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CYTOMORPHOLOGICAL STUDIES ON AMERICAN AND EUROPEAN *PHLEUM COMMUTATUM* GAUD. (POACEAE)

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The morphology, karyotype structure and nuclear DNA amount of European and American representatives of alpine cat's-tail *Phleum commutatum* Gaud. were analysed. The evolutionary relationships within this group of taxa are discussed. It was shown that the American and European tetraploids are both morphologically and karyologically similar to each other and to the diploid race of this species from the Polish Carpathians. Diploid relatives of tetraploid *P. commutatum* i.e., *P. commutatum* (2x) and *P. rhaeticum* (Humphries) Rauschert, showed intra- and interspecific differences in nuclear 2C DNA values, whereas tetraploids (of both European and American origin) showed highly uniform nuclear DNA amounts. The 2C DNA uniformity and the greater mean size of the basal chromosome set in tetraploid *P. commutatum* as compared with its diploid relatives suggest that the change in genome size occurred during early evolution of the tetraploid race of *P. commutatum*, before its migration to America.

Key words: *Phleum commutatum*, *P. alpinum*, *P. rhaeticum*, morphology, chromosomes, karyotype, C-banding, heterochromatin, flow cytometry, nuclear genome size.

INTRODUCTION

The taxon discussed in this paper was described by Linnaeus in 1753 in *Species plantarum* under the name *Phleum alpinum* (Humphries 1978). In 1808, Gaudin (cf. Nordenskiöld, 1945) distinguished two forms of the alpine cat's-tail in Switzerland: high-alpine *P. commutatum* and alpine *P. alpinum*. The name *P. commutatum* Gaud. was popularised by Nordenskiöld (1945), who suggested that this species is exclusively tetraploid and the oldest in the *Phleum* section. Apart from differences in ploidy levels, the two basal taxa of *P. alpinum* group differ slightly in morphology (Joachimiak and Kula, 1996, 1997; Joachimiak, 2005; Zernig, 2005); however, the main difference is in the range of their distribution (bipolar distribution for tetraploid and European mountain distribution for diploid). The current nomenclature of this group introduces considerable confusion and leads to misunderstandings (Joachimiak and Kula, 1996, 1997; Joachimiak, 2005; Zernig, 2005, and literature cited therein). Thus, we decided to use the broadly accepted name *P. commutatum* for all plants with glabrous awns, and the less ambiguous name *P. rhaeticum* (Humphries) Rauschert for plants with ciliate awns (Rauschert, 1979; Joachimiak and Kula, 1997; Mirek et al., 2002; Joachimiak, 2005; Zernig, 2005).

There have been many cytotaxonomic studies on European populations of *P. commutatum* (Gregor and Sansome, 1930; Michalski, 1955; Wilton and Klebesadel, 1973; Teppner, 1980; Kováts, 1981; Joachimiak and Kula, 1993, 1996). Nowadays it is known that two chromosomal races of this species can be distinguished in Europe: the diploid one (2n = 2x = 14) and the tetraploid one

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(2n = 4x = 28). The diploid race probably occurs in the south and centre of Europe (Litardiere, 1948; Teppner, 1980; Joachimiak and Kula, 1993, 1996, 1997), whereas the tetraploid one grows most probably in the north of the continent; presently the data on the karyotype structure of both diploid and tetraploid plants are limited to European populations of this species (Wilton and Klebesadel, 1973; Joachimiak and Kula, 1996).

There are, however, no data on the karyotype structure of the American tetraploid *P. commutatum*. They might be interesting, particularly because some 19th-century systematic publications noted morphological differences between American and European specimens of the alpine cat's-tail. The differences consisted in the formation of taller shoots, broader leaves and short-awn husks by the North American forms. Because of this, they were treated as a distinct variety, *P. commutatum* var. *americanum* Fourn., or even as a separate species – *P. haenkeanum* Presl (Nordenskiöld, 1945). The American tetraploid occurs in both North and South America, over a vast area. In North America it grows at many circumboreal localities in Alaska and Canada, then covers a wide strip in the western part of the continent, from the Rocky Mts. to the Sierra Nevada Mts. in California, extending to the Oriziba Mts. of Mexico (18°N), where the species reaches the southern border of its range in the northern hemisphere. Next, after a huge range gap it appears in South America, where it grows in the Andes from the Cordillera de la Costa range to Patagonia, and as far south as the island of South Georgia (Nordenskiöld, 1945; Callaghan, 1974; Wallace and Harrison, 1978). The taxon seems well adapted to harsh climate and to large temperature amplitudes.

Comparative studies on growth and development in populations from Norway and South Georgia showed only small differences in flowering response to daylength. However, the South Georgia populations tillered more profusely but flowered more sparsely, and also had lower growth rates and photosynthetic capacity than the Norwegian populations (Heide and Solhaug, 2001).

