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Research Paper

A karyological study of some *Dianthus* L. species (Caryophyllaceae) in Northeast of Iran

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Abstract

This study focuses on karyological investigation of four taxa of *Dianthus* (Caryophyllaceae) including *D. crossopetalus*, *D. orientalis* subsp. *stenocalyx*, *D. orientalis* subsp. *gorganicus*, and *Dianthus* sp. distributed in Northeast of Iran. The karyotype asymmetry/symmetry was evaluated using two methods: 1) CV_{CL} (coefficient of variation of chromosome length) and CV_{CI} (heterogeneity of the centromeric index); and 2) M_{CA} (mean centromeric asymmetry). The karyotype asymmetry was also used to investigate the relationships among the taxa. Results obtained from the current study revealed that there are two different ploidy levels ($2n = 2x = 30$ and $2n = 4x = 60$) among the investigated taxa. The indices CV_{CL} and M_{CA} described karyotype asymmetry correctly based on variation in chromosome length. Diagram of CV_{CL} vs. M_{CA} seems to be appropriate for karyological delimitation and taxonomic relationships among the *Dianthus* taxa under study.

Introduction

Dianthus L. (Caryophyllaceae) comprises a group of annual or perennial species worldwide.

The genus consists of 30 species in Iran. *Dianthus* with high intra-populational and interspecific variation of morphological and genetic characters has been recognized as a complex genus (BEHROOZIAN 2010). Therefore, morphological characters alone do not provide effective clues for delimitation of *Dianthus* species (BEHROOZIAN 2010). Additional studies such as cytology and molecular approaches could be useful for resolving taxonomic problems in plants (SEN 1975; LARSON et al. 2010; MARCUSSEN & BORGEN 2011; GAO et al. 2012). The last decades, karyological investigations provided fundamental characters for plant systematics and evolutionary analysis (STACE 2000). Recently, scientists have developed a variety of methods for karyotype asymmetry analysis (reviewed in ZUO & YUAN 2011; PERUZZI & EROĞLU 2013).

Dianthus with seven ploidy levels (2*x*, 3*x*, 4*x*, 5*x*, 6*x*, 8*x*, and 12*x*; reviewed in WEISS et al. 2002 and BALAO et al. 2009), is one of the genera with the most extensive ploidy series known in the subfamily Caryophylloideae. BALAO et al. (2010) suggested that different ploidy levels within and between populations

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of the genus are related to highly morphological variations. They declared that the evolution of cytological features within the genus has taken place in conjunction with morphological diversification. Furthermore, different ploidy levels have been reported for populations at the subspecific level (WEISS et al. 2002). Karyological studies on *Dianthus* are often restricted to just reporting of chromosome numbers, whereas karyological analyses were rarely reported. The aims of the present study are: 1) to report somatic chromosome numbers; 2) to assess karyotype asymmetry/symmetry; and 3) to reveal the karyotype relationships among some *Dianthus* species distributed in Northeast of Iran.

Material and methods

In order to study the karyological characteristics of *Dianthus* species, seeds were collected from various regions of Northeast of Iran. A minimum of five specimens were investigated for each species included in the current study (except for *D.* sp., see below). Depending on the number of populations per species, these individuals belong to one or more populations. At least five chromosome plates were analyzed per specimen of species. No intraspecific karyological variability has been found. Therefore, one voucher specimen per taxon was presented in Table 1. Voucher specimens are deposited in Ferdowsi University of Mashhad Herbarium (FUMH) (Table 1).

This study focuses on four taxa including *D. orientalis* subsp. *stenocalyx* (BOISS.) Rech.f., *D. orientalis* subsp. *gorganicus* Rech.f., the new record *D. crossopetalus* (FENZL ex BOISS.) GROSSH (GHAHREMANINEJAD et al. 2012), and an unknown *Dianthus* taxon. The last taxon was collected from an isolated and small population during field sampling (Table 1). The individuals of this taxon seem to be morphologically intermediate between *D. crinitus* subsp. *turcomanicus* (SCHISCHK.) Rech.f. and *D. orientalis* subsp. *stenocalyx*. Their vegetative habit is similar to the first subspecies while their floral characters tend more to the second. Karyological data of *D. crinitus* subsp. *turcomanicus,* which is published in previous study (JAFFARI & BEHROOZIAN 2010), are included in the current study to reveal its karyological relationship with the unknown taxon. One individual of this unknown taxon, however, is sampled to investigate its karyological characteristics. This taxon is named here after as *Dianthus* sp. in this study.

