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RESEARCH ARTICLE

The nuclear DNA content, ploidy, and chromosome numbers in some species of *Nitraria* and associations with pollen characteristics

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Abstract For the first time, nuclear genome size and ploidy of five *Nitraria* species from 49 populations were examined by flow cytometry. All populations were also analyzed for the chromosome number. We identified significant differences in the 2C nuclear DNA content among the analyzed species, and this parameter correlated with their ploidy. Diploid (2n = 2x = 24) species *N. sibirica* and *N. tangutorum* were found to have smaller genome size (1.24–1.34 and 1.57–1.65 pg) as compared to tetraploid (2n = 4x = 48) species *N. komarovii* (2.23–2.32 pg), *N. pamirica* (3.10–3.30 pg), and *N. schoberi* (2.93–3.39 pg). Intra-population genome size variation was found in examined species, varying from 1.01 to 1.08-fold. *Nitraria sibirica* has lower inter-population variation of the 2C (1.08-fold) as compared to *N. schoberi* (1.16-fold). All the *Nitraria* species are mixoploids. It turned out that an increase in the equatorial axis of *Nitraria* pollen is associated with an increase in 2C and 1Cx. An exception is *N. komarovii*, with its intermediate 2C DNA content and the smallest pollen grains. In general, our data confirm Bobrov's previous views on the system of the genus *Nitraria*, which distinguished ser. *Sibiricae* and ser. *Schoberianae* in sect. *Nitraria.*

Keywords C-value · Flow cytometry · Chromosome number · Mixoploidy · Pollen grain

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Introduction

The nucleus of plant cells contains the material carrying genetic information. Therefore, studying the nuclear DNA content and ploidy is of fundamental importance for answering complex biological questions. Genomes of organisms at the same level of organization are known to vary substantially in the DNA content. The genome size variation is characterized by a difference in the number of chromosomes, nuclear DNA content, and various repetitive DNA sequences (Sedelnikova 2015).

Research has demonstrated the correlations of genome size with breeding systems and species genesis (Albach and Greilhuber 2004; Weiss-Schneeweiss et al. 2005). Intraspecific variation of genome size has been found among plant specimens from geographically separated populations (Jakob et al. 2004; Schmuths et al. 2004; Smarda and Bures 2006), and the nuclear DNA content correlates with environmental factors (Kalendar et al. 2000; Knight and Ackerly 2002) and plant phenotypic traits (Knight

et al. 2005; Murray et al. 2005; Beaulieu et al. 2007). The amount of nuclear DNA can influence the phenotype through regulatory processes in the genome and via simple physical effects of the DNA material at the cellular level (Moeller 2018). These effects are known to result in changes in cell cycle duration, pollen maturation timing, and pollen grain size (Bennett 1972, 1987; Leitch and Bennett 2007; Beaulieu et al. 2008; Lomax et al. 2009), guard and epidermal cell size (Snodgrass et al. 2017), and seed size (Beaulieu et al. 2007). Plant ploidy is reported to be related to pollen grain size (Sousa et al. 2013; Srisuwan et al. 2019). An analysis of genome size in terms of phylogenetic relationships among individual taxa revealed that evolutionary trajectories of genome size and pollen size are probably unrelated (Moeller 2018).

Plants of the genus *Nitraria* L. are halophytes and are usually confined to intrazonal communities. The considerable isolation of numerous populations of *Nitraria* species from each other makes this genus a unique model plant for research on processes of genetic differentiation.

In this regard, despite the small number of taxa (10–12 species), no obvious patterns in the genus *Nitraria* (Bobrov 1965; Khalkuziev 1990; Pan et al. 1999; Banaev et al. 2023) and no clear idea of species genesis have been published so far. The issues about the center and time of origin of the genus *Nitraria* and pathways of species dispersal also remain debatable. The presence of its representatives in Australia (*N. billardierei* DC.) and the genus's biology led Komarov (1908) to believe that *Nitraria* had originated on saline sea coasts of Gondwana as part of tropical flora before the formation of the Asian and Australian deserts. Later on, almost all researchers believed in phylogenetic antiquity of the genus (Il'in 1944, 1958). At the same time, Korovin (1935) and Bobrov (1946) assumed that the center of origin of the genus *Nitraria* could be deserts of Central Asia, where at present, there is a center of its diversity. Popov (1927) suggested that a new habitat of the genus *Nitraria* had formed in Central Asia on the basis of data about *N. schoberi* L. and *N. retusa* (Forsk.) Aschers, already existing on the African continent in the Cretaceous period. Pan et al. (1999) assumed an African-Mediterranean origin of the genus in accordance with the distribution of diploid *Nitraria* species. Nonetheless, the same species has been reported to belong to a diploid or a tetraploid group (Pan et al. 2003; Temirbayeva and Zhang 2015; Marhold et al. 2020). The latest fossil pollen evidences suggest a new evolutionary history of Nitraria (Woutersen et al. 2023). Previous molecular genetic research of some *Nitraria* species from 31 populations in Siberia, the Republics of Kazakhstan and Tajikistan showed a clear separation of a diploid (ser. *Sibiricae* Bobrov) and a tetraploid (ser. *Schoberianae* Bobrov) species (Banaev et al. 2023). Biogeographical analysis suggest that the Central Asian species *N. sphaerocarpa* Maxim. is the oldest species (Paleocene), and the dispersal started from Central Asia to Africa (since the Oligocene) and to Siberia (5.95 Mya) (Late Miocene) and Australia (2.61 Mya) (Late Pliocene) (Zhang et al. 2015). However, as revealed by integration of fossil pollen morphology and molecular data, the split between *N. sphaerocarpa* and the other *Nitraria* types did not happen before the Miocene and modern species of *Nitraria* originate during the Late Miocene (Woutersen et al. 2023).

The nuclear DNA content, estimated by flow cytometry, is an essential genome feature together with the chromosome number (Doležel and Bartoš 2005). Karyotype analysis is an important method for revising species classification and studying phylogenetic relationships (Hong 2021). Flow cytometry can be considered a useful method for understanding taxonomic relationships (Bourge et al. 2018).

Available *Nitraria*-related cytological information that is important for understanding Nitrariaceae evolution is very scarce (Tarnavshi 1948; Reese 1958; Pan et al. 2003; Banaev et al. 2018a,b; Voronkova et al. 2018; Marhold et al. 2020, 2021, 2022). The Plant DNA C-values Database (https://cvalues.science.kew.org/; accessed on 20 June 2023) does not contain information on genome sizes of *Nitraria* species. Determination of genetic variability of wild plants helps to conserve and use them (Khaleghi and Khadivi 2023).

The purpose of this study was to analyze intra- and inter-population variations of the 2C DNA content and chromosome number and their correlations with pollen grain size of *Nitraria* species.

Materials and methods

Plant materials

Seeds of five species of the genus *Nitraria* (*N. sibirica* Pall., *N. schoberi*, *N. pamirica* L.I. Vassiljeva, *N.*

komarovii Iljin & Lava ex Bobrov, and *N. tangutorum* Bobrov) were collected in 49 natural populations in Russia, Tajikistan, and Kazakhstan from 2009 to 2021 (Table 1, Fig. 1). Twenty-four populations of *N. sibirica*, 22 populations of *N. schoberi*, and one population of each of three species (*N. pamirica*, *N. komarovii*, and *N. tangutorum)* were investigated. The vouchers are stored in herbarium NSK (the Dendrology Laboratory of the CSBG SB RAS, Novosibirsk, Russia) and available in the digital herbarium of the CSBG SB RAS, NSK (http://herb.csbg.nsc.ru:8081).