According to Nordenskiöld (1945), tetraploid *P. commutatum* is an allopolyploid which differs genetically not only from the two more distant species of the *P. pratense* group (*P. pratense* L. and *P. bertolonii* DC.), but also from its close diploid alpine relative, *P. rhaeticum*. Nordenskiöld, who did not know of the diploid race of *P. commutatum*, does not mention any hypothetical ancestors of this taxon. The present research is aimed at establishing possible cytogenetic or morphological differences between the North American and European tetraploid representatives of *P. commutatum* Gaud., and their relationship to the diploid, exclusively European race of this species.

MATERIALS AND METHODS

This research examined tetraploid specimens of *Phleum commutatum* cultivated from caryopses originating from two localities in California, U.S.A.: Point Reyes on the Pacific coast, and Levitt Lake at 2740 m a.s.l. in the Sierra Nevada Mountains. The plants grew in a controlled environmental room under long-day conditions at 15°C. Specimens of diploid *P. commutatum* [2x] from Poland, tetraploid specimens from Sweden and Scotland, and *P. rhaeticum* served as comparative material. The main characters analysed in particular forms of the alpine cat's-tail are presented in Table 1.

For the morphological analyses and measurements of the length of stomata, spikelets and awns, an Eclipse E800 (Nikon) microscope, SMZ 800 (Nikon) stereoscopic microscope and Lucia G image analysing system were used. Spikelet length was presented on the basis of averaging 100 measurements. For stomata length 600 measurements were averaged. Detailed morphological analysis of *P. commutatum* was performed on two tetraploid American accessions, and two tetraploid and two diploid accession from Europe. Our preliminary morphological analyses of different European *P. commutatum* accessions showed that plants representing the same ploidy level are very similar. The uniformity of diploid and tetraploid lines was confirmed by our molecular (nuclear ITS and cpDNA) studies (Stewart et al., 2005, and unpublished data).

Acetic orcein was used for conventional chromosome staining (Joachimiak, 1994). C-banding was performed according to Jouve et al. (1980), except that the slides were incubated in $Ba(OH)_2$ at 38°C. The metaphase plates were archived with the Lucia G image analysing system connected through a CCD camera (Panasonic) to an Eclipse E800 microscope (Nikon). Chromosome measurements and heterochromatin amounts were determined using CytoPlane v.1.2. The structure of classical and C-banding karyotypes in a given species was based on measurements of 20 conventionally banded and 10 C-banded metaphase plates. For collective karyotypes only relatively stable heterochromatin bands were considered, that is, those whose frequency was over 50% in the analysed chromosome collection. Polymorphic bands, except for those in the vicinity of NOR regions, were ignored.

Young leaves of *Phleum* were collected from three-week-old seedlings growing in a greenhouse and prepared for flow cytometric analysis according to Galbraith et al. (1983), with some minor modifications. Plant tissue (of the species in question and of the internal standard, simultaneously) was chopped with a sharp razor blade in a plastic Petri dish with 1 ml nucleus-isolation buffer (0.1 M Tris, 2.5 mM $MgCl_2 \cdot 6H_2O$, 85 mM NaCl, 0.1% v/v Triton

TABLE 1. Origin of the investigated *Phleum* accessions; + indicates the use of plants from a given locality for a particular analysis

	Taxon	Locality	Length of spikelets and awns	Length of stomata	Chromosome number	Heterochromatin distribution	Karyotype structure	Nuclear DNA amount
			/15/	/15/	/15/	/4/	/4/	/10/
[A]	*Phleum commutatum* (4x)	Point Reyes, California, USA	+	+	+	+	+	+
[B]	*Phleum commutatum* (4x)	Levitt Lake, Sierra Nevada Mountains, California, USA	+	+	+	+	+	+
[C]	*Phleum commutatum* (4x)	Umfors, Scandinavian Mountains, Sweden		+	+	+		+
[D]	*Phleum commutatum* (4x)	Åre, Scandinavian Mountains, Sweden	+		+	+	+	
[E]	*Phleum commutatum* (4x)	Highlands Mountains, Scotland		+	+	+		+
[F]	*Phleum commutatum* (2x)	Hala Krupowa, Beskidy Mountains, Poland *	+	+	+	+	+	+
[G]	*Phleum commutatum* (2x)	Jaworzyna, Gorce Mountains, Poland *		+	+	+	+	+
[H]	*Phleum rhaeticum* (2x)	Ipiros Ioannina, Pindos Mountains, Greece			+			+
[I]	*Phleum rhaeticum* (2x)	Polana na Stołach, Tatra Mountains, Poland **			+			+
[J]	*Phleum rhaeticum* (2x)	Retezat Mountains, Romania			+			+

/ / – number of plants per locality; the only exception was [J] – only one plant from this locality was analysed; * chromosome number for plants from this population has been already published (Joachimiak and Kula, 1993); ** one and only population of this species in Polish mountains (Zając and Zając, 2001) – relic of experiment with cultivation of Alpine species in the Tatra National Park (Mirek, 1995) most probably from Eastern Alps.

