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RAPD ANALYSIS POINTS TO OLD WORLD *BROMUS* SPECIES AS ANCESTRAL TO NEW WORLD SUBGEN. *FESTUCARIA*

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The genus *Bromus* subgen. *Festucaria* is a widespread Old World and New World taxon having genomes A, B and L, distinguished cytogenetically. Stebbins (1981) suggested that evolution in *Bromus* went from the small genomes A and B to the large genome L. Thus, Old World species with genomes A and B could be ancestral to New World species with genome L. To test this hypothesis we carried out RAPD analysis of a representative group of species from subgen. *Festucaria*. RAPD band patterns enabled resolution of 13 species after excluding the species-specific bands with high/moderate support. The basal position of *Bromus variegatus* M. Bieb. – an Old World species presumably having a B genome – in relation to some New World species with the genome L confirmed Stebbins' hypothesis of its ancestry in relation to the Old World species. The group of high bootstrap support, *B. cappadocicus* Boiss. et Balansa–*B. erectus* Huds.–*B. riparius* Rehm., with genome A. and a distinctly xerothermic ecological profile, was related to a New World species with genome L, *B. auleticus* Trin. ex Nees, pointing to their presumably common evolutionary history.

Key words: Genomes A and B, genome L, evolutionary migration, historical biogeography, phenetics.

INTRODUCTION

The species-rich brome grass genus *Bromus* L. occupies a very important place in the systematics and evolution of the Poaceae (Stebbins, 1981). The genus includes species with different ploidy levels (2n = 2x – 12x), and many taxa with higher than 2n = 2x chromosome number are of allopolyploid origin (Stebbins, 1981; Armstrong, 1991).

One of the most interesting but less known subgenera of the genus *Bromus* is subgenus *Festucaria*. It comprises about sixty Old World and New World species and possesses different ploidy levels. Cytogenetic study revealed that the American species, except for *B. pumpellianus* Scribn., possess genome L with large chromosomes, while the Eurasian species share genomes A and B with small chromosomes, except for the complex *B. ramosus* L. –*B. benekenii* (Lange) Trimen. Examining the history of the genus *Bromus*, Stebbins (1981) suggested that the subgenera *Ceratochloa*, *Festucaria*, and *Neobromus* emerged in Eurasia, and the spread of the subgenus *Festucaria* into the Americas and Africa started presumably in the Pliocene. In Eurasia, species with the polyploid complex, *Bromus erectus* and *B. inermis* Leyss., developed in the Pleistocene, as did the similar complex *B. marcanthus* Meyen in the Andes, South America.

The main sources of our knowledge of the genus *Bromus* are morphological, cytological-genomic and serological data. Smith's (1970) sectional treatment of the genus relies on serological data for *Bromus*, *Ceratochloa*, *Genea*, *Pnigma*, *Neobromus* and *Nevskiella*. Stebbins (1981) gave the taxonomic treatment at the rank of subgenera: *Ceratochloa*, *Boissiera*, *Bromus*, *Festucaria*, *Neobromus*, *Nevskiella* and *Stenobromus*.

DNA analysis of the genus *Bromus* has focused on non-encoding sequences of rDNA (Pillay, 1995, 1996; Ainouche et al., 1997), intraspecific and interspecific RAPD (Ainouche et al., 1999; Ferdinandez, 2001; Joachimiak et al., 2001; Sutkowska et al., 2002), ISSR (Sutkowska et al., 2002; Sutkowska and Mitka, 2005), chloroplast DNA (cpDNA) (Pillay and Hilu, 1990, 1995; Pillay, 1993, 1995; Pillay and

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Abbreviations: NJ – neighbor joining; OTU – operational taxonomic unit; PCo – principal coordinate; RAPD – Random Amplified Polymorphic DNA; UPGMA – unweighted pair-group method using arithmetic averages.

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Armstrong, 2001), and both chloroplast and nuclear DNA sequence data (Saarela et al., 2007). These analyses give deeper insight into the Linnaean taxonomic treatment and open new questions.

Restriction maps of rDNA have been worked out for some species of the genus *Bromus*, including *B. inermis* and *B. erectus* from subgenus *Festucaria*. Pillay (1996) suggested that restriction maps of this gene family might be useful for identification of species and interspecific hybrids. For this type of identification, RAPD and ISSR methods proved useful in confirming the hybrid nature of accessions (Sutkowska et al., 2002).

Pillay (1993) constructed restriction maps of cpDNA of *B. inermis* and compared them with similar maps for wheat, rye, barley, oat and rice. Cladistic analysis demonstrated that *B. inermis* (tribe *Bromeae*) is closely related to wheat, rye and barley (tribe *Triticeae*), and much less to oat (tribe *Aveneae*). In a complementary study, Pillay (1995) showed that *B. inermis* is more closely related to barley than to wheat and rye. In spite of the genetic differences between parents, progeny always strictly demonstrated a maternal pattern of cpDNA inheritance (Pillay and Armstrong, 2001).

RAPD (random amplified polymorphic DNA) technique enables analysis of anonymous, arbitrarily adopted sequences. For example, Ainouche et al. (1999) used RAPD technique in a comparative study of the tetraploid *B. hordeaceus* L. and diploid taxa from the subgenus *Bromus*.

PCR methods were used to study the postglacial migrations of *B. inermis* and *B. erectus* in Poland. The work showed that the present range of *B. erectus* probably originated from one source, and suggested the origin of *B. inermis* in Poland from two refugial areas (Sutkowska et al., 2002).

