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A karyosystematic study of Armenian *Muscari* and *Bellevalia (Hyacinthaceae)*

Abstract

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In parallel to the taxonomic treatment of *Muscari* and *Bellevalia* for the Flora of Armenia, the karyology of the Armenian representatives of these genera was studied. The obtained data confirm the cytological distinctness of *Muscari* ($x = 9$) and *Bellevalia* ($x = 4$). The karyotypes of all *Bellevalia* species investigated corresponds to the basic *Bellevalia* karyotype. Polymorphism was observed only with regard to satellites, which in *B. glauca* as a rule are found on one of the homologues of pairs 1 and 4 and vary in size, with a tandem satellite fixed on pair 1; in *B. paradoxa* a satellite is seen on the distal arm of pair 2. In octoploid *B. longistyla* a pericentric inversion and formation of a metacentric chromosome was found. *Muscari caucasicum* and *M. tenuiflorum* are characterised by the diploid chromosome number $2n = 18$, *M. sosnowskyi* has $2n = 36$, while in *M. neglectum* a hexaploid cytotype was found.

Introduction

Muscari Mill. and *Bellevalia* Lapeyr. belong to the taxonomically complex *Hyacinthaceae* genera. They are closely related both to each other and with some other genera once placed in *Hyacinthus* L. by Linnaeus. It is rather difficult to delimit these genera, in particular *Muscari, Bellevalia,* and *Hyacinthella* Schur, by using macromorphological features. Some *Bellevalia* species (*B. paradoxa* (Fisch. & C. A. Mey.) Boiss., *B. pycnantha* (K. Koch) Losinsk.) were once placed in *Muscari*. Although the plants of this group were thoroughly investigated (Feinbrun 1938-1939, Garbari 1967, Garbari & Greuter 1970, Stuart 1970, Bothmer & Wendelbo 1981, etc.), their taxonomy is still not completely clear, and they continue to attract the interest of many workers (e.g. Özhatay & al. 1991, Cowley & al. 1994, Özhatay & Johnson 1996). It has been shown that karyological features, in this group, are diagnostic at the generic level. Thus, *Bellevalia* species are characterised by the basic chromosome number $x = 4$ and have rather a stable karyotype. The chromosomes of pair 1 are largest, metacentric, with an arm ratio of 1.1-1.4; those of pair 2, second in size, are acrocentric; pairs 3 and 4 are of submetacentric chromosomes and can hardly be distinguished from each other by their size and arm ratio (Feinbrun 1938-1939, Persson & Wendelbo 1979, Bothmer & Wendelbo 1981, Özhatay & al. 1991). *Muscari* species have $x = 9$ chromosomes

and also a very peculiar, mainly bimodal karyotype (Garbari 1967, 1972, Garbari & Greuter 1970, Stuart 1970). The karyological data turned out to be crucial for taxonomic placement at the generic level, within this group, e.g. for including *Strangweja* Bertol. in the genus *Bellevalia* (Persson & Wendelbo 1979).

Different opinions have been held during the past 30 years concerning the circumscription of the genus *Muscari*. Some investigators (Garbari 1967, 1972, Garbari & Greuter 1970) have split *Muscari* into a series of separate genera, based on morphological (perianth shape, colour, etc.) and karyological features (mainly degree of bimodality), singling out *Muscarimia* Kostel. ex Losinsk., *Leopoldia* Parl., and *Pseudomuscari* Garbari & Greuter in particular. Other authors (Stuart 1970, 1985, Davis & Stuart 1980, 1984, Speta 1982) prefer to accept *Muscari* in a wider sense, recognising subgenera. The same basic number, $x = 9$, definitely distinguishes the representatives of *Muscari* s.l. from *Bellevalia* and some *Hyacinthella* species, and morphological features (shape of capsule and perianth, deciduous perianth, etc.) set them off from all *Hyacinthella* species (Stuart 1970). *Muscari* subg. *Muscari* (≡ subg. *Botryanthus* (Kunth) Zahariadi), subg. *Leopoldia* (Parl.) Peterm., and subg. *Moscharia* (Baker) Chouard are distinguished by karyotype morphology (degree of bimodality), but in some cases these distinctions are rather dim (Stuart 1970, Speta 1982, Cowley & al. 1994), and for the representatives of *M.* subg. *Pseudomuscari* D. C. Stuart – a group not yet sufficiently investigated and apparently polyphyletic (Davis & Stuart 1984) – different karyotypes have been reported (Stuart 1970).

