Title

Genome analysis of South American Elymus (Triticeae) and Leymus (Triticeae) species based on variation in repeated nucleotide sequences

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Genome analysis of South American *Elymus* (Triticeae) and *Leymus* (Triticeae) species based on variation in repeated nucleotide sequences

Jorge Dubcovsky, A.R. Schlatter, and M. Echaide

Abstract: Variation in repeated nucleotide sequences (RNSs) at the level of entire families assayed by Southern blot hybridization is remarkably low within species and is a powerful tool for scrutinizing the origin of allopolyploid taxa. Thirty-one clones from RNSs isolated from different Triticeae genera were used to investigate the genome constitution of South American *Elymus*. One of these clones, pHch2, preferentially hybridized with the diploid **H** genome *Hordeum* species. Hybridization of this clone with a worldwide collection of *Elymus* species with known genome formulas showed that pHch2 clearly discriminates *Elymus* species with the **H** genome (S^tH, S^tHH, S^tS^tH, and S^tHY) from those with other genome combinations (S^tY, S^tS^tY, S^tPY, and S^tP). Hybridization with pHch2 indicates the presence of the **H** genome in all South American *Elymus* species except *Elymus erianthus* and *Elymus mendocinus*. Hybridization with additional clones that revealed differential restriction fragments (marker bands) for the **H** genome confirmed the absence of the **H** genome in these species. Differential restriction fragments for the N^s genome of *Psathyrostachys* were detected in *E. erianthus* and *E. mendocinus* and three species of *Leymus*. Based on genome constitution, morphology, and habitat, *E. erianthus* and *E. mendocinus* were transferred to the genus *Leymus*.

Key words: Triticeae, *Elymus*, *Leymus*, repeated sequences.

Résumé : La variation des séquences nucléotidiques répétées (RNS) présente au niveau de familles botaniques entières, telle que révélée par hybridation Southern, est étonnamment faible entre espèces et constitue un outil puissant pour analyser l'origine de taxons allopolyploïdes. Trente et un clones RNS isolés chez différents genres appartenant aux Triticeae ont été employés pour analyser la composition génomique des espèces du genre *Elymus* en Amérique du Sud. Un de ces clones, pHch2, montre une hybridation préférentielle avec les espèces du genre *Hordeum* à génome **H** diploïdes. L'emploi de ce clone comme sonde lors d'hybridations avec une collection internationale d'espèces d'*Elymus* ayant une composition génomique connue a montré que pHch2 permet de distinguer nettement les élymes portant le génome **H** (S^tH, S^tHH, S^tS^tH et S^tHY) des élymes ne possédant pas un tel génome (S^tY, S^tS^tY, S^tPY et S^tP). L'hybridation avec la sonde pHch2 a montré la présence du génome **H** chez toutes les espèces sud-américaines à l'exception du *Elymus erianthus* et du *Elymus mendocinus*. Des hybridations additionnelles avec des clones qui révèlent des bandes spécifiques au génome **H** ont confirmé l'absence de ce génome parmi ces espèces. Des fragments de restriction spécifiques au génome N^s du *Psathyrostachys* ont été détectés chez les espèces *E. erianthus* et *E. mendocinus* de même que chez trois espèces du genre *Leymus*. En se fondant sur leur composition génomique, leur morphologie et leur habitat, les espèces *E. erianthus* et *E. mendocinus* sont considérées comme faisant partie du genre *Leymus*.

Mots clés : Triticeae, *Elymus*, *Leymus*, séquences répétées.

[Traduit par la Rédaction]

Introduction

The genus *Elymus* L. is the largest among the perennial Triticeae. It contains approximately 150 species, almost four times as many as the second largest genus, *Hordeum* L. (approximately 40 species). Species of *Elymus* are allopolyploids cytologically characterized by genome combinations of five basic genomes: S^t from *Pseudoroegneria* (Nevski) Á. Löve, **H** from *Hordeum* L., **Y** from an unknown diploid species, **P** from *Agropyron* Gaertn., and **W** from *Australopyrum* (Tzvelev) Á. Löve (Dewey 1984; Löve 1984; Jensen 1989; Jensen 1990*a*, 1990*b*, 1990*c*; Lu 1993). Genome symbols are in accordance with the system proposed at the 2nd International Triticeae Symposium (Wang et al. 1994).