The analyses showed a clear overall similarity in the band patterns of *B. hordeaceus* L., *B. arvensis* L., *B. pseudobrachystachys* H. Scholz, *B. carolihenrici* Greuter, *B. alopecuros* Poir. and *B. intermedius* Guss. The molecular similarity of the diploids *B. briziformis* Fisch. et C.A. Mey., *B. japonicus* Thunb., *B. squarrosus* L., *B. caroli-henrici* and *B. alopecuros* is accompanied by morphological similarities (Ainouche and Bayer, 1997; Smith, 1973). The RAPD method was also used in an analysis of hybridogenous genomes between *B. riparius* and *B. inermis* (Ferdinandez et al., 2001).

The RAPD method has successfully been used in other phylogenetic and population analyses, for example in *Aconitum* (Fico et al., 2003; Zhang et al., 2005), Dipterocarpaceae (Rath et al., 1997), *Morus* (Awasthi et al., 2004), *Phoenix* (Gonzales-Perez et al., 2004) and *Spartina* (Ayres and Strong, 2000).

This study uses molecular-based methods to study the relationships between New World and Old World species in subgen. *Festucaria*, and in particular the position of *B. variegatus*. Previous studies based on ISSR analysis (Sutkowska et al., 2007) suggested that *B. variegatus* has a genome different from *B. erectus*, which represents genome A. It is plausible that *B. variegatus* has genome B. We test this hypothesis. The molecular relationships between New World species of the genome L and their Old World counterparts may throw light on the mechanism of evolution within the taxon. Our working hypothesis is that the New World *Bromus* species of genome L originated from Old World (Asian) ancestors closely related to *B. variegatus*.

We established the RAPD banding patterns in the Old World species *B. biebersteinii* Roem. & Schult., *B. cappadocicus*, *B. ramosus*, *B. benekenii*, *B. erectus*, *B. inermis*, *B. riparius* and *B. variegatus*, and in the New World species *B. anomalus* Rupr. ex E. Four., *B. auleticus*, *B. ciliatus* L. and *B. parodii* Covas et & Itria and *B. pumpellianus*. Authors of plant names are according to Mirek et al. (2002), supplemented by www.ipni.org.

MATERIALS AND METHODS

Seeds of the species used in the study were obtained from the genetic seed bank at the USDA Plant Introduction Station, Pullman, WA, U.S.A. Thirty seeds of each species were sown in the greenhouse of the Department of Plant Breeding and Seed Science of the Agricultural University, Cracow. For molecular analysis, 2 to 5 plants were chosen from each population. The species, origin of plant population, and sample size of the examined species are given in Table 1.

Molecular analysis were done by the RAPD-PCR method. Here, the polymorphism is the result of mutations inside the sequences complementary to the 3' end and small indels between the hybridization sites of the starter. It is especially useful in seeking the markers used in the construction of genetic maps, and for evaluation of genetic similarity among plant taxa. RAPD-PCR allows one to obtain, in one amplification reaction, from a few to many products of various lengths (Tab. 2). RAPD primers generate species-specific markers, making them a useful tool in phylogenetic and systematic research (Oxelman, 1996).

DNA ISOLATION AND RAPD AMPLIFICATION

DNA was isolated from fully developed leaf lamina. Only healthy leaves were selected, without any signs of damage caused by insects, mold or pathogens that might cause contamination.

Prior to DNA isolation, plant material was ground in 2.5 M sodium acetate to prevent oxidation of cell sap compounds. The sample was then twice eluted with disinfected 0.9% natrium chloratum to eliminate sodium acetate and some compounds

TABLE 1. List of *Bromus* L. species, including chromosome and accession numbers, and origin of seed material. 1 – Armstrong 1981, 2 – Armstrong 1983, 3 – Armstrong 1984, 4 – Armstrong 1987, 5 – Armstrong 1991, 6 – Kozhuharov et al. 1981, 7 – Pillay and Hilu 1990, 8 – Tuna et al. 2001a, 9 – Tuna et al. 2001b, 10 – Tuna et al. 2005

Species	Chromosome number	Native distribution	Accession number of seed material (USDA, Pullman, USA)	Origin of seeds	No. of indiv.
			OLD WORLD		
Bromus variegatus	$2n = 14_3$	Temperate Asia	315395	Russian Federation	1
			300568	Former Soviet Union	8
B. erectus	$2n = 28_1,56_{2,3}$	N. Africa, Temperate Asia, Europe (N, E. E/S, Central)	253300	Former Yugoslavia	4
			234715	France	4
			418806	Italy	3
			598591	Kazakhstan	4
B. inermis	$2n = 28_1, 56_{1,3}, 62_4, 70_4$	Temperate Asia, Europe (E, Central)	619004	Mongolia	4
			598589	Kazakhstan	4
			598538	China	4
			?	Ukraine	4
			494692	Romania	3
B. ramosus	$2n = 14_4, 28_4, 42_4$	Temperate Asia, Europe (E, Central), N Africa	240152	Morocco	7
B. benekenii	$2n = 28_{3,4,5}$	Europe (E,S/E,S/W, Central), Temperate Asia, N Africa	314646	Former Soviet Union	10
B. riparius	$2n = 14_{4,8,9}, 56_{8,9}, 70_{6,8,9}$	Temperate Asia, Europe (E, S/E)	251683	Former Soviet Union	4
			314072	Russian Federation	4
			314363	Russian Federation	5
B. cappadocicus	$2n = 42_1$	Temperate Asia, Europe S/E	634283	Ukraine	4
			202532	Belgium	4
			325238	Russian Federation	4
			634284	Ukraine	5
B. biebersteinii	$2n = 56_8, 70_2$	Temperate Asia	341223	Canada	2
			372614	Canada	4
			341222	Canada	5
			325226	Russian Federation	5
			NEW WORLD		
B. ciliatus	$2n = 14_2, 28_{10}$	N America	232214	United States	10
B. anomalus	$2n = 14_7$	N America	232197	United States	1
			232199	United States	4
			232200	United States	3
B. auleticus	$2n = 42_7$	S America	477053	Uruguay	1
			598720	Uruguay	1
			598724	Argentina	6
B. parodii	$2n = 42_5$	S America	376931	Former Soviet Union	8
B. pumpellianus	$2n = 28_1, 56_1$	N/W America	371708	USA	3
			371709	USA	5
			236764	Canada	5