X–100; pH 7.0), supplemented with propidium iodide (50 μg/ml) and ribonuclease A (50 μg/ml). After chopping, the suspension was passed through a 50 μm mesh nylon filter. For each sample, 5000–7000 nuclei were analysed using a Partec CCA flow cytometer (Münster, Germany) equipped with an argon laser. For each cat's-tail form, 10 measurements of separate nucleus isolations from different plants were made. Histograms were analysed using DPAC v.2.2. *Zea mays* CEE–777 (2C=5.43 pg/nucleus; Lysák and Doležel, 1998) was used as the internal standard for diploids and *P. pratense* from Cracow-Kostrze (2C=8.92 pg/nucleus; value determined in a preliminary experiment at the Laboratory of Molecular Biology and Cytometry in Bydgoszcz, using *Zea mays* CEE–777) for tetraploids. Nuclear DNA content was calculated using the linear relationship between the *Phleum*/internal standard ratio of the 2C peak positions on the histogram of fluorescence intensities.

The results were processed statistically by ANOVA and the means obtained were compared by the post-hoc Duncan's test (Duncan, 1955). For statistical analyses the STATISTICA v.5.1 package (Statsoft, Inc.) was used.

RESULTS

ANALYSIS OF SELECTED MORPHOLOGICAL FEATURES

Plant habit

The specimens of alpine cat's-tail from the two localities in California did not present significant morphological differences. Nor did they differ in habit and size from the European forms of *P. commutatum*. They were 15–30 cm tall; they formed leaf blades about 10 mm wide, with membranous, blunt ligules, about 2 mm long.

Fig. 1. Morphology of spikelets in three forms of *P. commutatum*. (**a**) Tetraploid, U.S.A., (**b**) Tetraploid, Sweden, (**c**) Diploid, Poland. Millimeter scale at right.

TABLE 2. Morphology of spikelets in different *P. commutatum* accessions

Feature	Form	Mean (mm) ± SD
Mean spikelet length (mm)	*P. commutatum* (4x), USA [B]	4.96 ± 0.41^b
	P. commutatum (4x), Sweden [D]	5.61 ± 0.68^a
	P. commutatum (2x), Poland [G]	4.89 ± 0.86^b
Mean awn length (mm)	*P. commutatum* (4x), USA [B]	1.99 ± 0.25^b
	P. commutatum (4x), Sweden [D]	2.39 ± 0.46^a
	P. commutatum (2x), Poland [G]	1.80 ± 0.56^c
Ratio of spikelet length to awn length	*P. commutatum* (4x), USA [B]	2.48 ± 0.37^b
	P. commutatum (4x), Sweden [D]	2.46 ± 0.35^b
	P. commutatum (2x), Poland [G]	2.90 ± 0.58^a

Duncan's test – values followed by the same letter are not significantly different at $p \leq 0.05$

TABLE 3. Length of stomata in different *P. commutatum* accessions

Taxon	Mean stomata length (µm) ± SD	Min – max (µm)
P. commutatum (4x), USA [A, B]	40.66 ± 4.01^b	31.40 – 53.53
P. commutatum (4x), Sweden [C]	41.55 ± 2.85^a	32.22 – 49.94
P. commutatum (4x), Scotland [E]	38.82 ± 4.96^c	21.51 – 54.18
P. commutatum (2x), Poland [F]	31.86 ± 3.82^d	21.67 – 43.06

Duncan's test – values followed by the same letter are not significantly different at $p \leq 0.05$

TABLE 4. Conventional karyotypes of three forms of *P. commutatum* – comprehensive data

Taxon	2n	Ploidy level	Mean monoploid genome length	SD	Mean chromosome length (µm)	SD	Karyotype formula
P. commutatum [A, B]	28	4x	28.71	0.69	4.07	0.10	24 m + 4 sm
P. commutatum [D]	28	4x	24.07	2.25	3.32	0.31	20 m + 8 sm
P. commutatum [F, G]	14	2x	30.43	3.91	4.23	0.63	10 m + 4 sm

[ ] – accession (see Tab. 1); SD – standard deviation

Morphology of spikelets

In the systematics of the genus *Phleum*, the morphology of spikelets, particularly the length of awns and occurrence of hairs on the margins of husks and awns, is an important diagnostic feature (Humphries, 1980; Conert, 1998). For this reason the abovementioned features were analysed in detail, comparing the values obtained for the specimens from California with those for the plants from Sweden and Poland. The measurements of the spikelet length revealed that the studied forms of *P. commutatum* had long (about 5mm) spikelets with relatively long awns (Fig. 1, Tab. 2). The American and European tetraploids differed in spikelet and awn length but not in spikelet to awn length ratio, which significantly differed from that of the diploid form (Tab. 2).

