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ARTICLE

Cytology in *Silene*: From population diversity to section classification

Masoud Sheidai[1]*, Fahimeh Koohdar[1], Raheleh Tabaripoor[2], Jirar Karapetian[2], Abbas Gholipoor[3], Zahra Noormohammadi[4]

[1]Shahid Beheshti University, GC, Faculty of Biological Sciences, Tehran, Iran, [2]Biology Department, Urmiyeh University, Iran, [3]Biology Department, Payeme-noor University, Iran, [4]Faculty of Biological Sciences, Shahid Beheshti University, GC, Tehran, Iran

ABSTRACT Cytological studies were performed in 25 populations of six *Silene* species of the sections *Inflatae* Boiss., and *Auriculatae* Boiss., *S. odontopetala* Fenzi, *S. vulgaris* (Moench) Garcke, *S. pungens* Boiss., *S. aucheriana* Boiss., *S. sisianica* Boiss. & Buhse, and *S. pseudaucheriana* Melzh., showing 2n = 2x = 24 and 48. These are the first chromosome number reports for all six species. Among twelve populations of *S. aucheriana*, two populations had 2n=4x= 48 chromosome number, while the others had 2n=2x=24. ANOVA revealed significant chromosomal differences among *S. aucheriana*, *S. odontopetala* and *S. vulgaris*. ANOVA test also showed significant differences among sections, indicating the occurrence of significant quantitative changes in chromosome size during the species diversification. Meiotic analysis of *S. odontopetala* and *S. aucheriana* populations showed mainly bivalents and univalents in the metaphase of meiosis I, although some quadrivalents were observed too. Significant differences were formed for all meiotic characteristics among the sections studied, indicating a change in the number of genes controlling chromosome pairing and also heterozygote translocations as one of the adaptive strategies during the species diversification in *Silene*. **Acta Biol Szeged 55(1):27-39 (2011)**

KEY WORDS

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The genus *Silene* L. (Caryophylaceae) consists about 700 species world-wide but are mainly distributed in the northern hemisphere, Europe, Asia and northern Africa (Bari 1973; Greuter 1995). Based on morphological characters Chowdhuri (1957), classified the *Silene* species in 22 sections but molecular studies of Oxelman et al. (1997, 2000) and Burleigh and Holtsford (2003) do not support this sectional classifications, particularly not for the endemic North American taxa.

The *Silene* species are annual, biennial, or perennial herbs. Most are diploid having 2n = 2x = 24, or 2n = 2x = 20 chromosome (Swank 1932; Bari 1973; Oxelman and Lidén 1997). However, *S. fortunei* Vis., is triploid (2n = 3x = 30; Heaslip 1951), and some other species show tetraploid (2n = 4x = 48), hexaploid (2n = 6x = 72), and higher polyploidy levels, e.g. 2n = *c.* 96, 120 and 192 (Bari 1973). Moreover, 2n = 18 is reported for *S. conica* L., and *S. lacera* (Steven) Sims., (Sopova and Sekovski 1982), and 2n = 46 for *S. firma* Siebold & Zucc. (Zhang 1994), thus, x= 9, 10, 12 and 23 are the known basic chromosome numbers in *Silene*.

Extensive cytogenetic studies have been performed in *Silene* species from different parts of the world (Heaslip 1951; Bari 1973; Melzheimer 1978, 1980; Markova et al. 2006) but only recently a few cytological studies have been reported from Iran (Gholipour and Sheidai 2010a, 2010b; Sheidai et al. 2008, 2009, 2010). About 110 *Silene* species grow in Iran, of which about 35 species are endemic with very limited geographical distribution (Melzheimer 1980).

The aims of this study is to present cytological data for six unreported *Silene* species, revealing cytological variations leading to the species diversification and to show taxonomically usefulness of both karyotypic and meiotic features in circumscription of *Silene* sections. Therefore, the present study consists of two parts. In the first part, cytological diversity of 25 populations of six species in the sections *Inflatae* Boiss., and *Auriculatae* Boiss., (Melzheimer 1980) with regard to karyotype features, chromosome pairing, the occurrence of B-chromosomes and unreduced (2n) pollen grain formation will be presented and discussed, while in the second part, these new data will be combined with our previous reports in other sections of *Silene*, and the use of such data in the section classification will be discussed.

Materials and Methods

Cytological studies were performed in 25 populations of 6 *Silene* species of the sections *Inflatae* and *Auriculatae* (Table 1). Vouchers specimens are deposited in the Herbarium of Shahid Beheshti University (HSBU), Iran. The species studied are:

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*Corresponding author. E-mail: msheidai@yahoo.com, msheidai@sbu.ac.ir

Table 1. Karyotype features in *Silene* species and populations studied.

Species	Locality	Section	2n	Poloidy level	TL	L	S	L/S	X	St	A1	A2	TF%	CV	Karyotype formulae
Silene aucheriana	Oshtoran-kooh	Auriculatae	48	4x	82.20	4.53	2.58	1.76	3.42	2A	0.77	0.26	44.35	26.30	23m+1sm
S. aucheriana	Angooran-kooh		24	2x	38.65	4.03	2.40	1.68	3.22	1A	0.79	0.15	44.23	15.50	12m
S. aucheriana	Bijar		24	2x	43.97	2.66	2.19	1.21	3.66	1A	0.77	0.16	43.59	16.10	12m
S. aucheriana	Chakol Baza-kooh		24	2x	37.40	4.23	2.29	1.85	3.12	1A	0.78	0.17	43.64	17.60	11m+1sm
S. aucheriana	Firroz-kooh		24	2x	36.63	3.81	2.23	1.71	3.05	1A	0.78	0.15	43.73	15.10	12m
S. aucheriana	Tehran, Dizin		24	2x	33.44	3.60	1.90	1.89	2.79	1A	0.79	0.17	43.90	17.60	11m+1sm
S. aucheriana	Zarineh		48	4x	56.80	3.40	1.64	2.07	2.37	1B	0.78	0.17	43.85	16.90	24m
S. aucheriana	Zanjan		24	2x	32.66	3.53	1.93	1.83	2.72	1A	0.77	0.17	43.29	17.60	11m+1sm
S. aucheriana	Shahvar		24	2x	46.13	5.33	2.64	2.02	3.85	1B	0.73	0.21	41.95	20.80	11m+1sm
S. aucheriana	Manjil		24	2x	32.93	3.53	2.26	1.56	2.74	1A	0.76	0.13	46.41	13.50	11m+1sm
S. aucheriana	Neoor		48	4x	66.15	3.70	1.82	2.03	2.76	1B	0.72	0.16	41.48	15.90	23m+1sm
S. aucheriana	Haraz		24	2x	49.82	4.98	3.14	1.59	4.15	1A	0.76	0.14	43.10	14.00	12m
S. pseudoaucheriana	Oshtoran-kooh	Auriculatae	48	4x	87.03	5.33	2.41	2.21	6.96	1B	0.76	0.10	42.92	9.90	24m
S. sisianica	Ghooshchi	Auriculatae	48	4x	72.22	3.88	2.05	1.89	3.01	1A	0.74	0.15	42.36	15.00	23m+1sm
S. odontopetala	Binalood	Inflatae	24	2x	54.84	5.93	2.58	2.30	4.57	1A	0.72	0.18	42.00	18.00	11m+1sm
S. odontopetala	Chakol		24	2x	48.16	5.17	3.04	1.70	4.01	1A	0.71	0.15	42.00	15.00	11m+1sm
S. odontopetala	Tehran, Dizin		24	2x	52.76	5.87	3.01	1.95	4.40	1A	0.74	0.19	42.12	19.00	12m
S. odontopetala	Oshtoran-kooh		24	2x	38.21	4.41	3.02	1.46	3.18	1A	0.75	0.18	42.64	18.00	12m
S. odontopetala	Tehran, Touchal		24	2x	50.92	5.97	2.22	2.69	4.24	1B	0.67	0.23	39.93	23.00	9m+3sm
S. odontopetala	Shahdej		24	2x	54.34	5.82	2.60	2.24	4.53	1A	0.44	0.18	43.59	18.00	12m
S. odontopetala	Gajereh		24	2x	32.87	3.66	3.08	1.19	2.74	1A	0.73	0.16	41.96	16.00	12m
S. vulgaris	Siahbisheh	Inflatae	24	2x	50.51	5.42	2.88	1.88	4.21	1A	0.76	0.18	43.24	18.00	12m
S. vulgaris	Panjab		24	2x	40.38	4.35	2.23	1.95	3.36	1A	0.77	0.18	43.40	18.00	12m
S. vulgaris	Shahdej		24	2x	39.00	4.24	2.41	1.76	3.25	1A	0.79	0.16	44.08	16.00	12m
S. vulgaris	Firroz-kooh		24	2x	30.62	3.42	2.00	1.71	2.55	1A	0.78	0.15	43.96	15.00	12m
S. pungens	Urmiyeh	Inflatae	24	2x	31.93	3.59	1.87	1.92	2.66	1A	0.76	0.18	43.24	18.00	12m

Abbreviations: TL = Total chromosome length, L = Longest chromosome, S = Shortest chromosome, Ratio = Longest/shortest chromosome, X = Mean chromosome length, A1 and A2 = Romero-Zarco indices, TF = Total form percentage and CV = Coefficient of variation.