The seeds were germinated at 25 °C. Five to ten root tips of each taxon were pretreated with 8-hydroxyquinoline (0.002 M) for three hours at room temperature. Then, they were fixed in Carnoy's solution (3 : 1 absolute ethanol – glacial acetic acid) for 24 h at 4 °C. The fixed root tips were hydrolyzed in 1 N HCl at 60 °C for 5 – 7 min followed by washing them in distilled water. Then they were stained with aceto-orcein (1% w/v) for 2 h. Finally, the root tips were squashed in acetic acid 45%. The metaphase chromosomes were counted for at least 10 cells of each seedling root. The examined metaphase plates were photographed using Olympus BX52 microscope with automatic camera.

Chromosome measurements including long and short arms, total length of chromosome set, arm ratio index and relative chromosome length were performed for four taxa using the Nutype software. Chromosomes were identified according to the Levan method (LEVAN et al. 1964). The homologous chromosomes of diploid taxa were paired by their similarity in size and shape. A number of parameters were used to determine the karyotype asymmetry and symmetry according to PASZKO (2006): $CV_{CI} = [(S_{CI}/x_{CI}) \times 100$ (S_{CI} = the standard deviation of the centromeric index; x_{CI} = the mean centromeric index)], $CV_{CL} = A_2 \times 100$ (A_2 is proposed by ZARCO

Table 1
Voucher specimens included in the karyological study of four taxa of *Dianthus*

Voucher No.	Species	Location	Longitude (E)	Latitude (N)
35410	*D. crossopetalus*	Khorassan shomali province, E. Jajarm, between Khorashah and Jorbat	56°45′3.6″	37°06′32.4″
44075	*D. orientalis* subsp. *stenocalyx*	W. Bojnourd, Qorkhod protected area, Yakhtikalan pass	56°12′6.9″	37°24′2.7″
44111	*D. orientalis* subsp. *gorganicus*	Golestan province, NW of Islamabad	56°02′19.3″	37°43′0.2″
44119	*Dianthus* sp.	SW. Bojnourd, between Hessar-Hosseini and Rakhtian	57°09′24.2″	37°18′12.4″

Table 2
Karyotype formula according to LEVAN et al. (1964) used in the present work: SC – the shortest chromosome length; LC – the longest chromosome length; CL – mean length of chromosome; CI – mean centromeric index; SD – standard deviation; K.F. – karyotype formula. Karyological data of *D. crinitus* subsp. *turcomanicus* were used with permission from JAFFARI and BEHROOZIAN 2010

No.	Species	2*n*	Range SC-LS	Ratio LC/SC	CL (µm) Mean (±SD)	CI (µm) Mean (±SD)	K.F.
1	*D. crossopetalus*	30	1.94–3.45	1.78	2.61 (±0.37)	35.63 (±7.54)	10m + 20sm
2	*D. orientalis* subsp. *stenocalyx*	30	2.01–3.62	1.80	2.74 (±0.38)	40.30 (±6.57)	24m + 4sm + 2st
3	*D. orientalis* subsp. *gorganicus*	60	1.81–3.84	2.12	2.73 (±0.44)	37.38 (±9.84)	24m + 28sm + 8st
4	*Dianthus* sp.	60	2.16–3.35	1.77	2.67 (±0.29)	34.80 (±7.65)	24m + 32sm + 4st
5	*D. crinitus* subsp. *turcomanicus*	60	1.92–3.84	2.1	2.73 (±0.43)	47.11 (±5.54)	46m + 12sm + 2st

1986), and PERUZZI and EROĞLU (2013): $M_{CA} = A \times 100$ (A is proposed by WATANABE et al. 1999). CV_{CL} and M_{CA} indices provide the most suitable methods for estimating karyotype asymmetry, while the CV_{CI} did not describe karyotype asymmetry correctly (ZUO & YUAN 2011; PERUZZI & EROĞLU 2013). Therefore, a bidimensional scatter plot was drawn with the couple of parameters CV_{CL} and M_{CA} to reveal karyotype relationships among the organisms included in the current study. In addition, an idiogram was constructed for each taxa based on the average of the mean values calculated for the karyotypes using data taken from Table 2.