Elymus species with different genome combinations differ in their geographic distribution. The basic genome **W** has been identified only in one Australasian hexaploid (S^tYW; Torabinejad and Mueller 1993) and the basic genome **P** is restricted to some Central Asiatic hexaploids (S^tPY; Jensen

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1990*b*, 1990*c*). The **Y** genome has a wider distribution and is found in many Asiatic tetraploids and hexaploids (S^tY, S^tS^tY, S^tHY, and S^tPY; Lu 1993). Finally, species with S^t–**H** genome combinations (S^tH, S^tS^tH, S^tHH, and S^tS^tHH) have the widest geographic distribution, being found in Europe, Asia, North America, South America, Australia, and New Zealand (Dewey 1984; Löve 1984).

Genome formulas are known only for a small number of South American *Elymus* species. Genome analysis has resulted in the tetraploid species *Elymus agropyroides* Presl, *Elymus tilcarensis* (Hunziker) Á. Löve, and *Elymus magellanicus* (Desv.) Á. Löve being assigned the genome formula S^tH (Dewey 1970*b*, 1977; Jensen 1993) and the hexaploid species *Elymus patagonicus* Speg. and *Elymus scabriglumis* (Hakel) Á. Löve being assigned the genome formula S^tHH (Hunziker 1953, 1966; Dewey 1972*c*; Seberg 1991). Gupta et. al (1988) suggested that the **Y** genome was present in *E. scabriglumis*, but later concluded that the genome formula of this species was S^tHH (Lee et al. 1994). In spite of this apparent genome uniformity, poor chromosome pairing in meiosis of interspecific hybrids has revealed the presence of genome differentiation among some tetraploid South American *Elymus* species (Hunziker et al. 1965; Hunziker and Ferrari 1986).

Karyotype analysis of the South American *Elymus* species suggested that the S^tH genome was present in all South American tetraploids analyzed, but indicated differential characteristics in the karyotypes of the hexaploid *Elymus erianthus* Philippi and the octoploid *Elymus mendocinus* (Parodi) Á. Löve (Dubcovsky et al. 1989; Lewis et al. 1996). C-banding and N-banding of *E. erianthus* chromosomes (Seberg and Linde-Laursen 1996) and hybridization with a GAA satellite (Pedersen et al. 1996) and four repetitive DNA sequences cloned from barley (Svitashev et al. 1996) revealed additional differences and suggested the absence of the S^t and **H** genomes from this species (Seberg and Linde-Laursen 1996).

Clayton and Renvoize (1986; also, S.A. Renvoize, personal communication in Seberg and Linde-Laursen (1996)) suggested the inclusion of *E. erianthus* in *Leymus* Hochst., a genus of about 30 polyploid species occurring naturally in Eurasia and North America. More recently, *E. erianthus* was transferred from *Elymus* to a new monotypic genus, *Eremium* Seberg (Seberg and Linde-Laursen 1996), on the basis of its differential morphology and cytology. To decide between these alternative taxonomic treatments it is important to determine the genome constitution of *E. erianthus*. As *Leymus* is morphologically, cytologically, and genomically affiliated with the N^s genome (Dewey 1970*a*, 1972*a*, 1972*b*, 1976; Wang and Hsiao 1984; Zhang and Dvořák 1991; Sun et al. 1995; Mason-Gamer and Kellog 1996), it is particularly important, to establish the presence or absence of the N^s genome of the diploid genus *Psathyrostachys* Nevski in *E. erianthus*. The presence of a differential *Eco*RI site in the intergenic spacer (IGS) of the rDNA units of diploid *Psathyrostachys stoloniformis* C. Baden (Orgaard and Heslop-Harrison 1994) and *E. erianthus* (Dubcovsky et al. 1992), which is absent in the IGS of other diploid Triticeae and most South American *Elymus* species, provides preliminary evidence for the presence of the N^s genome in *E. erianthus*.

In this paper we investigate the distribution of the **H** and N^s genomes among South American *Elymus* species, using **H** and N^s genome specific repeated nucleotide sequences and **H** and N^s genome specific restriction fragments of repeated sequences (marker bands). We also investigate the use of these repetitive sequences as a tool for genome analysis in *Elymus* by using a worldwide set of species with known genome constitutions.

Materials and methods

Plant materials

The species analyzed, together with their genome constitution, accession number, and origin, are listed in Table 1. Voucher specimens of the South American *Elymus* species are deposited in the herbarium of the Instituto de Recursos Biologicas (IRB), Instituto Nacional de Tecnología Agropecuaria (INTA) (BAB, Castelar, Buenos Aires, Argentina). Herbarium acronyms are according to Holmgren et al. (Holmgren et al. 1990). Seeds of the *Elymus* species from North America, Europe, and Asia were provided by Dr. R.C. Johnson of the United States Department of Agriculture, Agricultural Research Service, Western Regional Plant Introduction Station, Pullman, Wash., U.S.A. Seeds of the *Hordeum* species were provided by Dr. R. von Bothmer of the Swedish University of Agricultural Sciences, Svalöv, Sweden.

