Nuclear DNA Content Variation among Central European *Koeleria* Taxa

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● *Background and Aims* Polyploidization plays an important role in the evolution of many plant genera, including *Koeleria*. The knowledge of ploidy, chromosome number and genome size may enable correct taxonomic treatment when other features are insufficient as in *Koeleria*. Therefore, these characteristics and their variability were determined for populations of six central European *Koeleria* taxa.
● *Methods* Chromosome number analysis was performed by squashing root meristems, and ploidy and 2C nuclear DNA content were estimated by flow cytometry.
● *Key Results* Three diploids (*K. glauca*, *K. macrantha* var. *macrantha* and var. *pseudoglauca*), one tetraploid (*K. macrantha* var. *majoriflora*), one decaploid (*K. pyramidata*) and one dodecaploid (*K. tristis*) were found. The 2C nuclear DNA content of the diploids ranged from 4·85 to 5·20 pg. The 2C DNA contents of tetraploid, decaploid and dodecaploid taxa were 9·31 pg, 22·89 pg and 29·23 pg, respectively. The DNA content of polyploids within the *K. macrantha* aggregate (i.e. within *K. macrantha* and *K. pyramidata*) was smaller than the expected multiple of the diploid genome (*K. macrantha* var. *macrantha*). Geography-correlated variation of DNA content was found for some taxa. Czech populations of *K. macrantha* var. *majoriflora* had a 5·06 % smaller genome than the Slovak ones. An isolated eastern Slovakian population of *K. tristis* revealed 8·04 % less DNA than populations from central Slovakia. In central and north-west Bohemia, diploid and tetraploid cytotypes of *K. macrantha* were sympatric; east from this region diploid populations, and towards the west tetraploid populations were dominant.
● *Conclusions* Remarkable intra-specific inter-population differences in nuclear DNA content were found between Bohemian and Pannonian populations of *Koeleria macrantha* var. *majoriflora* and between geographically isolated central and eastern Slovakian populations of *K. tristis*. These differences occur over a relatively small geographical scale.

Key words: Chromosome number, polyploidy, nuclear DNA content, genome size variation, flow cytometry, grasses, Poaceae, *Koeleria*.

INTRODUCTION

The genus *Koeleria* Pers. comprises 35–40 species of perennial, xerophilous to mesophilous and commonly heliophilous grasses widely distributed in Europe, North America, Australia, New Zealand and non-tropical regions of Africa, Asia and South America (Meusel *et al*., 1965). Some *Koeleria* species play an important role in many plant communities. The most widely distributed *K. macrantha* (Ledeb.) Schult. is considered to be one of the essential species in the steppes of Eurasia and prairies of the North American Great Plains (Looman, 1978; Tzvelev, 1983; Arnow, 1994). Other species are considered to be rare and/or threatened, e.g. *K. tristis* Domin is endemic in the West Carpathians and *K. arenaria* (Dumort.) Conert is an endangered taxon restricted to the coastal sand dunes of western Europe (Soják and Chrtek, 1963; Conert, 1998).

Although *Koeleria* has been subjected to several taxonomic revisions during the 20th century, many essential systematic questions remain unanswered (Domin, 1907;

Ujhelyi, 1972; Holub *et al*., 1972; Tzvelev, 1983; Molina, 1993). The main reason is the high morphological similarity of many taxa, especially in the *K. macrantha* aggregate (from the taxa discussed in this paper, the aggregate contains *K. macrantha* and *K. pyramidata*), accompanied by their large intraspecific phenotypic variability. Misunderstanding these facts and considering phenotypes as genotypes led some taxonomists to describe hundreds of intraspecific taxa (Domin, 1907; Ujhelyi, 1972), and thus provided the basis for many recent taxonomic problems. These problems seem to be very current, especially in central Europe, from where most of the taxa were described. Therefore this paper focuses in detail on this area. It also became obvious that for *Koeleria* a classification system based only on morphology was inadequate.