originating from cells which might partially or completely block DNA isolation. After this preparation procedure, DNA was isolated from samples with DNA-zol reagent (Invitrogen). DNA samples were separated electrophoretically with 1% low-melting-point agarose (Invitrogen) with ethidium bromide in

TABLE 2. RAPD marker primers used to distinguish *Bromus* subgen. *Festucaria* species

Primer	Primer sequence (5'→3')	Number of band scores	Mean number of bands per OTU	Range of bands per OTU
OPB01	GTTTCGCTCC	1012	6.6	3–11
OPB03	CATCCCCCTG	976	6.4	3–10
OPB14	TCCGCTCTGG	997	10.8	1–83
OPB15	GGAGGGTGTT	974	11.3	2–40
OPB18	CCACAGCAGT	1139	13.2	2–68
Total		5098	11.6	1–83

TAE buffer at 15 V for 3–4 h. After electrophoresis, the purified DNA was cut out in gel blocks. Smug intensity analysis allows estimation of DNA concentration and degradation levels.

The PCR reaction was conducted with reagents from an Invitrogen kit, except for the Taq polymerase which originated from Fermentas, at 2 units/μl concentration. Primer sequences (OPB01, OPB03, OPB14, OPB15, and OPB18) were taken from Oxelman (1996). Amplification was conducted with a 2720 thermal cycler (Applied Biosystems). Optimal conditions for the reaction were as follows: initial denaturation at 94°C for 5 min; 35 amplification cycles of denaturation at 94°C for 40 sec, annealing at 35°C for 40 sec and polymerization at 72°C for 40 sec; final polymerization at 72°C for 7 min.

PCR product separation was carried out in 2.5% agarose gel and archived with Imagemaster VDS (Pharmacia-Amersham). Original Liscap Capture ver. 1.0 software was also used.

For analysis of band patterns, GeleScan ver. 1.45 (Kucharczyk Techniki Elektroforetyczne) was used.

The molecular weight of the resulting amplification products was determined based on a calibration curve and manual checking.

NUMERICAL ANALYSES

The amplification products were scored as a presence/absence matrix of binary data. The initial matrix encompassed 440 RAPD bands and 156 *Bromus* operational taxonomic units (OTUs). Then it was reduced to 253 bands, excluding species-specific and singular bands, and 156 accessions were pooled to 13 *Bromus* species (Tab. 1). In both matrices, initial and reduced, 1-Jaccard similarity coefficients (Jaccard, 1908) were calculated to obtain secondary matrices of distances between accessions or species. The DISTANCE procedure based on 1000 bootstrap iterations was applied using PhylTools software (http://www.dpw.wau.nl/pv/PUB/pt; Buntjer, 1997–2001). The trees were obtained with the use of UPGMA (Sokal and Michener, 1958) and neighbor-joining (NJ) methods (Saitou and Nei, 1987) implemented in the Phylip package (Felsenstein, 2004). Relative bootstrap support values were computed with the CONSENSE option in the Phylip package. The trees were drawn with MEGA4 software (Tamura et al., 2007).

Ordination of OTUs based on the initial matrix was done with principal coordinate analysis (PCo) implemented in NTSYS-pc software (Rohlf, 2002). The ordination diagrams were produced with CanoDraw (Smilauer, 1990). The genetic distances between OTUs were calculated as the square root of the 1-Jaccard similarity coefficient. PCo shows distances between OTUs without assuming a hierarchical topology.

RESULTS

The UPGMA classification based on 440 amplified fragments and 156 OTUs, defined as individual *Bromus* accessions, pointed to high RAPD identity of particular species but gave a low level of group resolution (Fig. 1a). The bootstrap values at species level varied from 56% (*B. cappadocicus*) to 92% (*B. riparius*). The only group of species above 50% bootstrap value was *B. benekenii–B. ciliatus–B. ramosus*.

The UPGMA classification based on the reduced data set of 253 RAPD bands and 13 species displayed two groups of species (Fig. 1b). The first group was formed by *B. alueticus–B. cappadocicus –B. erectus–B. riparius* with bootstrap value 66%. The second group was formed by the remaining species, excluding *B. biebersteinii*, with 82% bootstrap support. The subgroup *B. ciliatus–B. pumpellianus–B. ramosus* had 50% bootstrap value.

