

Cytology and palynology of the *Clematis* L. species (Ranunculaceae) in Iran

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Cytological and palynological studies were performed on *Clematis* L. species (Ranunculaceae) of Iran indicating $2n = 2x = 16$ and $2n = 4x = 32$ in them. They formed only bivalents in metaphase of meiosis-I with some amount of chromosome stickiness and laggard formation in anaphase. The species possessed a symmetrical karyotype but differed in karyotypic formulae indicating the occurrence of structural changes in the chromosomes during species diversification. *Clematis* species usually possessed tricolpate pollens but differed in details of pollen morphology, colpi length, colpi width and apocolium length.

Keywords: *Clematis*, cytology, palynology, Iran

Introduction

The genus *Clematis* L. (Ranunculaceae) comprises about 250 species world wide. The species are well adapted to highland areas and are economically important for their chemical properties including the saponin glycosides available in their leaves and roots and also as ornamentals. *Clematis* is a vigorous climbing, with attractive flowers. Almost all species are found throughout the temperate regions of both hemispheres, and also in mountains in the tropics (TAMURA 1993). *Clematis* marks a peak of evolutionary development on the one hand and a high degree of speciation on the other. It is almost if not quite the most widely geographically distributed genus in the family and is very close to being the second largest genus in the family with 250 species. Although only about 40 of its 250 species have been studied, every one but two were simple diploids with $n = 8$. Obviously, polyploidy as a factor in both evolution and speciation has a limited role in this remarkable genus (KEENER 1967, YANO 1993).

Clematis is considered to be a monophyletic genus within Ranunculaceae. The genus *Clematis* has been divided into two subgenera of 1 – *Clematis* including the sections *Clematis*, *Cheiroopsis*, *Lasiantha*, *Aspidanthera* and *Naravelopsis*, and 2 – *Flammula* including the sections *Flammula*, *Pterocarpa*, *Viticella* and *Fruticella* (TAMURA 1987, TAMURA 1968, JOHANSSON 1995, CHRISTOPHER GREY-WILSON 2000, MAGNUS JOHNSON 2001)

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Based on calyx arrangement and pubescence of the stamens, the genus *Clematis* has been divided into 4 subgenera: 1 – Subgen. *Campanella* Tamura including the sections *Campanella* Tamura, *Tubulosae* Decne, *Atragen* (L.) DC., *Meclatis* (Spach) Tamura, *Bebaenanthera* Edgew and *Pseudanemone* Prantl., 2 – Subgen. *Viorna* (Reichb.) Tamura including only the section *Viorna* (Reichb.) Prantl., 3 – Subgen. *Clematis* including the sections *Clematis*, *Cheirppsis* DC., *Naraveliopsis* Hand.-Mazz., *Aspidanthera* Spach., and *Lasiantha* Tamura, 4 – *Flammula* (DC.) Peterm., including the sections *Flammula* DC., *Angustifoliae* (Tamura) Serov., *Fruticella* (Moench) DC., *Viticella* (Moench) DC., and *Pterocarpa* Tamura.

The section *Clematis* has been divided into 5 subsections such as *Angustifoliae*, *Rectae*, *Crassifoliae*, *Baoniniana* including 73 species and 47 varieties (WANG 2003a, b; 2006).

Biosystematic, phylogenetic and molecular studies have been performed to reveal the phylogenetic relationships of the *Clematis* species (POLINKOVA 1936, KEENER 1967, TAMURA 1968, TOWNSEND and EVANS 1980, ZIMAN 1981, YANO 1993, KIMBERLY *et al.* 2005, MIKEDA 2006, WANG 2006). Similar studies are lacking in Iran.

The number of *Clematis* species growing in Iran varies according to different authors. PARSA reports 8 *Clematis* species while RECHINGER *et al.* reported the occurrence of 6 species in the country: 1 – *C. viticella* L. from the section *Viticella*, 2 – *C. orientalis* L., 3 – *C. flammula* L., 4 – *C. isphahanica* Boiss., 5 – *C. songarica* Bunge., and 6 – *C. asplenifolia* Schrenk., all from the section *Clematis* (PARSA 1986, RECHINGER *et al.* 1992).

