Estimation of nuclear DNA content in *Sesleria* (Poaceae)

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SUMMARY - Taxonomy of the genus *Sesleria* Scop. is complicated by the fact that most of the typical characters are of quantitative nature and under strong environmental control. In some cases, unequivocal determination of individual species may be difficult. In this study, we have employed flow cytometry to estimate ploidy levels and to determine nuclear DNA content in populations of five central European *Sesleria* species *[S. albicans* Kit. ex Schult., S. *caerulea* (L.)Ard., S. *heufleriana* Schur, S. *sadleriana* Janka, S. *tatrae* (Deg.)Deyl]. Two DNA ploidy levels were found: tetraploid and octoploid. These ploidy levels (i.e. $2n = 4x = 28$ and $2n = 8x = 56$) were confirmed by chromosome counting. The 2C DNA content of tetraploid species ranged from 9.097 pg in S. *caerulea* to 9.585 pg in S. *heufleriana*. Octoploid taxa had approximately two times higher DNA content, ranging from 17.729 pg in S. *sadleriana* to 18.278 pg in S. *tatrae*. During the course of the study, two plants representing a so far unknown octoploid cytotype of S. *heufleriana* were discovered. Furthermore, it was found that octoploid *Sesleria* species had lower nuclear DNA content than a theoretical double of mean DNA content of related tetraploid species. In all species, the intraspecific variation of nuclear DNA content was small, not exceeding 3%. The relationship between *Sesleria* species and their evolution is discussed in the light of data on nuclear DNA content obtained for the first time in this study.

INTRODUCTION

The genus *Sesleria* Scop. includes perennial and heliophilous grasses, most of them preferring dry and rocky substrata. The species belonging to the genus are distributed over a large part of Europe, marginaly also in the North Africa and the Near East (cf. DEYL 1946). They play an important role in a number of plant communities as their basic and leading components. These *Sesleria* grasslands are often enriched by many rare relict or endemic taxa (cf. MUCINA *et al.* 1993; PIGNATTI and PIGNATTI 1975). In addition, some *Sesleria* species are

This work is dedicated to dr. Bohumil Travnlcek who stimulated our interest in the study of genus *Sesleria.*

considered endemic or endangered, *e.g.* S. *caerulea*, S. *heufleriana*, and S. *sadleriana* in Slovakia (MAGLOCKY and FERAKOVA 1993). Unfortunately, the analysis of various aspects of this genus is at present complicated by many unresolved taxonomic problems. Most of the typical characters are of quantitative nature and under strong environmental control. Taxonomic analysis and determination of distribution of individual species is thus very difficult. Considering the difficulties with the taxonomy of the *Sesleria* genus, we have suggested to employ flow cytometry to analyse ploidy levels in individual plants and plant populations (LYSAK *et al.* 1997). Flow cytometry is a technique which permits rapid estimation of nuclear DNA content (DOLEZEL 1991) and has been already found very useful in plant taxonomy to screen ploidy levels and to determine genome size (cf. DOLEZEL 1997). Here we report on the application of the technique for genome size analysis in *Sesleria*. Selected populations of five central European *Sesleria* species [S. *albicans* Kit. ex Schult., S. *caerulea* (L.) Ard., S. *heufleriana* Schur, S. *sadleriana* Janka, S. *tatrae* (Deg.) Deyl] were subjected to analysis of nuclear DNA content by flow cytometry. Special attention was given to the occurrence of intraspecific genome size variation and to the relationship between genome size and selected morphological characters (uppermost leaf length, spike length and width, glume length, palea length, lemma length and awn length of lemma) as estimated previously (LYSAK 1996).

MATERIALS AND METHODS

Plants from different populations of five *Sesleria* species from the territory of Central Europe were analysed (Table 1). Voucher specimens are deposited in Olomouc.

1. *Karyological analysis.* - Squash preparations were prepared as described previously (LYSAK *et al.* 1997). Briefly, root tips were pre-treated with 2mM 8-hydroxyquinoline and fixed in ethanol-acetic acid (3:1). After hydrolysis in 5N HCl at room temperature for 30 min, the root tips were stained in a Schiff reagent. Permanent squash preparations were made after maceration in 10% pectinase for 25 min. Chromosome counts were made on at least five intact metaphase plates in each plant.