While completing the taxonomic treatment of the genera *Muscari* and *Bellevalia* for Volume 10 of the Flora of Armenia (a treatment that was initiated by the late Dr A. Scjan, before her death) we revised critically the classification of the Armenian species. During the last decades, numerous representatives of *Bellevalia* and *Muscari* from neighbouring countries – Turkey, Iran, Iraq – had been studied karyologically (Al-Mudarris 1973, Wendelbo 1990, Dalgiç 1991, Cowley & al. 1994, Özhatay & Johnson 1996, etc.). Against this background, the karyological investigation of the Armenian representatives of these genera was a promising complement. There had been a number of karyological studies on Armenian material (Pogosjan 1965, 1966, 1967, 1975, 1981; Zahar'eva & Makusenko 1969, etc.). However, because of the extreme taxonomic complexity of the genera and the lack of a modern taxonomic treatment for Armenia, some data needed a verification. This is why we found it necessary to start our taxonomic treatment of the Armenian representatives of the genera by a karyological study.

Material and methods

Plants collected in the field in 1996 and 1997 were cultivated in Herbarium Department plot for study, then transferred the Garden of the Institute of Botany, Erevan. Older collections were taken from the Garden's holdings. The origin of the specimens studied is mentioned in Table 1. Vouchers have been deposited at ERE.

Mitotic metaphases of meristematic cells of bulb root tips were studied. The root tips were pre-treated for 2 h with colchicine (0.2 %), then fixed in Battaglia fluid 5: 1: 1: 1 for 10 min at room temperature. After hydrolysis in 1: 1 HCl (20 min at room temperature), the root tips were stained in Feulgen.

Table 1. Origin of the material studied, and chromosome numbers ($2n$) found.

Taxon	Origin	Coll. date	Collector	$2n$ =
Bellevalia longistyla	Ararat region, Urtz Mt chain	c. 1946	*Ahverdov & Mirzoeva*	32
glauca	Ararat region, Gorovan sands	1950	*Ahverdov & Mirzoeva*	8
	Tumanian region, Dsegh, edge of the gorge of Debet river	24 May 1997	*Nersesian*	8
	Idjevan region, Ahadzur	3 May 1997	*Gabrielian*	8
fominii	Tumanian region, Dsegh, edge of the gorge of Debet river	24 May 1997	*Nersesian*	8
paradoxa	Spitak pass, grassy slopes	23 May 1997	*Nersesian*	8
pycnantha	Vaik	1946	*Ahverdov & Mirzoeva*	16
	Aparan region, Mt Arailer	19 Jun 1997	*Nersesian*	16
	Sisian region, between Goraik and Vorotan pass	27 May 1997	*Gabrielian*	16
Muscari tenuiflorum	Geghard	1948	*Ahverdov & Mirzoeva*	18
	Tumanian region, Dsegh, edge of the gorge of Debet river	13 Jun 1997	*Nersesian*	18
	Aparan region, between Apnagjugh and Erindjatap, rocks	19 Jun 1997	*Manakian*	18
caucasicum	Erevan Botanical Garden	unknown	*Ahverdov & Mirzoeva*	18
	Ararat region, Eranos chain, Mt Ilkasar	30 Apr 1997	*Gabrielian & Manakian*	18
	Hosrov reserve	20 May 1996	*Oganezova*	18
sosnowskyi	Spitak pass, grassy slopes	23 May 1997	*Nersesian*	36
	Idjevan region, Vurghun, forest opening	16 Apr 1997	*Nersesian*	36
neglectum	Kafan	1945	*Mirzoeva*	54
	Erevan, Avan	20 Mar 1998	*Nersesian*	54