The present report is a part of biosystematic study of the genus *Clematis* in Iran, considering cytogenetic and palynological studies in three species of *C. orientalis*, *C. flammula* and *C. isphahanica*.

Materials and methods

Cytology

Cytological studies were performed on 9 populations of 3 species of *C. orientalis* L., *C. flammula* L. and *C. isphahanica* Boiss. For meiotic studies, young flower buds were collected randomly from 10 plants of each species/ population and fixed in glacial acetic acid: ethanol (1:3) for 24 hrs. Flower buds were washed and preserved in 70% ethanol at 4 °C until used. Cytological preparations were made using the squash technique and 2% aceto-orcein as the stain (SHEIDAI *et al.* 2006, 2007).

Fifty to one hundred pollen mother cells (PMCs) were analysed for chiasma frequency and distribution at diakinesis and metaphase stages while 500 PMCs were analysed for chromosome segregation during the anaphase and telophase stages. Pollen satiability as a measure of fertility was determined by staining a minimum of 500 pollen grains with 2 % acetocarmine: 50 % glycerin (1:1) for about 30 min. Complete pollens which were stained were taken as fertile, while incomplete, shrunken pollens with no stain were considered to be infertile (SHEIDAI *et al.* 2006).

In order to compare the meiotic characteristics among the species/ populations with different chromosome numbers, relative meiotic data were used (meiotic data/chromosome number). χ^2 test was performed to detect any significant difference in the relative chiasma frequency and distribution as well as chromosomes association as well as to detect any significant difference in meiotic abnormalities (SHEIDAI *et al.* 2007). Statistical analysis used SPSS ver. 9 (1998).

For karyotypic studies we collected freshly grown root tips from the seeds of at least ten randomly selected plants in each species. Details of the pre-treatment, fixation and squash technique as well as karyotype analyses followed the earlier report of SHEIDAI and RASHID (2007). The chromosomes were identified according to LEVAN et al. (1964), karyotype symmetry was determined according to STEBBINS (1971), while other karyotypic parameters like total form percentage (TF %) and coefficient of variation of the chromosome size were also determined (SHEIDAI and BAGHERI-SHABESTAREI 2007).

Pollen morphology

Fully matured anthers were removed from the specimens and prepared by the standard acetolysis method, after which they were mounted in glycerin jelly and sealed with paraffin wax for light microscopy (LM) (ERDTMAN 1969). Measurements and morphological observations of the pollen grains were performed with the use of a minimum of 20 pollen grains. For scanning electron microscopy (SEM), the pollen grains were attached to the aluminum stubs with double sided cellophane tape, air dried at room temperature and coated with gold. The specimens were examined with PHYLIPS XL 30, LEO 440i at 15 kV and 22 kV and photographed.

Results

Cytology

Meiotic and mitotic karyotype analyses of the *Clematis* species and populations studied revealed the presence of $2n = 2x = 16$ chromosome number in *C. orientalis* and $2n = 4x = 32$ chromosome number in *C. ispahanica* and *C. flammula* (Tabs. 1, 2; Figs. 1, 2).

The populations of *C. orientalis* formed ring and rod bivalents with the highest value of ring bivalents (5.50) in the Dizbad population and the lowest value of the same (4.00) in the Roodbar 84 population. The highest value of total chiasmata occurred in the Dizbad population (13.27) while the lowest value of occurred in the Roodbar 84 population (11.29). Two tetraploid species of *C. ispahanica* and *C. flammula* showed diplontic behavior and formed only bivalents in metaphase of meiosis-I.