The unrooted NJ tree gave a result complementary to the UPGMA classification (Fig. 1c). It reconstructed the group of *B. auleticus*, but with support below 50%. On the other hand, the group of *B. benekenii–B. pumpellianus–B. ramosus* gained relatively high 88% bootstrap support. The group

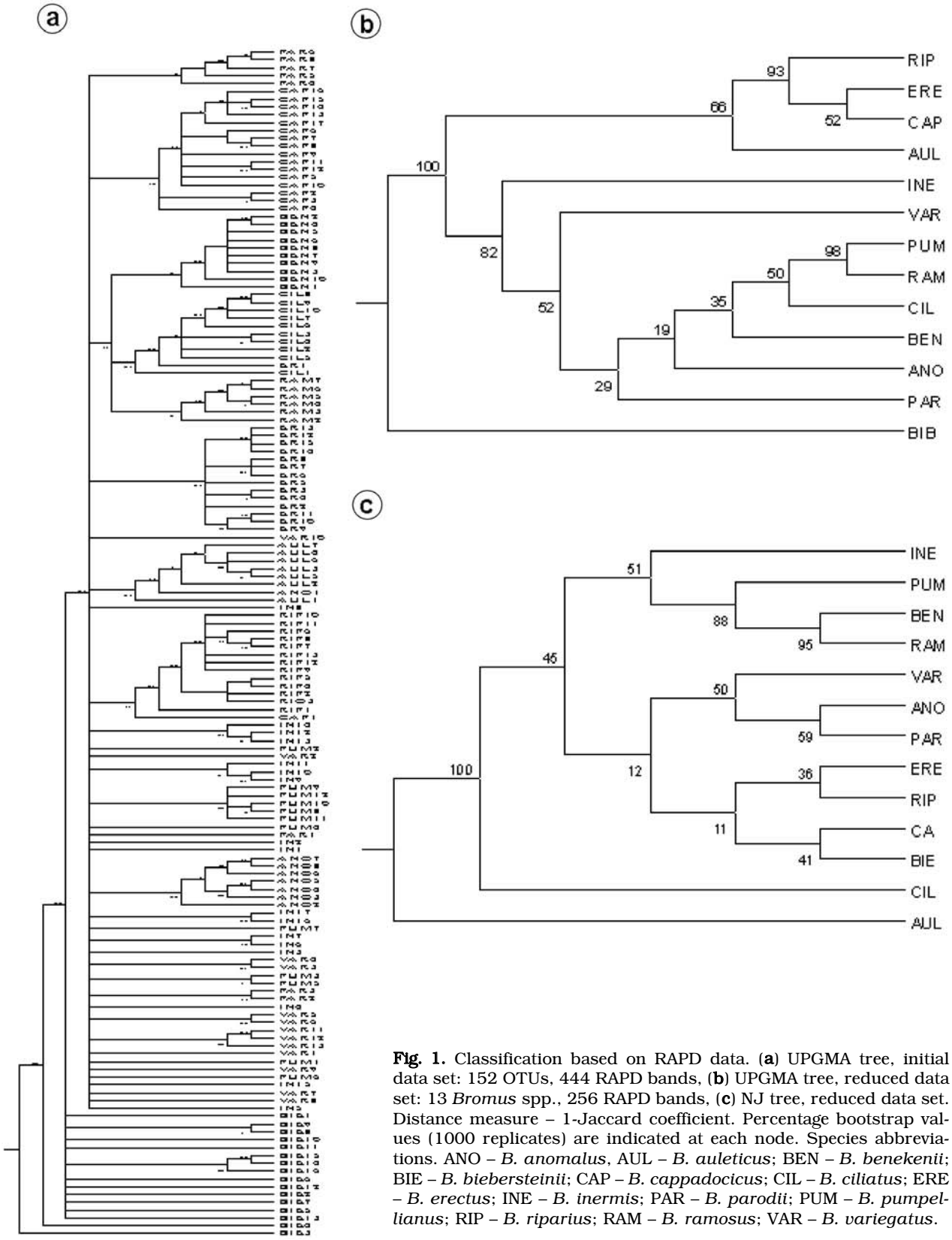

Fig. 1. Classification based on RAPD data. (**a**) UPGMA tree, initial data set: 152 OTUs, 444 RAPD bands, (**b**) UPGMA tree, reduced data set: 13 *Bromus* spp., 256 RAPD bands, (**c**) NJ tree, reduced data set. Distance measure – 1-Jaccard coefficient. Percentage bootstrap values (1000 replicates) are indicated at each node. Species abbreviations. ANO – *B. anomalus*, AUL – *B. auleticus*; BEN – *B. benekenii*; BIE – *B. biebersteinii*; CAP – *B. cappadocicus*; CIL – *B. ciliatus*; ERE – *B. erectus*; INE – *B. inermis*; PAR – *B. parodii*; PUM – *B. pumpellianus*; RIP – *B. riparius*; RAM – *B. ramosus*; VAR – *B. variegatus*.

B. anomalus–B. parodii–B. variegatus was partially reconstructed from the UPGMA analysis.

Generally, UPGMA and NJ showed three groups of species: the first *B. alueticus–B. cappadocicus-B. erectus–B. riparius* (UPGMA, 66%), the second *B. benekenii–B. ciliatus–B. inermis–B. pumpelianus–B. ramosus* (NJ, 88%, 50%) and the third *B. anomalus–B. parodii–B. variegatus* (NJ, 50%). In both analyses, *B. biebersteinii* remained unresolved.