Results

Chromosome numbers

Karyotype characters, mitotic metaphase chromosome, haploid idiogram and karyogram of the taxa investigated are shown in Tables 2 and 3, and Figures 1 and 2. Detailed karyotypes and idiograms of the taxa are reported here for the first time. Both *D. crossopetalus* and *D. orientalis* subsp. *stenocalyx* are diploid with a formula of $2n = 2x = 30$. The chromosome number for *Dianthus* sp. and *D. orientalis* subsp. *gorganicus* is $2n = 60$ and they are tetraploid. Karyograms of the two last taxa are not shown in Figure 2 due to lacking information concerning the origin (auto- or allopolyploidy) of these taxa.

Karyotype asymmetry analysis

The highest and lowest ratio of longest to shortest chromosomes is found in *D. orientalis* subsp. *gorganicus* and *Dianthus sp.*, respectively (Table 2). The mean value of the total chromosome length in *D. crossopetalus* (2.61) and *Dianthus sp.* (2.67) was comparatively lower than that of the both subspecies of *D. orientalis* (2.73 and 2.74). *Dianthus crossopetalus* consists of metacentric and sub-metacentric chromosomes, while the remaining taxa contain metacentric, sub-metacentric, and sub-telocentric chromosomes. In all examined taxa, metacentric chromosomes are the most common (56.65% of all the chromosomes),

Table 3
Asymmetry indices used for the classification of *Dianthus* taxa: CV_{CL} – coefficient of variation of chromosome length; CV_{CI} – heterogeneity of the centromeric index; M_{CA} – mean centromeric asymmetry. Karyological data of *D. crinitus* subsp. *turcomanicus* were used with permission from JAFFARI and BEHROOZIAN (2010)

No.	Species	CV_{CL}	CV_{CI}	M_{CA}
1	*D. crossopetalus*	14.23	21.16	29
2	*D. orientalis* subsp. *stenocalyx*	14.01	16.31	19
3	*D. orientalis* subsp. *gorganicus*	16.22	25.91	33
4	*Dianthus* sp.	12.98	21.99	30
5	*D. crinitus* subsp. *turcomanicus*	13.86	11.76	5.8

