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ARTICLE

Contribution to cytotaxonomy of *Silene*: chromosome pairing and unreduced pollen grain formation in sec. *Sclerocalycinae*

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ABSTRACT Meiotic studies of ploidy level, chromosome paring and chiasma frequency were performed on 24 populations of nine *Silene* species belonging to the section *Sclerocalycinae* growing in Iran. The species studied are: 1- *Silene bupleuroides* L., 2- *S. eremitica* Boiss., 3- *S. stapfii* Melzh., 4- *S. shahrudensis* Rech. (two populations), 5- *S. peduncularis* Boiss. (two populations), 6- *S. avromana* Boiss. (three populations), 7- *S. caesarea* Boiss. (seven populations), 8- *S. chlorifolia* SM. , 9- *S. swertiifolia* Boiss. (six populations). The species studied showed 2n = 2x = 24. The chromosome numbers of all species are reported here for the first time. The species and populations studied differed significantly in chiasma frequency and chromosomes pairing indicating partly their genetic differences. When the species were subjected to cluster analysis based on meiotic characters almost the populations of each species were grouped together indicating their distinctness. Meiotic abnormalities including multipolar cell formation formed unreduced pollen grains in some of the species while B-chromosomes occurred in some others.
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KEY WORDS

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The genus *Silene* L. (Caryophylaceae) with about 700 mostly hermaphrodite species and a few diocious or gynodioceious species shows world-wide distribution but are mainly distributed in the northern hemisphere, Europe, Asia and northern Africa (Bari 1973; Greuter 1995). Chowdhuri (1957), based on morphological characters classified *Silene* species in 22 sections but the results of molecular studies carried out by Oxelman et al. (1997, 2000) and Burleigh and Holtsford (2003) do not support such sectional classifications particularly for the endemic North American taxa.

The *Silene* species are annual, biennial, or perennial herbs most of which are diploid having 2n = 2x = 24, or 2n = 2x = 20 chromosome number (Swank 1932, Bari 1973; Oxelman and Lidén 1995). The species of *S. fortunei* is triploid (2n = 3x = 30; Heaslip 1951), but species showing tetraploid (2n = 4x = 48), hexaploid (2n = 6x = 72), and higher polyploidy levels for e.g. 2n = *c.* 96, 120 and 192 have also been reported (Bari 1973). Moreover 2n = 18 is reported for *S. conica* and *S. lacera* (Sopova and Sekovski 1982), Gvinianidze and Avazneli, 1982), and 2n = 46 is reported for *S. firma* (Zhang 1994), which make x= 9 and x = 23 along with x= 10 and 12, the known basic chromosome numbers for the *Silene*. Extensive cytogenetic studies have been performed on *Silene* species from different parts of the world (Heaslip 1951; Bari 1973; Melzheimer 1978; Markova et al. 2006) but similar studies on *Silene* species of Iran is only confined to karyotype analysis of few species (Gholipour and Sheidai 2008; Sheidai et al. 2008) and no report is available on chiasma frequency and distribution and other meiotic features of these species.

About 110 *Silene* species grow in Iran out of which about 35 species are endemic with very limited geographical distribution (Melzheimer 1980). The section *Sclerocalycinae* Boiss. contains 16 species and 3 subspecies in Iran (Melzheimer 1980), growing mainly in the west and north of the country. Our study reported in this paper is the first cytological analysis of 24 populations of 9 Iranian species of *Silene* species belonging to the section *Sclerocalycinae*.

Material and Methods

Cytological studies were performed on in 24 populations of nine *Silene* species of the section *Sclerocalycinae* Boiss., (Table 1), vouchers specimens are being deposited in the Herbarium of Shahid Beheshti University (HSBU), Iran. The species studied are: 1- *Silene bupleuroides* L., 2- *S. eremitica* Boiss., 3- *S. stapfii* Melzh., 4- *S. shahrudensis* Rech. (two populations), 5- *S. peduncularis* Boiss. (two populations), 6- *S. avromana* Boiss. (three populations), 7- *S. caesarea* Boiss. (seven populations), 8- *S. chlorifolia* SM. , 9- *S. swertiifolia* Boiss. (six populations).

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

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Table 1. Meiotic characteristics of *Silene* species studied.