Ordination of the OTUs along the first three principal coordinates, which explained 9.4% of total variance, showed genetic distinctness of some species under study. Axes 1 and 2 discriminated a group of species occupying the right side of the diagram (*B. benekenii*, *B. biebersteinii*, *B. ciliatus*, *B. ramosus*, *B. riparius*) from those on the left side (Fig. 2a). This set of species resembled the group previously recognized as the *B. benekenii* group, and added to it the previously unresolved *B. biebersteinii*. It also confirmed the close relationships between *B. benekenii* and *B. ramosus*. There is some separation between the Old World and New World species, but *B. anomalus*, *B. auleticus*, *B. erectus*, *B. pumpellianus* and *B. variegatus* overlapped with the Old World species (Fig. 2a). Axes 2 and 3 identified the *B. anomalus* group (Fig. 2b).

DISCUSSION

THE ANCESTRAL FORM OF GENOMES A AND B

The phylogeny of the genus *Bromus* is still far from fully understood, but one aspect seems well documented, that is, the typology of the A, B and L genomes based on classical studies on the meiotic conjugation of chromosomes at interspecific hybrids (Armstrong, 1981, 1983, 1984, 1991). The origin of these genomes and their relationships remains unresolved. Here the key is to recognize the diploids that might be ancestral or at least closely related to the ancestral form. The phylogeny of *Bromus* may be inferred from the distribution of genomes with large (L) and small (A and B) chromosomes among diploids and polyploids in the Old World and New World. According to Stebbins (1981) the cradle of *Bromus* is in the Old World. Thus, diploids from this region should be considered first.

The Eurasian species with small chromosomes are the diploid *B. variegatus*, autotetraploid *B. erectus* and allopolyploid *B. inermis* (Armstrong, 1991), *B. biebersteinii* and *B. cappadocicus* (Kozhuharov, 1981) and *B. riparius* (Armstrong, 1981). The present analysis showed the sister position of *B. cappadocicus–B. erectus*. They form a group with another Old World facultative diploid, *B. riparius*. They all share genome A with *B. erectus*. Thus, the complex *B. erectus–B. cappadocicus–B. riparius* with high UPGMA bootstrap support (93%) is presumably ancestral to the New World South American *B. auleticus*. The relations of *B. erectus* and *B. cappadocicus* with *B. auleticus* need further study. It is worth noting that the Old World species of the group have a similar ecological profile: all are moderately xerophilous, preferring open sites. They also have a relatively restricted geographical distribution as compared to other species of subgen. *Festucaria*, confined mostly to Europe. Among the species representing Eurasian small chromosomes, only the diploid genome A was identified in *B. erectus*, which as a rule is to be found in the autotetraploid form. In the present analysis it formed a sister group with *B. cappadocicus*. The latter species is polyploid; this accounts for its distinct position on the ordination diagram (Fig. 2b). All of these observations support the view that this is the oldest group of species in subgen. *Festucaria*.

The aim of the study was to determine whether *B. variegatus* could be a member of genome B. The search for the ancestral form or an existing diploid with genome B has not been fruitful. Small chromosomes A and B are difficult to distinguish from each other. Only indirect evidence of their existence can come from analysis of chromosome conjugation in hybrids. Our results show no relations between *B. variegatus* and *B. erectus*, representing genome A (Fig. 1a,b,c); thus they probably have different genomes.

Recently, Tuna et al. (2001a,b, 2004, 2006) studied the cytogenetic character and nuclear DNA content of the Eurasian species of the subgen. *Festucaria* and came to the conclusion that diploid *B. variegatus* or tetraploid *B. erectus* could be progenitors of tetra- and octoploid *B. inermis*, as suggested previously by Armstrong (1991).

The results of the present study incline towards Armstrong's hypothesis, at least in the case of *B. variegatus*. On the UPGMA diagram (Fig. 1b) *B. inermis* was joined, with moderate support of 82%, with *B. variegatus* and *B. erectus*. The ordination diagram also supports close relationships between the *B. variegatus* group and the respective taxa (Fig. 2b).

The PCo results (Fig. 2a) point to relations between the Eurasian *B. riparius* and *B. biebersteinii* and the complex *B. ramosus–B. benekenii–B. ciliatus*, representing genome L. *B. riparius* probably contains the same genomes as *B. inermis* (A, B) plus an additional genome (Armstrong, 1991). According to Tuna et al. (2001b) the similar average content of cDNA in *B. riparius* (22.15 pg) and *B. biebersteinii* (22.62 pg) suggests that these decaploids have similar genome structure and demonstrates a close genetic relationship.

Our results suggest that this "additional" genome could be closely related to genome L or

