Folia Geobotanica 40: 367–384, 2005

GENOME SIZE DISCRIMINATES BETWEEN CLOSELY RELATED TAXA *ELYTRIGIA REPENS* AND *E. INTERMEDIA* (*POACEAE*: *TRITICEAE*) AND THEIR HYBRID

Václav Mahelka[1)], Jan Suda[1,2)], Vlasta Jarolímová[1)], Pavel Trávníček[1,2)] & František Krahulec[1)]

1) Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice 1, CZ-252 43, Czech Republic, fax +420 2 6775 0031, e-mail mahelka@ibot.cas.cz

2) Department of Botany, Charles University, Benátská 2, CZ-128 01, Prague, Czech Republic

Abstract: Flow cytometric and karyological investigations were performed on the closely related taxa *Elytrigia repens* and *E. intermedia* (*Poaceae*: *Triticeae*) from the Czech Republic. DNA-hexaploids clearly prevailed among 238 examined plants and amounted to 96.2% of all samples. 2C-values ± s.d. for hexaploid *Elytrigia repens* and *E. intermedia* were estimated at 23.27 ± 0.20 pg and 27.04 ± 0.24 pg respectively. Genome size thus allowed reliable separation of the two species (difference ca. 16%) as well as the identification of hybrid individuals. Natural hybridization in *E. repens* – *E. intermedia* alliance seems to be quite a common phenomenon as indicated from a large proportion (one sixth) of hexaploid samples with intermediate 2C-values. Previously, the crosses were most probably overlooked or misidentified due to their weak morphological differentiation. New nonaploid cytotypes (2n=9x=63) were revealed for both species as well as for the hybrid (determined on the basis of morphological characters only), representing the first records from the field. Fusion of unreduced and reduced gametes of the hexaploids is the most plausible mode of nonaploid origin.

Keywords: C-value, Chromosome number, Cytotype, DNA content, Flow cytometry, Hybridization, Nonaploid, Wheatgrass

Nomenclature: LÖVE 1984

INTRODUCTION

Elytrigia repens (L.) NEVSKI [Syn.: *Agropyron repens* (L.) P. BEAUV., *Elymus repens* (L.) GOULD] and *Elytrigia intermedia* (HOST) NEVSKI [Syn.: *Agropyron intermedium* (HOST) P. BEAUV., *Thinopyrum intermedium* (HOST) BARKWORTH et D.R. DEWEY] are representatives of the family *Poaceae*, tribe *Triticeae* (wheatgrasses). *Triticeae* is a large group comprising approximately 500 taxa divided into 37 genera (LÖVE 1984). The relationships among, and the taxonomy and phylogeny of members of *Triticeae* have triggered a long-term dispute and are still in need of further targeted investigation. Among others, the complexity of the situation is caused by frequent allopolyploid origin of the species that partly share the same genomes (up to dodecaploid plants are known, each combining up to four more-or-less different genomes). Reticulate evolution manifesting itself in the majority of characters is another source of problems. The complexity of the group can easily be distinguished from the taxonomy of the two taxa studied: 137 and 132 synonyms were found for *E. intermedia* and *E. repens*, respectively (CLAYTON & WILLIAMSON 2003). Both

species are rhizomatous perennial grasses that are considered as out-crossing, wind pollinated, and reproduce by seeds and rhizomes (on a local scale).

Elytrigia repens is a native Eurasian species that has become established in most temperate zones of the world. It is one of the most troublesome weeds on cultivated land. In the Czech Republic, the plant is widespread throughout the whole territory from lowlands to the mountain belt, occasionally surviving even above the timberline. It occupies all man-made habitats and arable ground, but occurs also on such natural habitats as steppes, forest margins and tracks. The genome constitution of hexaploid cytotypes (2n=6x=42) was determined as StStH (where St and H designate *Pseudoroegneria* (NEVSKI) Á. LÖVE and *Hordeum* L. genomes respectively) (ASSADI & RUNEMARK 1995). Nevertheless, a more complex genome pattern seems to be plausible. Recent molecular phylogenetic study (MASON-GAMER 2004) revealed at least five distinct lineages, suggesting that allopolyploidy and introgression took place during the evolution of *E. repens*. Chloroplast DNA data identified three potential maternal genome donors (*Pseudoroegneria*, *Dasypyrum* (COSS. et DURIEU) T. DURAND and *Thinopyrum* Á. LÖVE), whilst nuclear DNA data confirmed the previously suggested *Pseudoroegneria* and *Hordeum* as genome contributors of hexaploid plants and, unexpectedly, three additional genome donors were identified: *Taeniatherum* NEVSKI and two donors of unknown identity.

Elytrigia intermedia occurs from France in the west to the Volga river region in the east, with further distribution forming a bend from Turkey and the Caucasus to Iran, Afghanistan, Pakistan, the Pamir Mts. and Altai Mts. in Central Asia (HEGI 1977). The species has also been introduced to North America. In the Czech Republic, its distribution strongly reflects the occurrence of steppe habitats (see Fig. 1). The species colonizes steppes, pine forests on sandy ground, vineyards, orchards and field margins in warm regions. Genome constitution was determined as E^eE^eSt (LIU & WANG 1993) or E^eE^bSt (CHEN et al. 1998), where E^e and E^b designate the closely related *Thinopyrum elongatum* (HOST) D.R. DEWEY and *Th. bessarabicum* (SAVUL. et RAYSS) Á. LÖVE genomes.

The principal morphological characters that distinguish between the cited *Elytrigia* species are as follows: (1) leaf sheath margins – hairy in *E. intermedia* vs. glabrous in *E. repens* (KUBÁT et al. 2002), and (2) glume shape – truncate or very shortly mucronate (never awn-tipped or gradually tapering) in *E. intermedia* vs. awn-tipped or gradually tapering (at least some of each inflorescence) in *E. repens* (BARKWORTH & DEWEY 1985). Nevertheless, many plants from the field combine both features, suggesting that hybridization might have occurred. Hybrid individuals were originally described as *Agropyron* ×*mucronatum* OPIZ (BERCHTOLD & OPIZ 1836) (syn. *Elytrigia mucronata* (OPIZ) PROKUDIN), however, their identification on the basis of morphological characters is uncertain due to large morphological variation of the putative parental species and frequent overlap of character values.

A survey of published karyological data has revealed considerable variation in ploidy levels (based on x=7) for both species (Table 1). Hexaploid cytotype (2n=42) prevails in *E. repens*, however tetraploid (2n=28) and octoploid (2n=56) individuals were also collected in the field (SAKAMOTO & MURAMATSU 1963). PETO (1930) reported 34 and 35 chromosomes for two individuals from Russia and considered these plants hybrids between hexaploid *E. repens* and some species with a lower chromosome set. In addition, one

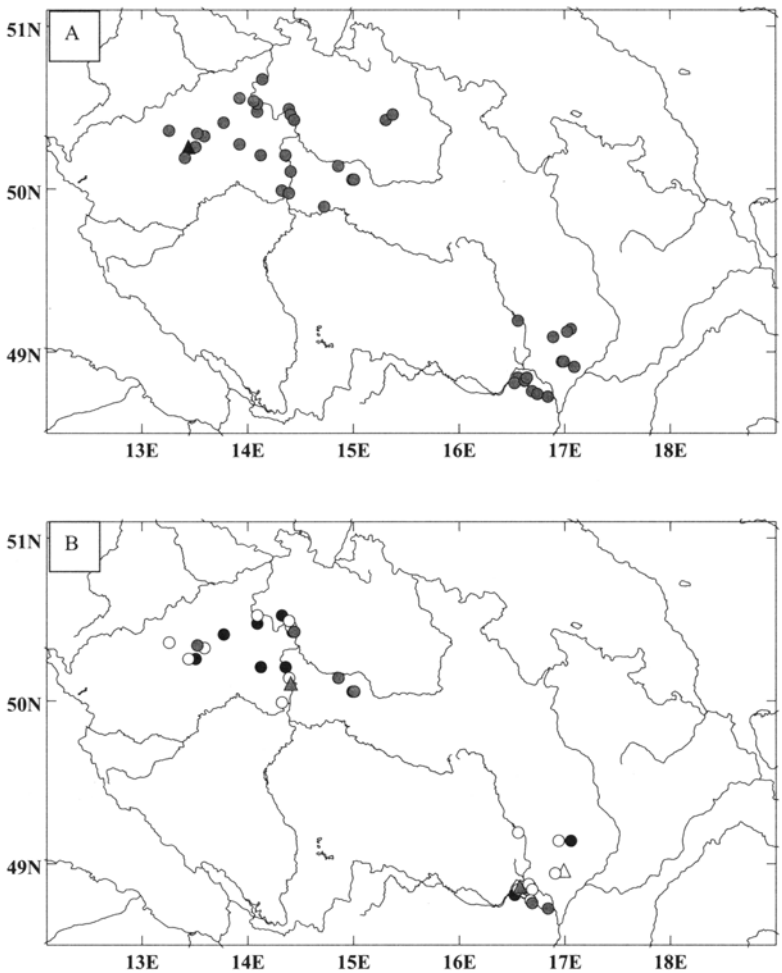

Fig. 1. A – Distribution of *Elytrigia repens* cytotypes in the area studied. Shaded dots designate localities of hexaploid plants, the black triangle designates the locality of a nonaploid individual (2n=9x=63). B – Distribution of *E. intermedia* cytotypes and putative hybrids. Open circles designate hexaploid *E. intermedia*, shaded circles designate hexaploid hybrids, black circles designate localities with the co-occurrence of both types. Triangles designate nonaploid plants (open – *E. intermedia*, shaded – hybrids). The Slovakian locality (no. 18) is omitted.

polyhaploid (2n=21) and one nonaploid (2n=63) plant were detected by DEWEY (1974) in the population of twin seedlings during the experimental germination study. An analogous situation was encountered in *E. intermedia*, where predominant hexaploid and minority tetraploid (BOWDEN 1965) cytotypes occurred. Along with the euploid plants, several aneuploids were detected for both species: 2n=40 for *E. repens* (PETROVA 1975), and 2n=41 and 43 for *E. intermedia* (HARTUNG 1946, BOWDEN 1965, ASSADI 1995). DNA 2C-values of the hexaploid individuals were estimated at 25.96 pg for *E. repens* (BENNETT et al. 1982), and

Table 1. List of published chromosome counts for *Elytrigia repens* and *E. intermedia*. The origin of the analyzed material is provided in brackets (where possible). Superscript numbers refer to the original plant names used in the article.