The basic chromosome number of *Koeleria* is $x = 7$. As shown in many studies, polyploidization has played a major role in the evolution of this genus. Karyologic analysis of approx. 20 taxa yielded a wide range of chromosome numbers $2n = 14, 28, 42, 56, 70, 84$ representing ploidy levels from $2x$ to $12x$ (Fedorov, 1969; Holub *et al*., 1972; Ujhelyi, 1972; Arnow, 1994). In general, auto- and allopolyploidization can lead to genotype changes during

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plant evolution. As a consequence of genome duplications in polyploids, new phenotypes showing altered gene expression may arise (in part this is caused by gene dosage regulation). These novel types may differ from their progenitors and successfully occupy new niches (reviewed in Osborn *et al*., 2003). Chromosome number, ploidy and C-values (1C corresponds to the DNA content of the unreplicated reduced chromosome complement *n*, while the 1C*x* value represents the DNA content of one non-replicated monoploid genome with the chromosome number *x*; Greilhuber *et al*., 2005) represent important characteristics for plants (reviewed in Bennett, 1998; Bennett *et al*., 2000). Moreover, genomic data can contribute in combination with morphological characters to intergeneric classification, taxa delimitation or hybrid identification (Zonneveld, 2001; Bureš *et al*., 2004; Morgan-Richards *et al*., 2004). For some plant groups positive correlations between nuclear DNA content and various features such as life-styles (Barow and Meister, 2002; Bureš *et al*., 2004; Jakob *et al*., 2004), environmental conditions and/or geographical distribution (reviewed in Price *et al*., 1988*a, b*; Ceccarelli *et al*., 1992; Bottini *et al*., 2000; Schmuths *et al*., 2004) were found.

This paper reports on the determination of chromosome numbers, flow cytometric analysis of 2C nuclear DNA content, and ploidy level of six central European *Koeleria* taxa to answer the following questions: (*a*) What is the nuclear genome size, ploidy level and chromosome number of investigated taxa? (*b*) What is the inter-population variability of the nuclear DNA content within the analysed taxa? (*c*) Is the variability positively correlated with their geographical distribution? (*d*) What are the implications for the taxonomic treatment of the genus?

MATERIALS AND METHODS

Experimental material

Six native *Koeleria* taxa from central Europe were examined: *K. glauca* (Spreng.) DC., *K. macrantha* (Ledeb.) Schult. subsp. *macrantha* var. *macrantha*, *K. macrantha* subsp. *macrantha* var. *pseudoglauca* (Schur) Trávnícek et Pecinka ined. (= *K. gracilis* var. *glabra* Domin, nom. illeg.), *K. macrantha* subsp. *macrantha* var. *majoriflora* (Borbás) Trávníček et Pecinka ined., *K. pyramidata* (Lam.) Beauv. and *K. tristis* Domin. Based on morphological characters, these taxa are placed into three—perhaps unnatural—lineages, usually treated as sections or subsections (Domin, 1905; Soják and Chrtek, 1963). *Koeleria macrantha* and *K. pyramidata* belong to nominal section *Koeleria*. *Koeleria glauca* belongs in subsection *Glaucae* Domin and *K. tristis* in subsection *Splendentes* Domin, both within section *Bulbosae* Domin. Classification of *K. tristis* as a synonym of *K. pyramidata* (cf. Humpries, 1980) is not acceptable. Both species can be distinguished on the basis of their morphology (*K. tristis*: leaf blades glabrous, serrate on the margins, dry leaf blades are convoluted, firm and nitid; *K. pyramidata*: leaf blades pubescent, with a prominent line of >2 mm long hairs on the sides, dry leaf blades are usually not convoluted, soft and glareless), have different chromosome numbers, ecology and geographical

distribution. *Koeleria tristis* differs also from *K. eriostachya* Pančić (by looser panicles without shortened branches and by lemmas only rarely covered by long hairs). However, the exact relationship of *K. tristis* with other *Koeleria* taxa is unknown.