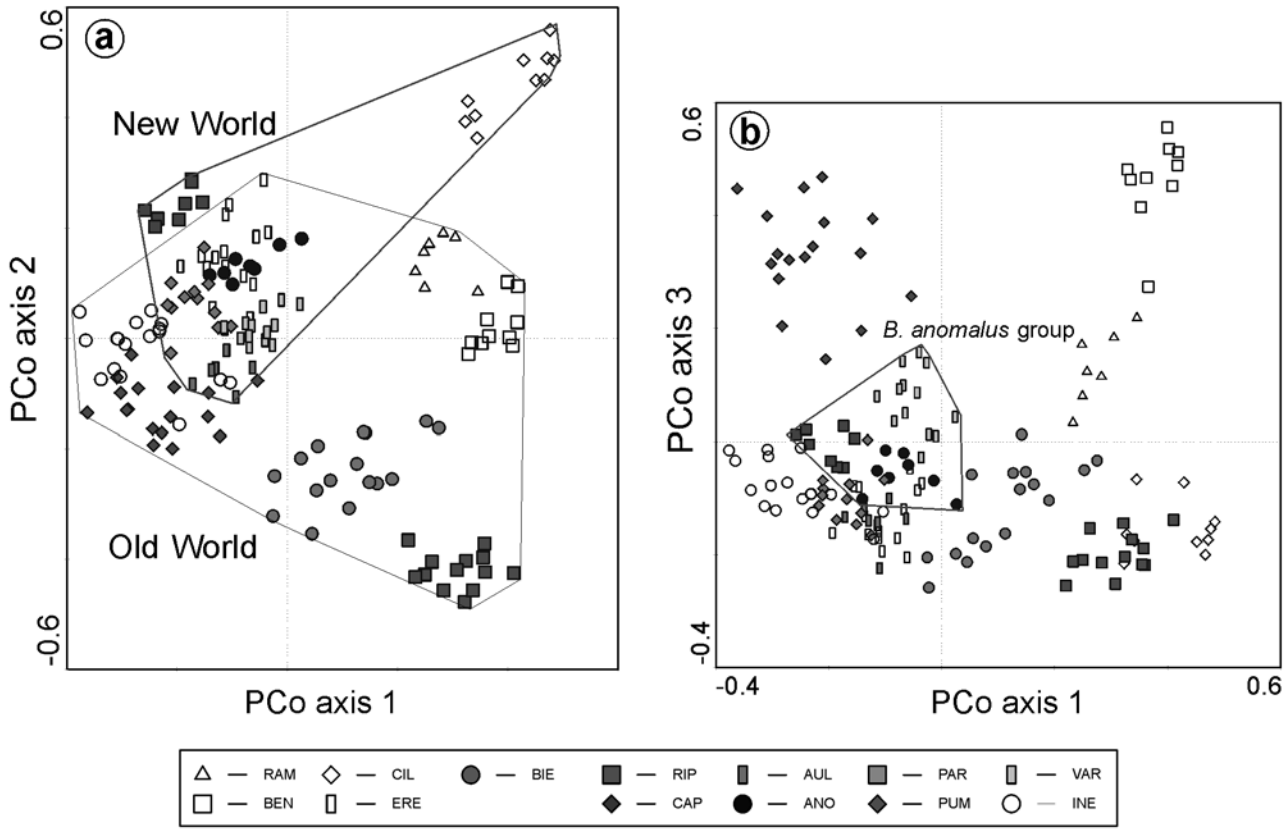

Fig. 2. PCo ordination of 152 OTUs based on 444 RAPD bands. (**a**) Distribution of OTUs along axes 1 and 2, (**b**) Distribution of OTUs along axes 1 and 3. Distance measure: squared (1-Jaccard coefficient). Species abbreviations as in Figure 1.

ancestral to it. This corroborates the previous supposition based on ISSR analyses (Sutkowska et al., 2007) that polyploids have an old phylogenetic history.

THE ORIGIN OF GENOME L

In our argument we adopted the view (Stebbins, 1981) that evolution proceeded from small to large chromosomes, thus that genome B is ancestral to L. The grouping *B. benekenii–B. ramosus–B. pumpellianus–B. inermis–B. ciliatus* (Fig. 1c) is interesting in this context. *B. ciliatus* and *B. pumpellianus* are New World species. The most coherent is the complex *B. benekenii–B. ramosus–B. pumpellianus* (88-98% bootstrap). *B. inermis* and *B. ciliatus* are weakly attached to the group (50% bootstrap). Three species (*B. ramosus*, *B. benekenii*, *B. ciliatus*) possess genome L. Based on satellite morphology, Armstrong (1981, 1983) regarded the Eurasian complex *B. ramosus–B. benekenii* as a possible progenitor or as related to the progenitor of the L genome of the American species. This scenario is corroborated in the present and other studies (Sutkowska and Mitka, 2005) regarding *B. pumpellianus*. The position of the diploid New World *B. ciliatus* is still unresolved. It is a good candidate for the ancestral form of the New World species of the subgenus, but it is weakly tied to the group. Additional information is needed to place it. The sister group *B. benekenii–B. ramosus* has a distinct ecological profile: they are mesophilous forest species. In this respect, *B. inermis* with small A and B chromosomes and a xerophilous ecological profile may be considered close to the hypothetical ancestor of the group. Based on cpDNA analysis, Pillay and Hilu (1990) reached similar conclusions regarding the monophyly of the group, in which *B. pumpellianus*, *B. biebersteinii* (not resolved in our analysis) and *B. inermis* form a clade sister to *B. ciliatus*.

The third group (*B. anomalus–B. parodii–B. variegatus*) recognized in the NJ classification with weak (50–59%) bootstrap support (Fig. 1c) and on

the ordination diagram (Fig. 2b) supports the origin of New World species from Old World genetic stock. In this respect the Old World diploid *B. variegatus* seems close (ancestral) to the group of New World species.

The basal position of *B. variegatus* among the American species (Sutkowska et al., 2007) suggests its ancestral (or related to ancestral) relation to New World species with genome L. This is consistent with the cytological results of Armstrong (1983, 1984) indicating that the American diploid species may have originated from the Eurasian diploids and that they are younger than Eurasian diploids.