Elytrigia repens 2n	n	Reference
42		STOLZE 1925[1], MOWERY 1929 (USA-Minnesota)[1], PETO 1929 (Canada)[1], 1930 (Western Canada[1], Denmark[1], Russia-Caucasus Mts.[2]), SCHIEMANN 1929[1], SIMONET 1935[1], VAKAR 1935[1], ROHWEDER 1937[1], SOKOLOVSKAYA & STRELKOVA 1939 (Russia)[1], ÖSTERGREN 1940a[1], b (Sweden)[1], SHARMAN 1943[1], SENN et al. 1947 (Canada)[1], 1949[1], PÓLYA 1948 (Hungary)[1], BEAUDRY 1951 (USA-Wisconsin)[1], HUNZIKER 1954[1], LÖVE & LÖVE 1956 (Iceland)[1], CAUDERON 1958 (France)[1], GILLETT & SENN 1960[1], JONES 1960[1], DEWEY 1961 (USA-Utah)[1], 1970 (USA)[1], 1972[1], 1980 (Iran)[1], BOWDEN 1965 (Canada)[1], GADELLA & KLIPHUIS 1966 (The Netherlands)[1], FERNANDES & QUEIROS 1969 (Portugal)[1], HENEEN 1972[3], KOZUHAROV & PETROVA 1973 (Bulgaria)[1], LÖVE & KJELLQVIST 1973 (Spain)[3], KRUSE 1974[1], ROOS 1975 (Estonia)[5], FREY et al. 1977 (Poland)[1], JOHNSON & JALAL 1977[1], DRULEVA in PROKUDIN et al. 1977 (Ukraine)[5], PETROVA in PROKUDIN et al. 1977 (Ukraine)[5], MURÍN in MÁJOVSKÝ et al. 1978 (Slovakia)[1], POGAN et al. 1980 (Poland)[1], LÖVE 1980a[5],b (Sweden)[6], 1984[8], 1986[9], PROBATOVA & SOKOLOVSKAYA 1980 (Russia-Altai Mts.)[7], 1982 (Russia-Far East)[5], NAPIER & WALTON 1981 (Canada)[1], BELAYEVA & SIPLIVINSKI 1981 (Russia)[1], AROHONKA 1982 (Finland)[3], GUZIK 1984 (Western Russia)[5], LU et al. 1990 (China)[5], SUN et al. 1992 (China)[5], SALOMON & LU 1994 (China-Xinjiang, Tiansan, Balguntai)[3], DEMPSEY et al. 1994 (Great Britain)[7], ASSADI 1995 (Iran)[3], ASSADI & RUNEMARK 1995 (Iran)[5], GOUKASIAN & NAZAROVA 1998[5], LÖVKVIST & HULTGÅRD 1999 (Sweden)[5]
28, 42		AVDULOV 1931[1], ROZANOVA 1940 (Russia-Altai Mts.)[1], HEISER & WHITAKER 1948 (USA-California)[1], SOKOLOVSKAYA & STRELKOVA 1948 (Russia-Altai Mts.)[1], JONES 1957[1], PARFENOV & DMITRIEVA 1988 (Belarus)[5], MIZIANTY et al. 2001 Poland)[3]
28		SINGH 1964 (Great Britain-RBG Kew)[1], GUZIK & LEVKOVSKII 1979 (East Russia)[1]
56		SAKAMOTO & MURAMATSU 1963[1]
21, 42, 63		DEWEY 1974 (USA-Utah)[1]
42, 40		PETROVA 1975 (42 Ukraine, 40 Moldova)[5]
34, 35		PETO 1930 (Russia-Caucasus Mts.)[1]
	21	DEWEY 1967 (USA)[1]
	14, 21	DEVESA et al. 1990 (Spain)[4]
	21+1-2 B	GERVAIS et al. 1999[5]

(original names: *Agropyron repens* (L.) P. BEAUV.[1], *A. repens* var. *glaucescens* ENGL.[2], *Elymus repens* (L.) GOULD[3], *Elymus repens* (L.) GOULD subsp. *repens*[4], *Elytrigia repens* (L.) NEVSKI[5], *Elytrigia repens* subsp. *arenosa* (PETIF) Á. LÖVE[6], *Elytrigia repens* (L.) NEVSKI subsp. *repens*[7], *Elytrigia repens* subsp. *pseudocaesia* (PACZ.) TZVELEV[8], *Elytrigia repens* subsp. *lolioides* (KAR. et KIR.) Á. LÖVE[9]).

Elytrigia intermedia (Table 1. – continued)

2n	n	Reference
42		PETO 1930[6], 1936[6], 1938[6], VAKAR 1934[6], 1935[6], 1936[6], PETO & BOYES 1940[6], SIMONET 1935[6], CHIZNYAK 1936[1], ARARATYAN 1938[6], 1939[6], JOHNSON 1938[6], KOSTOFF 1941[6], HARTUNG 1946 (Russia-Caucasus Mts.)[1], LITARDIÈRE 1948 (Corsica)[6], BELL 1950[6], THOMPSON & GRAFIUS 1950[8], PÓLYA 1950 (Hungary)[6], MATSUMURA 1951[6], 1952[6], MATSUMURA et al. 1958a[6], b[6], POPE & LÖVE 1952[2], SACHS 1952[6], BELL & SACHS 1953[6], GAUL 1953a[6], b[6], STEBBINS & PUN 1953 (Turkey)[1], CAUDERON 1954[6], 1958 (France)[6], 1962[1], MURAMATSU 1955[6], TATEOKA 1956[6], LÖVE & LÖVE 1961[6], SAKAMOTO & MURAMATSU 1963[1], SCHULZ-SCHAEFFER & JURA 1967 (Kazakhstan, Uzbekistan)[1], MURÍN in MÁJOVSKÝ et al. 1974 (Slovakia)[5], CHOPANOV & YURTSEV 1976 (Turkmenistan)[11], PETROVA in PROKUDIN et al. 1977 (Ukraine)[5], DRULEVA in PROKUDIN et al. 1977 (Ukraine)[11], PROBATOVA & SOKOLOVSKAYA 1978 (Russia-Caucasus Mts.)[8], LÖVE 1980 (Slovenia)[7], 1984[6,8,9,10], VÁCHOVÁ & FERÁKOVÁ 1980 (Slovakia)[11], NAPIER & WALTON 1981[3, 4], LÖVE & LÖVE 1982 (Italy)[5], PIAO 1982[1], POGAN et al. 1985 (Poland)[1], MIČIETA 1986 (Slovakia)[5], LIMIN & FOWLER 1988[16], LIU & WANG 1993 (Germany)[16], MUJEEB-KAZI et al. 1994[16], ASSADI & RUNEMARK 1995 (Iran)[16], PETROVA & STOYANOVA 1997 (Bulgaria)[12], MIZIANTY et al. 2001 (Poland)[12]
28, 42, 43		BOWDEN 1965 (Canada)[1]
41, 42, 43		ASSADI 1995 (Iran)[13, 14, 15]
43		HARTUNG 1946 (Turkey)[1]

(original names: *Agropyron intermedium* (HOST) P. BEAUV.[1], *A. trichophorum* (LINK) K. RICHT.[2], *A. trichophorum* (LINK) K. RICHT. cv. Greenleaf[3], cv. Chief[4], *Elytrigia intermedia* (HOST) NEVSKI[5], *E. intermedia* (HOST) NEVSKI subsp. *intermedia*[6], *E. intermedia* subsp. *barbulata* (SCHUR) Á. LÖVE[7], *E. intermedia* subsp. *trichophora* (LINK) Á. LÖVE et D. LÖVE[8], *E. intermedia* subsp. *pulcherrima* (GROSSH.) TZVELEV[9], *E. intermedia* subsp. *pouzolzii* (GODRON) Á. LÖVE[10], *E. trichophora* (LINK) NEVSKI[11], *Elymus hispidus* (OPIZ) MELDERIS[12], *E. hispidus* (OPIZ) MELDERIS var. *hispidus*[13], *E. hispidus* var. *podperae* (NÁBĚLEK) ASSADI[14], *E. hispidus var. villosus* (HACK.) ASSADI[15], *Thinopyrum intermedium* (HOST) BARKWORTH et D.R. DEWEY[16]).