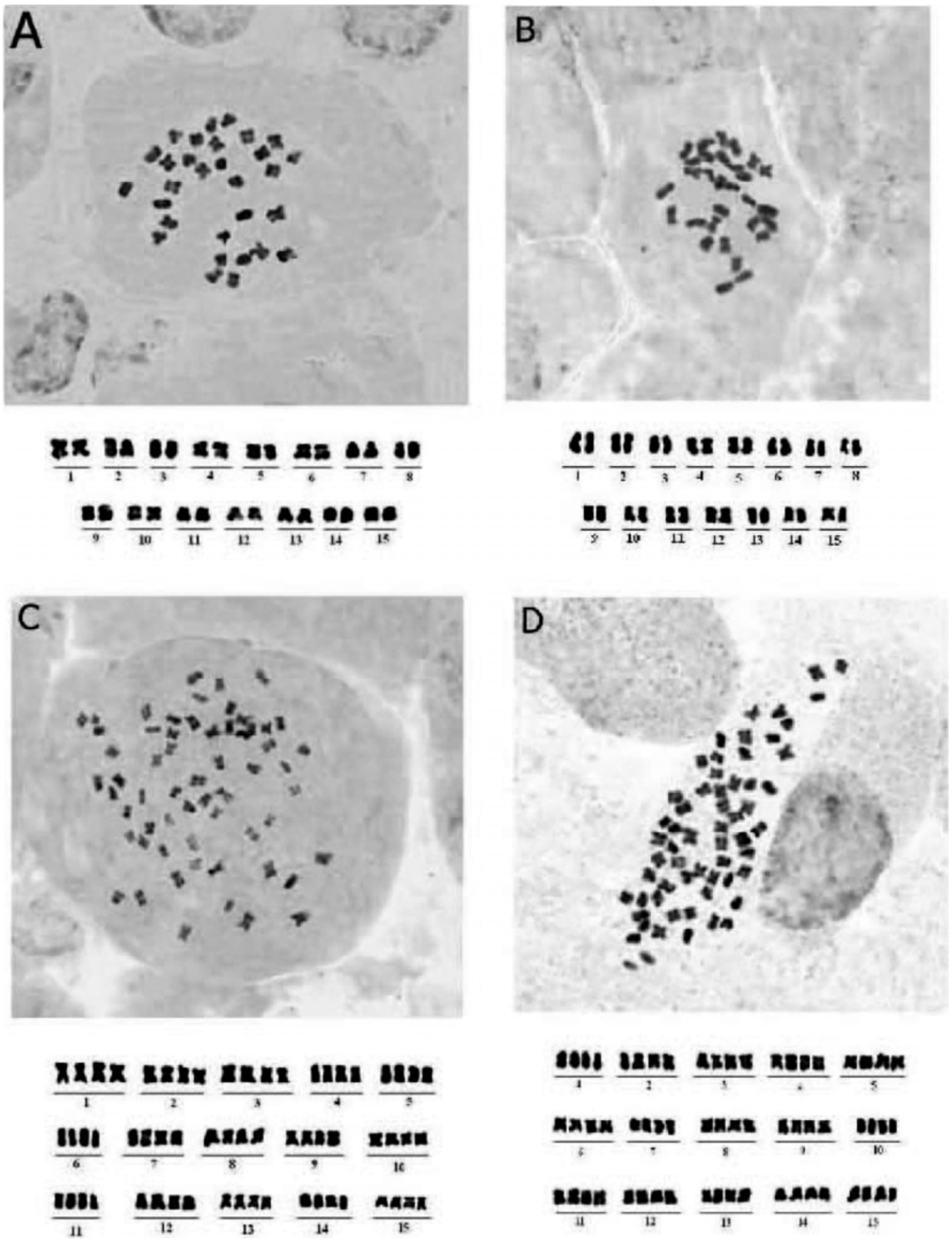

Fig. 1
Somatic chromosomes of four taxa of *Dianthus*: A) *D. crossopetalus*; B) *D. orientalis* subsp. *stenocalyx*; C) *D. orientalis* subsp. *gorganicus*; and D) *Dianthus* sp. Karyotypes of the two first species are illustrated below each metaphasic plate

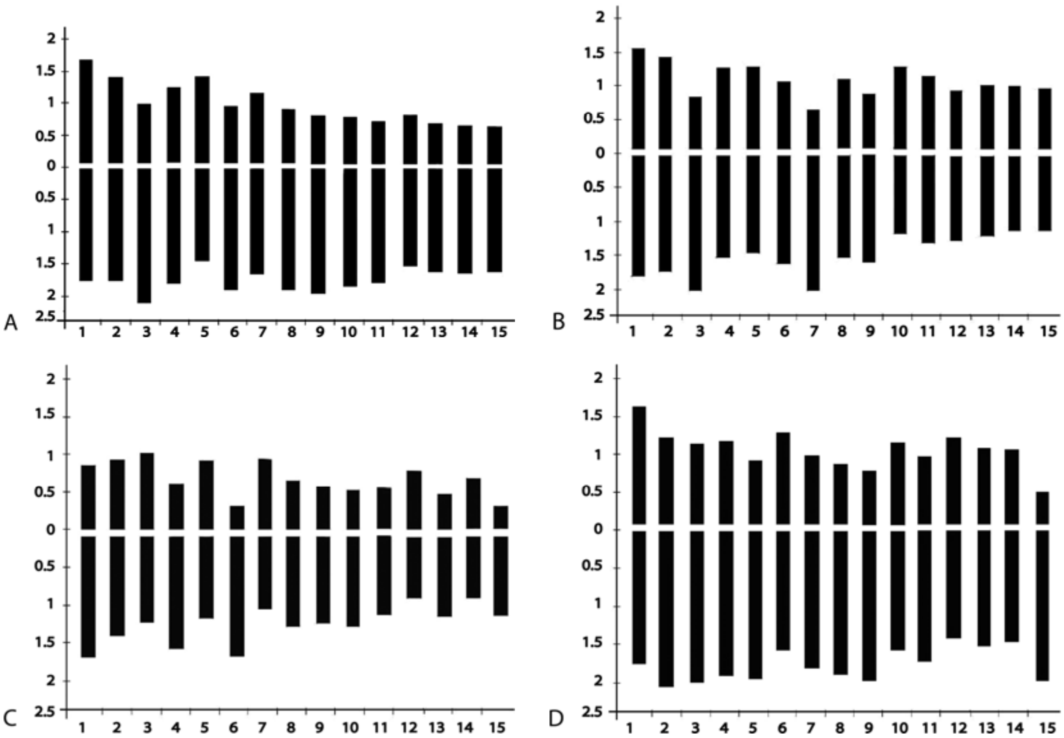

Fig. 2
Haploid idiograms of four taxa of *Dianthus*: A) *D. crossopetalus*; B) *D. orientalis* subsp. *stenocalyx*; C) *D. orientalis* subsp. *gorganicus*; and D) *Dianthus* sp.

followed by submetacentric chromosomes (36.32%), whereas subtelocentric chromosomes are rare (6.25%). The detailed information of karyotype formulae is shown in Table 2.