Fresh leaves of *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg) and *Raphanus sativus* L. 'Saxa'32 (2C = 1.11 pg) (Doležel et al. 1998) grown from seeds obtained from the Centre of Plant Structural and Functional Genomic at the Institute of Experimental Botany of the Academy of Sciences, AS CR (Olomouc-Holice, Czech Republic) (Doležel et al. 1992) were used as an internal standard.

Flow Cytometry (FCM)

All FCM procedures were performed in the Central Siberian Botanical Garden SB RAS (Novosibirsk, Russia). The analysis was performed on a Cy Flow Space instrument (Sysmex Partec, Norderstedt, Germany) with a 532 nm laser source. The DNA content of plants was determined by FCM with staining of isolated nuclei with propidium iodide (PI). The seeds of *Nitraria* were analyzed following a previously developed methodology (Banaev et al. 2018b). At least 10 plants were randomly selected in each population of *Nitraria* species for genome size variation analysis.

Nitraria plant embryos extracted from the seed were ground up using a razor blade in plastic Petri dishes together with an appropriate amount of an internal standard (*P. sativum* or *R. sativus*) in 500 µL of chilled extraction buffer (Nuclei Extraction Buffer) (Sysmex Partec, Norderstedt, Germany) according to the manufacturer's protocol. The plant tissue samples were incubated at room temperature for 2 min. After the extraction of the nuclei, the samples were passed through a 50 µm Celltrics Partec disposable filter (Sysmex Partec, Norderstedt, Germany), followed by the addition of 2 mL of the Staining Solution (for staining) consisting of Staining Buffer (Sysmex Partec, Norderstedt, Germany), PI (50 µg/mL), and RNase A (50 µg/mL). The staining was performed at room temperature in a dark place for 15 min. The prepared nuclei samples were stored in a refrigerator for no more than 4 h.

Next, 15,000 FCM events were collected [the required number is 5,000 to 20,000 (Galbraith et al. 1998; Doležel and Bartoš 2005)] three times per sample; the coefficient of variation of the mean was less than 5%. A relative nuclear DNA content was calculated based on a linear relation between fluorescence signals from stained nuclei of tested specimens and the internal standard (Doležel et al. 2007) and was expressed as an index.

The chromosome number (CHN)

Seeds were stratified on moist filter paper for 1 month and germinated at 27–28 °C. For fixation, roots 0.5–2.5 cm long were selected. After that, 30 individual plant specimens from each population of *Nitraria* were subjected to the determination of chromosome numbers (2n). Cytological procedures were performed on root meristem. Actively growing seedlings were kept for 3 h at room temperature in a 0.2% colchicine solution and fixed in an ethanol: acetic acid solution (3:1). Seeds were fixed between 10:00 and 11:00 AM (UTC + 7). The preparations were stained with acetohematoxylin according to Smirnov (1968). Chromosome examination and photodocumentation of metaphase plates were carried out under an Axioscope 40 microscope equipped with an AxioCam MRc 5 color digital camera and AxioVision v.4.8 software (Carl Zeiss Ltd., Göttingen, Germany) and under an Axioscope A1 microscope with an Axiocam 506 color digital camera and ZEN 2012 (blue edition) software (Carl Zeiss Ltd., Göttingen, Germany).

The most common number is taken as the value of the chromosome number. The following notation is used for chromosome numbers: modal number and numbers determined.

Morphometric results on pollen grains

For a comparative analysis of the DNA content and pollen characteristics, pollen morphometric data were borrowed from a previously published article (Tomoshevich et al. 2022). The following traits were characterized: polar axis (P, µm), equatorial axis (E, µm), and the P/E ratio.

Table 1 Voucher specimens of *Nitraria*

Name of the population	Voucher information	Herbarium specimen number
	N. sibirica	
Noven'koe	Russia, Altai Krai, vicinity of Noven'koe Village, 23 July 2020	NSK3001267
Kulunda	Russia, Altai Krai, on the shore of Lake Kulundinskoye, 08 August 2011	NSK3000987
Bele	Russia, Republic of Khakassia, the shore of Lake Bele, 08 August 2012	NSK3001261
Balansor	Russia, Altai Krai, on the shore of Lake Balansor, 26 July 2020	NSK3001257
Dzhira	Russia, Altai Krai, eastern shore of Lake Dzhira, 27 July 2020	NSK3001788
Gornyak	Russia, Altai Krai, vicinity of Gornyak Village, 23 July 2020	NSK3001268
Kuchuk	Russia, Altai Krai, vicinity of Nizhny Kuchuk Village, 27 July 2020	NSK3001778
Yarovoe	Russia, Altai Krai, southern shore of Bolshoye Yarovoe Lake, 28 July 2020	NSK3001253
Shara-Nur	Russia, Tuva Republic, the shore of Lake Shara-Nur, 04 August 2021	NSK3001733
Rubtsovsk	Russia, Altai Krai, vicinity of Rubtsovsk City, 06 August 2011	NSK3000992
Hadyn	Russia, Tuva Republic, northern shore of the Lake Hadyn, 26 July 2011	NSK3000912
Turan	Russia, Tuva Republic, vicinity of Turan village, the shore of Lake Beloe, 26 July 2011	NSK3001778
Ulug-Kol	Russia, Khakassia Republic, the shore of Lake Ulug-Kol, 08 August 2012	NSK3001262
Pospeliha	Russia, Altai Krai, vicinity of Pospelikha Village, 22 July 2020	NSK3001256
Uglovskoye	Russia, Altai Krai, vicinity of Uglovskoye Village, 26 July 2020	NSK3001498
Tassor	Russia, Altai Krai, the shore of Lake Big Tassor, 26 July 2020	NSK3001260
Chinkussor	Russia, Altai Krai, the shore of Lake Chinkussor, 26 July 2020	NSK3001252
Bagan	Russia, Novosibirsk Oblast, vicinity of Bagan Village, 29 July 2020	NSK3001251
Chagan	Russia, Novosibirsk Oblast, the shore of Lake Chagan, 03 August 2009	NSK3001288
Kosh-Agach (Chuya)	Russia, Altai Republic, 13 km from the Kosh-Agach Village, on the shore of a lake in the valley of the Chuya River, 10 August 2016	NSK3001007
Kosh-Agach (Chaganka)	Russia, Altai Republic, vicinity of Kosh-Agach Village, bank of the Chaganka River, 06 July 2018	NSK3001270
Balhash	Kazakhstan, Almaty Region, on the shore of Lake Balkhash, sandy desert, 25 July 2013	NSK3000921
Kurti	Kazakhstan, Almaty Region, north of Kurty Village, bank of the Kurty River, 20 July 2014	NSK3001782
Koktal	Kazakhstan, Almaty Region, vicinity of Koktal Village, 30 July 2013	NSK3000989
	N. schoberi	
Sariozek	Kazakhstan, Almaty Region, 30 km north of Saryozek Village, 29 July 2014	NSK3000969
Basshi	Kazakhstan, Almaty Region, vicinity of Bashshi Village, 30 July 2013	NSK3000998
Aidarli	Kazakhstan, Almaty Region, Zhambylskii District, 17 km south of Aydarly Village, 21 August 2017	NSK3000958
Koktal	Kazakhstan, Almaty Region, vicinity of Koktal Village, 30 July 2013	NSK3000999
Lepsi	Kazakhstan, Almaty Region, on the bank of the Lepsi River in outskirts of Lepsi Village, 28 July 2013	NSK3000997
Bagan	Russia, Novosibirsk Oblast, on the terrace of Lake Bagan, 29 July 2020	NSK3001254
Kaspii	Kazakhstan, Mangistauskaya Oblast, vicinity of Aktau City, on sandy mound, 12 June 2012	NSK3000979
Actau	Kazakhstan, Mangistauskaya Oblast, vicinity of Aktau City, 12 June 2012	NSK3000978
Tigen	Kazakhstan, Mangistauskaya Oblast, 6 km south of Tigen Village, 10 August 2017	NSK3000913
Krim	Crimea, on the sandy coast of the Black Sea in Dvuyakornaya Bay, 16 September 2013	NSK3000960
Pyandzh1	Tajikistan, on the sandy bank of the Panj River, 08 August 2014	NSK3000994
Pyandzh2	Tajikistan, 10 km north of Ishkashim Village, on the bank of the Panj River, 08 August 2014	NSK3000993