2. *Flow cytometric analysis of nuclear DNA content.* - Flow cytometric estimation of nuclear DNA content was performed using a Partec PAS **II** flow cytometer. For analysis of tetraploid plants, maize (*Zea mays* cv. CE- 777) was used as internal standard (2C = 5.433 pg, unpublished). In case of octoploid plants, *Sesleria heufleriana* Schur (population from Koniarska planina plateau, Tab. 1) was used as an internal standard. Nuclear DNA content of this population (2C = 9.547 pg) was estimated in a preliminary experiment using Z. *mays*. Young basal parts of leaf sheaths were used for isolation of intact nuclei. Small amounts of tissues (standard + sample) were simultaneously chopped in LB01 buffer (DOLEZEL *et al.* 1989), supplemented with propidium iodide and RNase (both 50 J.ll/ml). Suspension of isolated nuclei was filtered through a 50 ì lm

TABLE 1 - List of *Sesleria* populations in which ploidy and nuclear DNA content was analysed.

	No. of analysed plants	No. of measure-ments	Ploidy level
Sesleria albicans			
N Austria, valley of Thaya river, ca. 0.5 km east of Hardegg town, ca. 300 m a.s.l.	1	3	4x*
C Moravia, Javoříčko village, "Zkamenělý zámek" rock, ca. 500 m a.s.l.	2	5	4x**
S Moravia, Pálava Mts., Klentnice village, Mt. Pálava, ca. 400-450 m a.s.l.	3	8	4x**
W Slovakia, Malé Karpaty Mts., Mt. Vápenná (748 m)	4	11	4x**
C Slovakia, Slovenský raj Mts., Prielom Hornádu valley, ca. 550 m a.s.l.	1	3	4x*
S Slovakia, Slovenský kras Mts., Brzotínske skaly rocks, ca. 650 m a.s.l., locality A	5	12	4x**
S Slovakia, Slovenský kras, Brzotínske skaly rocks, ca. 650 m a.s.l., locality B	4	12	4x**
S Slovakia, Slovenský kras, ca. 0.5 km southeastern from Hačava village, ca. 700 m a.s.l.	3	7	4x**
S Slovakia, Slovenský kras, Zádielska planina plateau, Krkavčie skaly rocks, ca. 600 m a.s.l.	2	6	4x*
Sesleria caerulea			
C Bohemia, near Velenka village, ca. 200 m a.s.l.	1	3	4x*
E Bohemia, Bilé Poličany village, coast of Budínský rybník pond, ca. 290 m a.s.l.	1	3	4x*
W Slovakia, Vel'ká Fatra Mts., Blatnica village, Blatnická dolina valley, ca. 600 m a.s.l.	1	3	4x*
W Slovakia, Vel'ká Fatra, Blatnica, Selenec valley, ca. 750 m a.s.l.	2	4	4x*
Sesleria heufleriana			
S Slovakia, Slovenský kras, Plešivská planina plateau, Maštalná jaskyňa cave, ca. 600 m a.s.l.	2	5	4x*
S Slovakia, Slovenský kras, Plešivská planina, border of Gombasek quarry, ca. 550 m a.s.l.	1	2	4x*
S Slovakia, Slovenský kras, Plešivská planina, border of the plateau near Mt. Železné vráta, ca 700 m a.s.l.	1	2	4x*
S Slovakia, Slovenský kras, Koniarska planina plateau, near Mt. Záseky, ca. 500 m a.s.l.	4	11	4x*
S Slovakia, Slovenský kras, southeastern from Včeláre village, Mt. Dlhý vrch, ca. 400 m a.s.l.	8	24	4x*
S Slovakia, Slovenský kras, Plešivská planina, near turistic path above Vyšný hámor homestead, ca. 600 m a.s.l.	2	2	8x*
Sesleria tatrae			
W Slovakia, Malá Fatra Mts., mountain ridge from Mt. Malý Kriváň to Mt. Vel'ký Rozsutec, ca. 1500 m a.s.l.	5	15	8x*
C Slovakia, Vel'ká Fatra, Mt. Suchý vrch, ca. 1500 m a.s.l.	2	5	8x*
N Slovakia, Belianske Tatry Mts., Mt. Ždiarska vidla and adjacent slopes, ca. 1700-2000 m a.s.l.	5	11	8x*
Sesleria sadleriana			
E Austria, Hainburg a. d. Donau town, Mt. Braunsberg	4	12	8x**
W Slovakia, Biele Karpaty Mts., Vršatské Podhradie village, rocks facing the Vršatec castle, ca 750 m a.s.l.	4	10	8x**

* Ploidy level determined for the first time in this study;
** Ploidy level determined by Lysák *et al.* (1997).

nylon mesh and stored on ice prior to analysis. The gain of the instrument was adjusted so that the peak representing G1/GO nuclei of internal standard was positioned on channel l00. At least 10.000 nuclei were analysed in each sample and 2C nuclear DNA content of unknown sample was calculated according to a formula:

$$\text{Sample 2C DNA content} = \frac{\text{Sample peak mean}}{\text{Standard peak mean}} * \text{2C DNA content of a standard}$$

Nuclear DNA content of each was estimated three times, eventually twice. The results of DNA content estimation were analysed by one-way ANOV A andTukeyKramer multi-comparison test. Nonparametric Kruskal- Wallis one-way ANOVA on ranks was used when any doubt existed about data normality.