In total 286 plants from 126 populations were collected in Czech Republic, Germany, Hungary, Poland and Slovakia [Supplementary information; voucher herbarium specimens are deposited at the University of Olomouc (herbarium code: OL)]. The plants used for ploidy analysis were cultivated in the experimental garden of the Faculty of Biological Sciences, Palacký University, Olomouc. Plants that were used for chromosome number and nuclear DNA content analysis were grown in pots filled with perlite in a greenhouse at the Institute of Experimental Botany, Olomouc.

Chromosome counts

Young roots were incubated in a saturated solution of paradichlorbenzene in distilled water for 4 h, rinsed twice for 15 min in distilled water and fixed in ethanol–acetic acid (3 : 1) for at least 24 h. Fixed material was hydrolysed in 5 M HCl for 30 min and rinsed in distilled water. After Feulgen staining of chromosomes, roots were rinsed in distilled water, macerated in 10 % pectinase (Serva) for approx. 25 min at 37 °C and gently squashed in a drop of 45 % acetic acid. Chromosomes were counted in at least five intact metaphase plates per plant analysed.

Flow cytometric analysis

The analysis was performed by a Partec PAS II flow cytometer (Partec GmbH, Münster, Germany) equipped with a high pressure mercury arc lamp (100 W). Suspensions of intact nuclei were prepared by chopping approx. 0·05 g of basal parts of young leaves with a razor blade in 0·5 mL of Otto I buffer (Otto, 1990) and filtered through a 50-μm nylon mesh. Subsequently, 1 mL of Otto II buffer (Otto, 1990) containing propidium iodide and RNase (both 50 μg mL^{-1}) was added. After incubation for 10 min at 20 °C, relative fluorescence intensity of nuclei was analysed. For the ploidy analysis, the sample of unknown ploidy was measured together with a plant with known chromosome number. For 2C nuclear DNA content estimation, each plant was measured at least three times together with *Secale cereale* L. var. *cereale* ('Dankovske', 2C = 16·19 pg; Doležel *et al*., 1998) as an internal standard (Fig. 1A). The position of nuclei G_0/G_1 peaks in histograms of relative fluorescence intensity was used to estimate nuclear DNA content. The 2C DNA content of *Koeleria* was calculated as:

$$\text{\textit{Koeleria} 2C DNA content} = \frac{\textit{Koeleria}\ G_0/G_1 \text{peak mean}}{\text{Standard}\ G_0/G_1 \text{peak mean}}$$