Table 1 (continued)

Name of the population	Voucher information	Herbarium specimen number
	N. sibirica	
Kulunda	Russia, Altai Krai, on the shore of Lake Kulundinskoe, 08 August 2011	NSK3000987
Malinovoe	Russia, Altai Krai, on the shore of Lake Malinovoe, 27 July 2020	NSK3001250
Karalat	Russia, Astrakhan Oblast, vicinity of Karalat Village, 26 July 2018	NSK3000937
Baskunchak	Russia, Astrakhan Oblast, vicinity of Nizhnii Baskunchak village, 28 July 2018	NSK3000929
Balhash	Republic of Kazakhstan, Almaty Region, on the shore of Lake Balkhash, sandy desert, 25 July 2013	NSK3001000
Karatal	Kazakhstan, Almaty Region, vicinity of Ushtobe City, on the terrace of the Karatal River, 01 July 2015	NSK3000923
Alakol	Kazakhstan, Jambyl Region, the shore of Lake Alakol, 21 August 2017	NSK3000942
Taskarasu	Kazakhstan, Almaty Region, vicinity of Taskarasu Village, 01 August 2013	NSK3001001
Kuchuk	Russia, Altai Krai, vicinity of Nizhny Kuchuk Village, 27 July 2020	NSK3001265
Xinjiang	China, Xinjiang Uygur Autonomous Region, the vicinity of Altai City, saline land, 22 September 2012	NSK3000914
	N. komarovii	
Balhash	Kazakhstan, Almaty Region, on the shore of Lake Balkhash, sandy desert, 26 July 2013	NSK3000926
	N. tangutorum	
Ningxia-Hui	China, Ningxia-Hui Autonomous Region, sandy desert, 25 August 2015	NSK3000916
	N. pamirica	
Shaimak	Tajikistan, Eastern Pamir, on the cliff of the Djilga River, 10 August 2014	NSK3001238

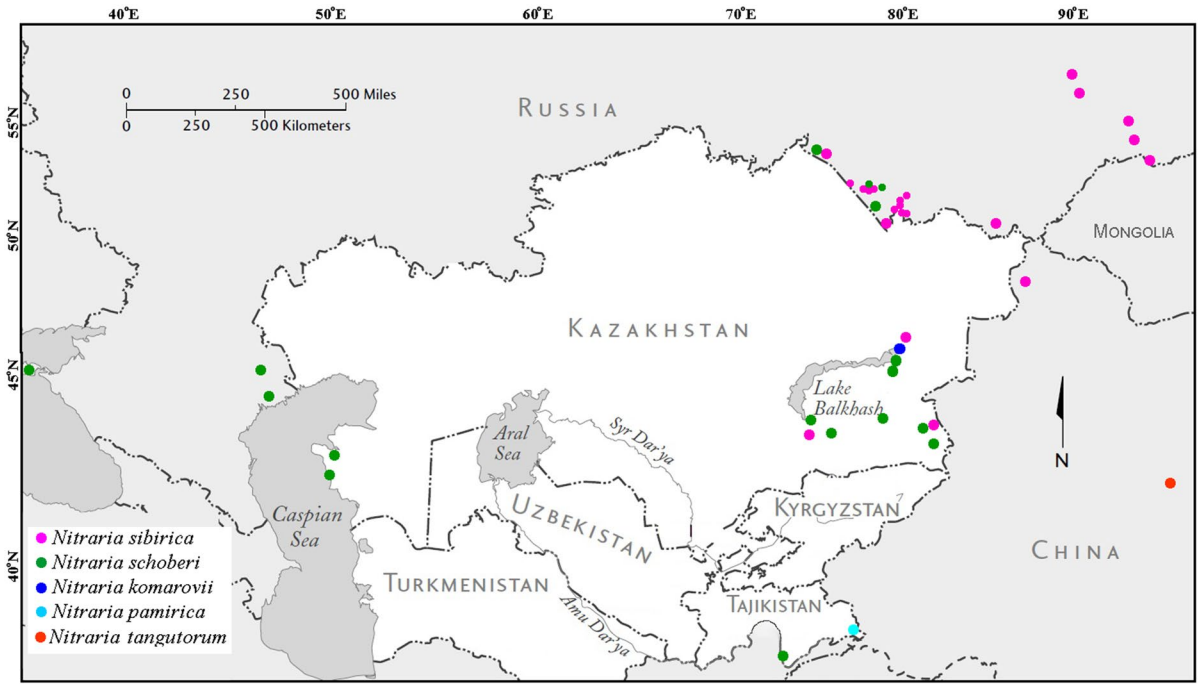

Fig. 1 The map of sampling sites of species from the genus *Nitraria*

Statistical analysis

These procedures were carried out in Microsoft Excel 7.0 and STATISTICA 6.0 (correlation analysis, LSD test, Newman–Keuls test, ANOVA, at $p \leq 0.05$) (StatSoft Inc., Tulsa, OK, USA). The images and data obtained by FCM were analyzed in the CyFlow® Space software (Sysmex Partec, Norderstedt, Germany). The findings are presented as mean values with standard error, standard deviation, and a coefficient of variation (mean ± SE; mean ± SD; CV, %). To calculate the 1C value, 2C was divided by two, and to calculate 1Cx, 2C was divided by ploidy.

Results

Genome size and ploidy

FCM revealed three peaks in *N. sibirica*, *N. komarovii*, *N. tangutorum*, and *N. pamirica* and four peaks in *N. schoberi* (Fig. 2). The first peaks in *N. schoberi* and *N. pamirica* proved to be internal standard peaks G1 and G2 (*R. sativus*). The next two peaks represented the G1 and G2 peaks of *Nitraria* (Fig. 2c, d). In *N. sibirica*, *N. komarovii*, and *N. tangutorum*, the first peaks were peaks G1 and G2 of the *Nitraria* plant itself, followed by the internal standard (*P. sativum*) (Fig. 2a, b, e). Figure 2f shows the histograms of *N. sibirica*, *N. komarovii*, and *N. schoberi* without the internal standard and illustrates the differences in genome size among these species.

