted for about 17% of the total variance, with the mean number of rod bivalents showing the highest positive correlation (>0.68). This analysis shows that these meiotic characters are the most variable among the n = 12 species, with the first factor characters separating *C. elbursensis* from the other species and the second factor characteristic separating *C. crispa* and *C. pinarocephala* from the other species (Figure 4).

The UPGMA dendrogram for the n = 13 species (Figure 5) shows three major clusters. The first major cluster contains 2 sub-clusters, one containing *C. adenostegia* and *C. irritans* and the other *C. concolor* and *C. sheidaei* which joining the first two species at some distance. The species *C. discolor* and *C. hypoleuca* are located a long way the first major cluster and each form a single cluster. Principal component analysis (Figure 6) of the n=13 species supports the cluster analysis showing separation of *C. discolor* and *C. hypoleuca* from the others and morphometric analysis of these species also produced similar results (unpublished data).

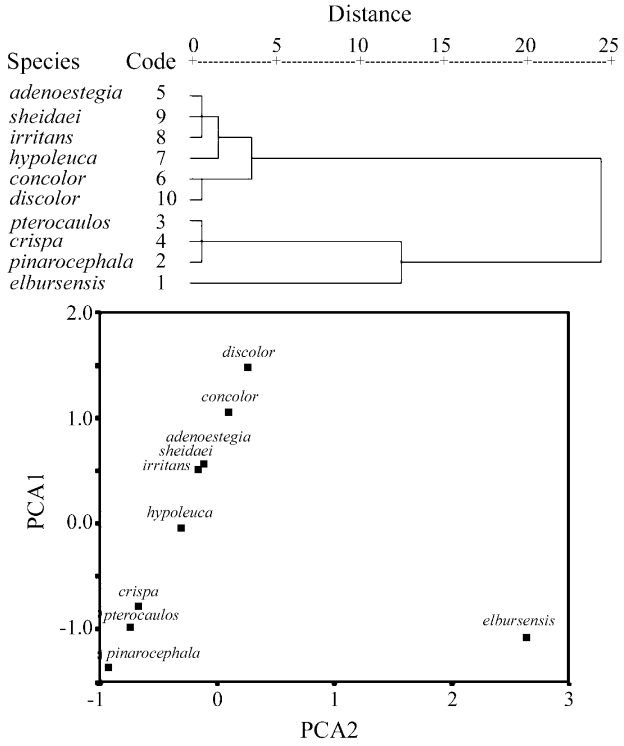
Principal component analysis of the meiotic data for the n = 13 species revealed that the first two PCA factors accounted for about 89% of the total variance. The first factor accounted for about 64% of the total variance, the highest positive correlation (>0.90) occurring with the mean number of ring bivalents and total and terminal chiasmata. For the second factor, Intercalary chiasmata showed the highest



Figures 3 and 4 - UPGMA cluster analysis and PCA ordination of n = 12 *Cousinia* species.



Figures 5 and 6 - UPGMA cluster analysis and PCA ordination of n = 13 *Cousinia* species.



Figures 7 and 8 - UPGMA cluster analysis and PCA ordination of n = 13 and n = 12 *Cousinia* species based on relative meiotic data.

correlation (>0.70). These results show that these meiotic characters are the most variable among the n = 13 species, with the first factor characters separating *C. elbursensis* from the other species and the second factor characteristic separating *C. discolor* and *C. hypoleuca* from the other species (Figure 4).

The cluster and PCA analysis of the joint n = 12 and n = 13 species data are presented in Figures 7 and 8, from which it can be seen that the n = 12 and n = 13 species are placed in two different groups which reflect their distinctive in meiotic characteristics. This grouping could indicate the presence of specific genomic control in each group. Comparative morphometric, anatomical and pollen morphology studies carried out by us on these species showed similar results to those reported above (unpublished data). The n = 12 and 13 *Cousinia* species have also been shown to be distinct in terms of the DNA sequences of their ITS regions (Susana *et al*., 2003) which showed a close relationship between n = 11 species and n = 13 species while the n = 12 formed a different group.

Our data indicates that in order to understand the evolutionary patterns operating in the genus *Cousinia* further studies should be carried out on species with different chromosome numbers.

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