Silene odontopetala Fenzi, (seven populations), *S. vulgaris* (Moench) Garcke, (four populations), *S. pungens* Boiss., (one population), from the sect. *Inflatae* Boiss., *S. aucheriana* Boiss., (twelve populations), *S. sisianica* Boiss., (one population), and *S. pseudaucheriana* Melzh., (one population), from the sect. *Auriculatae* Boiss., (Table 1).

Cytological methods

For the karyotype study, freshly grown root tips were collected from germinated seed of at least ten randomly selected plants in each species, pretreated with 0.002 mol 8-hydroxyquinolin (2-2.5 hrs.) and fixed in ethanol: acetic acid (3:1) for 24 hrs. The fixed tips were then washed thoroughly in distilled water, macerated in 60°C 1N HCl for about 5 min., and squash in 2% aqueous aceo-orcein stain solution. The somatic chromosome number and karyotype details were studied in at least 5 well-prepared metaphase plates. The chromosomes were photographed by digital camera and measured by Image Tools3 software (Sheidai et al. 2009).

Karyotype description

The chromosomes were described according to Levan et al. (1964), karyotype symmetry was determined according to Stebbins (1971), while other karyotype parameters like haploid total chromosome length (total sum of the size of the chromosomes by using only one chromosome from each pair), mean chromosome length (total haploid chromosome length/number of chromosome pairs), total form percentage (TF % = Sum of short arms of the chromosomes/ Total chromosome length), coefficient of variation (CV) of the chromosome size as well as the A1 and A2 indices of Romero-Zarco (1986) were determined (cf. Sheidai et al. 2009).

Meiotic studies

For meiotic 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. 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 100 pollen mother cells at diakinesis-metaphase I and 500 at anaphase and telophase. Complete pollens which were stained were taken as fertile, while incomplete, shrunken pollens with no stain were considered as infertile.

Table 2. Meiotic characteristics in *Silene* species and populations.

Species		Section	ROD	RB	IV	IX	TX	TOX
S. odontopetala	Khalil-Kooh	Inflatae	5.18	6.20	0.32	3.68	17.50	21.12
S. odontopetala	Gajereh	Inflatae	5.26	6.48	0.19	3.03	18.61	21.51
S. odontopetala	Touchal	Inflatae	5.00	6.73	0.23	3.77	18.80	22.42
S. odontopetala	Veresk	Inflatae	5.62	6.22	0.09	2.31	18.12	20.40
S. odontopetala	Shahdej	Inflatae	5.91	5.79	0.12	9.54	10.24	19.76
S. *pungens*	Urmeiyeh	Inflatae	4.52	6.55	0.32	4.51	15.52	20.03
S. aucheriana	Alamoot	Auriculatae	5.23	4.31	0.85	4.44	17.08	19.38
S. aucheriana	Semnan	Auriculatae	4.83	6.52	0.48	2.69	18.69	21.62
S. aucheriana	Sahand	Auriculatae	4.59	6.50	0.41	2.59	18.91	21.50
S. aucheriana	Dizin	Auriculatae	5.78	5.00	0.61	2.83	16.78	19.53
S. aucheriana	Golestan-kooh	Auriculatae	4.00	3.50	0.17	1.50	11.50	13.00
S. aucheriana	Nizva	Auriculatae	5.90	4.83	0.37	3.83	16.03	19.87
S. sisianica	Khalil-Kooh	Auriculatae	8.50	3.50	0.00	2.00	14.50	16.50

Abbreviations: ROD=Rod bivalents, RB=Ring bivalents, IV=Quadrivalents, IX=Intercalary chiasmata, TX=Terminal chiasmata, TOX=Total chiasmata.

Table 3. Pearson correlation among karyotype features in sect. *Auriculatae*.

		L	S	RATIO	X	ST	A1	A2	TF	CV
L	Pearson Correlation	1.000	-0.963**	0.146	0.291	-0.030	-0.283	0.346	-0.021	0.346
	Sig. (2-tailed)	.	0.001	0.619	0.313	0.918	0.326	0.225	0.942	0.225
	N	14	14	14	14	14	14	14	14	14
S	Pearson Correlation	0.963**	1.000	-0.123	0.184	-0.177	-0.049	0.370	0.201	0.370
	Sig. (2-tailed)	0.001	.	0.675	0.530	0.544	0.867	0.193	0.491	0.193
	N	14	14	14	14	14	14	14	14	14
RATIO	Pearson Correlation	0.146	-0.123	1.000	0.401	0.513	-0.876**	-0.112	-0.798**	-0.112
	Sig. (2-tailed)	0.619	0.675	.	0.155	0.61	0.001	0.703	0.001	0.703
	N	14	14	14	14	14	14	14	14	14
X	Pearson Correlation	0.291	0.184	0.401	1.000	0.333	-0.132	-0.387	-0.223	-0.387
	Sig. (2-tailed)	0.313	0.530	0.155	.	0.245	0.652	0.171	0.444	0.171
	N	14	14	14	14	14	14	14	14	14
ST	Pearson Correlation	-0.030	-0.177	0.513	0.333	1.000	-0.498	0.111	-0.459	0.111
	Sig. (2-tailed)	0.918	0.544	0.061	0.245	.	0.70	0.706	0.99	0.706
	N	14	14	14	14	14	14	14	14	14
A1	Pearson Correlation	-0.283	-0.049	-0.876**	-0.132	-0.498	1.000	0.010	0.622*	0.010
	Sig. (2-tailed)	0.326	0.867	0.000	0.652	0.070	.	0.972	0.017	0.972
	N	14	14	14	14	14	14	14	14	14
A2	Pearson Correlation	0.346	0.370	-0.112	-0.387	0.111	0.010	1.000	-0.011	1.000**
	Sig. (2-tailed)	0.225	0.193	0.703	0.171	0.706	0.972	.	0.971	0.001
	N	14	14	14	14	14	14	14	14	14
TF	Pearson Correlation	-0.021	0.201	-0.798**	-0.223	-0.459	0.622*	-0.011	1.000	-0.011
	Sig. (2-tailed)	0.942	0.491	0.001	0.444	0.99	0.017	0.971	.	0.971
	N	14	14	14	14	14	14	14	14	14
CV	Pearson Correlation	0.346	0.370	-0.112	-0.387	0.111	0.010	1.000**	-0.011	1.000
	Sig. (2-tailed)	0.225	0.193	0.703	0.171	0.706	0.972	0.001	0.971	.
	N	14	14	14	14	14	14	14	14	14

** = Correlation is significant at 0.01 level (2-tailed). * = Correlation is significant at 0.05 level (2-tailed).

Statistical analyses

In order to reveal significant difference among the species and populations studied, the analysis of variance (ANOVA) (cf. Sheidai et al. 2009) followed by the least significant difference test (LSD) (cf. Sheidai et al. 2009) were performed on the size of chromosomes, size of the long arms and size of the short arms as well as arms ratio among the species and populations studied (cf. Sheidai and Jalilian 2008).