According to the FCM results, the species in question can be classified into diploids (2n = 2x = 24) and tetraploids (2n = 4x = 48). Statistical analysis revealed four isolated groups based on 2C genome size (Fig. 3). The lowest DNA content was found in *N. sibirica* (1.24–1.34 pg), and the highest in *N. pamirica* (3.10–3.30 pg) and *N. schoberi* (2.93–3.39 pg).

The lowest intra-population variation of the DNA content is characteristic of *N. sibirica* (1.01–1.02-fold), and the highest variation is characteristic of *N. schoberi* (1.03–1.08-fold). In *N. komarovii*, *N. tangutorum*, and *N. pamirica*, the intra-population variation of genome size proved to be 1.04-, 1.05-, and 1.06-fold, respectively (Table 2). Additionally, *N. sibirica* has lower inter-population variation of the DNA content (maximum variation 1.08-fold) as compared to *N. schoberi* (maximum variation 1.16-fold). A DNA content (2C) analysis of variance (Newman–Keuls test, $p \leq 0.05$) subdivided *N. schoberi* populations into two groups: 2.93–3.10 and 3.17–3.38 pg. No significant differences were found among *N. sibirica* populations.

We noticed that in *N. pamirica*, *N. komarovii*, and *N. schoberi*, the modal number of chromosomes is 2n = 48, whereas in *N. sibirica* and *N. tangutorum*, it is 2n = 24, confirming the ploidy shown by FCM (Table 2, Fig. 2).

The characterization of the karyotype of *Nitraria* species helped us to determine the most frequent numbers of chromosomes (Table 2, Fig. 4). For instance, in *N. sibirica*, cells with 2n = 3x = 36, 2n = 4x = 48, 2n = 5x = 60 were found; in *N. tangutorum*, 2n≈26; in *N. schoberi*, 2n = 2x = 24, 2n = 5x = 60, 2n = 6x = 72, 2n = 8x = 96 (Fig. 5); in *N. pamirica*, 2n = 2x = 24. Cells with 2n≈40 and 2n≈80 were sometimes observed in *N. schoberi* and *N. sibirica*.

Different sets of chromosomes in *Nitraria* species were noted among different plants within populations and among cells of a single plant. The analysis of the nuclear DNA of *N. sibirica* revealed the presence of endopolyploid nuclei (up to 16C) in plants from different populations (Fig. 6).

Correlation of the nuclear DNA content with pollen grain size

The correlation analysis of datasets of pollen size and DNA contents uncovered a positive correlation between the pollen E and the 2C value (r = 0.52) and a negative correlation between the P/E ratio and 2C (r = –0.54) (Tables 3 and 4), indicating that E goes up with the increasing DNA content along with unchanged or decreasing pollen grain size. Monoploid genome size (1Cx) positively correlated with E even more strongly (r = 0.64).

Discussion

Genome size and ploidy

Interspecies variation of genome size is a well-known fact (Bennet et al. 2000; Doležel et al. 2007), but genome size within a species is thought to be stable (Greilhuber et al. 2005; Lomonosova et al. 2020). Lysak et al. (2000) documented a 1.06-fold variation

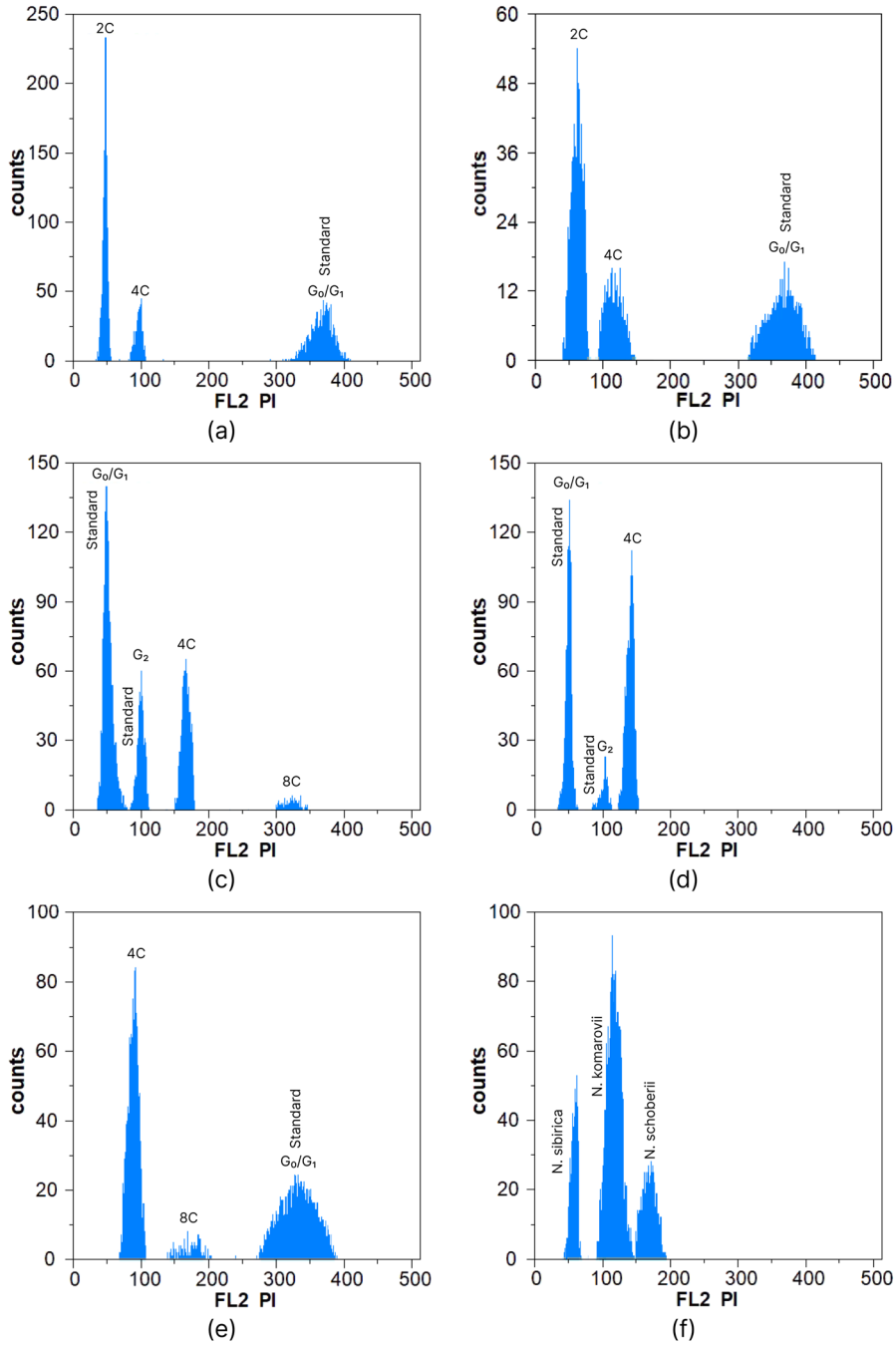

Fig. 2 Histograms of fluorescence intensity of PI. **a** *Nitraria sibirica*, **b** *N. tangutorum*, **c** *N. schoberi*, **d** *N. pamirica*, **e** *N. komarovii*, and **f** *N. sibirica*, *N. komarovii*, and *N. schoberi*

of genome size in European populations of *Sesleria albicans*. Similarly, lack of a significant variation, i.e., a 1.06-fold difference, was proved in *Pinus nigra* (Bogunic et al. 2007). In *Trifolium repens* and *T. fragiferum*, only a slight intraspecific variation of genome size was recorded, 1.05- and 1.03-fold, respectively (Lukjanová and Řepková 2021). In a paper about *Allium cepa* cultivars from different parts of the world, some authors reported exceptional intraspecific stability of genome size (Bennet et al. 2000).