Species	Locality	2n	TX	IX	TOX	RB	ROD
S. bupleurides ssp.							
bupleuroides	Damavand	24	14.7	15.17	19.88	9.58	2.42
S. eremtica	Khooy	24	17.00	5.23	22.23	9.03	2.93
S. stapfii	Kerman	24	17.18	3.24	20.42	8.06	3.94
*S. shahrudensis*1	Oshtorankooh	24	17.13	4.87	22.00	10.03	1.97
*S. shahrudensis*2	Semnan	24	15.63	6.00	21.63	9.13	2.88
*S. peduncularis*1	Ghooshchi	24	18.29	5.14	23.43	10.57	1.43
*S. peduncularis*2	Tabriz	24	17.74	4.17	21.91	9.09	2.91
*S. avromna*1	Arak	24	17.13	3.23	20.37	8.00	3.93
*S. avromna*2	Uremia	24	15.53	7.06	22.59	10.94	1.06
*S. avromna*3	Gajereh	24	12.41	7.93	20.26	10.11	1.89
*S. caesarea*1	Dena	24	18.84	2.77	21.65	8.61	3.39
*S. caesarea*2	Koohgol	24	17.58	3.87	21.45	8.52	3.48
*S. caesarea*3	Cheshmeh-Mishi	24	16.70	1.55	18.24	5.79	6.18
*S. caesarea*4	Yasooj	24	17.91	2.50	20.41	8.05	3.95
*S. caesarea*5	Khor village	24	16.36	4.50	20.86	8.57	3.43
*S. caesarea*6	Sharestanak	24	16.34	3.97	20.31	8.23	3.77
*S. caesarea*7	Nesa	24	14.69	4.54	18.85	6.23	5.77
S. chlorifolia	Ardakan	24	14.07	6.53	20.60	9.07	2.93
*S. swertiifolia*1	Arak	24	12.64	7.50	20.14	9.18	2.77
*S. swertiifolia*2	Oghlid	24	14.72	6.56	21.28	9.11	2.83
*S. swertiifolia*3	Darbandsar	24	14.54	4.37	18.91	6.94	5.06
*S. swertiifolia*4	Gachsar	24	18.18	2.82	21.00	9.27	2.73
*S. swertiifolia*5	Gajereh	24	14.91	7.91	22.82	8.91	3.00
*S. swertiifolia*6	Touchal	24	12.05	8.64	20.68	9.18	2.82

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

they were washed and preserved in ethanol at 4°C until used (Sheidai et al. 2006). 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 anaphase and telophase (Sheidai et al. 2008). Complete pollens which were stained were taken as fertile, while incomplete, shrunken pollens with no stain were considered as infertile (Sheidai and Rashid 2007).

In order to determine any significant difference in chiasma frequency and distribution among the species and populations we performed analysis of variance (ANOVA) by the least significant differences (LSD) method (Sheidai et al. 2008).

Grouping of the species based on meiotic characteristics was performed using various cluster analysis methods including single linkage, unweighted paired group with arithmetic average (UPGMA) and Neighbor Joining (NJ) method as well as ordination based on principal component analysis (PCA) (Podani 2000; Sheidai et al. 2008). Bootstrapping with 100 replications was performed on dendrograms obtained.

In order to detect significant difference between potential unreduced pollen grains and the normal (reduced pollens), t-test was performed. Statistical analyses used SPSS ver. 9 (1998), NTSYS ver. 2.1 (1998) and DARwin ver. 5.0.155 (2006) and PAUP ver. 4.0b10 (2001) software.