Results

So far, according to our results (as expressed in the synonymies given below), 7 species of *Bellevalia* (a W Mediterranean, SW Asian and Caucasian genus of c. 50 species) and 7 species of 2 subgenera of *Muscari* (c. 60 mainly European, Mediterranean and W Asian species) are known to grow in Armenia. Two Caucasian species, *M. alpanicum* Schchian and *M. dolichanthum* Woronow & Tron, were indicated from Armenia in error in Tahtadzjan (1990): the material, in fact, originated from elsewhere in the Caucasus.

Bellevalia fominii Woronow – This species, of which no Armenian material had been investigated cytologically before, was found to be a diploid ($2n = 8$; Fig. 1B).

Bellevalia glauca (Lindl.) Kunth (*B. ciliata* var. *glauca* (Lindl.) Boiss.; incl. *B. albana* Woronow, *B. wilhelmsii* (Steven) Woronow, *B. montana* (K. Koch) Boiss., and *B. makuensis* Woronow ex Grossh.). – We have confirmed the earlier, diploid ($2n = 8$) count by Pogosjan (1975, sub *B. makuensis*). As a rule, satellites are borne on one homologue of chromosome pairs 1 and 4. These satellites vary significantly in size. A tandem satellite is affixed to pair 1 (Fig. 1F).

Bellevalia longistyla (Miscz.) Grossh. (incl. *B. araxina* Woronow). – Found to be octoploid

($2n$ = 32), confirming an earlier count by Pogosjan (1975, sub *"B. wilhelmsii"*). The diploid number ($2n$ = 8) mentioned by Pogosjan (1975) for *B. longistyla* is probably due to labelling confusion and refers to *B. glauca*. A pericentric inversion (apparently in pair 2) and formation of a metacentric chromosome was observed (Fig. 1A).

Bellevalia paradoxa (Fisch. & C. A. Mey.) Boiss. (*Muscari paradoxum* (Fisch. & C. A. Mey.) K. Koch). – Our diploid count ($2n$ = 8) confirms the data of Voskanjan (1974). A satellite is found on the distal arm of pair 2 (Fig. 1E). Pogosjan's (1967) count of $2n$ = 18 for *M. paradoxum* was refuted as obviously incorrect by Voskanjan (1974) already.

Bellevalia pycnantha (K. Koch) Losinsk. (*Muscari pycnanthum* K. Koch). – Confirming an earlier count by Zahar'eva & Makusenko (1969) we found that this species is tetraploid ($2n$ = 16; Fig. 1D). Again, Pogosjan's (1967) count of 2n = 18 for *M. pycnanthum* was refuted as obviously incorrect by Zahar'eva & Makusenko (1969) already.

Bellevalia sarmatica (Georgi) Woronow (incl. *B.speciosa* Woronow). – This diploid species ($2n$ = 8, according to Pogosjan & al. 1969 and Pogosjan 1975) was not studied by us.

Bellevalia zygomorpha Woronow – Similarly, this diploid ($2n$ = 8, according to Pogosjan 1981) was not available to us for study.

Muscari (subg. *Leopoldia*) *caucasicum* (Griseb.) Baker – This species is characterised by a diploid chromosome number ($2n$ = 18; Fig. 1G), which confirms earlier counts on Armenian plants by Pogosjan (1966).

Muscari (subg. *Leopoldia*) *longipes* Boiss. (incl. *M. atropatanum* Grossh.). – Again a diploid ($2n$ = 18, according to Pogosjan 1965, 1967), which was not studied by us.

Muscari (subg. *Leopoldia*) *tenuiflorum* Tausch – A diploid ($2n$ = 18; Fig. 1C), as already mentioned by Pogosjan (1965, 1967).