Length of stomata

Stomata length in the various forms of alpine cat's-tail was correlated with ploidy level (Tab. 3). However, the differences in mean length of stomata between all the forms, regardless of ploidy level, proved statistically significant.

KARYOTYPE ANALYSIS

The conventional karyotypes of all the studied forms of alpine cat's-tail (as well as those of the majority of other species belonging to this genus) differed only

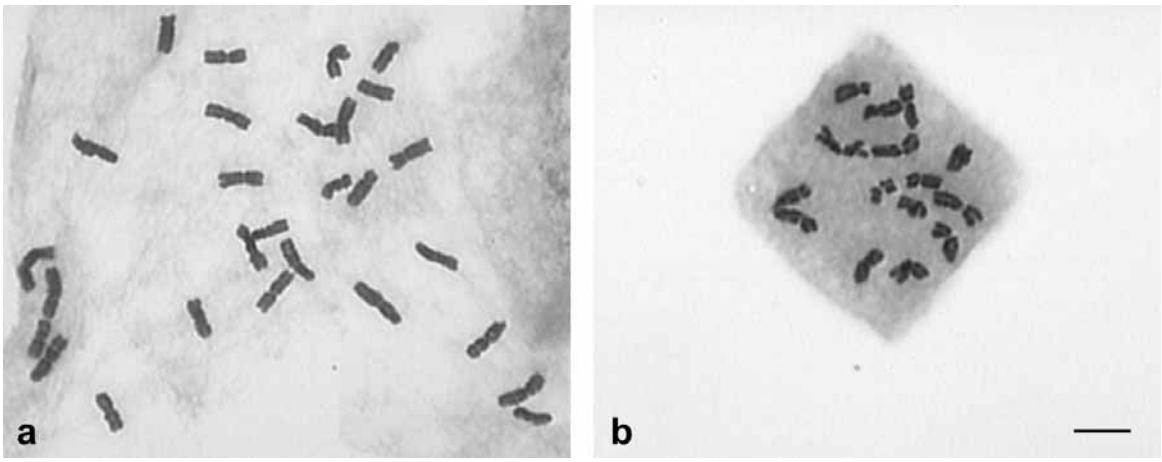

Fig. 2. Orceine-stained metaphase chromosomes of *P. commutatum*. (**a**) Point Reyes, U.S.A. (2n=28), (**b**) Beskidy Mountains, Poland (2n=14). Bar = 5 μm.

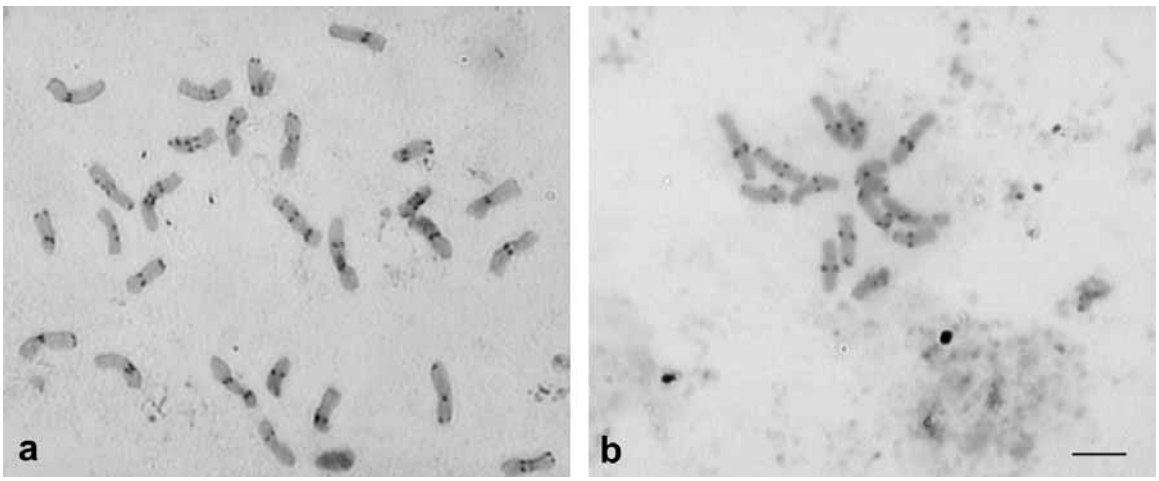

Fig. 3. C-banded metaphase chromosomes of *P. commutatum*. (**a**) Point Reyes, USA (2n=28), (**b**) Beskidy Mountains, Poland (2n=14). Bar = 5 μm.

slightly (Tab. 4). All the examined lines were characterised by the occurrence of SAT chromosomes easily distinguishable in the karyotype, as well as by the sporadic occurrence of 1–2 B chromosomes (Fig. 2).