Meiotic metaphase and anaphase chromosome stickiness occurred in the species and populations studied. The degree of chromosome stickiness ranged from stickiness among two or more chromosomes to the involvement of all metaphase chromosomes forming a complete clump (Fig. 1, C). The species and populations differed significantly ($p < 0.05$) in percentage of chromosome stickiness. The percentage of metaphase I cells showing stickiness varied from 2.50 in *C. flammula* to 7.50 in *C. ispahanica*. The percentage of metaphase I cells showing stickiness in different populations of *C. orientalis* ranged from 5.00% in the Roodbar 84 population to 6.60% in the Dizbad population. The percentage of metaphase I cells showing complete chromosome clumping varied from 2.50 in *C. flammula* to 20.00 in Shahrestanak population of *C. orientalis*.

One to a few anaphase-I laggard chromosomes occurred in *C. flammula*, *C. ispahanica* and the Roodbar 85 population of *C. orientalis*. The species and populations studied differed significantly ($p < 0.05$) in the mean number of laggard chromosomes too. The *Clematis* species showed >97 % pollen fertility.

Tab. 1. Meiotic characteristics in *Clematis* species and populations studied. Mean and standard deviations.

Species	Locality	2n	X	RB	ROB	IX	TEX	TOX	IXN	TXN	TOXN	RBN	ROBN
<i>Clematis orientalis</i>	Neyshaboor	16	2×	5.5 ±0.33	2.54 33/0±	5.4 ±0.45	7.86 ±0.56	13.27 ±0.33	0.68	0.98	1.66	0.69	0.32
<i>C. orientalis</i>	Shahrestanak Karaj	16	2×	4.81 ±0.30	3.18 ±0.18	4.81 ±0.31	8.13 ±0.57	13 ±0.32	0.6	1.02	1.63	0.6	0.4
<i>C. orientalis</i>	Roodbar 84	16	2×	4 ±0.41	4 ±0.41	4.88 ±0.40	7.11 ±0.76	11.29 ±0.75	0.61	0.89	1.41	0.5	0.5
<i>C. orientalis</i>	Roodbar 85	16	2×	4.25 ±0.34	3.75 ±0.34	5.1 ±0.33	7.3 ±0.64	12.4 ±0.36	0.64	0.91	1.55	0.53	0.47
<i>C. ispahantica</i>	Noor	32	4×	6.54 ±0.82	9.54 ±0.79	12.36 ±0.62	10.36 ±1.37	22.72 ±0.95	0.77	0.65	1.42	0.41	0.6
<i>C. flammula</i>	Roodbar	32	4×	7.5 ±0.53	8.5 ±0.53	11.68 ±0.49	12.18 ±0.95	23.87 ±0.59	0.73	0.76	1.49	0.47	0.03

Abbreviations:

x = Ploidy level, RB = Mean number of ring bivalents, ROB = Mean number of rod bivalents, IX = Mean number of intercalary chiasmata, TEX = Mean number of terminal chiasmata and TOX = Mean number of total chiasmata, RBN = Mean number of ring bivalents per cell, ROBN = Mean number of rod bivalents per cell, IXN = Mean number of intercalary chiasmata per bivalent, TEXN = Mean number of terminal chiasmata per bivalent, TOXN = Mean number of total chiasmata per bivalent.

Tab. 2. Karyotypic details of *Clematis* species.

Species	Locality	2n	S	L	TL	L/S	X	TF%	CV	KF
<i>Clematis orientalis</i>	Roodbar	16	5.43	11.34	64.96	2.08	8.12	32.90	13.17	4m+1sm + 1st + 2t
<i>C. orientalis</i>	Noor	16	5.47	10.13	62.86	1.85	7.85	36.20	19.87	5m+1st + 2t
<i>C. ispahanica</i>	Roodbar	32	3.69	11.36	116.91	3.07	7.30	35.20	14.65	10m+2st + 4t

Abbreviations: S = Size of the shortest chromosome (μm), L = Size of the longest chromosome (μm), TL = Total length of haploid chromatin length (μm), L/S = Ratio of the longest to shortest chromosome, X = The mean chromatin length, TF% = Total form percentage, CV = Coefficient of variation, KF = Karyotype formulae,