26.25 pg and 25.92 pg for *Thinopyrum intermedium* (HOST) BARKWORTH et D.R. DEWEY subsp. *intermedium* (syn.: *E. intermedia* subsp. *intermedia*) and *Th. intermedium* subsp. *barbulatum* (SCHUR) BARKWORTH et D.R. DEWEY (syn.: *E. intermedia* subsp. *barbulata* (SCHUR) Á. LÖVE) (VOGEL et al. 1999), respectively.

The aim of this study was to examine ploidy variation in two *Elytrigia* species native to the Czech Republic, to find species-specific marker(s) that allow reliable taxa identification, and to state whether the interspecific hybridization occurs as preliminarily assessed on the basis of morphological analysis.

MATERIAL AND METHODS

Field sampling

Two hundred and thirty-eight plants collected at 55 localities were included in the study (see Appendix, Fig. 1). Sampling design was targeted to cover the majority of morphological

variation within each locality. Using the two above-mentioned morphological characters, the material was preliminarily assigned to three groups: *E. repens*, putative hybrids, and *E. intermedia*. All plants were transferred to the experimental garden of the Institute of Botany at Průhonice for further investigation. Vouchers are deposited in the herbarium of the Institute of Botany (PRA).

Chromosome counting

At the first step, chromosomes of the 14 most typical plants representing all three groups (4 *E. repens*, 3 hybrids, 7 *E. intermedia*) from 12 localities were counted (see Appendix). Root tips of the cultivated samples were pre-treated with a saturated solution of p-dichlorbenzene, fixed in a mixture of alcohol : acetic acid (3 : 1) and stained with lacto-propionic orceine. All the plants were found to be hexaploid (2n=6x=42) and served as reference material for cytometric analyses. Karyological investigation was subsequently also applied to the 9 *Elytrigia* samples with relative fluorescence of nuclei deviating from that of hexaploid standards.

Genome size estimation

Genome size (either in relative or absolute units) was determined by flow cytometry using Partec PA II instrument. Sample preparation followed the two-step procedure originally described by OTTO (1990). *Triticum aestivum* var. *lutescens* (ALEF.) MANSF. 'Bezostaya 1' was chosen as an appropriate primary internal standard (with close, but not overlapping genome size); its 2C-value was estimated as 34.4 pg using *Vicia faba* L. (2C=26.9 pg; DOLEŽEL et al. 1992) as an internal standard. Intact leaf tissues of the analyzed plant (ca. 0.5 cm^2) and the internal standard were co-chopped with sharp razor blade in a Petri dish containing 1 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20). The suspension was filtered through a 42-µm nylon mesh and centrifuged (150 g/5 min). The supernatant was removed and the pellet was resuspended in 100 µl of fresh ice-cold Otto I buffer. Samples were incubated for 10 min at room temperature, and stained with 1 ml of Otto II buffer (0.4 M Na_2HPO_4 . 12 H_2O) supplemented with ß-mercaptoethanol (2 µl/ml) and fluorochrome. DAPI at a concentration of 4 µl/ml was used for ploidy level estimation (relative genome size), propidium iodide + RNase IIA (both at a concentration of 50 µl/ml) were used to determine absolute genome size. After a few minutes, relative fluorescence intensity (setting *Triticum* as the unit value) of the isolated nuclei was recorded (mostly 5 000 particles). Coefficients of variation of the G0/G1 peak of the plants analyzed varied from 1.23% to 3.11% and from 1.51% to 4.58% for analyses with DAPI and propidium iodide respectively. All samples with relative fluorescence deviating from the range defined for karyologically-confirmed reference material were re-analyzed using corresponding hexaploid *Elytrigia* as internal standard and all of them were also subjected to chromosome counting. For absolute genome size estimation, three hexaploid plants of both *Elytrigia* species were carefully selected according to the following criteria: (1) unambiguous determination on the basis of morphological characters, (2) spatial isolation from its counterpart, (3) cpDNA relevant to particular species (MAHELKA et al., unpubl. results), (4) relative nuclear DNA content matching the range for a given species. Each plant was

re-estimated at least three times on different days to minimize potential random instrumental drift. The genome size terminology follows GREILHUBER et al. (2004). As the ploidy of the samples was mostly inferred from their nuclear DNA content, it should be regarded as the DNA-ploidy level (HIDDEMAN et al. 1984).

RESULTS AND DISCUSSION

DNA-ploidy estimation/analysis

Three distinct groups were obtained when the relative fluorescence of nuclei of the 14 karyologically-proved hexaploids using *Triticum aestivum* var. *lutescens* (2C=34.4 pg) as internal standard was measured. These groups agreed fully with the previous plant determination (*E. repens*, putative hybrids, *E. intermedia*) based on morphological characters. Subsequent cytometric analyses of 224 individuals lacking any chromosome count further confirmed this genome size differentiation. The majority of the material (215 plants) were also DNA-hexaploids. A marked prevalence of the hexaploid cytotype was expected and agreed with records in the literature from other countries (cf. Table 1). Relative fluorescence of nuclei for the whole hexaploid assemblage were as follows (min.–max. (mean)): 0.710–7.728 (0.718), 0.755–0.782 (0.770), and 0.805–0.828 (0.816), corresponding to *E. repens*, putative hybrids, and *E. intermedia* respectively. The average difference between the parental species (using DAPI staining with A-T bases preference) thus amounted to 13.7%, i.e., *E. intermedia* possessed 1.137-fold higher values. The hybrids were located more or less mid-way (Figs. 2, 3). Very narrow intraspecific genome size variation was always found: 2.5% for *E. repens*, 2.9% for *E. intermedia* and 3.6% for the hybrids. Any intermediate fluorescence intensity between putative hybrids and parental species was not observed, indicating the lack of back-crosses due to potential hybrid sterility. The level of fertility in our collection of hexaploid hybrids has not yet been determined. However, ASSADI & RUNEMARK (1995) reported irregular meiosis with only one homologous pair of genomes and 20% pollen fertility in artificial crosses between *E. repens* and *Thinopyrum intermedium*. They supposed that since hybrids are relatively common in the field and reproduce asexually by rhizomes, at least some gene flow via backcrossing to the parents may be expected.

Species-specific DNA values permit the use of genome size as a reliable marker for taxa and hybrid delimitation in any *E. repens* – *E. intermedia* alliance. These data are certainly more robust and unbiased than morphological variation, which forms more a continuum (morphological discrimination between putative hybrids and parental species is vague and affected negatively by subjective judgement). Generally, in some species, ploidy level is therefore the main classification criterion, as demonstrated in *Festuca* (ŠMARDA & KOČÍ 2003). Inconsistencies between morphological and genome size data in our species set were encountered in 10.5% of the hexaploid samples (17 hybrid individuals, 3 individuals of *E. repens*, and 4 individuals of *E. intermedia* were morphologically misidentified). Within all the hexaploid cytotypes, *E. repens* was represented by 101 plants from 45 localities, putative hybrids by 38 plants from 20 localities, and *E. intermedia* by 90 plants from 31 localities (see Appendix, Fig. 1). Both species co-occurred at 21 localities, 8 of them were also inhabited by hybrid individuals. The large proportion of hybrid plants (16.6% of the hexaploids) is quite surprising and points to the weak reproduction barrier between *E. repens* and *E. intermedia*.

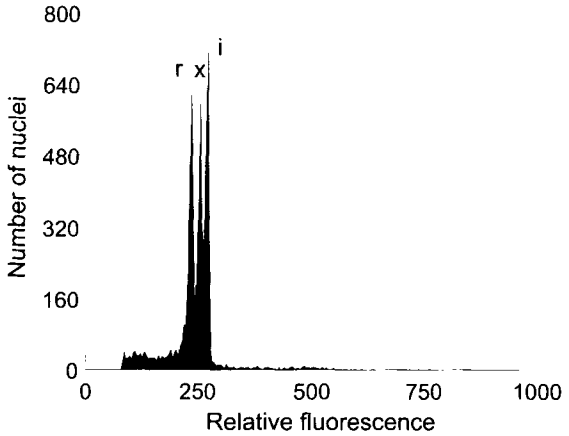

Fig. 2. Histogram of relative DNA content of *Elytrigia repens* (r), putative hybrid (x), and *E. intermedia* (i). Nuclei of the three plants were isolated, stained with DAPI and analyzed simultaneously.

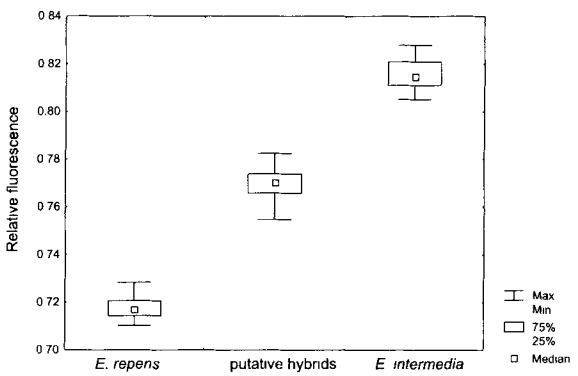

Fig. 3. Box plots illustrating relative fluorescences of nuclei (DAPI-stained) for hexaploid plants of *Elytrigia repens* (101 individuals), putative hybrids (38 individuals), and *E. intermedia* (90 individuals). *Triticum aestivum* was used as internal standard and its genome size was considered as unit value.