Analysis of C-banded chromosomes gave better insight into the karyotype structure of *P. commutatum*. In all analysed forms of alpine cat's-tail, both diploid and tetraploid, C-banding revealed a centromeric type of heterochromatin distribution (Fig. 3). In the karyotypes of tetraploid specimens from California and Sweden examined in this respect, seven groups of chromosomes were distinguished. The only slight chromosome diversity within the majority of the distinguished groups suggests that the chromosome complements of these plants consist of four rather similar genomes. However, some detected differences (especially within chromosome groups 2 and 6; Fig. 4) may signify hybrid (allotetraploid) origin or diploidisation after an autotetraploid event.

Collective karyotypes (Fig. 5) showed similar distributions of relatively stable heterochromatin segments in the genomes of three analysed forms: *P. commutatum* [2x] from Poland and *P. commutatum* [4x] from Sweden and the U.S.A. Such segments occur in close proximity to all centromeres, and two (in diploid plants) or two/three (in tetraploid plants) chromosome ends. The analysed forms differed in total heterochromatin content (stable + polymorphic blocks of heterochromatin). The American tetraploids clearly differed in this respect from their tetraploid relatives from Sweden, and had heterochromatin amounts similar to those of the diploids from Poland (Fig. 6).

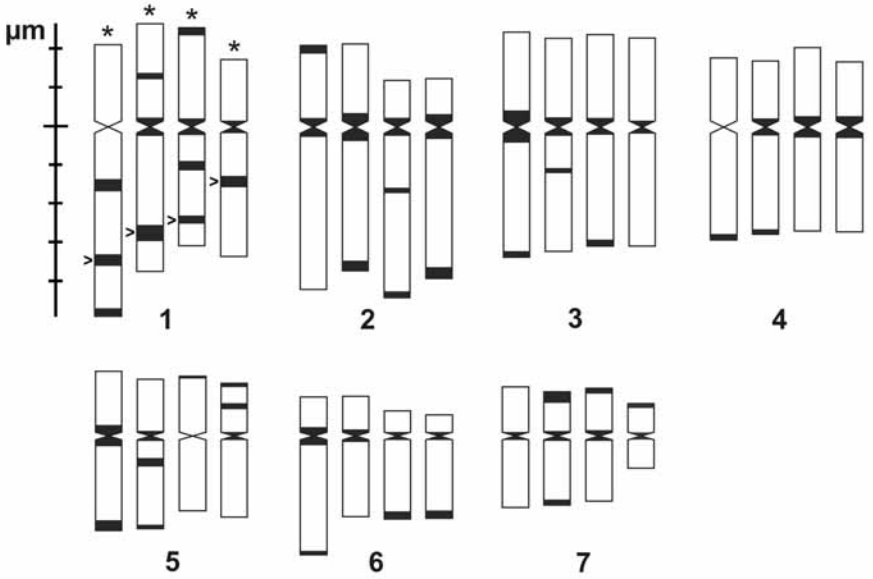

Fig. 4. C-band karyogram of metaphase plate of tetraploid *P. commutatum* (Levitt Lake, California, U.S.A.). * SAT chromosomes, > NOR-associated heterochromatin.

NUCLEAR DNA AMOUNT

Nuclear DNA amount measured in the Californian form of *P. commutatum* was compared with the data obtained for diploid and tetraploid cytotypes of *P. commutatum* from Europe and another European representative of the *P. alpinum* group, *P. rhaeticum* (Tab. 5; Fig. 7). The 2C DNA content of the tetraploid specimens was ~6.2 pg, and of the diploid ones varied from 2.4 pg to 2.9 pg.

The nuclear DNA amount measured in the specimens from the two Californian localities was almost identical (Tab. 5). Moreover, no significant statistical differences in 1Cx DNA content between the specimens from California and from Sweden and Scotland were recorded. However, the basal genome size of all studied diploid specimens was lower than that of the tetraploid ones, and different in the specimens from different locations. The lowest nuclear DNA content was found in *P. rhaeticum* originated from Romania.