In order to determine any significant difference in chiasma frequency and distribution among the species and populations

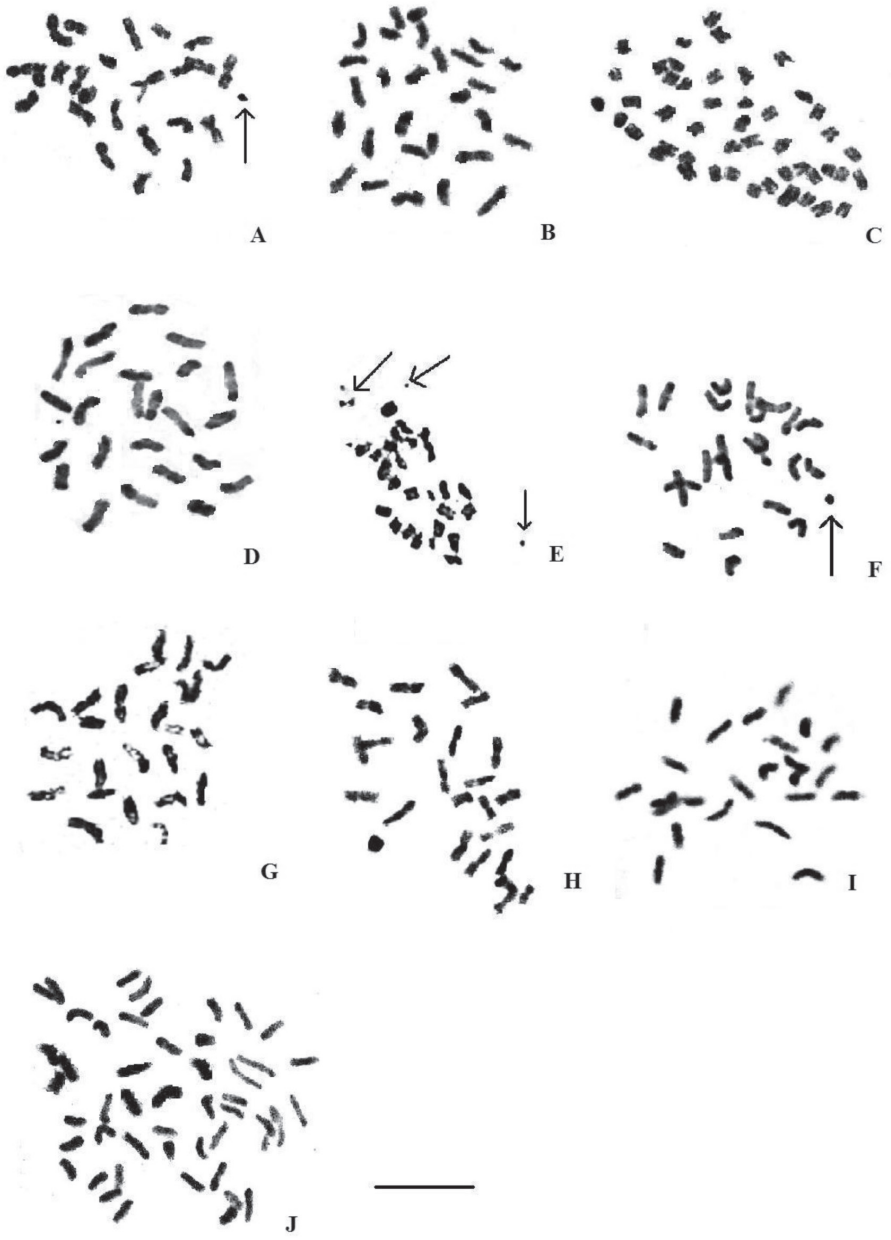

Figure 1. Representative somatic metaphase cells in the Silene species studied. A-C= Firooz-kooh, Noshahr and Neor populations of *S. aucheriana* showing 2n=24 (arrow indicates B-chromosome), 2n = 24 (A, B) and 2n=48 (C), respectively. D= Gajereh population of *S. odontopetala* showing 2n=24. E= Urmeiyeh population of *S. pungens* showing 2n=24 (arrow indicates B-chromosome). F and G= Firooz-kooh and Seiyahbisheh populations of *S. vulgaris* showing 2n=24 (arrow indicates B-chromosome). H and I = Angooran and Bijar populations of *S. aucheriana* showing 2n=24. J= Aomatic cell in *S. pseudaucheriana* showing 2n=48. Scale bar = 10 μm.

we performed analysis of variance (ANOVA) by the least significant differences (LSD) method (cf. Sheidai et al. 2008). In order to detect significant difference between potential unreduced pollen grains and the normal (reduced pollens), a t-test was performed (cf. Sheidai et al. 2009). Pearson correlation was determined among karyotype features.

In order to study the usefulness of cytological data for section circumscription in *Silene*, data obtained in the present study were combined with the results of similar studies made by us in other sections of the genus (Gholipour and Sheidai 2010, Sheidai et al. 2008, 2009). Therefore, in karyotype, based on sixty-three species and populations from four sections of *Inflatae* Boiss., *Lasiostemones* Boiss., *Sclerocalycinae* Boiss., and *Auriculatae* Boiss., (Appendix 1), and seven

Table 4. Pearson correlation among karyotype features in sect. Inflatae.

		L	S	RATIO	X	ST	A1	A2	TF	CV
L	Pearson Correlation	1.000	-0.972**	0.470	0.995**	0.313	-0.502	0.509	-0.478	0.509
	Sig. (2-tailed)	.	0.001	0.6123	0.001	0.321	0.097	0.0.91	0.116	0.091
	N	12	12	12	12	12	12	12	12	12
S	Pearson Correlation	0.972**	1.000	0.252	0.990**	0.144	-0.543	0.406	-0.264	0.406
	Sig. (2-tailed)	0.001	.	0.430	0.001	0.555	0.068	0.190	0.407	0.190
	N	12	12	12	12	12	12	12	12	12
RATIO	Pearson Correlation	0.475	0.252	1.000	0.401	0.734**	-0.068	0.553	-0.988**	0.553
	Sig. (2-tailed)	0.123	0.430	.	0.155	0.007	0.833	0.062	0.001	0.062
	N	12	12	12	12	12	12	12	12	12
X	Pearson Correlation	0.995**	0.990**	0.383	1.000	0.246	-0.524	0.470	-0.393	0.470
	Sig. (2-tailed)	0.001	0.001	0.219	.	0.443	0.081	0.123	0.206	0.123
	N	12	12	12	12	12	12	12	12	12
ST	Pearson Correlation	0.313	0.144	0.734**	0.246	1.000	-0.162	0.782**	-0.744**	0.783**
	Sig. (2-tailed)	0.321	0.555	0.007	0.443	.	0.614	0.003	0.006	0.003
	N	12	12	12	12	12	12	12	12	12
A1	Pearson Correlation	-0.502	-0.543	-0.068	-0.524	-0.162	1.000	-0.247	0.105	-0.247
	Sig. (2-tailed)	0.097	0.068	0.833	0.081	0.614	.	0.439	0.746	0.439
	N	12	12	12	12	12	12	12	12	12
A2	Pearson Correlation	0.509	0.406	0.553	0.470	0.782**	-0.247	1.000	-0.636**	1.000**
	Sig. (2-tailed)	0.0.91	0.190	0.062	0.123	0.003	0.439	.	0.026	0.001
	N	12	12	12	12	12	12	12	12	12
TF	Pearson Correlation	-0.478	-0.264	-0.988**	-0.393	-0.744**	0.105	-0.636**	1.000	-0.636**
	Sig. (2-tailed)	0.116	0.407	0.001	0.206	0.006	0.746	0.026	.	0.026
	N	12	12	12	12	12	12	12	12	12
CV	Pearson Correlation	0.509	0.406	0.553	0.470	0.783**	-0.247	1.000**	-0.636**	1.000
	Sig. (2-tailed)	0.091	0.190	0.062	0.123	0.003	0.439	0.001	0.026	.
	N	12	12	12	12	12	12	12	12	12

** = Correlation is significant at 0.01 level (2-tailed). * = Correlation is significant at 0.05 level (2-tailed).

karyotype features, a data matrix of 63 x 7 was formed. Similarly, for meiotic characteristics based on forthy-two species and populations from the four sections mentioned earlier (Appendix 2), and six meiotic features, a data matrix of 42 x 6 was formed.

Grouping of the species based on karyotype and meiotic characteristics was performed using various cluster analysis methods including unweighted paired group with arithmetic average (UPGMA) and Neighbor Joining (NJ) method (cf. Podani 2000). Statistical analyses used SPSS ver. 9 (SPSS Inc., 1998), NTSYS ver. 2.1 (Applied Biostatistics Inc., 1998) and DARwin ver. 5.0.155 (CIRAD, 2006) software.

Results and Discussion

Karyotype analysis

Details of the karyotype analyses in *Silene* species of sections *Auriculatae* and *Inflatae* are presented in Tables 1 & 2 and Figure 1, showing 2n = 2x = 24 and 48, as was also the case of species in the sections *Lasiostemones* Boiss., and *Scelerocalycinae* Boiss. (Appendix 1). However, 2n = 24 is the most frequent number among the species studied.