Asymmetry indices estimated on the basis of statistical data resolved the *Dianthus* karyotypes into the range of symmetrical to lowest asymmetrical values. Karyotype asymmetry also depends on both the relative variation in chromosome length (CV_{CL}) and heterogeneity of the centromeric index (CV_{CI}). *Dianthus orientalis* subsp. *gorganicus* is characterized by the highest value of both CV_{CL} and CV_{CI}, and the remaining species were characterized by lower values of both CV_{CL} and CV_{CI} (Table 3). In addition, the results indicate that the most asymmetrical karyotype belongs to *D. orientalis* subsp. *gorganicus* with the highest karyotype asymmetry of CV_{CL} = 16.22 and M_{CA} = 33.

According to the scatter plot (Fig. 3) obtained from the parameters CV_{CL} vs. M_{CA}, *D. crinitus* subsp. *turcomanicus* and *D. orientalis* subsp. *gorganicus* show the lowest and highest M_{CA}, respectively. Furthermore, *D. crossopetalus* and *D.* sp. exhibit relatively high M_{CA}. Among all taxa investigated in the present study, *D.* sp. and *D. orientalis* subsp. *gorganicus* demonstrate the lowest and highest CV_{CL}, respectively.

Discussion

The results obtained from the present study indicate that both subspecies of *D. orientalis* are identified with two different ploidy levels. *Dianthus orientalis* subsp. *stenocalyx* ($2n = 2x = 30$) has a widespread distributional range from east to west of Iran, whereas *D. orientalis* subsp. *gorganicus* ($2n = 4x = 60$) is distributed in narrow range in Northeastern Iran. JANAKI AMMAL and SELIGMAN (1956) reported co-occurrence of diploid and tetraploid individuals