$$\times \text{Standard 2C DNA content}$$

Intraspecific differences in genome size between populations of *K. macrantha* var. *majoriflora* and *K. tristis* were further tested to exclude the possibility that they represent an experimental artefact (e.g. due to presence of inhibitors in one sample). Material from two populations was
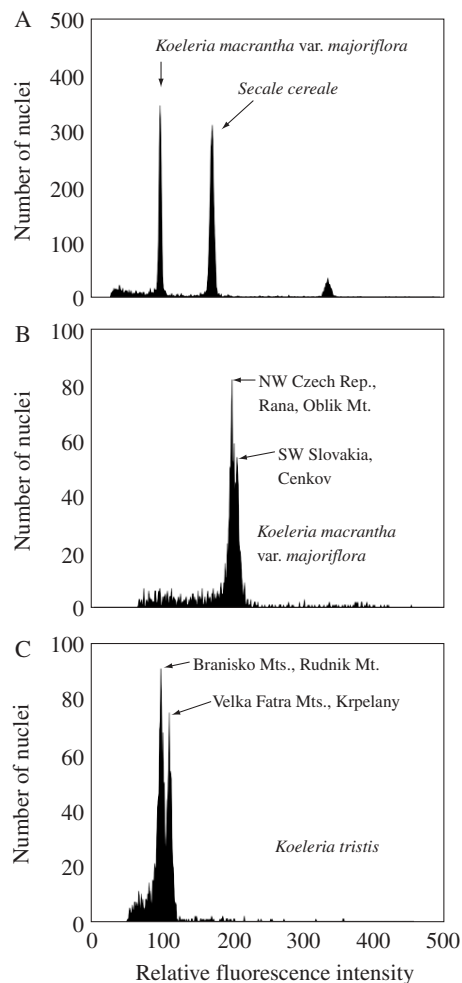
FIG. 1. Histograms of relative fluorescence intensity obtained after analysis of propidium-iodide stained nuclei of: (A) *Koeleria macrantha* var. *majoriflora* and *Secale cereale* 'Dankovske' (2C = 16·19 pg), which served as internal reference standard; (B) *K. macrantha* var. *majoriflora*; and (C) *K. tristis*. The simultaneous analysis of material from two populations of *K. macrantha* var. *majoriflora* with significantly different genome sizes resulted in a double peak, confirming the results of individual measurements (B). The same was true for *K. tristis* (C). Peaks of nuclei in G_0/G_1 phase of the cell cycle are marked by arrows.

co-chopped and analysed as described above. In the case of real differences, a double peak would be expected (Fig. 1B and C).

Statistical analysis

Nuclear DNA content data were analysed using the NCSS 97 statistics software (Statistical Solutions Ltd, Cork, USA). Hierarchical analysis of variance was performed to determine the variability within each taxon. The Tukey–Kramer and Bonferroni tests were used as multiple comparison tests of DNA values to discriminate dissimilar populations within each taxon. Using hierarchical cluster analysis, the relationships, based on nuclear DNA content, between individual populations of different

Koeleria taxa were analysed and the unweighted pair-group method was chosen.

RESULTS

Within the 126 populations analysed (see Supplementary information) belonging to six *Koeleria* taxa, three diploids ($2n = 14$), one tetraploid ($2n = 28$), one decaploid ($2n = 70$) and one dodecaploid ($2n = \sim84$) (Table 1) have been found. Chromosome numbers $2n = 56$ and $2n = 70$ previously reported for *K. tristis* from Rudnik Mt in the Branisko Mts (Májovský *et al.*, 1987; Murín and Májovský, 1987) could not be confirmed in the present study even when plants from the same population were examined.

Flow cytometric analyses revealed 2C DNA contents ranging from 4·85 to 29·23 pg. The mean 2C nuclear DNA content of *K. glauca* was 5·20 pg with only a small inter-population difference of 2·84 %. The low variation between four analysed populations was consistent with their phenotypic uniformity in the field and might reflect their close geographical origin.

Ploidy analysis and chromosome counting revealed diploid and tetraploid populations of *K. macrantha*. Within diploid populations, two different races were distinguished: a widely distributed type with pubescent leaves (var. *macrantha*) and a rare type with glabrous leaves occurring in relic vegetation on (ultra)basic soils in the Carpathians (var. *pseudoglauca*). The mean 2C nuclear DNA content estimated for nine populations of diploid *K. macrantha* var. *macrantha* was 4·95 pg without a significant inter-population difference (4·89 %, $P > 0.05$). For *K. macrantha* var. *pseudoglauca* only one population was analysed, therefore genome size variation could not be estimated. A morphometric study of diploid and tetraploid populations revealed significant differences as to quantitative characters between both cytotypes, i.e. length of spikelets, lower glume, anthers and stomata (Pecinka, 2001). Therefore the tetraploid populations were classified as a separate taxon, var. *majoriflora*. The mean 2C nuclear DNA content of eight populations of *K. macrantha* var. *majoriflor*a was 9·31 pg and showed a significant inter-population difference correlated with their geographic distribution in ANOVA and multiple comparison tests ($P < 0.05$). Six populations from the Czech Republic had significantly smaller genomes (5·06 %) than two populations from southern Slovakia (9·19 pg *vs*. 9·68 pg) (Fig. 1B).