Results and Discussion

Chromosome pairing and segregation

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

The highest total number chiasmata and ring bivalents

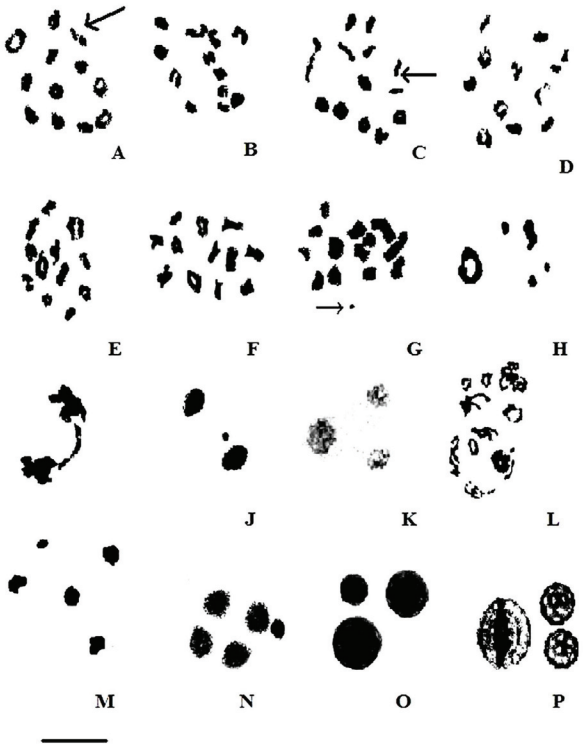

Figure 1. Representative meiotic cells in *Silene* species studied. A-F = Meiocytes showing 2n = 2x = 24 in Kerman population of *S. stapfii*, Tabriz population of *S. peduncularis*, Cheshmeh-Mishi population of *S. caesarea, S. bupleuroides* and *S. eremitica* respectively. G = Meiocyte showing one B-chromosome in *S. bupleuroides*. H = Metaphase chromosome stickiness in Khor village population of *S. caesarea*. I = Anaphase bridge in Darbandsar population of *S. swertiifolia*. J = Anaphase-I laggard in Arak population of *S. avromana*. K = Tripolar cell in Cheshmeh-Mishi population of *S. caesarea*. L = Meiocyte with double chromosome number in *S. eremitica*. M = Multipolar cell in *S. shahrudensis*. N = Multipolar cell in *S. eremitica*. O = Unreduced pollen grain (bigger size) in Yasooj population of *S. caesarea*. P = Unreduced pollen grain (bigger size) in Semnan population of *S. shahrudensis*. Scale bar = 10 µm.

occurred in Ghooshchi population of *S. peduncularis* (23.43 & 10.57 respectively), while the lowest values of the same occurred in Cheshmeh-Mishi population of *S. caesarea* (18.24 & 5.79 respectively). Dena population of *S. caesarea* showed the highest number of terminal chiasmata (18.84), while *S. swertiifolia* showed the highest number of intercalary chiasmata (8.64).

ANOVA followed by LSD test showed a significant difference for chiasma frequency and chromosome pairing among *Silene* species and populations studied, indicating that significant change has occurred in the number genes controlling chromosome pairing during species diand populations diversification. 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 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).

The UPGMA and NJ clustering and ordination based on principal component analysis (PCA) of the species produced similar results (Figs. 2 & 3). In general four major clusters or groups are formed; the populations of the species *S. caesarea* form two of the four major clusters. Their cytogenetic difference with the other species is well documented in the PCA plot (Fig. 3) as these populations are placed in the right side of the plot along PCA1 and PCA2 axes. The populations of *S. swertiifolia a*re distributed in two clusters or groups indicating the presence of intra-specific cytogenetic diversity in this species which also holds true for *S. avromana*. In Flora Iranica (Melzheimer 1980), the species of *S. bupleuroides*, *S. chlorifolia* and *S. swertiifolia* have been placed close to each other, the species of *S. caesarea* and *S. stapfii* being considered related and the same is suggested for the species of *S. peduncularis*, *S. avromana*, *S. shahrudensis* and *S. eremitica*. Grouping of the species based on cytological data reported by us supports the affinity of above said species (Figs. 2 & 3).

Meiotic abnormalities

Metaphase and anaphase chromosome stickiness occurred in some of the species like *S. peduncularis*, *S. shahrudensis* and *S. chlorifolia* (Fig. 1, H & 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 of bridges observed and the number of chromosomes involved in their formation varied among different meiocytes and in the species studied. Some of the species showed the occurrence of 1 to 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. 1, j). Genetic, environmental factors and their interaction have been considered as the possible reasons for the occurrence of chromosomes stickiness in different plant species and cultivars (Baptista-Giacomelli et al. 2000).

Multipolar cells and abnormal tetrads were observed in most of the species studied (Fig. 1, K, 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; Sheidai and Attaei 2005; Sheidai and Nouroozi 2005; Sheidai et al. 2005, 2006). 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 sub-grouping of