Our results indicate intra-population stability of the nuclear DNA content in all five examined species, ranging from 1.01- to 1.08-fold. A similar magnitude

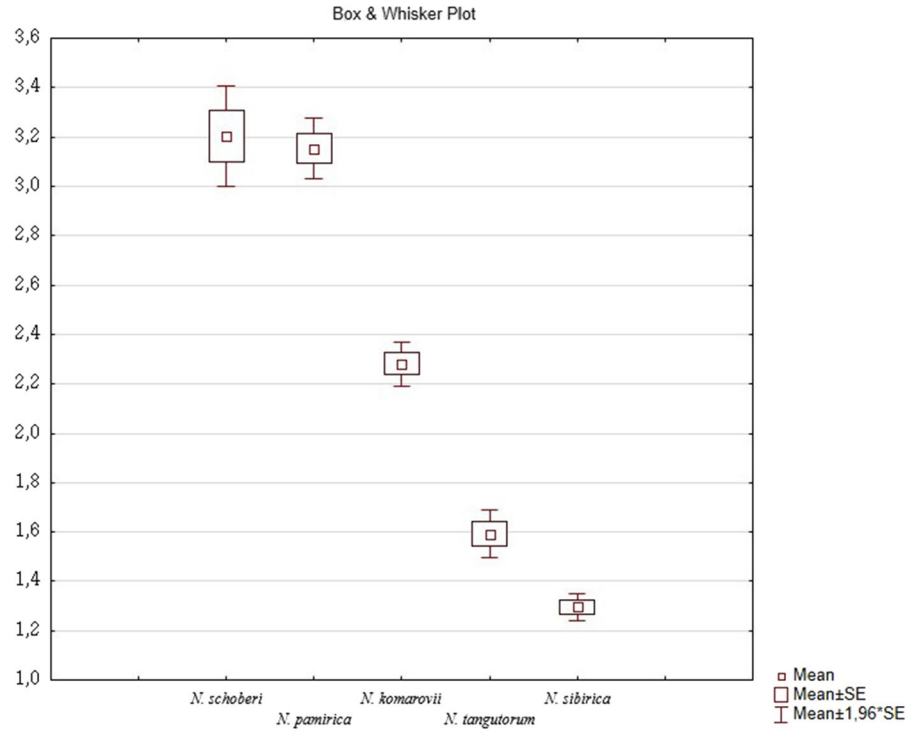

Fig. 3 A block diagram of the DNA content (2C) in five species of the genus *Nitraria*

of variation was observed within a single plant of each species of genus *Nitraria* (Banaev et al. 2018a). This result can be explained by the finding that seeds collected from one bush may have a male gametophyte from another plant (Voronkova et al. 2018).

We detected no obvious patterns regarding levels of variation of the DNA content across populations. In particular, among *N. schoberi* plants from the Eastern Pamir, the variation is 1.07-fold in the Pyandzh1 population and 1.01-fold in the Pyandzh2 population located 20 km away. Among *N. sibirica* plants, the highest variation of genome size (1.02-fold) was found in populations of Altai Krai (Uglovskoe), Altai Republic (Kosh-Agach), Tuva (Turan), and Kazakhstan (Koktal), located at a substantial distance from each other. Correlations between ecological differentiation and genome size have been reported at both interspecific and intraspecific levels (Knight et al. 2005; Knight and Ackerly 2002), for example, in plant species of genera *Larrea* (Poggio et al. 1989), *Cardiospermum* (Urdampilleta et al. 2012), *Berberis* (Bottini et al. 2000), *Coffea* (Razafinarivo et al. 2012), and *Psidium* (Tuler et al. 2019). For instance, it was shown that the species of *Larrea*, *Bulnesia*, and *Pintoa* that inhabit the most arid environments are the ones possessing the highest DNA content (Poggio et al. 1989). In other research, intraspecific DNA content variation has correlated with a geographic environment and ploidy in *Festuca pallens* (Smarda and Bures 2006) and *Miscanthus* sp. (Sheng et al. 2016). Our results suggest that among *N. schoberi* populations, the DNA content is lower in plants growing in the Balkhash-Alakol basin and along the coasts of large water bodies, the Black Sea and Caspian Sea.

Our analysis of DNA content variation showed higher stability of genome size in *N. sibirica* than in *N. schoberi*. This finding is consistent with available data on increased variation of genome size in polyploids (Tuna et al. 2017). As stated above, *N. sibirica* is diploid (2n = 2x = 24), and *N. schoberi* is tetraploid (2n = 4x = 48).

Our results show that monoploid genome size of *Nitraria* varies 1.40-fold (0.57–0.80 pg), with the smallest value in *N. komarovii*. Genome downsizing in the process of polyploidization may increase a plant's environmental adaptive fitness and facilitate competition with their diploid species. For example, it was demonstrated that altered 1Cx values reflect plasticity of the polyploid genome in various *Miscanthus* species (Sheng et al. 2016). Furthermore, a