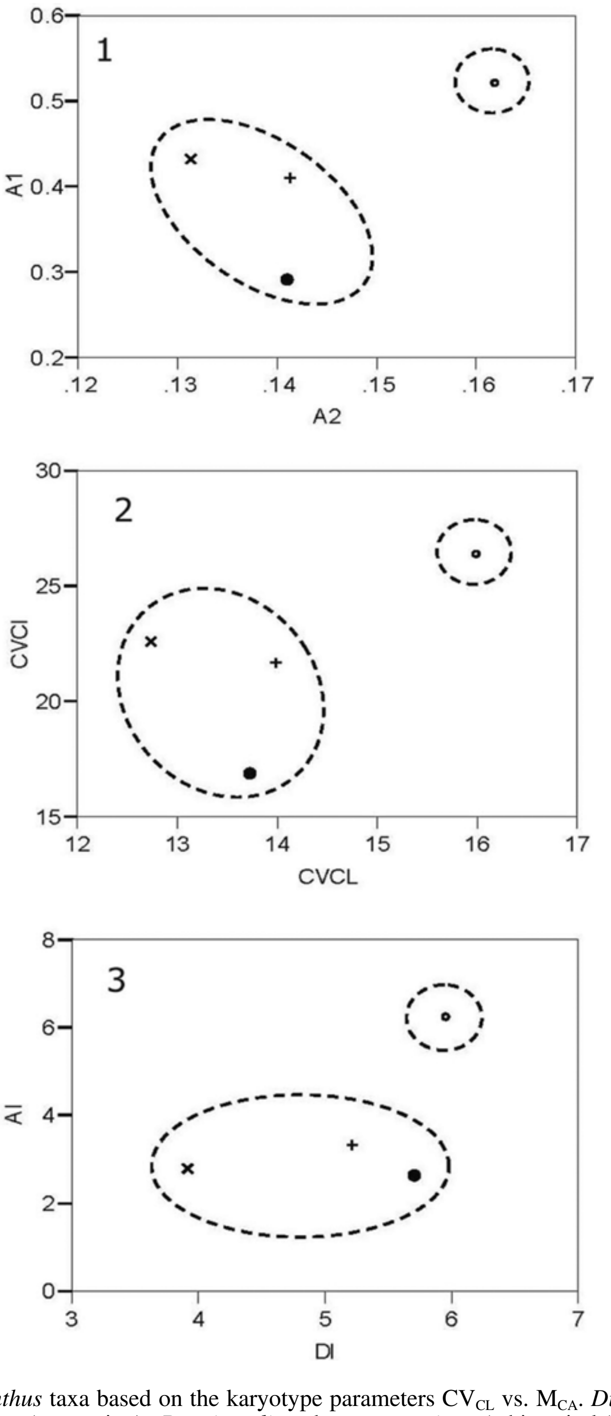

Fig. 3
Scatter diagram of *Dianthus* taxa based on the karyotype parameters CV_{CL} vs. M_{CA}. *Dianthus crossopetalus* (plus sign), *Dianthus* sp. (cross sign), *D. orientalis* subsp. *gorganicus* (white circle), *D. orientalis* subsp. *stenocalyx* (dark circle), *D. crinitus* subsp. *turcomanicus* (triangle). Karyological data of *D. crinitus* subsp. *turcomanicus* were used with permission from JAFFARI and BEHROOZIAN (2010)

within a population of *Dianthus monspessulanus* L. ($2n = 2x = 30$ and $2n = 4x = 60$). Likewise, WEISS et al. (2002) reported occurrence of tetraploid, pentaploid and hexaploid cytotypes in two subspecies including *Dianthus plumarius* subsp. *blandus* (RCHB.) HAYEK ($2n = 4x = 60$, $2n = 5x = 75$, and $2n = 6x = 90$) and *D. plumarius* subsp. *hoppei* (Port.) Hegi ($2n = 6x = 90$). They also expressed that an autopolyploidization model agrees with the occurrence of polyploids within diploid species such as *D. monspessulanus*, *D. superbus* L., *D. acicularis* FISCH. ex LEDEB., *D. petraeus* WALDST. & KIT., and putatively diploid species *D. arenarius* L., and *D. gratianopolitanus* Vill. Moreover, the report of GATT et al. (1998) on significant production of unreduced pollen grains in diploid species *D. knappii* (PANT.) ASCH. & KANITZ ex BORBÁS further strengthens this hypothesis. However, the occurrence of polyploidy and genome duplication as two major evolutionary phenomena seem to play prominent roles in speciation within *Dianthus* (CAROLINE 1957; WEISS et al. 2002; BALAO et al. 2010). Results obtained from the scatter diagrams (Fig. 3) showed that *D. orientalis* subsp. *gorganicus* could be identified by its highly asymmetric karyotypes while *D. orientalis* subsp. *stenocalyx* separated from the other taxa with the lowest asymmetry karyotype.

Karyotypes and systematics

The CV_{CL} vs. M_{CA} diagram is rather preferable than scatter diagrams based on other indices because it is better suited to demonstrate karyotype relationships among taxa, especially when chromosome size variation is negligible (PERUZZI & EROĞLU 2013). *Dianthus orientalis* subsp. *gorganicus* and *Dianthus* sp. displayed a high asymmetry index value with a high level of karyotype asymmetry (Table 3). In the other hand, *D. orientalis* subsp. *stenocalyx* and *D. crossopetalus* showed a low asymmetry index value indicating greater karyotype symmetry and stabilized genome (SHAMURAILATPAM et al. 2012). Two subspecies of *D. orientalis* were not grouped together in the scatter plot (Fig. 3). *Dianthus orientalis* subsp. *stenocalyx* with the lowest karyotype asymmetry (among the taxa included in the present study) is separated from the other taxa (Fig. 3). Conversely, *D. orientalis* subsp. *gorganicus* with the highest karyotype asymmetry shows obviously difference in morphological features of chromosomes as compared to those of the subspecies *D. orientalis* subsp. *stenocalyx*. It was also reported that increasing karyotype asymmetry could occur via a shift of the centromere position from median to subterminal or terminal chromosome and differences in the size of individual chromosomes (LIU et al. 2006). According to the karyotype difference between *D. orientalis* subsp. *stenocalyx* and *D. orientalis* subsp. *gorganicus*, it seems, however, that the taxonomic position of *D. orientalis* subsp. *gorganicus* could be considered as an independent species. Nonetheless, the exactly taxonomic position of this taxon should be investigated by further data.