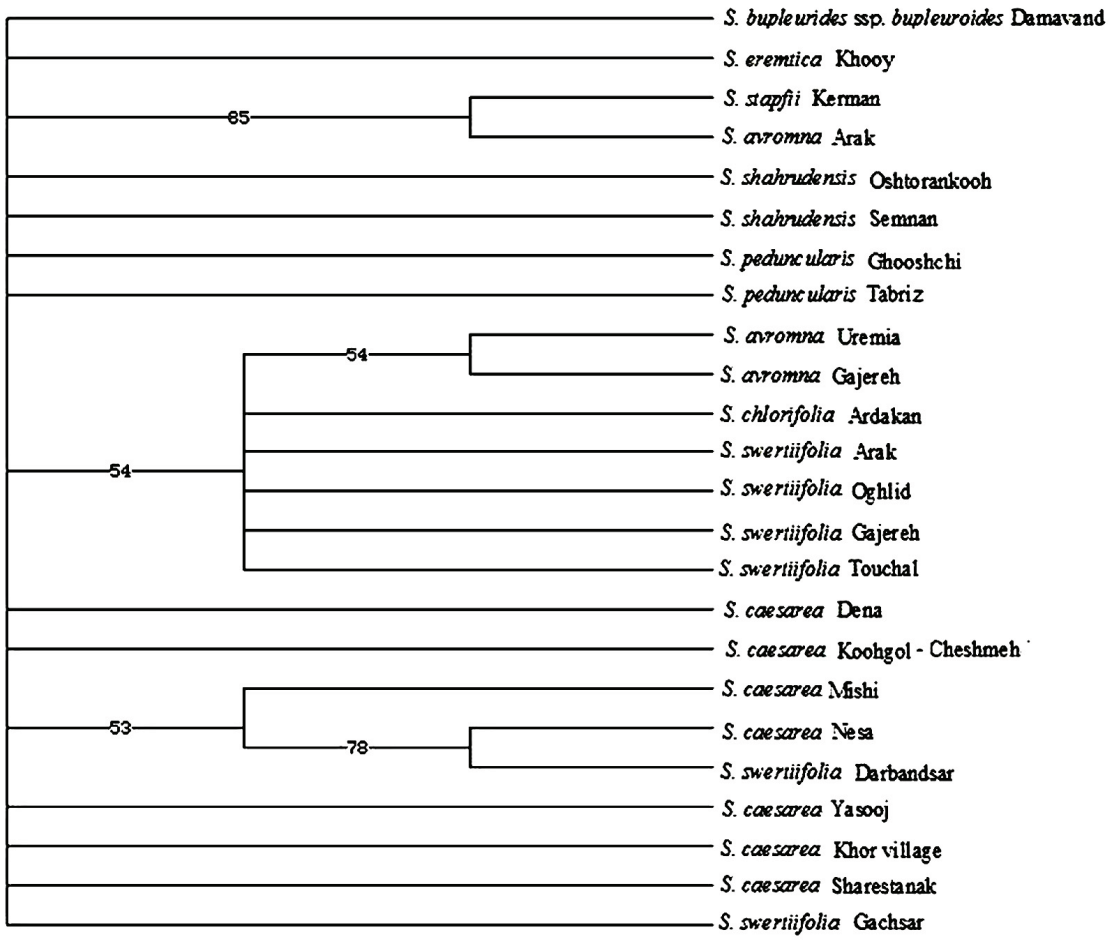

Figure 2. NJ dendrogram of *Silene* species studied. (Numbers above clusters are bootstrap values)

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).

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. 1, L, O & P). A numerically unreduced diploid or 2n gamete is a meiotic product bearing the sporophytic rather than the gametophytic chromosome number. Such gametes result from abnormalities during either microsporogenesis (2n pollen) or megasporogenesis (2n eggs). Unreduced gametes are known to produce individuals with higher ploidy level through a process known as sexual polyploidization (Villeux 1985). Sexual polyploidization has been considered a major route to the formation of naturally occurring polyploids. 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 *Silene* species studied revealed that the main cytological mechanisms for production of potential 2*n* gametes are: 1- anaphase-II failure leading to the formation of triads at the end of telophase-II instead of tetrad (one is unreduced, Fig. 1, K); 2- multipolar spindles as discussed earlier and 3- syncyte formation (Fig. 1, L).