Table 2 Relative genome sizes (2C) and CHNs (2n) in *Nitraria*

Name of the population	DNA content (pg)					CHN	
	Mean	Min	Max	SD	CV%	Modal number	Numbers determined
N. schoberi							
Karalat	3.12	3.09	3.14	0.023	0.75	48	42*,46*,48,50*,56*,64*
Baskunchak	3.24	3.23	3.25	0.010	0.31	48	48
Krim	3.09	3.06	3.11	0.017	0.55	48	24, 36, 40, 42*, 48, 57, 60, 66, 69
Kaspii	3.23	3.21	3.25	0.015	0.46	48	48
Actau	3.20	3.16	3.23	0.020	0.63	48	48
Tigen	3.10	3.06	3.13	0.029	0.92	48	36, 48, 54, 56*, 60, 64*
Pyandzh2	3.25	3.22	3.26	0.016	0.49	48	24, 36, 48, 56, 72, 76, 92
Pyandzh1	3.21	3.09	3.32	0.050	1.56	48	24, 44, 48, 54, 60, 74
Aidarli	3.07	3.05	3.09	0.012	0.39	48	24*, 26, 36, 42*, 48, 54, 58*, 62
Lepsi	3.25	3.18	3.34	0.040	1.23	48	24, 36, 48, 60, 68
Balhash	2.98	2.93	3.02	0.031	1.04	48	48, 60, 72
Karatal	2.97**	2.95	2.99	0.013	0.45	48	42,46,48,50,64
Alakol	2.97	2.94	2.99	0.044	0.54	48	48
Sariozek	3.17	3.16	3.21	0.019	0.60	48	36, 48, 50, 60*, 66, 74
Taskarasu	3.29	3.25	3.32	0.028	0.88	48	48
Basshi	3.32	3.30	3.34	0.015	0.45	48	38, 40, 48, 72
Koktal	3.27	3.23	3.30	0.027	0.83	48	24, 36, 48, 50, 56, 64
Kuchuk	3.33	3.28	3.38	0.035	1.06	48	48
Malinovoe	3.21	3.16	3.27	0.032	1.00	48	24, 36, 42*, 46*, 48, 54, 60, 70, 76*, 82
Kulunda	3.22	3.16	3.28	0.035	1.09	48	24, 36, 42*, 48, 68
Bagan	3.34	3.28	3.39	0.036	1.08	48	24, 34*, 36, 42*, 48, 60, 64*
Xinjiang	3.26	3.23	3.34	0.029	0.89	48	24, 36, 48, 54, 60, 62
Mean	**3.19**	**2.93**	**3.39**	**0.104**	**3.25**		
N. sibirica							
Gornyak	1.32	1.32	1.34	0.007	0.52	24	24, 36, 48
Noven'koe	1.28	1.27	1.28	0.003	0.25	24	18, 24, 28
Pospeliha	1.25	1.24	1.26	0.003	0.32	24	24
Balansor	1.27	1.27	1.28	0.004	0.33	24	24, 36, 48
Uglovskoye	1.32	1.30	1.33	0.008	0.60	24	24, 36, 48
Tassor	1.27	1.26	1.28	0.003	0.33	24	24
Chinkussor	1.25	1.24	1.26	0.004	0.29	24	24
Rubtsovsk	1.30	1.29	1.30	0.004	0.28	24	24, 36, 48
Kuchuk	1.29	1.27	1.29	0.005	0.30	24	24
Dzhira	1.27	1.26	1.28	0.006	0.45	24	16, 24, 36, 48
Kulunda	1.27	1.27	1.28	0.004	0.33	24	18, 20, 24, 36, 48
Yarovoe	1.28	1.27	1.29	0.006	0.41	24	24
Bagan	1.28	1.27	1.29	0.004	0.30	24	24, 36, 54, 60
Chagan	1.33	1.32	1.33	0.003	0.24	24	24
Bele	1.27	1.26	1.28	0.007	0.55	24	24, 48
Ulug-Kol	1.33	1.32	1.34	0.005	0.35	24	24, 32, 40, 49
Turan	1.34	1.32	1.34	0.008	0.63	24	24
Hadyn	1.28	1.27	1.29	0.007	0.52	24	24, 36, 38
Shara-Nur	1.32	1.31	1.32	0.003	0.24	24	24

Table 2 (continued)

Name of the population	DNA content (pg)					CHN	
	Mean	Min	Max	SD	CV%	Modal number	Numbers determined
Kosh-Agach (Chuya)	1.31	1.31	1.32	0.004	0.32	24	24, 36
Kosh-Agach (Chaganka)	1.26	1.25	1.28	0.011	0.86	24	24, 36
Balhash	1.31	1.31	1.32	0.007	0.54	24	24, 27, 34, 36, 39
Kurti	1.26	1.24	1.27	0.007	0.59	24	24, 36, 48
Koktal	1.26	1.25	1.27	0.009	0.69	24	24
Mean	**1.30**	**1.24**	**1.34**	**0.029**	**2.25**		
N. pamirica	3.15	3.10	3.30	0.062	1.97	48	24, 48
N. komarovii	2.28	2.23	2.32	0.045	1.96	48	48, 60
N. tangutorum	1.59	1.57	1.65	0.041	1.45	24	24, 26

* population already studied by Marhold et al. (2021) concerning CHN, ** population already studied by Voronkova et al. (2018) concerning FCM

Average values are boldfaced

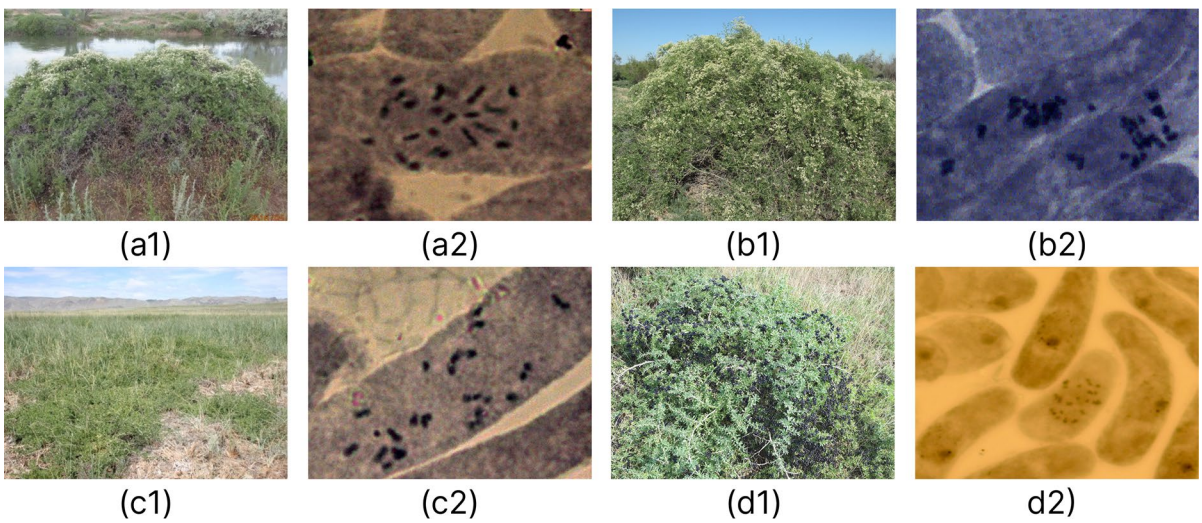

Fig. 4 The habitus and mitotic chromosomes of*Nitraria sibirica* from different populations: **a1, a2** Kurti, (2n = 24), **b1, b2** Koktal (2n = 24), **c1, c2** Shara-Nur (2n = 24), and **d1, d2** Gornyak. Photos by E.V. Banaev and M.A. Tomoshevich

decrease in the size of a monoploid genome indicates that the species in question is evolutionarily young (Šmarda et al. 2008). Our finding that the monoploid genome of *N. komarovii* is the smallest supports the point of view of Bobrov (1946), according to whom *N. komarovii* is the youngest species associated with the recent history of the Caspian Basin.