TABLE 5. Nuclear 2C DNA amount and basal genome size (1Cx) in *P. commutatum* and *P. rhaeticum*

Species/ploidy level	Origin	Mean amount of nuclear DNA ± SD (pg)	
		2C	1Cx
P. commutatum (4x)	California [A]	6.165 ± 0.093	1.541 ± 0.023^a
P. commutatum (4x)	California [B]	6.137 ± 0.080	1.534 ± 0.020^a
P. commutatum (4x)	Sweden [C]	6.196 ± 0.095	1.549 ± 0.024^a
P. commutatum (4x)	Scotland [E]	6.193 ± 0.045	1.548 ± 0.011^a
P. commutatum (2x)	Poland [F]	2.727 ± 0.038	1.364 ± 0.019^e
P. commutatum (2x)	Poland [G]	2.630 ± 0.066	1.315 ± 0.033^d
P. rhaeticum (2x)	Greece [H]	2.926 ± 0.109	1.463 ± 0.055^b
P. rhaeticum (2x)	Poland [I]	2.489 ± 0.048	1.245 ± 0.024^e
P. rhaeticum (2x)	Romania [J]	2.407 ± 0.052	1.204 ± 0.026^f

[ ] – accession (see Tab. 1); Duncan's test – values followed by the same letter are not significantly different at $p \leq 0.05$; 1Cx – DNA content of one non-replicated monoploid genome with chromosome number x (according to Greilhuber et al., 2005).

DISCUSSION

MORPHOLOGY AND CYTOLOGY

Morphological and cytological observations showed that the American tetraploid *P. commutatum* does not deviate from the typical form of this taxon from the north of Europe. The tetraploids did differ in spikelet to awn length ratio from the diploid *P. commutatum*. This result is interesting because there is still a lack of good morphological characters enabling the diploid and tetraploid forms of this species to be distinguished (Joachimiak and Grabowska-Joachimiak, 2000). The two *P. commutatum* cytotypes differ clearly also in stomata length,

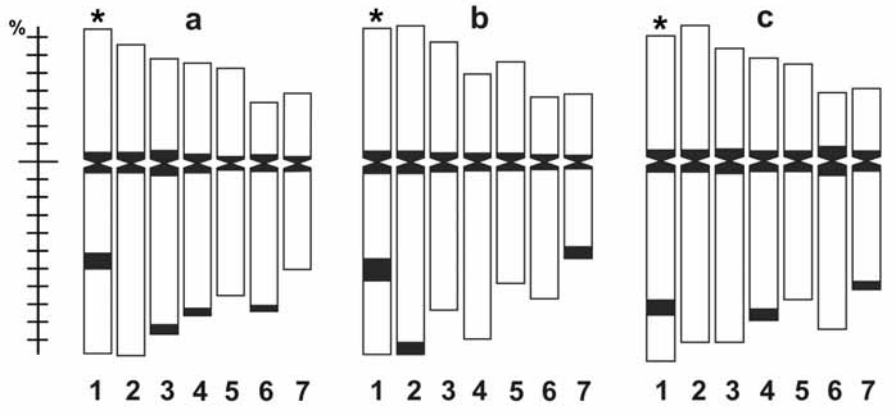

Fig. 5. C-band karyotypes of three forms of *P. commutatum*. (**a**) Tetraploid, U.S.A., (**b**) Tetraploid, Sweden, (**c**) Diploid, Poland. * SAT chromosomes. Variable terminal bands not indicated.

but this character also shows significant inter-population variability. This suggests that apart from ploidy level (amount of nuclear DNA), stomata length can also be influenced to a certain extent by other factors. Similar inter-population differences in the stomata length have already been recorded in reference to hexaploid *P. pratense* (Joachimiak and Grabowska-Joachimiak, 2000).

American and European tetraploids show very similar karyotype composition. The only cytologically detectable difference between these lines concerns the total amount of heterochromatin. However, substantial inter-population differences in the heterochromatin amount were observed in diploid *P. commutatum*, *P. rhaeticum* and some other *Phleum* taxa analysed so far (Joachimiak and Kula, 1993, 1996; Joachimiak et al., 1997). Variation in the number and size of polymorphic heterochromatic segments has been found in many plants, and possible mechanisms of this variation have been suggested: spontaneous amplifications, asymmetric crossing over, transpositions, etc. (Guerra, 2000; Bennetzen, 2000). The evolutionary role of such polymorphisms is largely unknown. It should not be concluded, however, that polymorphic blocks of heterochromatin are unnecessary surplus of no functional importance, nor that their persistence in the genomes of some plants is completely accidental (see Joachimiak et al., 1997; Hemleben et al., 2000, and references cited therein).

The centromeric type of heterchromatin distribution observed in two *P. commutatum* cytotypes is a rather unusual karyotype feature within the genus *Phleum*. In all other representatives of sect. *Phleum* and almost all representatives of sect. *Chilochloa*, telomeric distribution of heterochromatin prevails. The only exception is the annual *P. arenarium* from sect. *Chilochloa* (Kula and Kutyna, 2005; Joachimiak 2005).