Muscari (subg. *Muscari*) *neglectum* Guss. (incl. *M. leucostomum* Woronow) was found to be hexaploid ($2n$ = 54; Fig. 1H). This number had already been reported by Pogosjan (1965, sub *M. leucostomum*) for a population from the Sorza in the Sevan region, while for other Armenian populations he gave $2n$ = 45). Surprisingly, in a later paper (Pogosjan 1967), he did not again mention the Sorza plant and considered his former pentaploids as tetraploid ($2n$ = 36).

Muscari (subg. *Muscari*) *pallens* (M. Bieb.) Fisch. – This tetraploid species ($2n$ = 36; Pogosjan 1966) was not available to us for study.

Muscari (subg. *Muscari*) *sosnowskyi* Schchian (*'M. polyanthum'* auct. fl. caucas., non Boiss.). – This species appears to be consistently tetraploid ($2n$ = 36), as already reported by Pogosjan (1965).

Muscari (subg. *Muscari*) *szovitsianum* Baker (*M. armeniacum* var. *szovitsianum* (Baker) Stuart). – We have not so far studied this plant, which was found to be tetraploid or pentaploid ($2n$ = 36, 45) by Pogosjan (1965, 1967), Pogosjan & al. (1969), and Zahar'eva & Makusenko (1969).