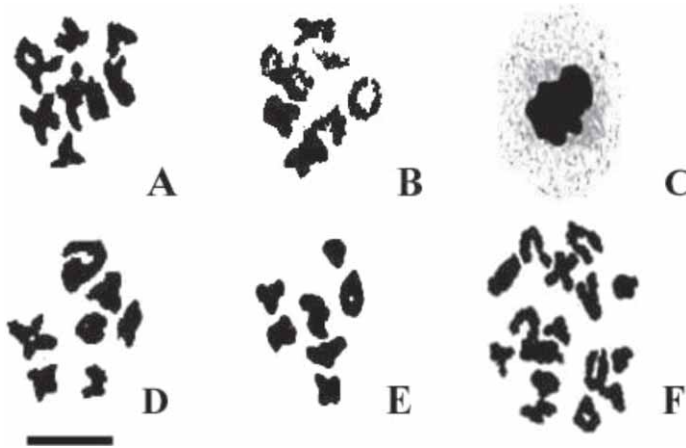


Fig. 1. Meiotic cells in *Clematis* species. A = Meiotic cell showing $n = 8$ in Dizbad population of *C. Orientalis*, B – Meiotic cell showing $n = 8$ in Roodbar 84 population of *C. Orientalis*, C – Meiotic cell showing complete clump in metaphase of meiosis-I in Roodbar 84 population of *C. Orientalis*, D – Meiotic cell showing $n = 8$ in Sharestanak population of *C. Orientalis*, E – Meiotic cell showing $n = 8$ in Roodbar 85 population of *C. Orientalis*, F = Meiotic cell showing $n = 16$ in *C. flammula*. Scale bar = $10 \mu\text{m}$.

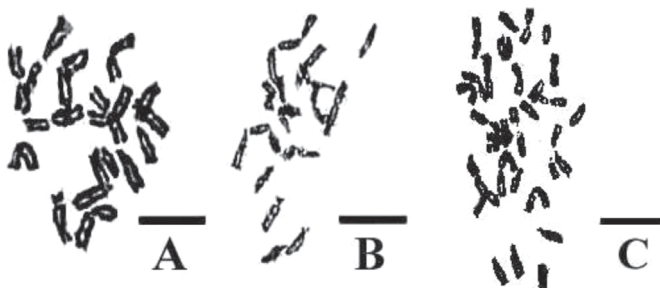


Fig. 2. Somatic metaphase cells in *Clematis* species. A – Roodbar 84 population of *C. orientalis* showing $2n = 16$, B – Yoosh population of *C. orientalis* showing $2n = 16$, C – Roodbar population of *C. ispahanica* showing $2n = 32$. Scale bar = $10 \mu\text{m}$.

The somatic chromosome numbers obtained also show a tetraploid chromosome number ($2n = 4 \times = 32$) for *C. orientalis* (Tab. 2, Figs. 2, 3). The size of chromosomes varied from 3.69 μm to 11.35 μm in *C. ispanica*. The highest value of CV (36.20) for the size of