The chromosomes were mostly metacentric and submetacentric (Fig. 2). Seven populations of *S. odontopetala* had 2n=24 chromosomes but differed in their karyotype formulae. Three populations of Binalood, Chakol, and Touchal had both meta and submetacentric chromosomes, while other populations had only metacentric chromosomes. Five populations of *S. vulgaris* had 2n=24 chromosome and metacentric chromosomes.

Among the twelve populations of *S. aucheriana*, two populations, Oshtoran-kooh and Neor, had 2n=4x= 48 chromosome numbers, while other populations had 2n=2x=24. Most of these populations had meta and submetacentric chromosomes. Subtelocentric and telocentric chromosomes occurred in the species of the sect. *Scelerocalycinae* (Appendix 1).

Among the *S. odontopetala* populations, the highest values of total and mean haploid chromosome length occurred in the Binalood population (54.84 & 4.57 µm, respectively) while, the lowest value of the same parameters occurred in the Gajereh population (32.87 & 2.74 µm, respectively). The size of the longest chromosome varied from 1.60 µm in the Gajereh population to 2.6 µm in Binalood population.

Among populations of *S. vulgaris*, the Siahbisheh population had the highest values of total and mean haploid chromosome length (50.51 and 4.21 µm, respectively) and the the size of the longest chromosome varied from 1.52-2.40 µm.

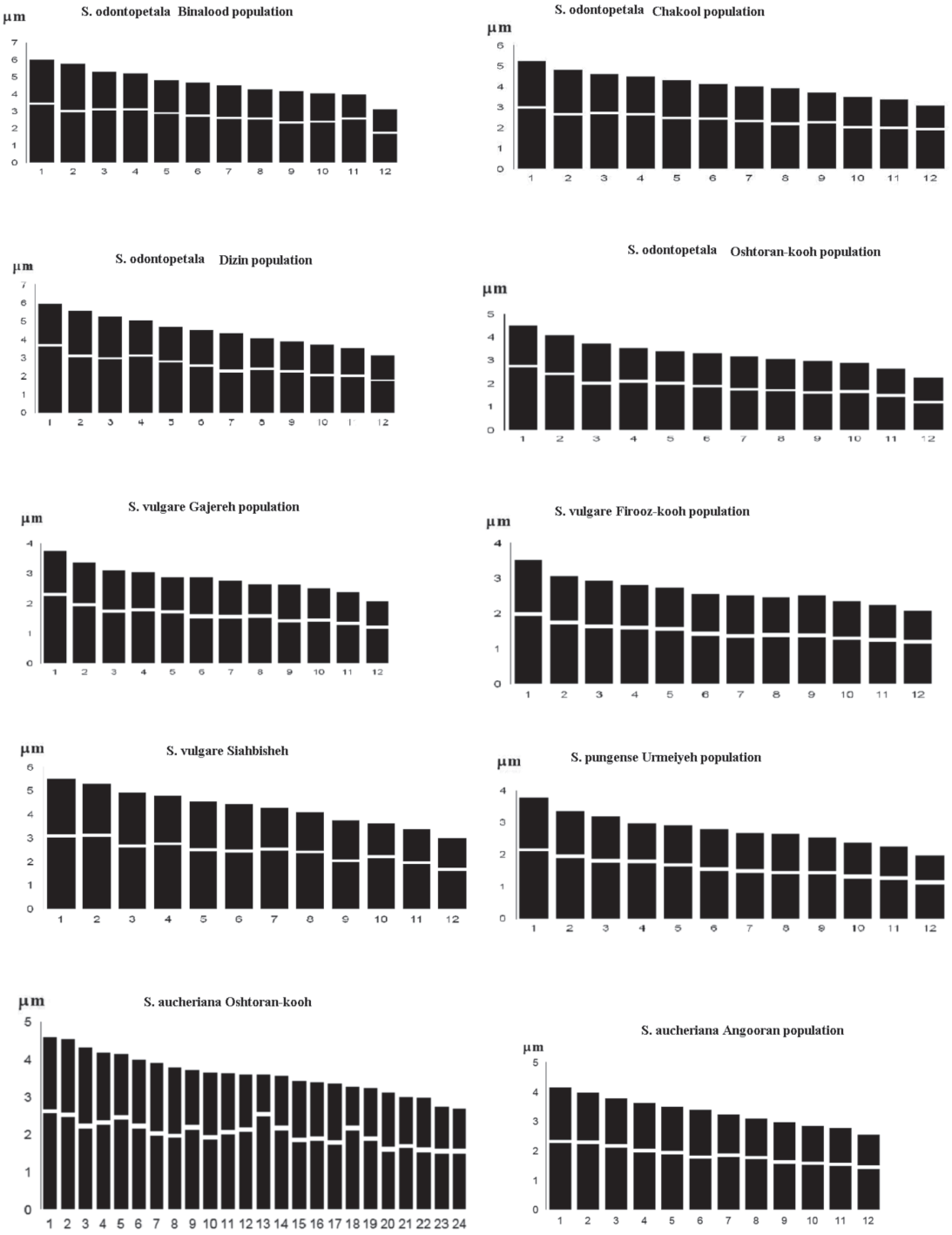

Figure 2. Representative ideograms of karyotypes in some populations of the six *Silene* species studied (cf. Table 1).

Similarly, among *S. aucheriana* populations, the Oshtoran-kooh population, which is tetraploid with 2n=48, had the highest total chromosome length (82.20 µm), while the diploid Haraz population, had the highest mean chromosome length (4.15 µm). The the size of the longest chromosome varied from 1.33 µm in the Zarineh population to 2.22 µm in the Shahvar population.

Among the *S. odontopetala* populations, the highest coefficient of variation (CV) (23.00) for the chromosomes size occured in the Touchal population, while in *S. aucheriana*,