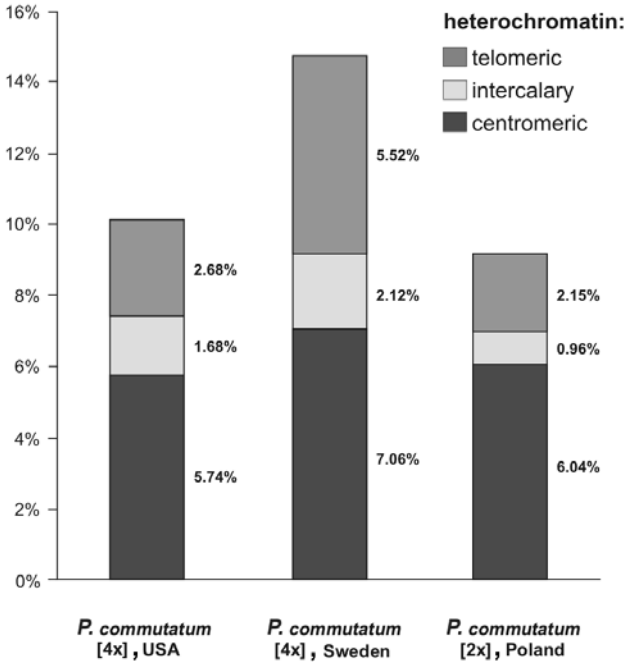

Fig. 6. Heterochromatin amount (expressed as % of total karyotype length) in three *P. commutatum* forms. Both stable and polymorphic segments were considered.

NUCLEAR DNA AMOUNT

The differences in 2C DNA content between American and European *P. commutatum* races are not significant; the DNA value of all analysed tetraploid plants stands at about 6.2 pg. Most probably, the vast bipolar region where this cytotype can be found at present was captured by one primeval tetraploid form, emerging in Europe, which has not changed much until now. Interestingly, different accessions of diploid *P. commutatum* and *P. rhaeticum*