However, it should be noted that this is not the frequency of hybrids in the field, as the sampling was not random but targeted to cover the majority of morphological variation within each locality. We are convinced that comprehensive cytometric investigation would reveal hybrid individuals also at other localities within the distribution area of the parental species. Considering the susceptibility of *Elytrigia* to hybridization, the discovery of crosses with other related taxa cannot be ruled out. It is a plausible assumption that genome size data might be very useful in this mission and thus open new prospects in *Triticeae* research.

2C genome size estimation

Average 2C-values ± s.d. estimated using intercalating fluorochrome propidium iodide were 23.27 ± 0.20 pg and 27.04 ± 0.24 pg for *E. repens* and *E. intermedia* respectively, giving a difference of 16.2% (Table 2). A markedly different number of samples measured and/or the unequal proportion of AT/GC base pairs in the two taxa might be responsible for certain discrepancies between the results for DAPI and propidium iodide staining.

Our genome size estimate for *E. intermedia* (2C=27.04 pg) matches quite well the flow-cytometric data published by VOGEL et al. (1999), who reported virtually identical 2C-values (25.92–26.25 pg) for two subspecies of *Thinopyrum intermedium* (syn.: *Elytrigia intermedia*). However, no intraspecific division was possible in our material owing to continuous variation in the morphological characters used for subspecies delimitation; moreover, the formerly distinguished morphotypes lack any separate geographical distribution. On the contrary, the

Table 2. Genome size data for hexaploid *Elytrigia repens* and *E. intermedia* estimated by propidium iodide flow cytometry. *Triticum aestivum* (2C=34.4 pg) calibrated against *Vicia faba* (26.9 pg; DOLEŽEL et al. 1992) was used as internal standard. Three individuals of each species were measured on three different days in order to minimize potential random instrumental drift.

Species	Chromosome number (2n)	2C-value ± s.d. (pg)	C-value (pg)	Cx-value (pg)
Elytrigia repens	42	23.27 ± 0.20	11.64	3.88
Elytrigia intermedia	42	27.04 ± 0.24	13.52	4.51

previous genome size for *E. repens* (2C=25.96 pg; BENNETT et al. 1982) as determined by Feulgen microdensitometry shows 1.12-fold higher values compared to our result, and approaches the estimate for *E. intermedia*. Three theories can be invoked to explain this discrepancy: (1) the existence of genome size variation between *E. repens* from Georgia and the Czech Republic, (2) methodological problems during the preparation of samples for densitometry (related primarily to the temperature and duration of hydrolysis; GREILHUBER 1998), and (3) the use of different internal standard with deviating 2C-value.

Nonaploid plants (2n=9x=63)

Nine individuals (ca. 3.8% of the samples) showed significantly higher fluorescence of nuclei than the reference material of any hexaploid taxon. They also formed three distinct groups, although with smaller inter-group differences than observed among the hexaploids. The mean ratios between the *Triticum* standard and these group members were 1.057 (1 individual from 1 locality, determined morphologically as *E. repens*), 1.107 (range 1.104–1.114, 6 individuals from 2 localities, determined morphologically as putative hybrids), and 1.144 (2 individuals from 1 locality, determined morphologically as *E. intermedia*). Overall variation in relative DNA content (DAPI staining) at nonaploid level thus reached 8.2%. The novel cytotypes were assumed to be DNA-nonaploids. Subsequent chromosome counting confirmed the expected ploidy level and revealed 63 chromosomes in all of them. The mean ratios between nonaploid/hexaploid fluorescence of nuclei for particular groups were 1.473 in *E. repens*, 1.444 in putative hybrids, and 1.406 in *E. intermedia*.

The nonaploid plants were discovered in the field for the first time. The only previous report of this cytotype refers to a single individual arising in artificial conditions. DEWEY (1974) detected one plant with 2n=63 during the cytological investigation of twin seedlings (arising from polyembryonic seeds). The mode of origin of the nonaploid plant was not mentioned by the author. All the plants analyzed in his study represented inbreds from one generation of selfing. The question arises whether the artificial nonaploid would also survive in the natural habitat.

The nonaploids most probably arose by the fusion of reduced and unreduced gametes of the corresponding hexaploids. Relative genome size data for *E. repens* corroborated this hypothesis; the actual 2C-value of the nonaploid was only 1.9% smaller than expected.The relative 2C-value of 9x *E. intermedia*, however, was considerably smaller (by 7.0%) than one

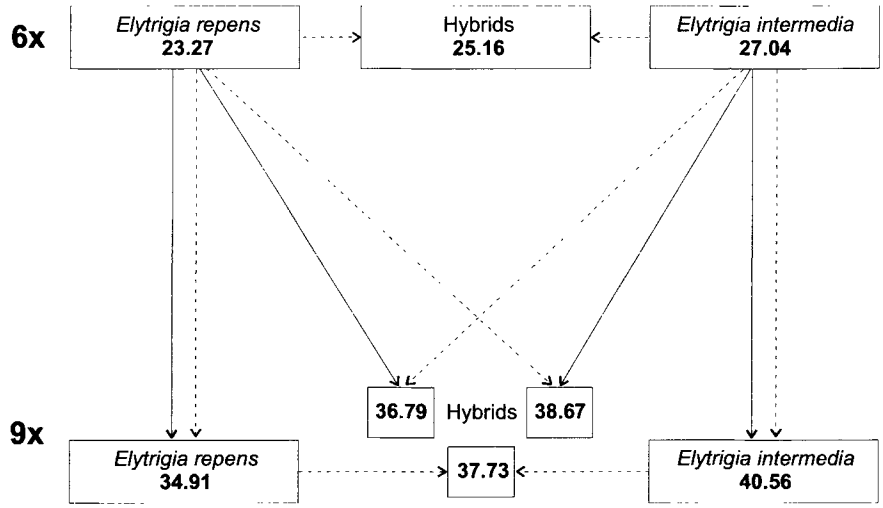

Fig. 4. Potential genesis of nonaploid plants (2n=9x=63). Both *E. repens* and *E. intermedia* are considered as putative parental taxa; involvement of hexaploid hybrids is omitted for lack of evidence of fertility. Solid and dashed lines designate the contribution of unreduced and reduced gametes respectively. Theoretical genome sizes (2C-values; pg) are given.

would predict from simple summation of reduced + non-reduced hexaploid gametes. Regarding the genesis of hybrid individuals, several pathways may theoretically be reconstructed, reflecting the origin of unreduced gametes, the ploidy level of parental plants, and the involvement of hexaploid hybrids (Fig. 4). It seems that the donor of the unreduced gamete in our 9x plants morphologically determined as hybrids was always *E. repens* (under this scenario, the mean 2C-value of the nonaploids was only 1.7% smaller than expected). An alternative hybrid origin after a crossing of 9x *E. repens* with 9x *E. intermedia* is less plausible owing to their sporadic occurrence (though fully consistent with relative genome size data). Similarly, the participation of hexaploid hybrids is questionable because of their apparent sterility, inferred from the lack of 6x back-crosses. One may even on the basis of 2C-values speculate that nonaploid plants morphologically determined as *E. intermedia* might actually have also been hybrid individuals sharing the unreduced genome of hexaploid *E. intermedia* and the reduced genome of *E. repens* (the difference between actual and theoretical values would be 2.7%). Generally, genome size data do not provide the best marker for testing the origin of polyploids. It has been repeatedly demonstrated that both genome size reduction and increase may occur after polyploid formation, resulting in deviations from simple summation of the genome sizes (ECKARDT 2001, LEVY & FELDMAN 2002, BENNETT 2004, LEITCH & BENNETT 2004). Irrespective of the mode of nonaploid origin, some fall in the nuclear DNA content must have taken place during their evolution. Similarly, rapid elimination of some DNA sequences after polyploidization was recorded in related *Triticum* genus (FELDMAN et al. 1997).

Although no dodecaploid plants (2n=12x=84) were found in our study, their occurrence (though very rare) might be expected owing to the production of unreduced gametes by both species of *Elytrigia*.

It is certain that at least some of the nonaploids are partially fertile (MAHELKA, unpubl.). One nonaploid individual yielded several fully developed seeds indicating either backcrossing to the putative hexaploid parents or some degree of self-pollination (considering the rarity of nonaploids in the field). Molecular analysis of all nonaploid plants, their potential progeny as well as hexaploid hybrids is essential in order to elucidate their genesis more certainly. GISH seems to be the most powerful tool to identify actual genome composition.

Acknowledgement: We thank David Short, who kindly improved our English, E. Ibermajerová and E. Morávková for their careful maintenance of the plants in the garden and V. Matějovičová for help with the literature search. Three anonymous reviewers are appreciated for their helpful comments. This study was supported by grant project no. QC 1362 of the Ministry of Agriculture of the Czech Republic, with further support provided by the Czech Science Foundation (grants nos. 206/05/0778 and 206/03/H137), Academy of Sciences of the Czech Republic (grant no. AV0Z60050516) and the Ministry of Education, Youth and Sports (project MŠM0021620828).