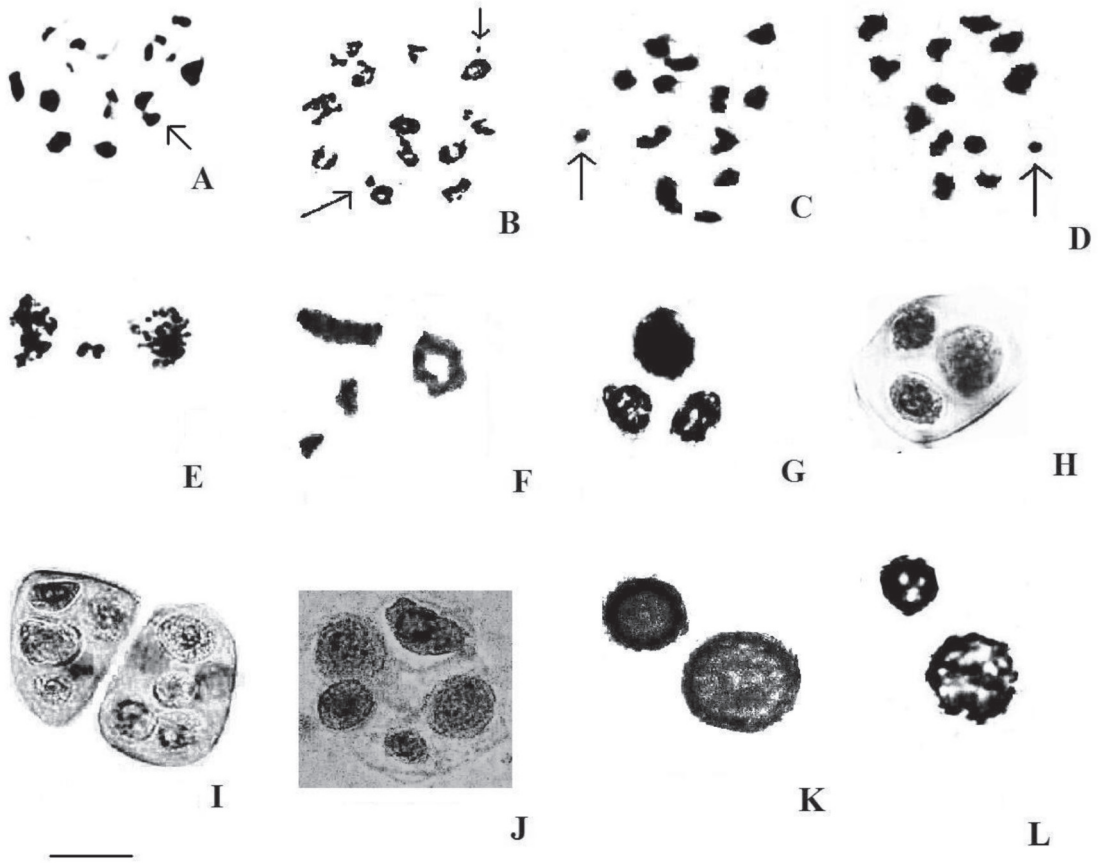

Figure 3. Representative meiotic cells in the Silene species studied. A= Metaphase I cell showing quadrivanet formation (arrow) in Dizin population of *S. aucheriana*. B= Metaphase I cell showing B-chromosomes (arrow) in Shadej population of *S. odontopetala*. C= Metaphase I cell showing B-chromosomes (arrow) in Urneiyeh population of *S. pungens.* D= Metaphase I cell showing B-chromosomes (arrow) in *S. pungens*. E= Anaphase-I cell showing laggard chromosomes in Gajereh population of *S. odontopetala*. F= Meiocyte showing chromosome clumping in *S. aucheriana*. G and H= Tripolar cells in *S. pungens* and *S. odontopetala*, repectively. I and J= Multipolar cells in *S. odontopetala* and *S. aucheriana*, repectively. K and L= Potential unreduced pollen grains (bigger size pollen grains) in *S. odontopetala* and *S. aucheriana*, respectively. Scale bar = 10 µm

the Oshtoran-kooh population showed the highest coefficient of variation (26.30). Among the *S. aucheriana* populations, ANOVA test revealed a significant difference ($p < 0.05$) for the long arms of the chromosome pairs number 2, 6-10, size of the short arms in the chromosome pairs number 4, 7-12 and total size of the chromosome pairs number 2-12 as well as arm ratios of the chromosome pairs number 4 and 9. Similarly, *S. odontopetala* populations differed significantly in total size, size of the long arms and short arms of the chromosome pairs number 1-12 and also in arms ratio of chromosome pairs number 1 and 2. In *S. vulgaris*, size of the long arms of the chromosome pairs number 2-4 and 10, short arms of the chromosome pairs number 3 and 5-11 and total size of the chromosome pairs number 2-11, as well as the arm ratio of chromosome pairs number 10, differed significantly among the populations.

ANOVA test for karyotype features among the sections showed a significant difference for total chromosome length, size of the longest and shortest chromosomes, the mean size of chromosomes and TF%, with the sect. *Sclerocalycinae* having the highest values of size parameters and lowest value of TF%, while sect. *Lasistemones* showed the lowest values of size parameters and *Auriculatae* the highest TF%. These results indicate the occurrence of significant quantitative changes in size of chromosomes during species diversification.

Pearson correlation determined among karyotype features showed a positive significant correlation between the mean chromosome length, size of the longest chromosome and size of the shortest chromosome in species of the sect. *Inflata*. The ratio of the chromosomes arms showed a significant positive correlation ($r=0.73$, $p=0.007$) with Stebbins· karyotype symmetry classes, and a significant negative correlation with total form percentage (TF%). Coefficient of variation also showed a significant positive correlation ($r=0.78$, $p=0.003$) with Stebbins· classes but had a significant negative correla-

Appendix 1. Karyotype features in *Silene* species.

	Species	Locality	Setion	2n	TL	L	S	L/S	X	A1	A2	TF%	ST	KF
1	*Silene aucheriana*	Oshtoran-kooh	Auriculatae	48	82.2	4.5	2.6	1.8	3.4	0.77	0.263	44.35	2A	23m+1sm
2	*S. aucheriana*	Angooran-kooh	Auriculatae	24	38.7	4	2.4	1.7	3.2	0.79	0.155	44.23	1A	12m
3	*S. aucheriana*	Bijar	Auriculatae	24	44	2.7	2.2	1.2	3.7	0.77	0.161	43.59	1A	12m
4	*S. aucheriana*	Chakol Baza-kooh	Auriculatae	24	37.4	4.2	2.3	1.9	3.1	0.78	0.176	43.64	1A	11m+1sm
5	*S. aucheriana*	Firroz-kooh	Auriculatae	24	36.6	3.8	2.2	1.7	3.1	0.78	0.151	43.73	1A	12m
6	*S. aucheriana*	Tehran, Dizin	Auriculatae	24	33.4	3.6	1.9	1.9	2.8	0.79	0.176	43.90	1A	11m+1sm
7	*S. aucheriana*	Zarineh	Auriculatae	48	56.8	3.4	1.6	2.1	2.4	0.78	0.169	43.85	1B	24m
8	*S. aucheriana*	Zanjan	Auriculatae	24	32.7	3.5	1.9	1.8	2.7	0.77	0.176	43.29	1A	11m+1sm
9	*S. aucheriana*	Shahvar	Auriculatae	24	46.1	5.3	2.6	2	3.9	0.73	0.208	42	1B	11m+1sm
10	*S.aucheriana*	Manjil	Auriculatae	24	32.9	3.5	2.3	1.6	2.7	0.76	0.135	46.4	1A	11m+1sm
11	*S. aucheriana*	Neoor	Auriculatae	48	66.2	3.7	1.8	2	2.8	0.72	0.159	41.48	1B	23m+1sm
12	*S. aucheriana*	Haraz	Auriculatae	24	49.8	5	3.1	1.6	4.2	0.76	0.140	43.10	1A	12m
13	*S. pseudoaucheriana*	Oshtoran-kooh	Auriculatae	48	87	5.3	2.4	2.2	7	0.76	0.099	42.92	1B	24m
14	*S. sisianica*	Ghooshchi	Auriculatae	48	72.2	3.9	2.1	1.9	3	0.74	0.150	42.36	1A	23m+1sm
15	*S. odontopetala*	Binalood	Inflatae	24	54.8	5.9	2.6	2.3	4.6	0.72	0.18	42	1A	11m+1sm
16	*S. odontopetala*	Chakol	Inflatae	24	48.2	5.2	3	1.7	4	0.71	0.15	42	1A	11m+1sm
17	*S. odontopetala*	Tehran, Dizin	Inflatae	24	52.8	5.9	3	2	4.4	0.74	0.19	42.1	1A	12m
18	*S. odontopetala*	Oshtoran-kooh	Inflatae	24	38.2	4.4	3	1.5	3.2	0.75	0.18	42.6	1A	12m
19	*S. odontopetala*	Tehran, Touchal	Inflatae	24	50.9	6	2.2	2.7	4.2	0.67	0.23	39.9	1B	9m+3sm
20	*S. odontopetala*	Shahdej	Inflatae	24	54.3	5.8	2.6	2.2	4.5	0.44	0.18	43.6	1A	12m
21	*S. odontopetala*	Gajereh	Inflatae	24	32.9	3.7	3.1	1.2	2.7	0.73	0.16	42	1A	12m
22	*S. vulgaris*	Siahbisheh	Inflatae	24	50.5	5.4	2.9	1.9	4.2	0.76	0.18	43.2	1A	12m
23	*S. vulgaris*	Panjab	Inflatae	24	40.4	4.4	2.2	2	3.4	0.77	0.18	43.4	1A	12m
24	*S. vulgaris*	Shahdej	Inflatae	24	39	4.2	2.4	1.8	3.3	0.79	0.16	44.1	1A	12m
25	*S. vulgaris*	Firroz-kooh	Inflatae	24	30.6	3.4	2	1.7	2.6	0.78	0.15	44	1A	12m
26	*S. pungens*	Urmiyeh	Inflatae	24	31.9	3.6	1.9	1.9	2.7	0.76	0.18	43.2	1A	12m
27	*S. shahrudensis*	Kerman	Sclerocalycinae	24	44.5	4.8	2.4	2.4	3.7	0.375	0.23	38.4	2B	7M+5sm
28	*S. shahrudensis*	Semnan	Sclerocalycinae	24	56.9	6.4	2.8	2.3	4.7	0.44	0.23	36.9	2B	6M+6sm
29	*S. stapfii*	Kerman1	Sclerocalycinae	24	58.3	6.2	3.8	1.6	4.9	0.506	0.14	33.2	3A	1M+11sm
30	*S. stapfii*	Kerman2	Sclerocalycinae	24	74.2	8	4.8	1.6	6.2	0.53	0.14	30.5	2A	5M+3sm+3st+1t
31	*S. stapfii*	Yazd	Sclerocalycinae	24	59.4	6.5	3.6	1.8	4.9	0.465	0.17	34.5	2A	3M+9sm
32	*S. caesarea*	Yasooj1	Sclerocalycinae	24	70	8.1	3.8	2	5.8	0.488	0.19	32.4	3B	1M+11sm
33	*S. caesarea*	Yassoj2	Sclerocalycinae	24	58.1	6	3.1	1.8	4.8	0.473	0.21	34.7	2A	3M+9sm
34	*S. caesarea*	Khor	Sclerocalycinae	24	62	6.6	3.8	1.7	5.2	0.514	0.16	32.6	3A	1M+11sm
35	*S. caesarea*	Oshtorankooh	Sclerocalycinae	24	50.5	5.1	3.3	1.6	4.2	0.142	0.5	33	2A	2M+10sm
36	*S. caesarea*	Shajoo	Sclerocalycinae	24	71	8	4.5	1.8	5.9	0.526	0.16	31.3	2A	3M+7sm+2st
37	*S. caesarea*	Sharestanak	Sclerocalycinae	24	67.3	7.3	4.2	1.7	5.6	0.481	0.16	33.4	2A	2M+9sm+1st
38	*S. peduncularis*	Dizin	Sclerocalycinae	24	48.4	5	2.9	1.4	4	0.405	0.15	37.4	2A	6M+6sm
39	S. peduncularis	Zanjan	Sclerocalycinae	24	48.3	5.6	2.9	1.6	4.5	0.404	0.21	39.8	1A	7M+5sm
40	*S. peduncularis*	Hamedan	Sclerocalycinae	24	50.6	5.5	2.8	1.9	4.2	0.38	0.18	38.5	1A	6M+6sm
41	*S. peduncularis*	Urmeyeh	Sclerocalycinae	48	85.2	4.8	2.4	2	3.5	0.48	0.25	36.7	2A	13M+11sm
42	*S. avromana*	Gajereh	Sclerocalycinae	24	60.1	6.2	3.3	1.9	5	0.497	0.18	33.9	2A	2M+10sm
43	S. bupleuroides	Tonekabon	Sclerocalycinae	24	52.4	5.8	3.4	1.7	4.4	0.451	0.15	34.8	2A	5M+6sm+1st
44	*S. chlorifolia*	Margoon	Sclerocalycinae	24	71.8	7.4	4.4	1.7	6	0.52	0.14	32	2A	1M+11sm
45	*S. swertiifolia*	Oshtorankooh	Sclerocalycinae	24	59.5	6.5	3.6	1.8	5	0.44	0.16	36	2A	3M+9sm