There has been some uncertainty regarding the taxonomic status of *D. crossopetalus*. In Flora Kavkaza (GROSSHEIM 1930) this species is regarded as synonymous with *D. crinitus* Sm. In Flora of Turkey (DAVIS 1967) this species is recognized as a variety of *D. crinitus*, while in Flora Orientalis (BOISSIER 1975), Flora of USSR (SHISHKIN 1936), Flora Iranica (RECHINGER 1986), and ASSADI (1985), it is considered as an autonomous species. *Dianthus crinitus* is a tetraploid species ($2n = 2x = 60$) with symmetrical karyotype (JAFFARI and BEHROOZIAN 2010), while results of the present study demonstrated that *D. crossopetalus* ($2n = 2x = 30$) could be assessed as an intermediate karyotype asymmetry when compared to the other taxa (Table 3). In addition, the karyological position of *D. crossopetalus* in scatter diagram (Fig. 3) is obviously discriminated from that of *D. crinitus*. As a result, based on the karyotype asymmetry analyses (JAFFARI & BEHROOZIAN 2010 and the results of the current study) and morphometric investigation (unpublished results), our karyological interpretation on the taxonomic position of *D. crossopetalus* is that this taxon is probably an autonomous species rather than a variety or synonym of *D. crinitus*.

Dianthus sp. with an unknown taxonomic position is morphologically intermediate between *D. crinitus* subsp. *turcomanicus* and *D. orientalis* subsp. *stenocalyx.* Results of this study show that this species has its own karyotype characteristics (Fig. 3). Although karyotype features comparably provide valuable insights into taxonomic relationship (SILJAK-YAKOVLEV & PERUZZI 2012) but we suggest that more investigations are necessary to clarify its exactly taxonomic position.

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References

ARANO, H. 1963: Cytological studies in sub family *Carduoideae* (Compositae) of Japan. IX. The karyotype analysis and phylogenic consideration of *Pretya* and *Ainsliaea*. – Bot. Mag. Tokyo **79**: 32–39.

ASSADI, M. 1985: The genus *Dianthus* L. (Caryophyllaceae) in Iran. – Iranian J. Bot. **3**(1): 9–54.

BALAO, F.; SORIGUER, R. C.; TALAVERA, M.; HERRERA, J. & TALAVERA, S. 2009: Distribution and diversity of cytotypes in *D. broteri* as evidenced by genome size variation. – Ann. Bot. **104**: 965–973.

BALAO, F.; VALENTE, L. M.; VARGAS, P.; HERRERA, J. & TALAVERA, S. 2010: Radiative evolution of polyploidy races of the Iberian carnation *Dianthus broteri* (Caryophyllaceae). – New Phytol. **187**(2): 542–551.

BEHROOZIAN, M. 2010: Genetic polymorphism investigation between and within populations of *Dianthus* L. using RAPD technique and comparing with ploidy levels of populations in Khorassan Razavi province. MSc dissertation, Mashhad branch, Azad Islamic University (in Persian with summary in English).

BOISSIER, E. 1975: *Dianthus* L. in Flora Orientalis, **1**: 479–515. A. Asher & CO B.V. Amsterdam.

CAROLINE, R. C. 1957: Cytological and hybridization studies in the genus *Dianthus*. – New phytol. **56**: 81–97.

DAVIS, P. H. 1967: *Dianthus* in Flora of Turkey, **2**: 99–131, Edinburg University Press. England.

GAO, Y. D.; ZHOU, S. D.; HE, X. Y. & WEN, J. 2012: Chromosome diversity and evolution in tribe Lilieae (Liliaceae) with emphasis on Chinese species. – J. Plant Res. **125**: 55–69.

GATT, M.; HAMMETT, K. R. W.; MARKHAM, K. R. & MURRAY, B. G. 1998: Yellow pinks: intraspecific hybridization between *Dianthus plumarius* and related species with yellow flowers. – Sci. Hortic. **7**: 207–218.

GHAHREMANINEJAD, F.; JOHARCHI, M. & VITEK, E. 2012: New plant records for Khorassan province, Iran, V, with complementary notes to its flora. – Ann. Naturhist. Mus. Wien, B **114**: 59–94.