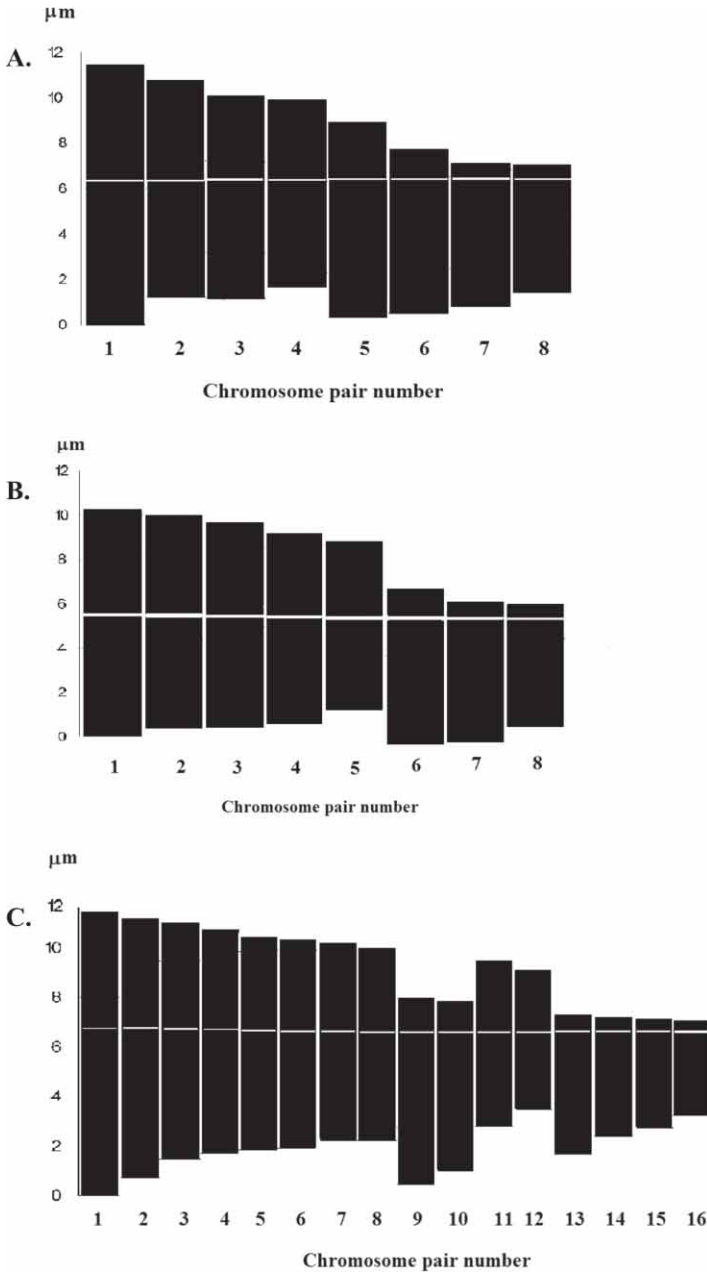


Fig. 3. Ideograms of *Clematis orientalis* Roodbar population (A), *C. orientalis* Yoosh population (B) and *C. ispanica* (C).

chromosomes occurs in the Noor population of *C. orientalis* indicating the highest degree of size variation among its chromosomes.

The *clematis* species studied differ in their karyotypic formulae (Tab. 2), possess TF% of >32 and are placed in 2A and 2B classes of Stebbins system (Tab. 2).

Pollen morphology

Since *C. orientalis* populations showed morphological variations and were grouped in four clusters (data not given), representative populations were included in the palynological study (Tab. 3, Fig. 4).

Tab. 3. Pollen measurements in *Clematis* species. (The rows of data are: Minimum, Mean and maximum respectively).

Species	P	E	P/E	Colpi length	Colpi width	Apocolpium	Apo Index
O1	30.03	24.13	1.19	15.70	1.74	3.59	0.20
	32.40	27.07	1.19	16.15	2.27	3.98	0.22
	34.82	29.18	1.24	16.85	2.78	4.52	0.24
O2	28.76	23.77	1.20	16.59	1.63	2.18	0.16
	22.92	27.25	1.20	19.29	2.54	4.98	0.23
	36.03	29.50	1.22	21.40	3.43	6.10	0.31
O3	29.98	20.71	1.33	21.56	1.12	6.60	0.36
	33.18	24.70	1.34	25.92	1.63	5.80	0.39
	37.27	27.11	1.44	29.30	2.15	7.11	0.47
F	24.99	24.15	1.03	22.69	2.36	3.48	0.17
	26.79	24.70	1.08	24.86	4.04	4.98	0.20
	28.00	25.63	1.09	26.58	4.90	6.21	0.25
I	30.13	27.18	1.10	26.10	2.02	3.85	0.18
	35.00	29.18	1.20	24.18	2.99	5.18	0.24
	38.23	30.75	1.24	28.63	3.68	6.37	0.31

Abbreviations: O1–O3 = *Clematis orientalis* Dizbad, Roodbar and Karaj populations respectively, F = *C. flamula*, I = *C. ispanica*. Apo Index = Apocolpium index, P = Polar view, E = Equatorial view.