Appendix 1. cont.

46	*S. swertiifolia*	Gajereh	Sclerocalycinae	24	67.9	7.2	4.3	1.7	5.7	0.5	0.15	33	2A	1M+11sm
47	S. swertiifolia	Sharestanak	Sclerocalycinae	24	64.3	7.3	4	1.8	5.4	0.41	0.19	37	2A	6M+6sm
48	*S. swertiifolia*	Kerman	Sclerocalycinae	24	66.5	7.1	4	1.8	5.5	0.52	0.16	32	3A	3M+8sm+1st
49	*S. laxa*	Shahoo	Sclerocalycinae	24	52.4	5.8	3	1.9	4.4	0.38	0.19	38	2A	8M+4sm
50	*S. longipetala*	Ilam	Lasistemones	24	32.2	3.2	2	1.6	2.7	0.25	0.14	42	1A	11m+1sm
51	S. tenella	Gadook	Lasistemones	24	32.2	3.3	2	1.7	2.7	0.24	0.14	42	1B	11m+1sm
52	*S. tenella*	Sabalan	Lasistemones	24	32.4	3.6	1.7	2.1	2.7	0.28	0.2	42	2A	12m
53	*S. tenella*	Chashm	Lasistemones	24	37.1	3.8	2.2	1.7	3.1	0.27	0.18	42	1A	11m+1sm
54	*S. tenella*	Shahdej	Lasistemones	24	32.6	3.5	2	1.8	2.7	0.23	0.15	43	1A	12m
55	S. tenella	Neor	Lasistemones	24	26.3	2.5	1.5	1.7	2.2	0.26	0.13	42	1A	12m
56	*S. claviformis*	Soodkooh	Lasistemones	24	40.9	4.7	2.7	1.7	3.4	0.31	0.16	42	1A	11m+1sm
57	*S. claviformis*	Payesib	Lasistemones	24	40.8	4.5	2.7	1.6	3.4	0.37	0.15	39	2A	8m+4sm
58	*S. Marschallii*	Gadook	Lasistemones	24	35.8	3.7	2.4	1.6	3	0.24	0.24	43	1A	12m
59	S. Marschallii	Damavand	Lasistemones	24	38.2	4.3	2.3	1.8	3.1	0.32	0.17	40	1A	11m+1sm
60	*S. Marschallii*	Lavasanat	Lasistemones	24	47.3	4.8	3	1.6	3.9	0.35	0.14	39	1A	11m+1sm
61	*S. Marschallii*	Bakhtiari	Lasistemones	24	36.2	3.9	2.3	1.7	3	0.26	0.14	42	1A	12m
62	*S. Marschallii*	Tonekabon	Lasistemones	24	37.3	3.9	2.1	1.9	3.1	0.29	0.19	41	1A	11m+1sm
63	S. Marschallii	Manjil	Lasistemones	24	42.9	4.6	2.2	2.1	3.6	0.25	0.19	42	1B	12m

Abbreviations: TL = Total chromosome length, L = Longest chromosome, S = Shortest chromosome, Ratio = Longest/shortest chromosome, X = Mean chromosome length, A1 and A2 = Romero-Zarco indices, TF = Total form percentage, ST = Stebbins class and KF = Karyotype formulae.

tion (r=-0.63, p=0.026) with Total form percentage (TF%). In sect. *Auriculatae*, also a positive significant correlation was obtained between the mean chromosome length, size of the longest chromosome and size of the shortest chromosome while, significant negative correlations were observed between chromosome arms ratio, TF% (r=-0.79, p=0.001), and Romero-Zarco A1 index (r=-0.07, p=0.001).

Total form percentage (TF%) varied from 32.93 in Touchal population of *S. odontopetala* from the sect. *Inflatae*, to 46.41 in Manjil population of *S. aucheriana* in sect. *Auriculatae* (Table 1), a higher value of TF% indicates the presence of relatively more symmetrical karyotype. Similar karyotype data of the species in two sections of *Lasiostemones* and *Scalrocalycinae* are given in Appendix 1. As the species studied occupy 1A-3A and 1B-3B classes of Stebbins classification (Appendix 1), we conclude that *Silene* species have relatively symmetrical karyotype.

Chromosome pairing and segregation

The *Silene* species and populations studied showed the presence of 2n = 2x = 24 and 2n = 4x = 48 chromosomes (Table 1, Fig. 1A-G). The chromosome numbers of all species are reported for the first time.

Meiotic analysis of five *S. odontopetala* populations showed n = 12 (2n=24), supporting the karyotype study. The chromosomes formed mainly bivalents and univalents in the metaphase of meiosis I, although some amount of quadrivalents were observed too (Table 2). The highest values for total and terminal chiasmata (21.51 and 18.61, respectively) were found in the Gajereh population, while the lowest values occurred in the Shahdej population (19.76 and 10.24, respectively).

Six *S. aucheriana* populations also showed 2n=24 chromosomes, forming mainly bivalents and univalents in the metaphase of meiosis I, along with a few quadrivalents (Table 2). The highest values for total and terminal chiasmata occurred in the Semnan population (21.62 and 18.69, respectively), while the lowest values occurred in the Golestan population (13.00 and 11.50, respectively). The ANOVA test did not show significant difference for chiasma frequency and chromosome pairing among the *Silene* populations in each section. However a significant difference ($p<0.01$) occurred for all meiotic characteristics including mean number of ring and rod bivalents as well as quadrivalents and chiasma frequency and distribution, among the sections studied.

The species in sections *Auriculatae* and *Inflaltae* showed significantly higher values of meiotic parameters compared to sections of *Lasiostemones* and *Scalrocaycinae*. However, the latter sections differed significantly from each other in characters like, mean number of ring bivalents, quadrivalents, and total number of chiasmata. These results indicate a change in the chromosome pairing and chiasma formation during species diversification. Moreover, the occurrence of quadrivalents

Appendix 2. Meiotic characteristics in *Silene* species.