REFERENCES

ASSADI M. (1995): Meiotic configurations and chromosome number in some Iranian species of *Elymus* L. and *Agropyron* GAERTNER (*Poaceae*: *Triticeae*). *Bot. J. Linn. Soc.* 117: 159–168.

ASSADI M. & RUNEMARK H. (1995): Hybridisation, genomic constitution and generic delimitation in *Elymus* s.l. (*Poaceae*: *Triticeae*). *Pl. Syst. Evol.* 194: 189–205.

ARARATYAN A.G. (1938): Chisla khromosom nekotorykh vidov i form *Agropyrum* (Chromosome numbers of some species and forms in the genus *Agropyrum*). *Sovetsk. Bot.* 6: 109–111.

ARARATYAN A.G. (1939): K kariologicheskomu izucheniyu dyni (A contribution to caryological knowledge of melons). *Dokl. Akad. Nauk S.S.S.R.* 25: 777–780. (n. v., cit. sec. BOLKHOVSKIKH et al.1969).

AROHONKA T. (1982): Chromosome counts of vascular plants of the island Seili in Nauvo, SW Finland. *Turun Yliopiston Biol.-Laitoksen Julkaisula* 3: 1–12.

AVDULOV N.P. (1931): Kario-sistematicheskoe issledovanie semeistva zlakov (Karyo-systematic study of the grasses). *Trudy. Prikl. Bot.* 44: 1–428.

BARKWORTH M.E. & DEWEY D.R. (1985): Genomically based genera in the perennial *Triticeae* of North America: identification and membership. *Amer. J. Bot.* 72: 767–776.

BEAUDRY J.R. (1951): Seed development following the mating *Elymus virginicus* L. × *Agropyrum repens* (L.) Beauv. *Genetics* 36: 109–133.

BELAYEVA V.A. & SIPLIVINSKI V. (1981): [Reports]. In: LÖVE Á. (ed.), Chromosome number reports LXXIII, *Taxon* 30: 857–860.

BELL G.D.H. (1950): Investigations in the *Triticinae*. I. Colchicine techniques for chromosome doubling in interspecific and intergeneric hybridization. *J. Agric. Sci. (Cambridge)* 40: 9–18.

BELL G.D.H. & SACHS L. (1953): Investigations in the *Triticinae*. II. The cytology and fertility of intergeneric and interspecific F1 hybrids and their derived amphidiplois. *J. Agric. Sci. (Cambridge)* 43: 105–115.

BENNETT M.D. (2004): Perspectives on polyploidy in plants – ancient and neo. *Biol. J. Linn. Soc.* 82: 411–423.

BENNETT M.D., SMITH J.B. & SMITH L.R.I. (1982): DNA amounts of angiosperms from the Antarctic and South Georgia. *Environm. Exp. Bot.* 22: 307–318.

BERCHTOLD G.F. & OPIZ P.M. (1836): *Oekonomisch-technische Flora Böhmens I/2*. J.H. Pospischil, Prag.

BOLKHOVSKIKH Z., GRIF V., MATVEIEVA T. & ZAKHARYEVA O. (1969): *Khromosomnye chisla tsvetkovykh rastenii (Chromosome numbers of flowering plants)*. Nauka, Leningrad.

BOWDEN W.M. (1965): Cytotaxonomy of the species and interspecific hybrids of the genus *Agropyron* in Canada and neighboring areas. *Canad. J. Bot.* 43: 1421–1448.

CAUDERON Y. (1954): Obtention d'amphidiploides a partir de croisement entre des *Triticum* a 2n=28 et *Agropyrum glaucum* (2n=42). In: *VIIIth International Botanical Congress, Rapp. Commun. Parvenus Avant Congr. Sect. 9 & 10*, Paris, pp. 199–200. (n. v., cit. sec. BOLKHOVSKIKH et al. 1969).

CAUDERON Y. (1958): Étude cytogénétique des *Agropyrum* français et de leurs hybrides avec les blés. *Ann. Inst. Natl. Rech. Agron., Sér. B.* 8: 389–543.

CAUDERON Y. (1962): Étude cytogénétique du genre *Agropyrum*. *Rev. Cytol. Biol. Vég.* 25: 287–301.

CHEN Q., CONNER R.L., LAROCHE A. & THOMAS J.B. (1998): Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic in situ hybridisation. *Genome* 41: 580–586.

CHIZNYAK N.A. (1936): Formoobrazovanie u pshenichno-pyreinykh gibridov i vyvedenie mnogoletnikh pshenits (Formation of *Triticum-Elytrigia* hybrids and production of perennial wheats). *Selekts. & Semenov.* 12: 20–33.

CHOPANOV P. & YURTSEV B.N. (1976): Khromosomnye chisla nekotorykh zlakov Turkmenii. II. (Chromosome numbers of some grasses of Turkmenia. II.). *Bot. Zhurn.* 61: 1240–1244.

CLAYTON W.D. & WILLIAMSON H. (2003): *World grass flora: Nomenclature*. Royal Botanic Gardens, Kew.

DEMPSEY R.E., GORNALL R.J. & BAILEY J.P. (1994): Contributions to a cytological catalogue of the British and Irish flora, 4. *Watsonia* 20: 63–66.

DEVESA J.A., RUIZ T. & TORMO R., MUNOZ A., VIERA M.C., CARRASCO J., ORTEGA A. & PASTOR J. (1990): Contribución al conocimiento cariológico de las *Poaceae* en Extremadura II. *Bol. Soc. Brot., ser. 2.* 63: 153–205.

DEWEY D.R. (1961): Hybrids between *Agropyron repens* and *Agropyron desertorum*. *J. Heredity* 52: 13–21.

DEWEY D.R. (1967): Synthetic hybrids of New World and Old World *Agropyrons*: III. *Agropyron repens* × tetraploid *Agropyron spicatum*. *Amer. J. Bot.* 54: 93–98.

DEWEY D.R. (1970): A cytogenetic study of *Agropyron stipaefolium* and its hybrids with *Agropyron repens*. *Bull. Torrey Bot. Club* 97: 315–320.

DEWEY D.R. (1972): Cytogenetics of *Elymus angustus* and its hybrids with *Elymus giganteus*, *Elymus cinereus*, and *Agropyron repens*. *Bot. Gaz.* 133: 57–64.

DEWEY D.R. (1974): Cytogenetics of a polyhaploid *Agropyron repens*. *Bull. Torrey Bot. Club* 101: 266–271.

DEWEY D.R. (1980): Morphological, cytological and taxonomic relationships between *Agropyron repens* and *A. elongatiforme* (*Gramineae*). *Syst. Bot.* 51: 61–70.

DOLEŽEL J., SGORBATI S. & LUCRETTI S. (1992): Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiol. Pl. (Copenhagen)* 85: 625–631.

ECKARDT N.A. (2001): A sense of self: The role of DNA sequence elimination in allopolyploidization. *Pl. Cell* 13: 1699–1704.

FELDMAN M., LIU B., SEGAL G., ABBO S., LEVY A. & VEGA J.M. (1997): Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. *Genetics* 147: 1381–1387.

FERNANDES A. & QUEIROS M. (1969): Contribution a la connaissance cytotaxonomique des Spermatophyta du Portugal. I. *Gramineae*. *Bol. Soc. Brot., Ser. 2*, 43: 3–140.

FREY L., MIREK Z. & MIZIANTY M. (1977): Contribution to the chromosome numbers of Polish vascular plants. *Fragm. Florist. Geobot.* 23: 317–325.

GADELLA Th.W.J. & KLIPHUIS E. (1966): Chromosome numbers of flowering plants in the Netherlands II. *Proc. Kon. Ned. Akad. Wetensch, Ser. C, Biol. Med. Sci.* 69: 541–556.

GAUL H. (1953a): Über meiotische Fragment- und Brückenbildung der Bastarde *Secale* und *Triticum* × *Agropyrum*. *Chromosoma* 6: 314–329.

GAUL H. (1953b): Genomanalytische Untersuchungen bei *Triticum* × *Agropyron intermedium* unter Berücksichtigung von *Secale cereale* × *A. intermedium*. *Z. Vererbungsl.* 85: 505–546.

GERVAIS C., TRAHAN R. & GAGNON J. (1999): IOPB chromosome data 14. *Newslett. Int. Organ. Pl. Biosyst.* 30: 10–15.

GILLETT J.M. & SENN H.A. (1960): Cytotaxonomy and intraspecific variation of *Agropyron smithii* RYDB. *Canad. J. Bot.* 38: 747–760.

GOLDBLATT P. (ed.) (1984): Index to plant chromosome numbers 1979–1981. *Monogr. Syst. Bot. Missouri Bot. Gard.* 8.

GOLDBLATT P. (ed.) (1988): Index to plant chromosome numbers 1984–1985. *Monogr. Syst. Bot. Missouri Bot. Gard.* 23.

GOUKASIAN A. & NAZAROVA E. (1998): Mediterranean chromosome number reports 8 (1009–1025). *Fl. Medit.*. 8: 307–313.

GREILHUBER J. (1998): Intraspecific variation in genome size: a critical reassessment. *Ann. Bot. (Oxford)* 82 (Supplement A): 27–35.

GREILHUBER J., DOLEŽEL J., LYSÁK M.A. & BENNETT M.D. (2004): The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. *Ann. Bot. (Oxford)* 94: 1–6.