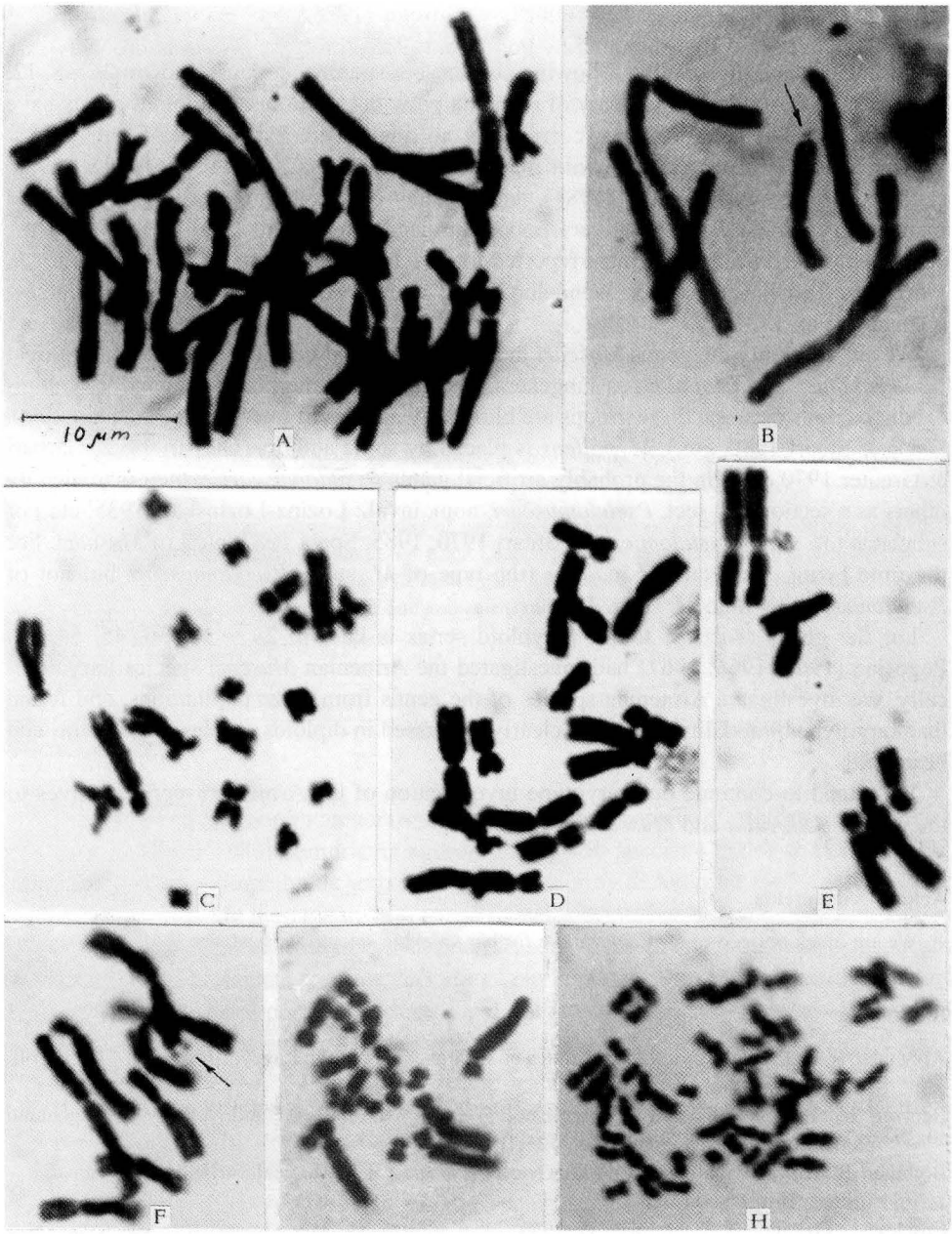

Fig.1. Somatic metaphase plates of Armenian representatives of *Bellevalia* and *Muscari*. – A, *B. longistyla* (Misz.) Grossh. ($2n = 32$); B, *B. fominii* Woronow ($2n = 8$); C, *Muscari tenuiflorum* Tausch ($2n = 18$); D, *Bellevalia pycnantha* (C. Koch) Losinsk. ($2n = 16$); E, *B. paradoxa* (Fisch et C. A. Mey.) Boiss. ($2n = 8$); F, *B. glauca* (Lindl.) Kunth ($2n = 8$); G, *M. caucasicum* (Griseb.) Baker ($2n = 18$); H, *M. neglectum* Guss. ($2n = 54$). – Satellites are indicated by arrows. Scale bar = 10 µm (valid for all figures).

Discussion and conclusions

For the genus *Bellevalia* the following chromosome number series is known: $2n = 8$, 12, 16, 24, 32, and 40. On the territory of Armenia grow di-, tetra- and octoploid plants have been found. Their karyotypes correspond to the basic karyotype of the genus. Polymorphism is observed only with regard to satellites, but as noted by Bentzer & al. (1972), Bothmer & Wendelbo (1981), and Özhatay & al. (1991), satellite position and number, in *Bellevalia,* are not a very important diagnostic feature. Satellites on the acrocentric chromosomes (pair 2), here reported for *B. paradoxa,* was so far only known for *B. tabriziana* Turrill (Persson & Wendelbo 1979) and *B. edirnensis* Özhatay & Mathew (Özhatay & al. 1991).

We are accepting the genus *Muscari* in a wide sense and consider it reasonable to treat *"Botryanthus"* and *Leopoldia* as subgenera, since the macromorphological and karyological distinctions between these groups are blurred by a series of intermediates (Stuart 1970, Davis & Stuart 1980, etc.). *M. pallens* is placed by some authors (Garbari 1972, Garbari & Greuter 1970, etc.) in the probably artificial genus *Pseudomuscari,* which is treated by others as a section (*M.* sect. *Pseudomuscari,* nom. inval.: Lozina-Lozinskaja 1935, etc.) or subgenus (*M.* subg. *Pseudomuscari:* Stuart 1970, 1985, Speta 1982, etc.) of *Muscari.* For the time being, we retain *M. pallens* (the type of *M.* subg. *Pseudomuscari* but not of *Pseudomuscari*) within *M.* subg. *Muscari.*

For the genus *Muscari,* too, a polyploid series is known: $2n = 18$, 36, 45, 54, 72. Pogosjan (1965, 1966, 1967) had investigated the Armenian *Muscari* species karyologically. We investigated Armenian species of the genus from other populations, and found that karyotype bimodality was more clearly expressed in diploids and less so in tetra- and hexaploids.

We intend to continue our karyotype investigation of the Armenian representatives of the genera *Bellevalia* and *Muscari.*

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