RESULTS

We have estimated nuclear DNA content in five *Sesleria* species representing 24 European populations. Two DNA ploidy levels were found: tetraploid and octoploid. These ploidy levels *(i.e. 2n =4x* =28 and *2n =8x* =56) were confirmed by chromosome counting. The results of nuclear DNA content analysis are summarized in Table 2. The 2C DNA content oftetraploid species ranged from 9.097 :±: 0.068pg in S. *caerulea* to 9.585 :±: 0.056 pg in S. *heufleriana.* Octoploid taxa had approximately two times higher DNA content, ranging from 17. 729 :±: 0.133 pg in S. *sadleriana* (Hainburg) to 18.278 :±: 0.142pg in S.*tatrae.*

1. *Sesleria albicans.*

   Tetraploid S. *albicans* is the commonst European *Sesleria* species. The Slovak populations of S. *albicans* represent the northeastern limit of its distri

TABLE 2 - Mean nuclear DNA content, genome size and intraspecific genome size variation in *Sesleria.*

Species	Number of analysed populations	Ploidy level	2C nuclear DNA content (pg)		Genome size (Mbp per haploid genome, x)*	Maximum interpopulation difference (%)
			mean	± S.D.		
S. albicans	9	4x	9.256	0.076	2233	1.84**
S. caerulea	4	4x	9.097	0.068	2195	1.73
S. heufleriana	5	4x	9.585	0.056	2312	0.84
S. heufleriana	1	8x	18.952	—	2286	—
S. tatrae	3	8x	18.278	0.142	2205	0.68
S. sadleriana ("Vršatec")	1	8x	18.282	0.218	2205	—
S. sadleriana ("Hainburg")	1	8x	17.729	0.133	2139	—

* 1 pg DNA = 965 Mbp (BENNETT *et al.* 1997);
** Satistically significant at $P < 0.01$.

bution area. This basiphilous taxon predominantely grows on rocks from hill countries to alpine zone. In the territory of Slovakia and the Czech republic S.*albicans* occurs in calcareous or dolomitic areas, which are more or less isolated from each other .
Mean 2C nuclear DNA content of S.*albicans* was 9.256 pg (Tab. 2). Although the maximum interpopulation difference in DNA content was as small as 1.84%, theintraspecific variation in genome size was found statiscally significant ($F_{8,58}=7.56$, $P<0.01$). Possibleclinal character of observed variation could not be confirmed. Genome size variation did not correlate with any independent variable *(e.g.* altitude, latitude) . Similarly, a relationship between DNA content and the values of selected morphological characters (uppermost leaf length and six inflorescence characters) was non-significant.

2. *Sesleria caerulea.*

5. *caerulea* belongs to the most interestingtaxa of the genus for its growth on basic swamps or wet meadows. The species occures mainly in planar altitudes but may be found up to submountain zone in Slovakia. We have investigated plants representing two populations from Bohemia and two Slovak populations. Mean 2C nuclear DNA content of S*caerulea* was 9.097 pg (Tab. 2, Fig. **1** A). Intraspecific variation in genome size was found statistically non-significant (maximum difference 1.73%,*i.e.* 1.018-fold, $P>0.01$).

3. *Sesleria heufleriana.*

5. *heufleriana* represents a Carpathian endemit distributed in the easternCarpathians (Rumania, Ukraine) and in adjacent Pannonian area (Hungary, Slovakia) (DEYL 1946) . The species is spread from hilly countries to mountain altitudes. In Slovakia, S*heufleriana* occurs in Slovensky kras Mts. Populations from threekarst plateau were investigated. Mean 2C nuclear DNA content of 5. *heufleriana* was 9.585 pg (Tab. 2). Although karst plateaus are more or less isolated from each other by deep valleys, our data did not prove the presence ofintraspecific genome size variation in S. *heufleriana* ($P > 0.01$). Maximal difference between populations was 0.84%, i.e. 1.008-fold.
Until now, only tetraploid *($2n = 4x = 28$)* chromosome number has been reported in this species. However, during the course of the study two plants representing a so far unknownoctoploid cytotype of 5. *heufleriana* were revealed in Slovensky kras (Plesivska planina plateau). The 2C nuclear DNA content of 18.952pg was estimated for theseoctoploid plants (Tab. 2).