The potential unreduced pollen grains, ranged in size from 64-77 μm in diameter and differed from the smaller sized pollen grains (reduced pollen grains) ranging in size from 38-57 μm in diameter. 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

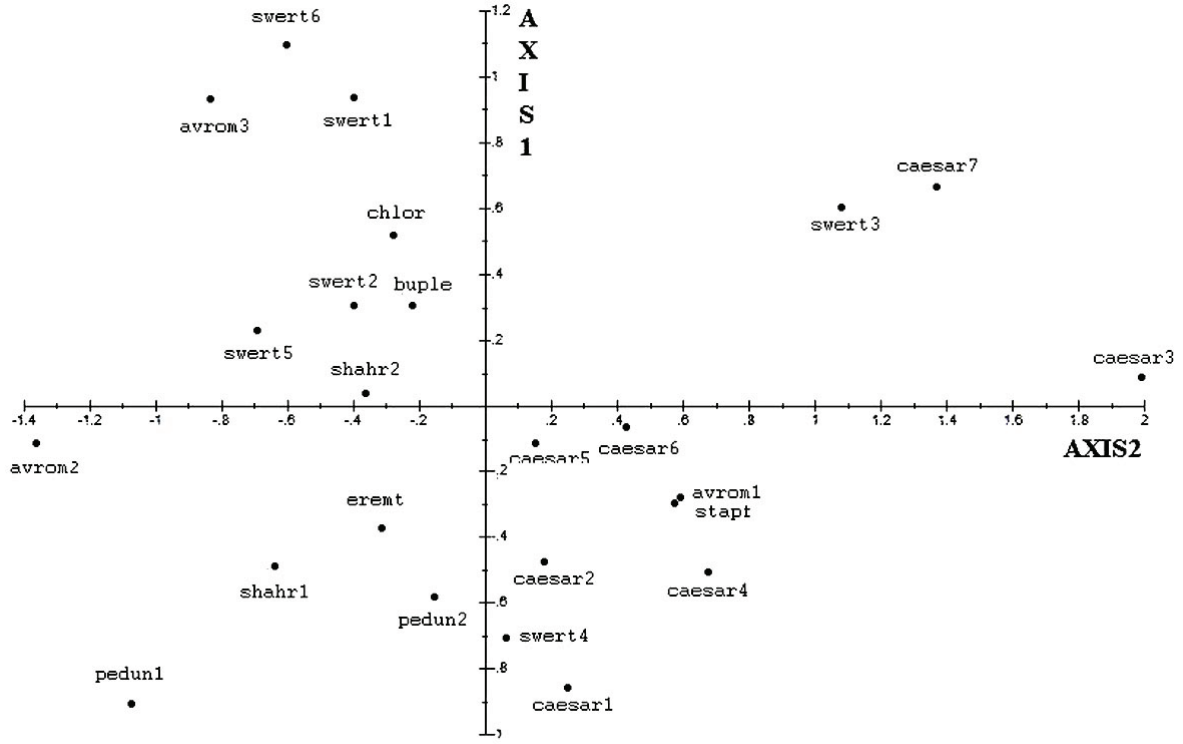

Figure 3. PCA plot of *Silene* species studied. Species abbreviations: buple = *S. bupleuroides*, shar1 & 2 = Oshtorankooh and Semnan populations of *S. shahrudensis*, swert1-6 = Arak, Eghlid, Darbandsar, Gachsar, Gajereh and Touchal populations of *S. swertiifolia*, avrom1-3 = Arak, Uremia and Gajereh populations of *S. avromana*, eremt = *S. eremitica*, pedun1 & 2 = Ghooshchi and Tabriz populations of *S. peduncularis*, stapf = *S. stapfii*, ceasar1-7 = Dena, Koohgol, Cheshmeh-Mishi, Yasooj, Khor village, Sharestanak and Nesa populations of *S. caesarea*, chlor = *S. chlorifolia*.

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 differed from 2-3% in the species studied. The occurrence of unreduced gametes has been considered important in the evolution of polyploids and also is of economic importance for example in potato for obtaining natural tetraploid by crossing 4x ×2x lines (Bretagnolle and Thompson 1995).

B-chromosomes

The species of *S. chlorifolia*, *S. bupleuroides*, *S. stapfii*, *S. caesarea* and *S. peduncularis showed* the presence of 0-2 B-chromosomes (Bs) (Fig. 1, G). The B-chromosomes were smaller than the A-chromosomes and did not form any meiotic association with them. B-chromosomes are accessory chromosomes reported in many plant and animal species. They show numerical polymorphism and, when present in high numbers, negatively affect the growth and vigor of the plants, while in low numbers they may be beneficial to the plant possessing them (Camacho et al. 2000). B-chromosomes may affect the frequency and distribution of chiasmata as well as chromosome association, however due to low number of meiocytes showing presence of Bchromosomes in the species studied; their effects on chiasma frequency and chromosome associations could not be worked out.

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