GUZIK M.B. (1984): Khromosomnye chisla nekotorykh dikorastushchikh zlakov Predural'ya (Chromosome numbers of some spontaneous grass species from the Preduralic region). *Ekol. Opyl. Rast.* 8: 82–86. (n. v., cit. sec. GOLDBLATT 1988).

GUZIK M.B. & LEVKOVSKII V.P. (1979): Khromosomnye chisla dikorastushchikh zlakov stepei Zabaikal'ya i Khakasii (Chromosome numbers of spontaneous grasses of Baikal and Khakassia steppe). *Ekol. Opyl.* 4: 26–32. (n. v., cit. sec. GOLDBLATT 1984).

HARTUNG M.F. (1946): Chromosome numbers in *Poa*, *Agropyron* and *Elymus*. *Amer. J. Bot.* 33: 516–532.

HEGI G. (1997): *Illustrierte Flora von Mitteleuropa 1/3.* Ed. 3. Parey Buchverlag im Blackwell Wissenschafts-Verlag, Berlin, Wien.

HEISER C.B. & WHITAKER T.W. (1948): Chromosome number, polyploidy and growth habit in California weeds. *Amer. J. Bot.* 35: 179–186.

HENEEN W.K. (1972): Separation difficulties during anaphase I in *Elymus* (*Agropyron*) species. *Bot. Not.* 125: 430–438.

HIDDEMAN W., SCHUMANN J., ANDREEF M., BARLOGIE B., HERMAN C.J., LEIF R.C., MAYALL B.H., MURPHY R.F. & SANDBERG A.A. (1984): Convention on nomenclature for DNA cytometry. *Cytometry* 5: 445–446.

HUNZIKER J.H. (1954): Estudios citológecos en las Hordeas (Gramíneas). I. *Revista Invest. Agric.* 8: 99–104.

JOHNSON G.A. & JALAL S.M. (1977): Meiotic behavior and fertility interrelationships in *Agropyron* and *Agrohordeum* species. *Cytologia* 42: 263–272.

JOHNSON L.P.V. (1938): Hybridization of *Triticum* and *Agropyron*. IV. Further crossing results and studies on the F1 hybrids. *Canad. J. Res., Sect. C., Bot. Sci.* 16: 417–444.

JONES K. (1957): Some aspects of plant variation: the grasses. In: LOUSLEY J.E. (ed.), *Progress in the study of the British flora*, Bot. Soc. British Isles, London, pp. 45–55.

JONES K. (1960): Cytology. *Rep. (Annual) Welsh Pl. Breed. Sta. 1959*: 68.

KOSTOFF D. (1941): Wheat phylesis and wheat breeding from a cytogenetic point of view. *Bibliogr. Genet.* 13: 149–224.

KOZHUHAROV S.I. & PETROVA A.V. (1973): [Reports]. In: LÖVE Á. (ed.), IOPB chromosome number reports XL, *Taxon* 22: 285–291.

KRUSE A. (1974): *Hordeum* × *Agropyrum* hybrids. *Hereditas* 78: 291–294.

KUBÁT K., HROUDA L., CHRTEK J. jun., KAPLAN Z., KIRSCHNER J. & ŠTĚPÁNEK J. (eds.) (2002): *Klíč ke květeně České republiky (Key to the Flora of the Czech Republic)*. Academia, Praha.

LEITCH I.J. & BENNETT M.D. (2004): Genome downsizing in polyploid plants. *Biol. J. Linn Soc.* 82: 651–663.

LEVY A.A. & FELDMAN M. (2002): The impact of polyploidy on grass genome evolution. *Pl. Physiol. (Lancaster)* 130: 1587–1593.

LIMIN A.E. & FOWLER D.B. (1988): Cold hardiness expression in interspecific hybrids and amphiploids of the *Triticeae*. *Genome* 30: 361–365.

LITARDIÈRE R. de (1948): Nouvelles contributions a l'étude de la Corse (Fascicule 7). *Candollea* 11: 175–227.

LIU Z.-W. & WANG R. R.-C. (1993): Genome analysis of *Elytrigia caespitosa*, *Lophopyrum nodosum*, *Pseudoroegneria geniculata* ssp. *scythica*, and *Thinopyrum intermedium* (*Triticeae*: *Gramineae*). *Genome* 36: 102–111.

LÖVE Á. (1980a): [Reports]. In: LÖVE Á. (ed.), IOPB chromosome number reports LXVI, *Taxon* 29: 166–169.

LÖVE Á. (1980b): [Reports]. In: LÖVE Á. (ed.), Chromosome number reports LXVII, *Taxon* 29: 350–351.

LÖVE Á. (1984): Conspectus of the *Triticeae*. *Feddes Repert.* 95: 425–521.

LÖVE Á. (1986): [Reports]. In: LÖVE Á. (ed.), Chromosome number reports XC, *Taxon* 35: 198.

LÖVE Á. & KJELLQVIST E. (1973): Cytotaxonomy of Spanish plants. II. Monocotyledons. *Lagascalia* 3: 147–182.

LÖVE Á. & LÖVE D. (1956): Cytotaxonomical conspectus of the Icelandic flora. *Acta Horti Gothob.* 20: 65–291.

LÖVE Á. & LÖVE D. (1961): Chromosome numbers of central and northwest European plant species. *Opera Bot.* 5: 1–581.

LÖVE Á. & LÖVE D. (1982): [Reports]. In: LÖVE Á. (ed.), IOPB chromosome number reports LXXVI, *Taxon* 31: 583–587.

LÖVKVIST B. & HULTGÅRD U.-M. (1999): Chromosome numbers in south Swedish vascular plants. *Opera Bot.* 137: 1–42.

LU B.-R., YAN J. & YANG J.-L. (1990): Cytological observations on *Triticeae* materials from Xinjiang, Qinghai and Sichuan. *Acta Bot. Yunnan.* 12: 57–66.

MÁJOVSKÝ J. et al. (1974): Index of chromosome numbers of Slovakian flora. Part 4. *Acta Fac. Rerum Nat. Univ. Comenianae, Bot.* 23: 1–23.

MÁJOVSKÝ J. et al. (1978): Index of chromosome numbers of Slovakian flora (Part 6). *Acta Fac. Rerum Nat. Univ. Comenianae, Bot.* 26: 1–42.

MASON-GAMER R.J. (2004): Reticulate evolution, introgression, and intertribal gene capture in an allohexaploid grass. *Syst. Biol.* 53: 25–37.

MATSUMURA M. (1951): Genome-analysis in *Agropyrum*, a genus related to *Triticum*. *Rep. (Annual) Natl. Inst. Genet.* 1: 25.

MATSUMURA M. (1952): Studies on *Agropyrum*, a genus related to *Triticum*. *Rep. (Annual) Natl. Inst. Genet.* 2: 26.

MATSUMURA S., MURAMATSU M. & SAKAMOTO S. (1958a): Genome analysis in *Agropyron*, a genus related to *Triticum*. *Proc. X Int. Congr. Genet.* 2: 181–182. (n. v., cit. sec. LÖVE A. & LÖVE D. 1961: 365).

MATSUMURA S., MURAMATSU M. & SAKAMOTO S. (1958b): Genome analysis in *Agropyron*, a genus related to *Triticum*. *Seiken Ziho* 9: 39–48.

MIČIETA K. (1986): Karyological study of the Slovak flora XI. *Acta Fac. Rerum Nat. Univ. Comenianae, Bot.* 33: 51–55.

MIZIANTY M., FREY L. & SZCZEPANIAK M. (2001): The *Agropyron-Elymus* complex (*Poaceae*) in Poland: biosystematics. In: FREY L. (ed.), *Studies on grasses in Poland*, PAN, Krakow, pp. 25–77.

MOWERY M. (1929): Development of the pollen grain and the embryo sac of *Agropyron repens*. *Bull. Torrey Bot. Club.* 56: 319–324.

MUJEEB-KAZI A., JAHAN Q. & VAHIDY A.A. (1994): Application of a somatic and meiotic cytological technique to diverse plant genera and species in the *Triticeae*. *Pakistan J. Bot.* 26: 353–366.

MURAMATSU M. (1955): Karyological studies on the F1 hybrid between *Triticum aegilopoides* (autotetraploid) and *Agropyron intermedium*. *Seiken Ziho* 7: 75–85.

NAPIER K.V. & WALTON P.D. (1981): New interspecific hybrids in the genus *Agropyron*. *Euphytica* 30: 459–466.

ÖSTERGREN G. (1940a): Cytology of *Agropyron junceum*, *A. repens* and their spontaneous hybrids. *Hereditas* 26: 305–317.

ÖSTERGREN G. (1940b): On the morphology of *Agropyron junceum* (L.) P.B., *A. repens* (L.) P.B. and their spontaneous hybrids. *Bot. Not.* 133–143.

OTTO F. (1990): DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA. In: CRISSMAN H.A. & DARZYNKIEWICZ Z. (eds.), *Methods in cell biology 33*, Academic Press, New York, pp. 102–110.

PARFENOV V.I. & DMITRIEVA S.A. (1988): Kariologicheskaya kharakteristika predstavitelei flory sosudistykh rastenii Berezinskogo biosfernogo zapovednika (Karyological characteristics of the vascular plants of "Berezinskoi zapovednik" biosphere reserve). *Zapovedniki Belorussii, Issl.* 12: 3–8.