The general shape of pollen in the Roodbar 85 (Figs. 4, A–D) and Dizbad (Figs. 4, E–F) populations of *C. orientalis* is prolate spheroidal in equatorial view and is round in polar view (Figs. 4, Q–T). The pollen is tricolpate, isopolar and radiosymmetric, the furrows are long, relatively wide and the furrow margins unfolded. Tectum is soft, smooth with microchinate projections.

The pollen shape in the Sharestanak population of *C. orientalis* is equiprolate in equatorial view and round in polar view (Figs. 4, I–L), trizonocolpate, isopolar and radiosymmetric. The furrows are long, slender and their margins are folded inwards and thickened. The furrow membrane is granulate with microchinate projections.

In *C. ispahunica* (Figs. 4, M–P), the pollen shape is equiprolate in equatorial view and round in polar view, trizonocolpate, isopolar and radiosymmetric. The furrows are syncolpate, almost long, folded and wide with regular, upright margins which are blunt at the end. The furrow membrane is granulate with microchinate projections.

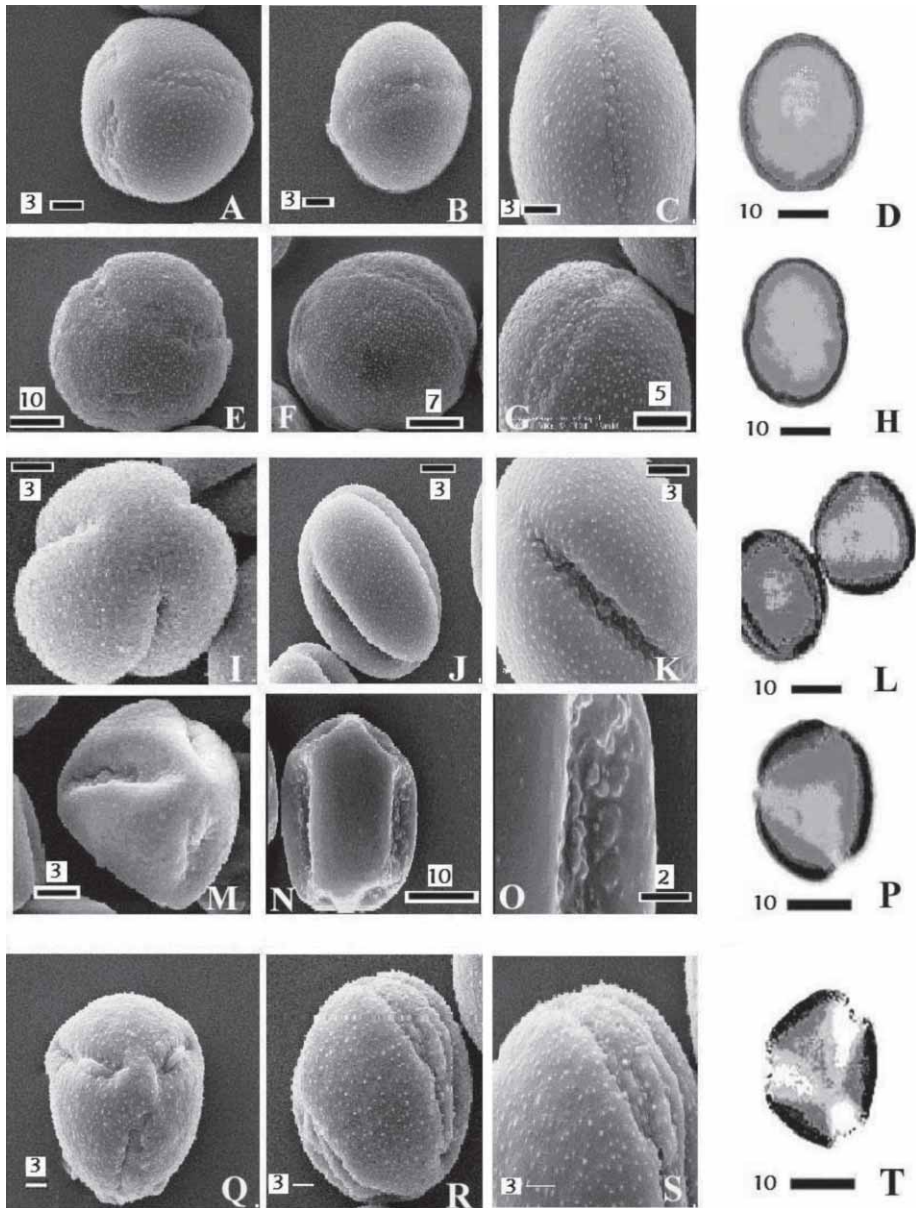


Fig. 4. Pollen shape and measurements in *Clematis* species. A–D – Roodbar 85 population of *C. orientalis*., E–F – Dizbad population of *C. orientalis*, I–L – Sharestanak population of *C. orientalis*, M–P – *C. ispahunica*. Q–T – *C. flammula*. Scale bar = μm .

In *C. flammula* (Figs. 4, Q–T), the pollen shape is prolate spheroidal in equatorial view and almost triangular in polar view, trizonocolpate, isopolar and radiosymmetric. The furrows are long, slender, not folding; their margins are irregular, pointed at the tip. The groove membrane is granulate with microchinate projections.

Discussion

The earlier cytological studies report $2n = 2x = 16$ and 32 for *C. orientalis* and $2n = 2x = 16$ for *C. flammula* and *C. ispahanica* (BESKARAVAYNAJA et al. 1979, BIR et al. 1987, PASTOR et al. 1988, SEROV 1989), therefore a tetraploid chromosome number is new for two species of *C. ispahanica* and *C. flammula*.

Considering the diplontic behavior of the species of *C. ispahanica* and *C. flammula* we can not say for sure if it is due to the presence of a controlling mechanism for chiasma formation or due to the allopolyploid nature of the species studied. χ^2 test showed no significant difference in the relative meiotic characteristics of the *Clematis* species and populations studied, suggesting the close affinity of these species.