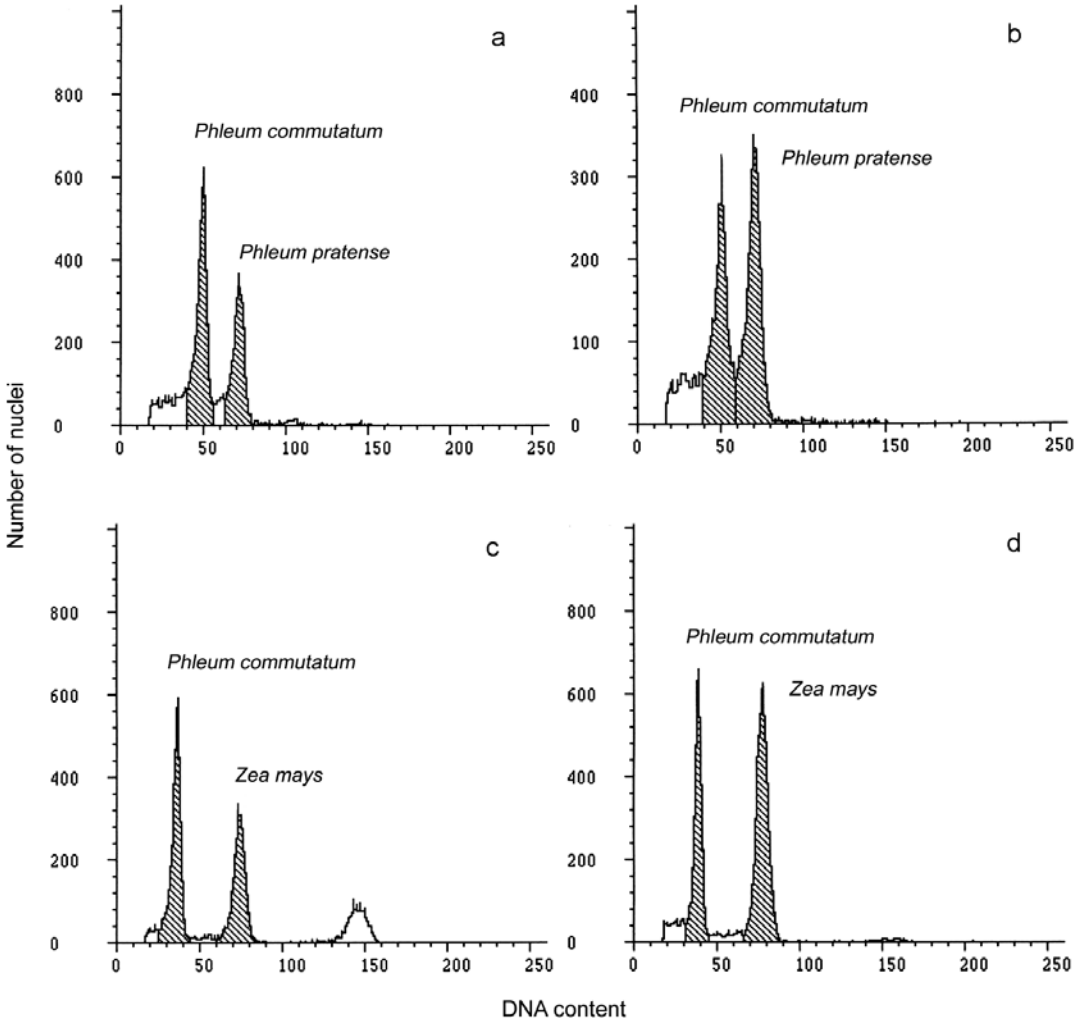

Fig. 7. DNA histograms of nuclei isolated simultaneously from leaves of *P. commutatum* and internal standard. (**a**) Tetraploid, U.S.A. [B], FR = 0.69, CV = 4.53%, (**b**) Tetraploid, Sweden [C], FR = 0.70, CV = 4.68%; (**c**) Diploid, Poland [G], FR = 0.47, CV = 4.39%, (**d**) Diploid, Poland [F], FR = 0.50, CV = 4.25%. [ ] indicates origin of *Phleum* samples (Tab. 1); FR – fluorescence ratio of G_1 peak means of *P. commutatum* and the internal standard; CV – coefficient of variation for the internal standard.

are not so stable in this respect and show significant DNA-value differences. It seems probable that these differences are connected with the high variability in the amount of polymorphic heterochromatin usually observed in these taxa (Joachimiak and Kula, 1993, 1996; Joachimiak et al., 1997). On the other hand, there is no relationship between genome size and heterochromatin amount in tetraploid *P. commutatum* – American and European accessions of this form show substantially the same DNA value despite a clear (5%) difference in heterochromatin amount.

This is the first flow-cytometric estimation of nuclear DNA amount in *P. commutatum* [2x] and *P. rhaeticum*. The mean 2C DNA value of four *P. commutatum* [4x] accessions analysed here (6.17 pg) is 0.957-fold lower than reported by Bennett and Leitch (http://www.rbgkew.org.uk/cval/homepage.html) (6.45 pg).

THE ORIGIN OF TETRAPLOID *P. COMMUTATUM*

In light of the current morphological and karyological data available, it is clear that diploid *P. commutatum* (CC genome formula), or a close ancestral relative, has contributed at least one of the genomes of *P. commutatum* [4x]. Indeed, if the latter is an autotetraploid (CCCC), it may have contributed both genomes. However, if *P. commutatum* [4x] is an allotetraploid (CCXX), the other genome could be

assumed to be from ancestral *P. rhaeticum* (RR genome formula) or its close (probably extinct) relative. Although chromosome data suggest rather an autopolyploid origin for this most widespread *Phleum* representative, hybrid origin cannot be excluded. It is well known that allopolyploids are often more vigorous than their diploid parents and may acquire geographical distributions ranging far beyond those of their ancestors (Stebbins, 1947, 1950).

Unfortunately, auto-/allopolyploid origin of the tetraploid form cannot be unambiguously deduced from the available karyosystematic data, nor from nuclear DNA measurements. The 2C DNA amount of *P. commutatum* [4x] (~6.2 pg) distinctly deviates from additivity (the calculated DNA value for the genomic constitution CCCC is ~5.4 pg, and for CCRR is ~5.2 pg). There are two possible explanations of the problem: (1) The genome size of the diploid ancestral form was ~1.5 pg, that is, the same or very similar to that recorded in the present tetraploid races of *P. commutatum*; modern diploids, both *P. commutatum* and *P. rhaeticum* (genome size ~1.3 pg) were descendants of this (extinct) ancestral species. (2) The ancestor (or ancestors) of tetraploid *P. commutatum* had smaller genomes, and the amount of DNA increased in the emerging tetraploid. This seems very possible, because many natural polyploids show different, unexpected changes in 2C DNA amount (Ohri, 1998). It has been suggested that non-additive changes on the DNA level are typical for allopolyploid species, because of 'genomic shock' induced by hybridity and the need for rapid adjustment of two different genomes joined together in one nucleus (Ozkan et al., 2001). On the other hand, it has been shown that the nuclear DNA amount is rather less than expected in the majority of natural polyploids (Levy and Feldman, 2002; Leitch and Bennett, 2004).

Recently, it was shown that some DNA sequences (nuclear ITS and trnL gene intron of chloroplast DNA) accurately discriminate between four diploid taxa recognized in sect. *Phleum* (*P. commutatum* [2x], *P. rhaeticum*, *P. bertolonii* and *P. echinatum*) (Stewart et al., 2005). Future molecular studies of different tetraploid cat's-tail lines should shed light on the origin of *P. commutatum* [4x] and the relationship between the American and European races of this taxon.

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