PETO F.H. (1929): Chromosome numbers in *Agropyron*. *Nature* 124: 181–182.

PETO F.H. (1930): Cytological studies in *Agropyron*. *Canad. J. Res.* 3: 428–448.

PETO F.H. (1936): Hybridization of *Triticum* and *Agropyron*. II. Cytology of the male parents and F1 generation. *Canad. J. Res., Sect. C., Bot. Sci.* 14: 203–214.

PETO F.H. (1938): Hybridization of *Triticum* and *Agropyron*. V. Doubling the chromosome number in *T. vulgare* and F1 of *T. vulgare* × *A. glaucum* by temperature treatments. *Canad. J. Res., Sect. C., Bot. Sci.* 16: 516–529.

PETO F.H. & BOYES J.W. (1940): Hybridization of *Triticum* and *Agropyron*. VI. Induced fertility in vernal emmer × *A. glaucum*. *Canad. J. Res., Sect. C., Bot. Sci.* 18: 230–239.

PETROVA O.A. (1975): O polimorfizme pyreya polzuchego *Elytrigia repens* (L.) DESV. i ego khromosomnom chisle (On polymorphism of *Elytrigia repens* (L.) DESV. and its chromosome number). *Tsitol. Genet.* 9: 126–128.

PETROVA A. & STOYANOVA K. (1997): Mediterranean chromosome number reports 7 (783–802). *Fl. Medit.* 7: 204–213.

PIAO Z.S. (1982): The studies on the chromosomal morphology and banding pattern in *Agropyron intermedium*. *Acta Genet. Sin.* 9: 350–356.

POGAN E., CZAPIK R. & JANKUN A. (1985): Further studies in chromosome numbers of Polish angiosperms. Part XVIII. *Acta Biol. Cracov., Ser. Bot.* 27: 57–74.

POGAN E., RYCHLEWSKI J. et al. (1980): Further studies in chromosome numbers of Polish angiosperms. *Acta Biol. Cracov., Ser. Bot.* 22. 129–153.

PÓLYA L. (1948): Chromosome numbers of certain alkali plants. *Arch. Biol. Hung. II.* 18: 145–148.

PÓLYA L. (1950): Magyarországi növényfajok kromoszómaszámai. II (Chromosome numbers of Hungarian plants. II.). *Ann. Biol. Univ. Debrecen* 1: 46–56.

POPE W.K. & LÖVE R.M. (1952): Comparative cytology of colchicine-induced amphidiploids of interspecific hybrids: *Agropyron trichophorum* × *Triticum durum*, *T. Timopheevi* and *T. macha*. *Hilgardia* 21: 411–429.

PROBATOVA N.S. & SOKOLOVSKAYA A.P. (1978): Khromosomnye chisla i taksonomiya nekotorykh zlakov Kavkaza (Chromosome numbers and taxonomy of some grasses in Caucasus). *Bot. Zhurn.* 63: 1121–1131.

PROBATOVA N.S. & SOKOLOVSKAYA A.P. (1980): K kariotaksonomicheskomu izucheniyu zlakov gornogo Altaya (A karyotaxonomic study of the grasses of the Altai Mts.). *Bot. Zhurn.* 65: 509–520.

PROBATOVA N.S. & SOKOLOVSKAYA A.P. (1982): Konspekt khromosomnykh chisel *Poaceae* Sovetskogo Dalnego Vostoka. I. Triby *Oryzeae*, *Brachypodieae*, *Triticeae* (Synopsis of chromosome numbers in *Poaceae* from the Soviet Far East. I. The tribes *Oryzeae*, *Brachypodieae*, *Triticeae*). *Bot. Zhurn.* 67: 62–70.

PROKUDIN YU. N., VOVK A. G., PETROVA O.A., ERMOLENKO E.D. & VERNICHENKO YU.V. (1977): *Zlaki Ukrainy (Grasses of the Ukraine)*. Naukova Dumka, Kiev.

ROHWEDER H. (1937): Versuch zur Erassung der mengenmässigen Bedeckung des Darss und Zingst. *Planta* 27: 501–549.

ROOS A.V. (1975): Khromosomnye chisla nekotorykh vidov zlakov (Chromosome numbers of some grass species). *Bot. Zhurn.* 60: 860–864.

ROZANOVA M.A. (1940): O biologicheskoi izolyatsii i skrytykh vidakh (On biological isolation and "cryptic species"). *Bot. Zhurn. S.S.S.R.* 25: 304–308.

SACHS L. (1952): Chromosome mosaics in experimental amphidiploids in *Triticinae*. *Heredity* 6: 157–170.

SAKAMOTO S. & MURAMATSU M. (1963): Chromosome numbers of *Gramineae* species collected in Pakistan, Afganistan and Iran. *Rep. (Annual) Natl. Inst. Genet. (1962)* 13: 48–50.

SALOMON B. & LU B.-R. (1994): Genomic relationships between species of the *Elymus semicostatus* group and *Elymus* sensu lato (*Poaceae*). *Pl. Syst. Evol.* 191: 199–201.

SCHIEMANN E. (1929): Zytologische Beiträge zur Gattung *Aegilops*. III. Chromosomenzahlen und Morphologie. *Ber. Deutsch. Bot. Ges.* 47: 164–181.

SCHULZ-SCHAEFFER J. & JURA P. (1967): Biosystematic investigations in the genus *Agropyron*, IV. Species karyotype analysis, phytogeographic and other studies. *Z. Pflanzenzücht.* 57: 146–166.

SENN H.A., HEIMBURGER M.L. & MOORE R.J. (1947): The cytotaxonomy of Canadian species of *Agropyron*. *Amer. J. Bot.* 34: 607.

SENN H.A., BOWDEN W.M. & MOORE R.J. (1949): La citotaxonomia del genero *Agropyron* (Cytotaxonomy of the genus *Agropyron*). *Lilloa* 19: 119–120.

SHARMAN B. C. (1943): Nucleoli in *Agropyron repens* BEAUV. *Nature* 151: 170.

SIMONET M. (1935): Contribution a l'étude cytologique et génétique de quelques *Agropyrum*. *Compt. Rend. Acad. Sci. Paris* 201: 1210–1212.

SINGH D.N. (1964): Cytological studies in the *Gramimeae*. III. *Sci. & Cult.* 30: 396–397.

SOKOLOVSKAYA A.P. & STRELKOVA O.S. (1939): Geograficheskoe raspredelenie poliploidov. I. Issledovanie rastitelnosti Pamira (The geographical distribution of polyploids. I. Studies of the Pamir's vegetation). *Uchen. Zap. Leningr. Gosud. Univ., Ser. biol.* 35: 42–62.

SOKOLOVSKAYA A.P. & STRELKOVA O.S. (1948): Geograficheskoe raspredelenie poliploidov. II. Issledovanie flory Altaya (The geographical distribution of polyploids. II. Studies of the flora of the Altai Mts.). *Uchen. Zap. Ped. Inst. Im. Gercena.* 66: 179–193.

STEBBINS G.L. & PUN F.T. (1953): Artificial and natural hybrids in the *Gramineae*, tribe *Hordeae*. VI. Chromosome pairing in *Secale cereale* × *Agropyron intermedium* and the problem of genome homologies in the *Triticinae*. *Genetics* 38: 600–608.

STOLZE K.V. (1925): Die Chromosomenzahlen der hauptsächlichsten Getreidearten nebst allgemeinen Betrachtungen über Chromosomen, Chromosomenzahlen und Chromosomengrösse im Pflanzenreich. *Bibliogr. Genet.* 8: 1–71.

SUN Y.K., ZHAO Y.T., DONG Y.C., ZHOU R.H. & XU S.J. (1992): Karyotypes of eleven species of *Triticeae* in Northeast China. *Acta Phytotax. Sin.* 30(4): 342–345.

ŠMARDA P. & KOČÍ K. (2003): Chromosome number variability in Central European menbers of the *Festuca ovina* and *F. pallens* groups (sect. *Festuca*). *Folia Geobot.* 38: 65–95.

TATEOKA T. (1956): On morphological convergence between *Brachypodium sylvaticum* and *Agropyron yezoense*. *Cytologia* 21: 146–152.

THOMPSON D.L. & GRAFIUS J.E. (1950): Cytological observations of the F1 and two backcross generations of *Triticum vulgare* × *Agropyron trichophorum*. *Agron. Jour.* 42: 298–303.

VÁCHOVÁ M. & FERÁKOVÁ V. (1980): [Reports]. In: LÖVE Á. (ed.), Chromosome number reports LXIX, *Taxon* 29: 722–723.

VAKAR B.A. (1934): Bastarde zwischen Arten der Gattung *Triticum* und Arten der Gattung *Agropyrum*. *Züchter* 6: 211–215.

VAKAR B.A. (1935): *Tsitologiya pshenichno-pyreinykh gibridov (Cytology of the* Triticum-Agropyron *hybrids)*. Omskoje obl. izd., Omsk.

VAKAR B.A. (1936): Cytologische Untersuchung über F1der Weizen-Queckengras Bastarde. *Cytologia* 7: 293–312.

VOGEL K.P., ARUMUGANATHAN K. & JENSEN K.B. (1999): Nuclear DNA content of perennial grasses of the *Triticeae*. *Crop Sci. (Madison)* 39: 661–667.