It is documented in the literature that in *Nitraria* species, most frequent chromosome numbers are divisible by the principal number x = 12 or nondivisible by it (Zakharyeva and Astanova 1968; Pan et al. 2002, 2003). For *N. schoberi* from mountain deserts of Central Asia, 2n = 24 was reported (Reese 1958), and for *N. schoberi* from southern Romania, it is 2n = 66 (Tarnavshi 1948). The data on chromosome sets for most plants are now readily available and collected in publicly available resources such as the Chromosome Counts Database (CCDB; http://ccdb.tau.ac.il/ (accessed on 20 August 2023), where information (2n) on six species of the genus *Nitraria* is available: *N. sibirica* (24, 30, and 60), *N. schoberi* (24, 34, 42, 48, 60, ~66, 72, and 96), *N. pamirica* (24

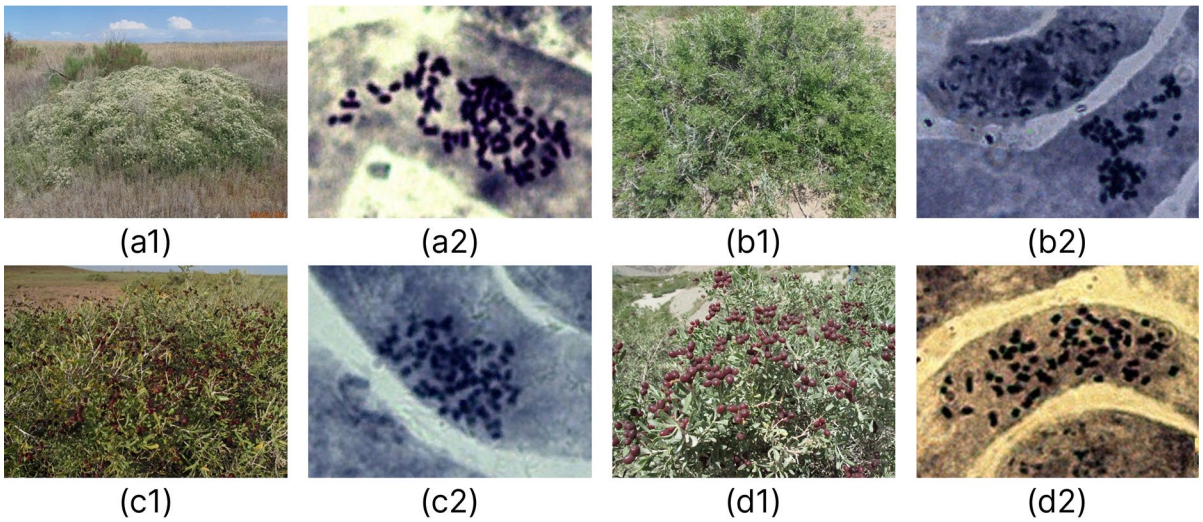

Fig. 5 The habitus and mitotic chromosomes of *Nitraria schoberi* from different populations: **a1, a2** Balhash (2n = 72), **b1, b2** Lepsi (2n = 60), **c1, c2** Sariozek (2n = 60), **d1, d2** Pyandzh1 (2n = 72). Photos by E.V. Banaev and M.A. Tomoshevich

and 48), *N. komarovii* (24), *N. tangutorum* (24), and *N. retusa* (18 and 24). In the Index to Plant Chromosome Numbers (IPCN, http://legacy.tropicos.org/Project/IPCN; accessed on 20 August 2023), data (2n) are given for *N. sibirica* (24 and 60) and *N. retusa* (24).

Earlier, various cytotypes in the genus *Nitraria* have been documented; for instance, in *N. pamirica*, the typical chromosome number proved to be $2n = 48$, $2n \approx 4x$, and 2C = 3.15 pg, but only a few specimens showed $2n = 24$, $2n \approx 2x$, 2C = 1.50 pg; in *N. schoberi* the typical number is $2n = 48$, $2n \approx 4x$, 2C = 2.98 pg, but some specimens have $2n \approx 8x \approx 96$, 2C = 5.75 (Marhold et al. 2020). Furthermore, different chromosome numbers have been detected among cells of a single plant, thus pointing to mixoploidy. The latter is a phenomenon characteristic of many woody plant species (Butorina 1989; Butorina and Gavrilov 2001), in particular, it is typical for representatives of families with small chromosomes, including *Nitraria* (Muratova et al. 2011, 2013). Proportions (%) of cells having different ploidy levels is one of the factors of plant adaptation to new or extreme habitat conditions, e.g., drought, strong light intensity, and high salinity (Cookson et al. 2006; Kunakh 2011; Gegas et al. 2014; Scholes and Paige 2015). According to Sedelnikova (2015), mixoploidy and aneuploidy in Pinaceae family species are often seen in extreme intrazonal bog and mountain ecotypes, and the highest level of mixoploidy for conifers is found in populations of *Larix sibirica* at the northern border of the species range. The level of endoreduplication may be species-specific or may differ between populations or even between individuals of the same species (Barow and Meister 2003). When researching the family Chenopodiaceae, Skaptsov et al. (2017) reported that endopolyploidy is usually observed in diploid species of the genera *Chenopodium*, *Dysphania*, *Oxybasis*, and *Suaeda* and not found in polyploid specimens of *Suaeda*.

The correlation of the nuclear DNA content with pollen grain size

The positive correlation between pollen grain size and the nuclear DNA content is probably the most easily explained because pollen contains only the components necessary for the initiation and maintenance of pollen tube growth and carries a haploid genome. A direct correlation between the nuclear DNA content and pollen size has been found in many plant species and groups (Bennett 1987; Bennett et al. 2005; Sinjushin 2021). Of interest are correlations between the DNA content, ploidy, and pollen grain size. For example, among 17 species of the genus *Lippia*, a positive association was detected between the chromosome number and pollen grain size as well as between ring length and ring width (Sousa et al.