The most robust groups in the RAPD analyses were *B. ramosus–B. pumpellianus* (UPGMA) and *B. benekenii–B. ramosus* (NJ; Fig. 1b,c). The latter pair of species are sister to another New World species, *B. pumpellianus*. They share the large chromosomes described as genome L. It is thought that genome L is of Eurasian origin and that its occurrence in *B. ramosus* and *B. benekenii* is a relict (Sutkowska and Mitka, 2005). The linkage of *B. inermis* and *B. pumpellianus* to *B. benekenii* and *B. ramosus* suits the hypothesis that genomes A or B are ancestral to genome L. The close relation of *B. ciliatus*, albeit with weak support in both classifications, to the complex *B. ramosus–B. benekenii* – the only group in Eurasia with genome L – suggests that the species can also possesses genome L (Sutkowska and Mitka, 2005).

Previous ISSR analyses (Sutkowska and Mitka, 2005) of selected species of the subgenus *Festucaria* (*B. pumpellianus*, *B. inermis*, and *B. erectus*, Eurasian species with small chromosomes; *B. ramosus* and *B. benekenii*, Eurasian species with genomes similar to the American genome L) confirmed the close relations between species with small chromosomes, and pointed to the Eurasian origin of *B. pumpellianus*. Those results also suggest the old history of genome L, that it is of Eurasian origin, from the relict genome complex of *B. benekenii–B. ramosus*.

None of the groups of species from subgen. *Festucaria* resolved by RAPD and numerical analyses was homogenous in respect of genome type and geographical distribution. Such a pattern seems to be the result of the complicated history of the taxon, involving not only polyploidization but evolutionary migration accompanied by several interspecific multiple hybridizations, as recognized, for example, in sect. *Genea* (Fortune et. al., 2008). It seems likely, based on RAPD analysis, that *B. variegatus* possesses genome B. The view that genome L originated from genome B seems relevant in light of our results. The hypothesis should be verified by other molecular techniques based on sequencing of chloroplast and nuclear DNA.

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REFERENCES

Ainouche ML, and Bayer RJ. 1997. On the origins of the tetraploid *Bromus* species section *Bromus* (Poaceae): insights from internal transcribed spacer sequences of nuclear ribosomal DNA. *Genome* 40: 730–743.

Ainouche ML, Bayer RJ, Gourret J-P, Defontaine A, and Misset MT. 1999. The allotetraploid invasive weed *Bromus hordeaceus* L. (Poaceae): genetic diversity, origin and molecular evolution. *Folia Geobotanica* 34: 405–419.

Armstrong KC. 1981. The evolution of *Bromus inermis* and related species of *Bromus* sect. *Pnigma*. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 102: 427–443.

Armstrong KC. 1983. The relationship between some Eurasian and American species of *Bromus* section *Pnigma* as determined by the karyotypes of some F1 hybrids. *Canadian Journal of Botany* 61: 700–707.

Armstrong KC. 1984. Chromosome pairing affinities between Old and New World species of *Bromus* section *Pnigma*. *Canadian Journal of Botany* 62: 581–585.

Armstrong KC. 1987. Chromosome numbers of perennial *Bromus* species collected in the USSR. *Canadian Journal of Plant Science* 87: 267–269.

Armstrong KC. 1991. Chromosome evolution of *Bromus*. In: Tsuchiya T and Gupta PK [eds.], *Chromosome engineering in plants: Genetics, breeding, evolution, Part B*, 363–377. Elsevier, Amsterdam, the Netherlands.

Awasthi AK, Nagaraja GM., Naik GV, Kanginakudru S, Thangavelu K, and Nagaraju J. 2004. Genetic diversity and relationships in mulberry (genus *Morus*) as revealed by RAPD and ISSR marker assays. *BCM Genetics* 5: 1.

Ayres DR and Strong DR. 2000. Origin and genetic diversity of *Spartina anglica* (Poaceae) using nuclear DNA markers. *American Journal of Botany* 88: 1863–67.

Buntjer JB. 1997–2001. Phylogenetic Computer Tools v. 1.3., Wageningen Univ., The Netherlands.

Felsenstein J. 2006. PHYLIP (phylogeny inference package), ver. 3.66. Computer program distributed by author, website http://evolution.genetics.washington.edu/phylip.htlm. Department of Genome Science, Univ. of Washington, New York, USA.

Ferdinandez YSN, Somers DJ, and Coulman BE. 2001. Estimating the genetic relationship of hybrid bromegrass to smooth bromegrass and meadow bromegrass using RAPD markers. *Plant Breeding* 120: 149–153.

Fico G, Spada A, Braca A, Agradi E, Morelli I, and Tomé F. 2003. RAPD analysis and flavonoid composition of *Aconitum* as an aid for taxonomic discrimination. *Biochemical Systematics and Ecology* 31: 293–301.

FORTUNE PM, POURTAU N, VIRON N, and AINOUCHE ML. 2008. Molecular phylogeny and reticulate origins of the polyploid *Bromus* species from section *Genea* (Poaceae). *American Journal of Botany* 95: 454–464.

GONZALES-PEREZ MA, CAUJAPE-CASTELLAS J, and SOSA PA. 2004. Molecular evidence of hybridisation between the endemic *Phoenix canariensis* and the widespread *P. dactylifera* with RAPD markers. *Plant Systematics and Evolution* 247: 165–175.

JACCARD P. 1901. Distribution de la flore alpine dans le Bassin des Dranses et dans quelques régions voisines. *Bulletin de la Sociéte Vaudoise des Sciences Natureless* 37: 239–272.