GREIHUBER, J. & SPETA, F. 1976: C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica* and *Puschkinia* (Liliaceae). – Pl. Syst. Evol. **126**: 149–188.

GROSSHEIM, A. A. 1930: *Dianthus crossopetalus* in Flora Kavkaza, **2**: 428. Tiflis, Narodnyi Komissariat Zemledeliya SSR Armenii, Baku, Izd. Az. Otd. Zak. Fil. Ak. Nauk.

HUZIWARA, Y. 1962: Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosome of Aster. – Amer. J. Bot. **49**: 116–119.

JAFFARI, A. & BEHROOZIAN, M. 2010: A cytotaxonomic study on *Dianthus* species in northeast of Iran. – Asian J. Plant Sci. **9**(1): 58–62.

JANAKI AMMAL, E. K. & SELIGMAN, R. 1956: Notes on occurrence of chromosome races in *Dianthus monspessulanus* in Northern Italy. – J. Roy. Hort. Soc. **77**: 221–223.

LARSON, S. R.; CULUMBER, C. M.; SCHWEIGERT, R. N. & CHATTERTON, N. J. 2010: Species delimitation tests of endemic *Lepidium papilliferum* and identification of other possible evolutionarily significant units in the *Lepidium montanum* complex (Brassicaceae) of western North America. – Conserv. Genet. **11**: 57–76.

LAVANIA, U. C. & SRIVASTAVA, S. 1992: A simple parameter of dispersion index that serves an adjunct to karyotype asymmetry. – J. Biosci. **17**: 179–182.

LEVAN, A. K.; FREDGA, K. & SANDBERG, A. A. 1964: Nomenclature for centromic position on chromosomes. – Hereditas **52**: 201–220.

LIU, H.; YAN, G.; SHEN, F. & SEDGLEG, R. 2006: Karyotype in *Leucodendron* (Protaceae): evidence of the primitiveness of the genus. – Bot. J. Linn. Soc. **151**: 387–394.

MARCUSSEN, T. & BORGEN, L. 2011: Species delimitation in the Ponto-Caucasian *Viola sieheana* complex, based on evidence from allozymes, morphology, ploidy levels, and crossing experiments. – Pl. Syst. Evol. **291**: 183–196.

PASZKO, B. 2006: A critical review and a new proposal of karyotype asymmetry indices. – Pl. Syst. Evol. **258**: 39–48.

PERUZZI, L. & EROĞLU, H. 2013: Karyotype asymmetry: again, how to measure and what to measure? – Comp. Cytogen. **7**(1): 1–9.

RECHINGER, K. H. 1986: Caryophyllaceae II, Flora Iranica, no. 163 – Graz.

ROMERO ZARCO, C. 1986: A new method for estimating karyotype asymmetry. – Taxon **35**: 526–530.

SEN, S. 1975: Cytotaxonomy of Liliales. – Feddes Repert. **86**(5): 255–305.

SHAMURAILATPAM, A.; MADHANAV, L.; YADAV, R. R.; BHAT, K. V. & RAO, S. R. 2012: Chromosome diversity analysis in various species of *Vigna* Savi from India. – Nucleus **55**(2): 107–114.

SHISHKIN, B. K. 1936: *Dianthus.* – In: V. L. KOMAROV (ed.), Flora of the U.S.S.R, Vol. 6: 804–861. Moskva & Leningrad.

SILGAK-YAKOVLEW, S. & PERUZZI, L. 2012: Cytogenetic characterization of endemics: past and future. – Plant Biosyst. **146**: 694–702.

STACE, C. A. 2000: Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21th centuries. – Taxon **49**: 451–477.

WATANABE, K.; YAHARA, T.; DENDA, T. & KOSUGE, K. 1999: Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae): statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. – J. Plant Res. **112**: 145–161.

WEISS, H.; DOBES, C. H.; SCHNEEWIESS, G. M. & GRIEMLER, J. 2002: Occurrence of Tetraploid and Hexaploid Cytotypes between and within Populations in *Dianthus* Sect. *Plumaria* (Caryophyllaceae). – New Phytol. **156**: 85–94.

ZUE, L. & YUAN, Q. 2011: The difference between the heterogeneity of the centromeric index and intrachromosomal asymmetry. – Pl. Syst. Evol. **297**: 141–145.