	Species	Section	ROD	RB	IV	IX	TX	TOX
1	*S. odontopetala*	Inflaltae	5.18	6.20	0.32	3.68	17.5	21.12
2	*S. odontopetala*	Inflaltae	5.26	6.48	0.19	3.03	18.61	21.51
3	*S. odontopetala*	Inflaltae	5.00	6.73	0.23	3.77	18.8	22.42
4	*S. odontopetala*	Inflaltae	5.62	6.22	0.09	2.31	18.12	20.40
5	S. odontopetala	Inflaltae	5.91	5.79	0.12	9.54	10.24	19.76
6	*S. pungens*	Inflaltae	4.52	6.55	0.32	4.51	15.52	20.03
7	*S. aucheriana*	Auriculatae	5.23	4.31	0.85	4.44	17.08	19.38
8	*S. aucheriana*	Auriculatae	4.83	6.52	0.48	2.69	18.69	21.62
9	*S. aucheriana*	Auriculatae	4.59	6.50	0.41	2.59	18.91	21.50
10	*S. aucheriana*	Auriculatae	5.78	5.00	0.61	2.83	16.78	19.53
11	*S. aucheriana*	Auriculatae	4.00	3.50	0.17	1.50	11.50	13.00
12	*S. aucheriana*	Auriculatae	5.90	4.83	0.37	3.83	16.03	19.87
13	*S. sisianica*	Auriculatae	8.50	3.50	0.00	2.00	14.50	16.50
14	*S. bupleurides ssp.*	Scalrocalycinae	2.42	9.58	0.00	1 5.17	14.70	19.88
15	*S. bupleuroides*	Scalrocalycinae	2.93	9.03	0.00	5.23	17.00	22.23
16	*S. eremtica*	Scalrocalycinae	3.94	8.06	0.00	3.24	17.18	20.42
17	*S. stapfii*	Scalrocalycinae	1.97	10.03	0.00	4.87	17.13	22.00
18	*S. shahrudensis*	Scalrocalycinae	2.88	9.13	0.00	6.00	15.63	21.63
19	*S. shahrudensis*	Scalrocalycinae	1.43	10.57	0.00	5.14	18.29	23.43
20	*S. peduncularis*	Scalrocalycinae	2.91	9.09	0.00	4.17	17.74	21.91
21	*S. peduncularis*	Scalrocalycinae	3.93	8.00	0.00	3.23	17.13	20.37
22	*S. avromna*	Scalrocalycinae	1.06	10.94	0.00	7.06	15.53	22.59
23	*S. avromna*	Scalrocalycinae	1.89	10.11	0.00	7.93	12.41	20.26
24	*S. avromna*	Scalrocalycinae	3.39	8.61	0.00	2.77	18.84	21.65
25	S. caesarea	Scalrocalycinae	3.48	8.52	0.00	3.87	17.58	21.45
26	*S. caesarea*	Scalrocalycinae	6.18	5.79	0.00	1.55	16.7	18.24
27	*S. caesarea*	Scalrocalycinae	3.95	8.05	0.00	2.5	17.91	20.41
28	*S. caesarea*	Scalrocalycinae	3.43	8.57	0.00	4.5	16.36	20.86
29	*S. caesarea*	Scalrocalycinae	3.77	8.23	0.00	3.97	16.34	20.31
30	*S. caesarea*	Scalrocalycinae	5.77	6.23	0.00	4.54	14.69	18.85
31	*S. caesarea*	Scalrocalycinae	2.93	9.07	0.00	6.53	14.07	20.6
32	*S. chlorifolia*	Scalrocalycinae	2.77	9.18	0.00	7.5	12.64	20.14
33	*S. swertiifolia*	Scalrocalycinae	2.83	9.11	0.00	6.56	14.72	21.28
34	*S. swertiifolia*	Scalrocalycinae	5.06	6.94	0.00	4.37	14.54	18.91
35	*S. swertiifolia*	Scalrocalycinae	2.73	9.27	0.00	2.82	18.18	21.00
36	*S. swertiifolia*	Scalrocalycinae	3.00	8.91	0.00	7.91	14.91	22.82
37	*S. swertiifolia*	Scalrocalycinae	2.82	9.18	0.00	8.64	12.05	20.68
38	*S. swertiifolia*	Lasiostemones	0.25	0.23	0.00	0.06	0.66	0.72
39	*S. marschallii*	Lasiostemones	0.35	0.13	0.00	0.06	0.81	0.86
40	*S. marschallii*	Lasiostemones	0.33	0.16	0.00	0.08	0.76	0.84
41	*S. marschallii*	Lasiostemones	0.37	0.13	0.04	0.14	0.80	0.94
42	*S. propinqua*	Lasiostemones	0.34	0.15	0.00	0.12	0.67	0.79

Abbreviations: ROD=Rod bivalents, RB=Ring bivalents, IV=Quadrivalents, IX=Intercalary chiasmata, TX=Terminal chiasmata, TOX=Total chiasmata.

in diploid species due to heterozygote translocation may be an adaptive strategy in the *Silene* species diversification as also reported in *Brassica napus* (Sheidai et al. 2006), *Festuca* (Sheidai and Bagheri-Shabestari 2007) and *Bromus* (Sheidai and Nouroozi 2005). Such heterozygote translocations lead to the formation of new linkage groups (Rees and Dale 1974). Variation in chiasma frequency and localization is genetically controlled (Quicke 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 among species and populations with the same chromosome number is considered a mean for generating new forms of recombination influencing the variability within natural populations in an adaptive way (Rees and Dale 1974).

Metaphase and anaphase chromosome stickiness occurred in all populations of *S. aucheriana*, *S. sisianica*, *S. odontopetala*, and *S. pungens* (Fig. 1H & I). The degree of chromosome stickiness ranged from stickiness among two or more chromosomes to the involvement of all metaphase chromosomes forming a complete clump. Chromosome bridges resulting from stickiness were observed in anaphase-I and II as well as telophase-I and II stages (Fig. 1, H). The thickness

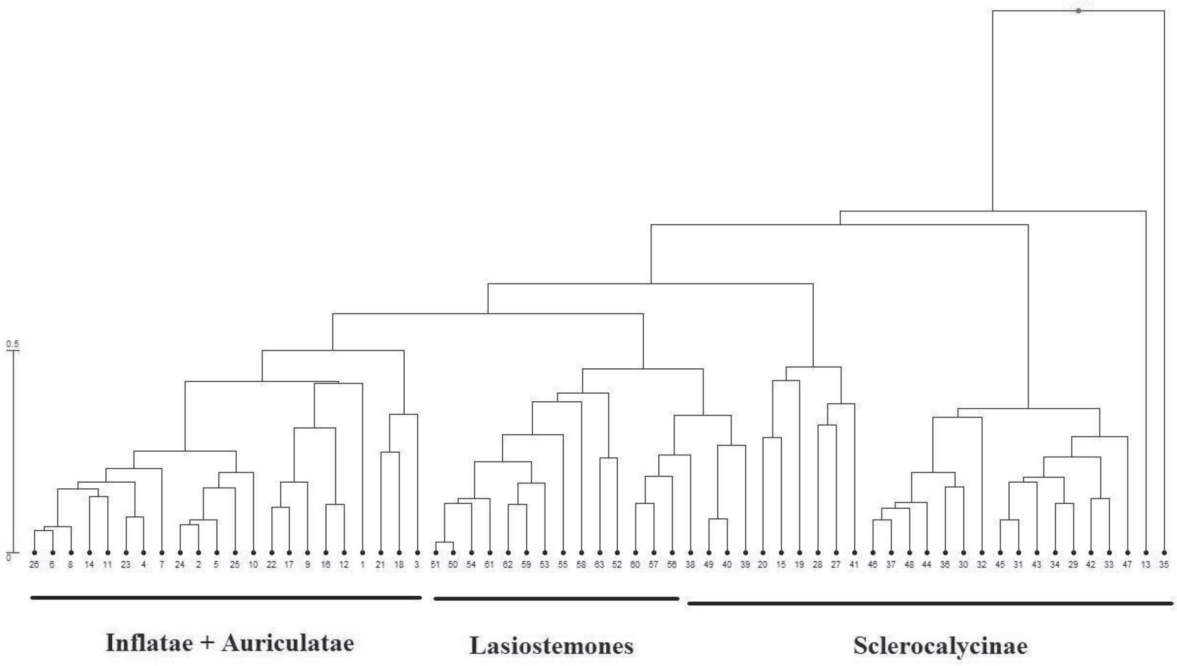

Figure 4. UPGMA dendrogram of *Silene* species and sections based on karyotype data. OUT numbers correspond to the numbers in Appendix 1.

of bridges and the number of chromosomes involved in their formation varied among different meiocytes and in the species studied. Interactions between genetic and environmental factors have been considered the possible reasons for the occurrence of chromosomes stickiness in different plant species and cultivars (Baptista-Giacomelli et al. 2000).