The mean 2C nuclear DNA content of decaploid *K. pyramidata* was 22·89 pg with statistically significant interpopulation differences ($P < 0.05$) in ANOVA. The multiple comparison Tukey–Kramer test found differences between the populations: (*a*) Nové Dobrkovice *vs*. Rvištĕ (also confirmed by Bonferroni test), Kurdějov and Krnov; (*b*) Rvištĕ *vs*. Abrod. However, it is difficult to explain these differences on the basis of geographical or reproductive isolation, since all populations are located within the continuous distribution area of the species.

The mean 2C nuclear DNA content of the five populations of dodecaploid *K. tristis* analysed was 29·23 pg. The multiple comparison tests revealed significant differences

TABLE 1. *Chromosome numbers, ploidy and nuclear DNA content in central European* Koeleria *taxa*

	No. of populations analysed (chromosomes/ploidy)	2n	Ploidy	Absolute 2C DNA content		
				No. of populations/ samples/measurements	Mean ± s.d. (pg)	1C*x* genome size (Mbp)*
K. glauca	4/6	14	2*x*	4/5/15	5·20 ± 0·06	2543
K. macrantha						
var. *macrantha*	4/59	14	2*x*	9/28/86	4·95 ± 0·08	2421
var. *pseudoglauca*	1/4	14	2*x*	1/4/12	4·85 ± 0·05	2372
var. *majoriflora*	1/22	28	4*x*	8/26/83	9·31 ± 0·25	2277
K. pyramidata	4/30	70	10*x*	10/26/79	22·89 ± 0·50	2239
K. tristis	1/7	∼84	12*x*	5/13/44	29·23 ± 1·12	2382

* 1 pg DNA = 978 Mbp (Doležel *et al*., 2003).

between the population from Rudník Mt and all the other populations ($P < 0.05$). The plants from Rudník Mt (27·32 pg; eastern Slovakia) had significantly less nuclear DNA (8·04 %) than those from the Vel'ká Fatra Mts and the Chočské vrchy Mts (29·71 pg; central Slovakia) (Fig. 1C).

DISCUSSION

Within the known range of genome size in plants (Bennett *et al*., 2000), the analysed *Koeleria* taxa occupy an intermediate position. As far as is known, only four *Koeleria* species have been analysed previously for genome size. Using Feulgen microdensitometry, Grime *et al*. (1985) determined 2C nuclear DNA content of *K. macrantha* from Scotland to be 9·20 pg; the ploidy and chromosome number of the plants analysed were not mentioned. Later on, the value was included in the 'Plant C-values database' and annotated as 2C nuclear DNA content of the diploid (Bennett and Leitch, 2003). However, so far, only tetraploid populations of *K. macrantha* have been reported from the British Isles (Dixon, 2000). Therefore it is assumed that the analysed plants were most likely tetraploid and their 2C nuclear DNA content is comparable with the value of 9·31 pg found for tetraploid populations of central European *K. macrantha* by flow cytometry (this study). Recently, the 2C nuclear DNA content of three tetraploid species, *K. novozelandica* Domin, *K. cheesemanii* (Hack.) Petrie and *K. riguorum* Edgar et Gibb, endemic to New Zealand, has been estimated by flow cytometry to be 9·95 pg, 9·95–9·82 pg and 9·82 pg, respectively (Murray *et al*., 2005). The nuclear DNA content of 5·90 pg previously reported for *K. novozelandica* (Murray *et al*., 2003) was not confirmed. The genome size of 9·82–9·95 pg of tetraploid New Zealand species corresponds rather well to the genome size of central European tetraploids (9·31 pg).