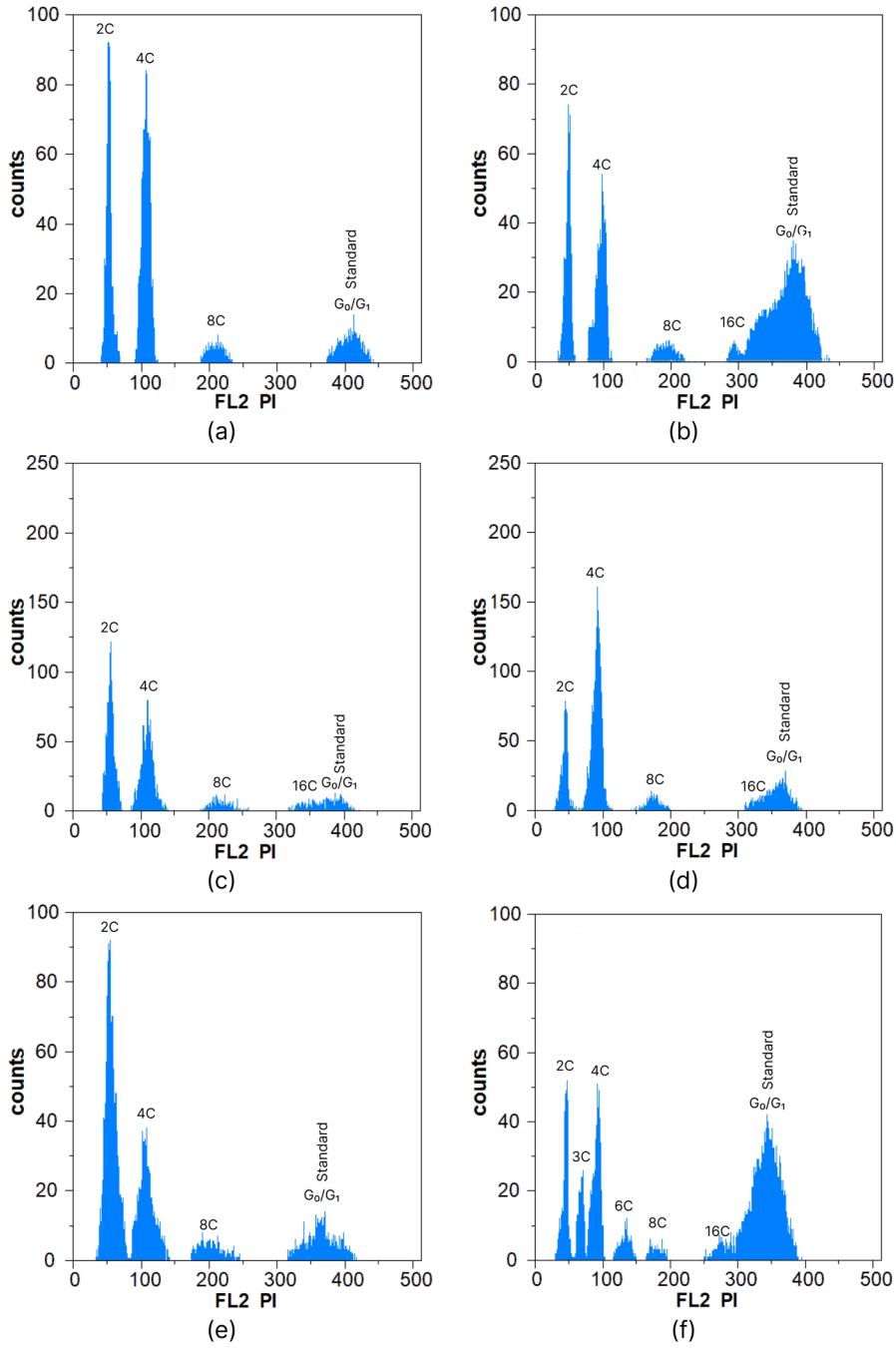

Fig. 6 Histograms of PI fluorescence intensity of endopolyploid nuclei in plants from different populations of *Nitraria N. sibirica*. **a** Kosh-Agach (Chuya); **b** Gornyak; **c** Shara-Nur; **d** Balansor; **e** Uglovskoye; and **f** Kulunda

2013). In *Ipomoea batatas* and *I. trifida*, both genome size and ploidy correlate with pollen size (Srisuwan et al. 2019). For species of the genus *Plantago*, pollen diameter was shown to correlate with 2C but not necessarily with ploidy (Wong and Murray 2012).

According to Amer and Amany (2014), there is a strong correlation between polyploidy and pollen morphological variation in *Atriplex halimus*. By contrast, Knight et al. (2010) found no association between pollen size and genome size after examining phylogenetic history of 464 plant species.

We did not see enlargement of the pollen grain with increasing ploidy or increasing genome size in the examined species of *Nitraria.* Our results indicate

Table 3 Morphometric results on pollen grains and on the DNA content of *Nitraria*

Species	P (µm)	E (µm)	P/E	2C (pg)	Ploidy and CHN	1C (pg)	1Cx (pg)
N. sibirica	38.34 ± 0.27c	20.97 ± 0.16c	1.84 ± 0.01b	1.30 ± 0.002d	2n = 2x = 24	0.65	0.65
N. schoberi	40.45 ± 0.33b	24.83 ± 0.26b	1.65 ± 0.02c	3.19 ± 0.013a	2n = 4x = 48	1.59	0.80
N. komarovii	27.15 ± 0.31e	16.42 ± 0.30d	1.66 ± 0.03c	2.28 ± 0.009b	2n = 4x = 48	1.14	0.57
N. tangutorum	42.76 ± 0.48a	21.14 ± 0.19c	2.02 ± 0.02a	1.59 ± 0.011c	2n = 2x = 24	0.78	0.78
N. pamirica	32.29 ± 0.32d	26.50 ± 0.28a	1.22 ± 0.01d	3.15 ± 0.009a	2n = 4x = 48	1.58	0.79

Means followed by the same letter are not significantly different according to the LSD test at $p \leq 0.05$

Table 4 A correlation matrix for the pollen characteristics and DNA content of the *Nitraria* species

Variable	Boldfaced correlations are significant at $p \leq 0.05$					
	Means	SD	P	E	P/E	2C
P	38.14	5.25				
E	22.40	3.82	**0.53**			
P/E	1.73	0.25	**0.33**	**–0.62**		
2C	2.18	0.89	0.02	**0.52**	**–0.54**	
1Cx	0.71	0.08	**0.43**	**0.64**	**–0.29**	**0.75**

boldfaced correlations are significant at p ˂ 0.05

a direct correlation between the 2C DNA content, chromosome number, and E of pollen grains in species *N. pamirica*, *N. schoberi*, *N. sibirica*, and *N. tangutorum*. *N. komarovii* is an exception because it is a tetraploid but is characterized by the smallest pollen E. The P/E ratio, which is a parameter of pollen grain shape, is lower in tetraploids *N. pamirica*, *N. schoberi*, and *N. komarovii* than in diploids *N. sibirica* and *N. tangutorum*.

Conclusions

Our work shows that in species of the genus *Nitraria*, genome size, ploidy, and pollen grain size are interrelated. We can rank the species under study by genome size and ploidy as follows: diploid (2n = 2x = 24): *N. sibirica* (1.30 pg) and *N. tangutorum* (1.59 pg); tetraploid (2n = 2x = 48): *N. komarovii* (2.28 pg), *N. pamirica* (3.15 pg), *N. schoberi* (3.19 pg). By pollen E and 2C (E; 2C), the ranking is as follows: *N. sibirica* (20.97 µm; 1.30 pg), *N. tangutorum* (21.14 µm; 1.59 pg) < *N. schoberi* (24.83 µm; 3.19 pg), *N. pamirica* (26.50 µm; 3.15 pg). An exception is *N. komarovii* (16.42 µm; 2.28 pg), having an intermediate DNA content and the smallest pollen width. A comparison of pollen E and 1Cx (E; 1Cx) results in the following ranking: *N. komarovii* (16.42; 0.57) < *N. sibirica* (20.97; 0.65) < *N. tangutorum* (21.14; 0.78) < *N. schoberi* (24.83; 0.8) < *N. pamirica* (26.50; 0.79). An increase in E clearly correlates with 1Cx. Our findings indicate the correctness of Bobrov's (1946) views on relationships within the genus *Nitraria*, who distinguished two series in sect. *Nitraria*: ser. *Sibiricae* (*N. tangutorum* and *N. sibirica*) and ser. *Schoberianae* (*N. schoberi* and *N. komarovii*). *N. pamirica*, which we believe should be placed in the ser. *Schoberianae*, was not known to E.G. Bobrov because it was described by Vasilieva (1974) later. It is obvious that the five species under study differ in their genesis. As pointed out by Bobrov, the species of the ser. *Sibiricae* are probably related in origin to ancient deserts of Central Asia, whereas *N. schoberi* originated in the Aral-Caspian lowlands and spread eastward and southeastward to Central Asia and Western Siberia.

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Author contributions EVB: conceptualization, project administration, methodology, resources, and writing–review and editing. MAT: conceptualization, writing–original draft

preparation, resources, methodology, investigation, and visualization. AAE: software, visualization, and formal analysis. All authors read and approved the final manuscript.

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Declarations

Competing interests The authors declare no competing interests.

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