Some of the species showed the occurrence of one or a few laggard chromosomes in anaphase I and II as well as telophase I and II. Such laggard chromosomes formed micronucleus in telophase of meiosis II (Fig. 1j). The presence of meiocytes having the double gametic chromosome number as well as bigger pollen grains size (potential unreduced (2*n*) pollen grains), were noticed in most of the *Silene* species studied (Fig. 1L, O & P).

B-chromosomes

The species and populations of *S. odontopetala*, *S. vulgaris* and *S. pungens* from the sect. *Inflaltae*, and *S. aucheriana* and *S. sisianica* from the sect. *Auriculatae* showed the occurrence of B-chromosomes. The B-chromosomes were smaller than the A-chromosomes and did not form any meiotic association with them. Similarly, the species of *S. chlorifolia*, *S. bupleuroides*, *S. stapfii*, *S. caesarea* and *S. peduncularis* of the sect. *Sclerocalycinae* as well as *S. claviformis*, *S. Marschallii* and *S. propinqua* of the sect. *Lasiostemones* also show the occurrence of B-chromosomes (Sheidai et al. 2009).

B-chromosomes are accessory chromosomes reported in many plant and animal species. They show numerical polymorphism and when present in high numbers, they negatively affect the growth and vigor of the plants, while in low numbers they may be beneficial to the plant (Camacho et al. 2000). B-chromosomes may affect the frequency and distribution of chiasmata as well as chromosome association. However, due to the low number of meiocytes showing presence of B-chromosomes in the species studied, their effects on chiasma frequency and chromosome associations could not be worked out.

Unreduced pollen grains formation

Multipolar cells and abnormal tetrads were observed died (Fig. 1K, M & N). Multipolar cells may be formed due to spindle abnormalities. Such meiotic abnormalities may lead to the formation of abnormal tetrads and aneuploid gametes (Villeux 1985; Nirmala and Rao 1996). The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in chromosome alignment during metaphase. Any distortion or breakage in the spindle may result in random subgrouping of the chromosomes which function independently (Nirmala and Rao 1996). In several instances, spindle abnormalities have led to the production of aneuploid gametes, for example in polyploidy hybrids and derivatives of *Aegilops* ×*Triticum* hybrids, amphiploid Triticineae, amphiploids of *Solanum* hybrids, etc. Different reasons have been suggested for the occurrence of spindle abnormalities including: duality of nucleus in foreign cytoplasm, environmental influence and disharmonious gene interaction (Nirmala and Rao 1996).

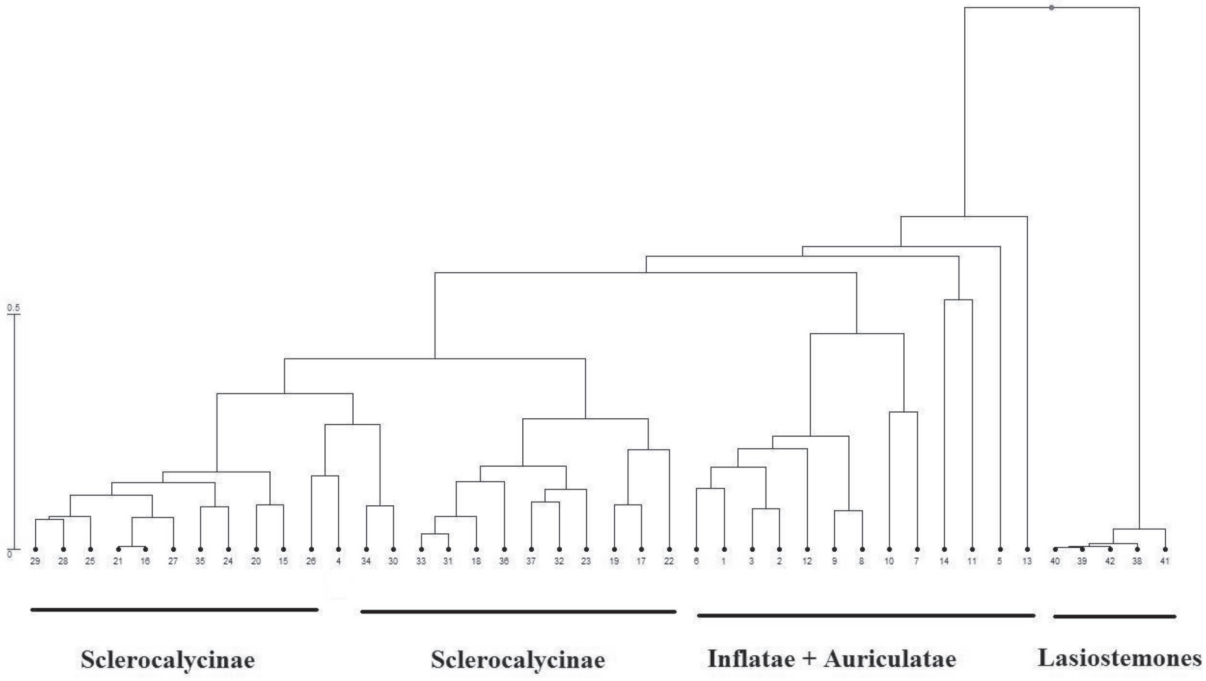

Figure 5. UPGMA dendrogram of *Silene* species and sections based on meiotic data. OUT numbers correspond to the numbers in Appendix 2.

The presence of meiocytes having double the gametic chromosome number as well as bigger size pollen grains (potential unreduced (2*n*) pollen grains), were noticed in most of the *Silene* species studied (Fig. 1L, O & P). Different cytological mechanisms are responsible for the production of 2n gametes, including premiotic doubling of the chromosomes, omission of the first and second meiotic division, post-meiotic division, abnormal spindle geometry, abnormal cytokinesis and desynapsis (Villeux 1985). Detailed cytological investigation of the *Silene* species included here revealed that the main cytological mechanisms for production of potential 2*n* gametes are: either anaphase-II failure leading to the formation of triads at the end of telophase-II instead of tetrad (one is unreduced, Fig. 1K); or multipolar spindles as discussed earlier and 3- syncyte formation (Fig. 1L).

The size of normal (reduced) pollen grains was 26-32.86 µm in the species of the sect. *Inflatae*, while size of the potential unreduced pollen grains was 34.56-45.21 µm. Similarly, the size of normal (reduced) pollen grains was between 11.10-19.50 µm in the species of the sect. *Auriculatae* and size of the potential unreduced pollen grains ranged between 19.60-25.50 µm.

The mean diameter of normal (reduced) pollen grains ranged from 19.78 µm to 27.80 µm in the species of the sect. *Lasiostemones*, while size of the potential unreduced pollen grains was between 29.10-48.28 µm. The same data were 38-57 and 64-77 µm respectively in the species of the sect. *Sclerocalycinae*.

The presence of giant pollen grains has been used as an indication of the production of 2n pollen grains. A variety of methods have been used to detect 2*n* gametes, including morphological screening of 2*n* pollens and flow cytometry analysis of pollen along with cytological investigations (Bretagnolle and Thompson 1995). The measurement of the pollen grains in the species with unreduced meiocytes revealed the presence of a bimodal distribution of pollen grain size. T-test analysis also showed a significant difference between the pollen grains, indicating the possible 2*n* constitution of the larger pollen grains. The frequency of potential unreduced pollen grains was 2-3% in the species studied. The occurrence of unreduced gametes is important in the evolution of polyploids and is also of economic importance; for example in potato for obtaining natural tetraploid by crossing 4x and 2x lines (Bretagnolle and Thompson 1995).

Section classification

The UPGMA and NJ clustering of the species based on karyotype (Appendix 1), and meiotic features (Appendix 2), gave similar results (Figs. 4 & 5). In general three major clusters were formed; the species of the sections *Auriculatae* and *Inflatae* showed close affinity and were placed among each other in both analyses, while the species of the sect. *Lasiostemones* and *Sclerocalycinae*, formed separate groups, indicating karyotypic and meiotic distinctness. Therefore, we suggest that cytological characteristics including karyotype and meiotic features may be used to show *Silene* species

affinities along with molecular and morphological characteristics, particularly at the sectional level.

The present study also showed significant differences in the size of chromosomes as well as the frequency and distribution of chiasmata among the sections and also the occurrence of heterozygote translocations, all of which might have accompanied the speciation process in *Silene*.

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