The genome sizes of polyploid taxa belonging to the *K. macrantha* aggregate (Domin, 1907; Holub *et al*., 1972; Tzvelev, 1983): *K. macrantha* var. *majoriflora* (9·31 pg) and *K. pyramidata* (22·89 pg) were found to be lower than expected on the basis of the *K. macrantha* var. *macrantha* genome (5·96 % and 7·51 %, respectively). A similar decrease in genome size of polyploids in comparison to diploids has also been observed for other genera (Lysák

and Doležel, 1998; Hörandl and Greilhuber, 2002; Kotseruba *et al*., 2003). The possible reason could be partial elimination of repetitive DNA sequences in the polyploid genomes (Greilhuber, 1998; Kotseruba *et al*., 2003). Several recent studies have shown that sequence elimination by various mechanisms may occur rapidly in newly established polyploid plant genomes (Song *et al*., 1995; Voytas and Naylor, 1998; Ozkan *et al*., 2001; Devos *et al*., 2002).

European diploid and tetraploid cytotypes in *K. macrantha* show distribution correlated with longitude. In eastern Slovakia, only diploid populations were found, whereas, in central Germany tetraploid populations seem to be dominant. According to previous flow cytometric analyses and the study of voucher herbarium specimens (Pecinka, 2001), central and north-west Bohemia appears to be a transition zone with a frequent occurrence of both cytotypes, sometimes even with mixed populations in one locality. In spite of this, no triploids indicating hybridization between var. *macrantha* and var. *majoriflora* were observed within >100 plants sampled in this region. However, occurrence of tetraploid hybrid plants originating from a fusion of a non-reduced gamete of a diploid and a reduced gamete of a tetraploid cannot be excluded.

Within the populations of tetraploid *K. macrantha* var. *majoriflora* analysed, the populations from Czech Republic had significantly lower nuclear DNA than those from southern Slovakia (Fig. 1B). This difference was paralleled by minute differences in their morphology such as colour and hairiness of lemmas (Pecinka, 2001), and might indicate their different evolutionary history. Geography-correlated inter-population variation in nuclear DNA content was also found for *K. tristis*. This West Carpathian endemic occurs only in 14 localities, unevenly distributed within a small area of 150 × 25 km (Soják and Chrtek, 1963; Pecinka, 2001). All populations except one are clustered in central Slovakia (Vel'ká Fatra Mts, Chočské vrchy Mts and the western part of the Nízké Tatry Mts). The remaining single population, which is located ∼110 km eastwards in the Branisko Mts (eastern Slovakia), had a significantly lower genome size (8·04 %) than those from central Slovakia (Fig. 1C). This difference suggests a long-term isolation of central and eastern Slovakian populations, which could be maintained over time by extreme substrate and vegetation requirements of this species. *Koeleria tristis*

occurs exclusively on dolomites or dolomitic-limestones which are quite rare in Slovakia. Moreover, it grows only in spatially relatively small isolates of perialpine and dealpine vegetation which are separated by large areas of continuous forest at least since the end of the last glaciation, i.e. approx. 10 000 years ago (Krippel, 1986). The difference in genome size observed between populations of *K. tristis* is in contrast with their uniformity in morphological characters (Pecinka, 2001). Inter-population variation in genome size has also been reported for more-or-less distant populations of other plants like *Armeria maritima*, *Prospero autumnale*, *Hordeum* or *Arabidopsis thaliana* (Ebert *et al.*, 1996; Vekemans *et al.*, 1996; Jakob *et al.*, 2004; Schmuths *et al.*, 2004). However, it does not seem to be a general rule, since it was not found for isolated populations of other species such as *Sesleria albicans* or *Abies fraseri* (Lysak *et al.*, 2000; Auckland *et al.*, 2001).

SUPPLEMENTARY MATERIAL

Supplementary information available online (http://aob.oxfordjournals.org) includes a list of all populations investigated, numbers of plants analysed for chromosome number, ploidy and genome size from each population and the mean population genome size.

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