4. *Sesleria sadleriana.*

5. *sadleriana* largely dominates in thermophilous grasslands on limestones of colline and supracolline zone, respectively. Distribution area ofoctoploid

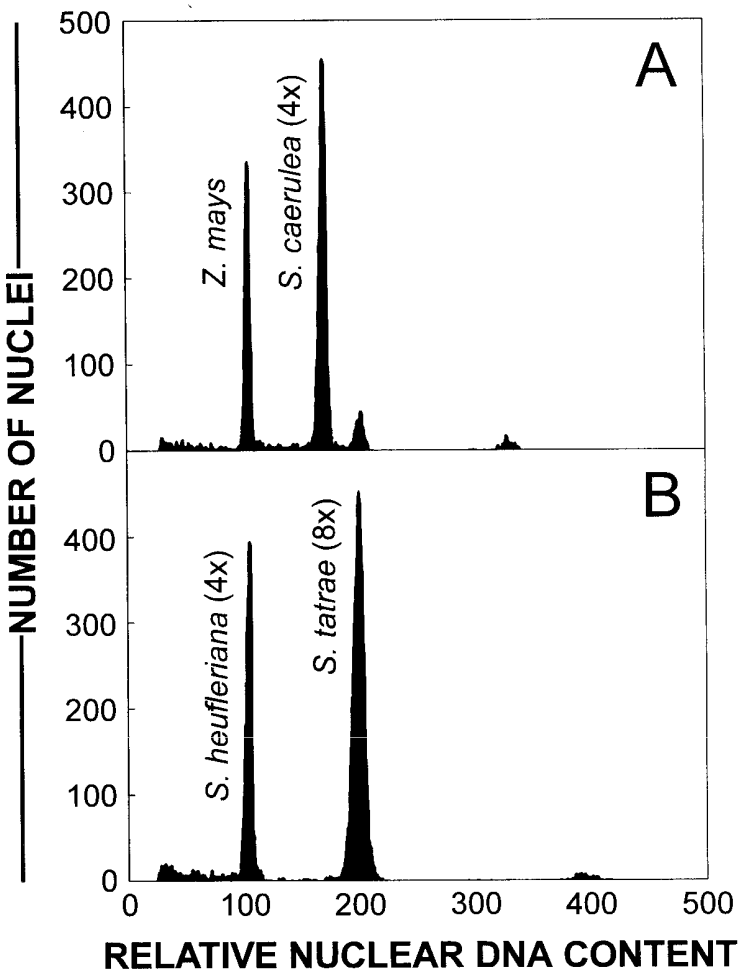

Fig. 1. Estimation of nuclear DNA content using flowcytometry. (A) Simultaneous analysis of nuclei isolated from *Sasleria caerulea (2n = 4x* = 28) and from *Zea mays* cv. CE- 777 used in this study as an internal standard for tetraploid *Sesleria* species. (B) Simultaneous analysis of nuclei isolated from S.*tatrae (2n = 8x* = 56) and from S. *heufleriana (2n = 4x* = 28) used in this study as an internal standard foroctoploid *Sesleria* species

species S. *sadleriana* is of a disruptive character. The species is distributed in eastern Alps (Slovenia, Croatia), Pannonian area (Austria, Hungary), and in westernCarpathians (Slovakia) (LYSAK *et al.* 1997) . Plants from two populations only were available and studied. We investigated the population fromHainburg a.d. Donau vi1l. (eastern Austria) and the population from "Vrsatec" cliff (western Slovakia). The population fromHainburg is considered a 'classi-

cal" locality of S. *sadleriana* (DEYL 1946). On the other hand, the locality "Vrsatec" is a newly discovered occurrence of the species (1YsAK *et al.* 1997). The results based on analysis of nuclear DNA content indicate a significant difference (3.02 %) between the two populations (F I ,20 = 53 .71 , P < 0.01) . The plants from "Vrsatec" have a higher DNA content than those from Hainburg (18.282 vs. 17. 729pg).

5. *Sesleria tatrae* .

The octoploid species S. *tatrae* grows on limestones and dolomites from montane to alpine zone of the West Carpathians (Slovakia, Poland). Plants from three separate high-mountains in Slovakia were analysed. Mean 2C nuclear DNA amount of 5 *tatrae* was 18.278 pg (Tab. 2, Fig. **1** B). Although gene exchange does not exist between studied populations, genome size variation was found to be non-significant, F2,28 = 0.67, P> 0.01 (maxdiff. 0.68%, 1.007 -fold) .