JOACHIMIAK A, SUTKOWSKA A, and MITKA J. 2001. RAPD studies in *Bromus* (Poaceae) from the Old and New Worlds – preliminary results. *Acta Botanica Cracoviensia Series Botanica* 43: 79–86.

KOZHUHAROV S, PETROVA A, and EHRENDORFER F. 1981. Evolutionary patterns in some brome grass species (*Bromus*, Gramineae) of the Balkan Peninsula. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 102: 381–391.

MIREK Z, PIĘKOŚ-MIRKOWA H, ZAJĄC A, and ZAJĄC M. 2002. *Flowering plants and Pteridophytes of Poland. A checklist.* W. Szafer Institute of Botany, Polish Academy of Science, Cracow.

OXELMAN B. 1996. RAPD patterns, nrDNA ITS sequences and morphological patterns in *Silene* section *Sedoideae* (Caryophyllaceae). *Plant Systematics and Evolution* 201: 93–116.

PILLAY M. 1993. Chloroplast genome organization of bromegrass, *Bromus inermis* Leyss. *Theoretical and Applied Genetics* 86: 281–287.

PILLAY M. 1995. Chloroplast DNA similarity of smooth bromegrass with other pooid cereals: implications for plant breeding. *Crop Science* 35: 869–875.

PILLAY M. 1996. Genomic organization of ribosomal RNA genes in *Bromus* (Poaceae). *Genome* 39: 198–205.

PILLAY M, and ARMSTRONG KC. 2001. Maternal inheritance of chloroplast DNA in interspecific crosses of *Bromus*. *Biologia Plantarum* 44: 47–51.

PILLAY M, and HILU KW. 1990. Chloroplast DNA variation in diploid and polyploid species of *Bromus* (Poaceae) subgenera *Festucaria* and *Ceratochloa*. *Theoretical and Applied Genetics* 80: 326–332.

PILLAY M, and HILU KW. 1995. Chloroplast-DNA restriction site analysis in the genus *Bromus* (Poaceae). *American Journal of Botany* 82: 239–249.

RATH P, RAJASEGER G, GOH CJ, and KUMAR PP. 1998. Phylogenetic analysis of dipterocarps using random amplified polymorphic DNA markers. *Annals of Botany* 82: 61–65.

SAITOU N, and NEI M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.

SAARELA JM, PETERSON PM, KEANE RM, CAYOUETTE J, and GRAHAM SW. 2007. Molecular phylogenetics of *Bromus* (Poaceae: Pooideae) based on chloroplast and nuclear sequence data. *Aliso* 23: 450–467.

SOKAL RR, and MICHENER CD. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Scientific Bulletin* 38: 409–1438.

SMITH PM. 1970. Taxonomy and nomenclature of the Bromegrasses (*Bromus* L. s. l.). *Notes from the Royal Botanic Garden, Edinburgh* 30: 361–376.

SMITH PM. 1973. Observations on some critical Bromegrasses. *Watsonia* 9: 319–332.

STEBBINS GL. 1981. Chromosomes and evolution in the genus *Bromus* (Gramineae). *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 102: 359–379.

SUTKOWSKA A, WILK Ł, MITKA J, and JOACHIMIAK A. 2002. DNA polymorphism in *Bromus erectus* and *Bromus inermis* from Poland's territory. *Zeszyty Problemowe Postępów Nauk Rolniczych* 488: 187–195. (In Polish with English summary).

SUTKOWSKA A, and MITKA J. 2005. Molecular PCR-ISSR analysis in *Bromus* subgenus *Festucaria* – preliminary results. In: Frey L [ed.], *Biology of grasses*, 207–216. W. Szafer Institute of Botany, Polish Academy of Science, Cracow.

SUTKOWSKA A, MITKA J, GLIMOS E, and WARZECHA T. 2007. Phylogenetic relations among selected species of genus *Bromus*, subgenus *Festucaria* (Poaceae). In: Frey L [ed.], *Biological issues in grasses*, 69–179. W. Szafer Institute of Botany, Polish Academy of Science, Cracow.

SMILAUER P. 1990. CANODRAW. Scientia Publishing, Budapest.

TAMURA K, DUDLEY J, NEI M, and KUMAR S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–1599.

TUNA M, GILL KS, and VOGEL KP. 2001a. Karyotype and C-banding pattern in the chromosomes of inbred lines and open pattern of mitotic chromosomes in diploid bromegrass (*B. riparius* Rehm.). *Crop Science* 41: 831–834.

TUNA M, VOGEL KP, ARUMUGANATHAN K, and GILL KS. 2001b. DNA content and ploidy determination of bromegrass germplasm accessions by flow cytometry. *Crop Science* 41: 1629–1634.

TUNA M, VOGEL KP, GILL KS, and ARUMUGANATHAN K. 2004. C-banding analyses of *Bromus inermis* genomes. *Crop Science* 44: 31–37.

TUNA M, VOGEL KP, and ARUMUGANATHAN K. 2005. Genome size and Giemsa C-banded karyotype of tetraploid *Bromus ciliatus* L. *Euphytica* 146: 177–182.

TUNA M, VOGEL KP, and ARUMUGANATHAN K. 2006. Cytogenetic and nuclear DNA content characterization of diploid *Bromus erectus* and *Bromus variegatus*. *Crop Science* 46: 637–641

ZHANG FM, CHEN WL, YANG QE, and GE S. 2005. Genetic differentiation and relationship of populations in the *Aconitum delavayi* complex (Ranunculaceae) and their taxonomic implications. *Plant Systematics and Evolution* 254: 39–48.