Variation in chiasma frequency and localization is genetically controlled. Such a variation in the species and populations with the same chromosome number is considered a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (REES and DALE 1974, QUICKE 1993).

The significant difference for the percentage of chromosome stickiness and laggards observed among *Clematis* species and populations may indicate their genomic differences. Genetic and environmental factors as well as genomic-environmental interaction have been considered the reason for chromosome stickiness in different plant species (NIRMALA and RAO 1996, BAPTISTA-GIACOMELLI et al. 2000). The meiotic abnormalities observed may be the reason for the low pollen infertility observed in the species studied.

Variations observed in the karyotypic formulae among the *clematis* species may indicate the occurrence of structural changes in their chromosomes. These species are placed in relatively primitive classes of 2A and 2B of the Stebbins system indicating the presence of a symmetrical karyotype in *clematis* species studied. Therefore in general, the variations observed in both meiotic chromosome pairing as well as the details of karyotypes (although the study considers only 3 species) may indicate the role of cytological changes in *clematis* species diversification.

The occurrence of a granulate membrane in furrows in *C. Ispahanica* has already been reported; however, 4 and 6 colpate pollens were not previously recorded (SEROV and TRASEVICH 1986).

Differences observed in palynological characteristics of the *clematis* species may be of use in the species identification and systematics. The populations of *C. orientalis* also showed some variation in their pollen characteristics. The section *Meclatis* has been considered as *C. orientalis sensu lato* as the most variable complex within the genus *Clematis*, possessing the widest geographical distribution, and thus divided into seven varieties. The previously cited author believes that *C. orientalis* var. *orientalis* grows in Iran (GREY-WILSON 1989). The morphological, cytological and palynological findings support such a consideration; however, at present we are not attempting to make any infraspecific division for *C. orientalis*, as this would need further detailed investigation.

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