DISCUSSION

To our knowledge, this is the first report on genome size estimation in the genus *Sesleria.* Compared to a known range of genome size in plants (BENNETT *et al.* 1997), the *5esleria* species should be considered taxa with a medium size genome. In all species analysed in this study the variation of genome size was very limited, not exceeding 3%. This observation is in contrast with a significant variation which has been observed within some species (MICHAELSON *et al.* 1991; CECCARELLI *et al.* 1992; GRAHAM *et al.* 1994). Whether the variation reported in these and other studies reflects a plasticity of nuclear genome or is rather due to methodological errors is still a matter of discussion. For instance, CAVALLINI and NATALI (1990) and CAVALLINI *et al.* (1993) observed a significant intraspecific genome size variation in *Pisum sativum.* However, this finding was not confirmed by other independent studies (BARANYI and GREIL-HUBER 1995, 1996).

In S. *albicans,* we have observed nuclear DNA content variation up to 1.84% . Although small, this variation was statistically significant. This species is considered to be the closest to a hypothetical ancestral type of the *Sesleria* section. Isolation of individual populations for a long time has been suggested to occur during its evolution (DEYL 1946). Considering this fact, the variation in genome size was surprisingly low. Nevertheless, the small differences observed could reflect diversification of individual populations of S. *albicans.* LLOYD and WOOLHOUSE (1978) described interpopulation differences in the rates of photosynthesis and transpiration in S. *albicans.* Also interpopulation varia tion in some morphological characters was revealed by DIXON (1982) .

However, no relationship between DNA content and the values of selected morphological characters was observed in this study.

The observation of intraspecific DNA content variation (3%) in S. *sadleriana* is interesting. Both populations ("Vrsatec" and Hainburg) analysed have been classified as S. *sadleriana* based on identical ploidy level (*2n = 8x* = 56) and similarity of morphological characters (LYSAK *et al.* 1997). The difference in genome size variation may be explained by possible separate origin (from tetraploid S. *albicans* ?) and separate evolution of these populations. Polytopic origin of polyploids was documented in some taxa and it is not a rare event (SOLTIS and SOLTIS 1995). The species S. *sadleriana* and S. *tatrae* are noted for considerable similarity and may be classified in a rank of subspecies (cf. DEYL 1980). In this context, it is interesting to note that the *sadleriana* plants from "Vrsatec" locality had almost the same nuclear 2C DNA content (18.282 pg) as the population of S. *tatrae* (18.278 pg). These results indicate a close relationship between the "Vrsatec" population of S. *sadleriana* and S. *tatrae.* Even if previous morphometric analysis lead us to classify the "Vrsatec" population as S. *sadleriana* (LYSAK *et al.* 1997), differentiation of the Vrsatec population and S. *tatrae* populations from a common ancestral (tetraploid) type seems to be quite probable. Also geographical position of the "Vrsatec" population in relation to distribution of S. *tatrae* seem to support this hypothesis. Other techniques will be needed to resolve this problem.

We have found that octoploid *Sesleria* species had lower nuclear DNA content than a theoretical double of mean DNA content of related tetraploid species. The decrease in genome size with higher ploidy has been reported also in other genera and could be a consequence of selection to reduce the negative effect of increased DNA content in polyploids *(e.g.* BENNETT and THOMAS 1991). The results of CHENUIL *et al.* (1997) obtained in the *Barbus* (Cyprinidae) indicate that shortening of microsatellites and reduction of their number could be one of possible molecular mechanisms of eliminating excessive DNA from organisms with higher ploidy levels. Thus, considering the difference in size of genomes in tetraploids and octoploids in *Sesleria* a possible autopolyploid origin of polyploids *(e.g.* S. *sadleriana,* UJHELYI and FELFOLDY 1948) cannot be verified by a DNA content estimation alone.

The occurrence of an octoploid cytotype of S. *heufleriana* was an unexpected result. The cytotype could have arisen from tetraploid plants of the same species, which were growing within the same population. This hypothesis is supported by the fact that octoploid plants were morphologically indistinguishable from tetraploid ones (LYSAK 1996). However, further study will be necessary to understand the origin and frequency of octoploid plants within the populations of S. *heufleriana.*

In some genera, genome size was found to correlate with generally accepted taxonomic structure *(e.g.* MAXTED *et al.* 1991). However, in this study

we found no correlation between systematic position of analysed species as suggested by DEYL (1946) and their genome size. Also, DEYL' s hypotheses on probable origins of some *Sesleria* polyploids cannot be verified using data on nuclear DNA content. Thus we conclude that although flow cytometry can be used to estimateploidy and nuclear DNA content in the *Sesleria*, the data on genome size alone cannot explain unequivocally the evolution and relationship of *Sesleria* species.

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