Received 16 December 2004, revision received 24 February 2005, last revision received and accepted 28 March 2005

Encl. Appendix pp. 383–384

APPENDIX

List of populations studied with the number of plants analyzed for each group. The ploidy level of all samples was estimated by flow cytometry. The number of karyologically-confirmed individuals is given in brackets. The location and habitat type of each locality are given. All localities are in the Czech Republic except of locality number 18, which is in Slovakia.

No.	Locality Name (location, habitat)	Coordinates	*E. repens* 6x	*E. repens* 9x	*E. intermedia* 6x	*E. intermedia* 9x	hybrid 6x	hybrid 9x
01.	Rubín 1 (3 km NE of Podbořany town, top of Rubín hill, steppe)	50°15′13.2″ N 13°26′12.4″ E	3		3		0	
02.	Rubín 2 (2.5 km NE of Podbořany town, field margin)	50°15′15.0″ N 13°26′03.3″ E	1	1 (1)	0		0	
03.	Raná (0.5 km SW of Raná village, bottom of Raná hill, steppe)	50°24′34.9″ N 13°46′38.9″ E	3		4		5	
04.	Cikánka (Prague city, near Cikánka bus stop, slope along the road)	49°59′53.8″ N 14°19′32.9″ E	2		2		0	
05.	Brno (Brno city, Kamenný hill, roadside)	49°11′02.5″ N 16°33′05.1″ E	2		1		0	
06.	Kobeřice (Kobeřice village, wasteland near the church)	49°05′34.0″ N 16°53′26.7″ E	1		0		0	
07.	Čejč 1 (1.5 km SE of Čejč village, wasteland)	48°56′16.9″ N 16°59′04.1″ E	1		0		0	
08.	Růžový kopec (2 km NNW of Mikulov, top of Růžový hill, steppe)	48°49′14.3″ N 16°37′31.2″ E	7		14		0	
09.	Jánská hora (2 km W of Dolní Dunajovice village, top of Jánská hora hill, steppe and vineyard)	48°50′54.4″ N 16°33′29.1″ E	1		2		0	
10.	Paví vrch (2 km S of Sedlec village, Paví hill, steppe and field margin)	48°45′50.8″ N 16°41′33.1″ E	1		0		1	
11.	Skalky (1 km W of Sedlec village, Skalky hill, steppe)	48°46′36.2″ N 16°40′23.2″ E	0		1		0	
12.	Malé Žernoseky (Malé Žernoseky village, near the station, grassland)	50°32′24.8″ N 14°03′21.8″ E	1		0		0	
13.	Zlončice (0.5 km S of Dolánky village, steppe)	50°12′40.9″ N 14°21′31.4″ E	2		1		1	
14.	Knovíz (0.5 km NW of the church at Knovíz village, pine forest)	50°12′48.1″ N 14°07′46.4″ E	2		2		1	
15.	Humenský vrch (1 km W of Keblice village, top of Humenský hill, alongside the footpath)	50°28′53.5″ N 14°05′10.1″ E	1		2 (1)		2	
16.	Zebín (2 km NE of Jičín town, top of Zebín hill, steppe)	50°27′12.8″ N 15°22′26.0″ E	4 (1)		0		0	
17.	Vrbčany 1 (1.5 km NE of Vrbčany village, steppe)	50°03′43.5″ N 14°59′56.0″ E	1		1		4 (2)	
18.	Hajnáčka (4 km NE of Hajnáčka village, oak forest)	48°14′50.0″ N 19°59′00.0″ E	0		1		0	
19.	Ješovice 1 (2 km N of Liběchov village, field margin)	50°25′26.0″ N 14°26′17.2″ E	3 (1)		0		1	
20.	Ješovice 2 (1 km WWS of Ješovice village, pine forest)	50°25′45.4″ N 14°25′02.6″ E	0		1		2	
21.	Stračí (0.3 km E of Stračí village, pine forest)	50°27′04.2″ N 14°24′30.7″ E	1		2		0	
22.	Radouň (0.5 km S of Křešov village, steppic slope along the roadside)	50°29′17.8″ N 14°23′59.3″ E	1		4		0	
23.	Trnovany (0.2 km W of Trnovany village, Robinia grove)	50°19′02.3″ N 13°35′45.7″ E	2		1		0	
24.	Úhošť (0.5 km NNW of Úhošťany village, Úhošť hill, steppe)	50°21′36.4″ N 13°15′15.8″ E	9 (1)		3		0	
25.	Povrly (1 km W of Povrly village, orchard)	50°40′32.6″ N 14°08′21.8″ E	1		0		0	
26.	Radobýl (1 km NE of Žalhostice	50°31′44.5″ N	2		5 (1)		0	

	village, Radobýl hill, steppe)	14°05′30.7″ E						
27.	Sedlecké skály (Prague city, Sedlecké skály reserve, steppe)	50°08′25.1″ N 14°23′36.9″ E	0	6		0		
28.	Praha, Pecka (Prague city, Pecka reserve, *Robinia* grove)	50°06′26.8″ N 14°24′21.9″ E	1	0		0		2 (2)
29.	Záhoří (2 km NW of Žatec town, steppe)	50°20′24.3″ N 13°31′09.4″ E	5	0		2		
30.	Bezděkov (3 km E of Žatec town, steppe)	50°19′42.1″ N 13°35′22.7″ E	2 (1)	4		0		
31.	Libořice (0.5 km SW of Libořice village, orchard)	50°15′11.0″ N 13°30′26.3″ E	1	1		2		
32.	Přerovský vrch (1.5 km E of Nový Přerov village, Přerovský hill, *Robinia* grove)	48°48′41.6″ N 16°31′00.4″ E	1	2 (1)		1		
33.	Milovická stráň (0.2 km S of Milovice village, Milovická stráň reserve, steppe)	48°50′53.3″ N 16°41′34.8″ E	0	5 (2)		0		
34.	Pavlov – Děvín (0.5 km W of Pavlov village, Děvín hill, steppe)	48°52′27.7″ N 16°39′41.7″ E	0	5		0		
35.	Klentnice (0.5 km SW of Klentnice village, Stolová hora hill, steppe)	48°50′25.2″ N 16°38′24.4″ E	1	5		0		
36.	Valtice (1.5 km SW of Valtice town, vineyard)	48°44′13.1″ N 16°44′13.9″ E	1	0		0		
37.	Poštorná (3 km SSW of Poštorná village station, pine forest)	48°43′34.6″ N 16°50′06.4″ E	2	0		2 (1)		
38.	Hodonín 1 (2 km N of Hodonín town, pine forest margin)	48°54′10.7″ N 17°05′16.1″ E	1	0		0		
39.	Hodonín 2 (1.5 km S of Dubňany village, pine forest)	48°54′08.2″ N 17°05′14.2″ E	2	0		0		
40.	Čejč 2 (0.5 km NE of Čejč village, roadside)	48°56′54.7″ N 16°58′37.4″ E	2	2 (1)		0		
41.	Hovorany (1.5 km NW of Hovorany village, Hovoranské louky reserve, steppe)	48°57′54.2″ N 16°58′25.7″ E	0	2	2 (2)	1		
42.	Kobylí (2 km NE of Kobylí village, Kobylská skála hill, shrubs)	48°56′30.7″ N 16°55′19.1″ E	0	3 (1)		1		
43.	Nesovice (1.5 km W of Nesovice village, Malhotky reserve, steppe)	49°08′53.5″ N 17°03′23.7″ E	2	2		1		
44.	Kloboučky (0.5 km E of Kloboučky village, Baračka reserve, shrubs)	49°07′45.0″ N 17°01′12.5″ E	1	0		0		
45.	Křižanovice (0.5 km E of Křižanovice village, steppe)	49°08′44.6″ N 16°56′54.8″ E	0	2		3		
46.	Milešovka (2 km N of Milešov village, Milešovka hill, along the footpath)	50°33′16.6″ N 13°55′52.4″ E	2	0		0		
47.	Starý Vestec (hill 0.5 km SSE of Starý Vestec village, pine forest)	50°08′17.5″ N 14°51′04.1″ E	2	0		2		
48.	Dolní Dunajovice (2.5 km W of Dolní Dunajovice village, vineyard and field margin)	48°51′22.7″ N 16°34′03.9″ E	5	0		2		4 (4)
49.	Vrbčany 2 (0.5 km N of Vrbčany village station, field margin)	50°03′35.5″ N 15°00′05.6″ E	7	0		2		
50.	Valov (2 km S of Podbořany town, field margin)	50°12′34.5″ N 13°24′54.4″ E	6	0		0		
51.	Zichovec (0.5 km N of Zichovec village, pine forest margin)	50°16′32.2″ N 13°55′27.4″ E	1	0		0		
52.	Drahobuz (0.5 km E of Drahobuz village, roadside)	50°31′32.1″ N 14°19′41.6″ E	0	1		2		
53.	Veliš (0.2 km SE of Podhradí village, Veliš hill, shrubs)	50°25′01.0″ N 15°18′57.9″ E	2	0		0		
54.	Senohraby (Senohraby village, garden)	49°53′56.4″ N 14°43′40.0″ E	1	0		0		
55.	Zbraslav (Prague city, forest margin)	49°58′32.9″ N 14°23′23.4″ E	1	0		0		
Total			101 (4)	1 (1)	90 (7)	2 (2)	38 (3)	6 (6)