South American *Elymus* species were identified using the keys of Nicora (1978) and Hunziker (1966). *Elymus angulatus* Presl did not flower and it was not possible to verify the identification provided by the collector, Dr. O. Seberg (University of Copenhagen, Denmark).

Delimitation of the genus *Elymus* followed Löve (Löve 1984; Zuloaga et al. 1994), and *Hordeum* was considered in sensu Bothmer (Bothmer et al. 1991). When all the South American species of *Agropyron* were transferred to *Elymus* (Löve 1984), *Agropyron scabrifolium* (Döll) Parodi was included as a subspecies of *Elymus breviaristatus* (A.S. Hitchc.) Á. Löve. This is probably incorrect, because *A. scabrifolium* and *Agropyron breviaristatum* A.S. Hitchc. are reproductively isolated species (Hunziker et al. 1965). Since *E. breviaristatus* ssp. *scabrifolious* (Döll) Á. Löve (Löve 1984; Zuloaga et al. 1994) is the only published name for this taxon under *Elymus*, we temporarily use it for the sake of consistency. A taxonomic review of this species is in preparation (O. Seberg, personal communication).

Clones

The clones used in this study, together with the origin of the genomic libraries, vector, cloning site, size, and references are listed in Table 2.

Methods

Nuclear DNAs were isolated from leaves of single plants following the procedure of Dvořák et al. (1988) and digested with *Taq*I and *Hae*III. DNA loading in the gel was adjusted by ploidy level (diploids, 2 μg; tetraploids, 4 μg; hexaploids, 6 μg; and octoploids, 8 μg). DNAs were electrophoretically fractionated in 1.7% agarose gel and transferred to Hybond N+ nylon membrane (Amersham, Ill., U.S.A.) by capillary transfer. Fluorescence intensity of ethidium bromide under UV light was integrated for each lane after electrophoretic fractionation with an IBM PC based image processing system (Foto/Eclipse, Fotodyne) with the program NIH Image (version 1.55). Prehybridization and hybridization were performed in a rotary hybridization oven at 65°C in a solution containing 1% SDS, 2.5× SSPE buffer (1× SSPE: 0.18 M NaCl, 10 mM $NaPO_4$, plus 1 mM EDTA, pH 7.7), 0.1% polyethylene sulfonic acid, and 0.01% sodium pyrophosphate. The immobilized DNAs were hybridized overnight with [α-^{32}P]-labeled probes by the random primer method (Feinberg and Vogelstein 1983). The membranes were washed in 2× SSC (1× SSC: 1.15 M NaCl plus 0.015 M sodium citrate) plus 0.5% SDS for 30 min, 1× SSC plus 0.5% SDS for 30 min, and 0.2× SSC

Table 1. Plant species used in this study.

					H-genome bands[a]				Barley bands[b]			**N**^{s}-genome bands[c]				
	Genome	Accession	Origin	Identification No.	pHch2	pHch93	pHch1.3	AK60A9	pTIUCD2	pHVUCD16	pHVUCD32	pPjUCD5	pPjUCD6	pPjUCD11	pLmUCD1	pTa71
Hordeum vulgare L.	**I**	cv. Morex	U.S.A.	1	–	–	–	–	+	++	++	–	–	–	–	–
Hordeum bulbosum L.	**I**	DV1322–3	Unknown	2	–	–	–	+	+	+	+	–	–	ns	–	ns
Hordeum marinum ssp. *marinum* Huds.	**X**^{a}	H 90-I	Greece	3	–	–	–	–	+	+	+	–	–	–	–	ns
Hordeum marinum ssp. *marinum*	**X**^{a}	H 515	Spain	4	–	–	–	–	+	+	+	–	–	ns	–	ns
Hordeum marinum ssp. *gussonoeanum* (Parl.) Thell.	**X**^{a}	H 299	Bulgaria	5	–	–	–	–	+	+	+	–	–	–	–	ns
Hordeum murinum L. ssp. *glaucum* (Steud.) Tzvelev	**X**^{u}	H 3325	Georgia	6	–	–	–	–	+	+	+	–	–	ns	–	ns
Hordeum murinum ssp. *glaucum*	**X**^{u}	PI 211045	Afghanistan	7	–	–	–	–	+	+	+	–	–	–	–	ns
Hordeum bogdanii Wilensky	**H**	H 7065	People's Republic of China	8	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum roshevitzii Bowden	**H**	H 10070	Russia	9	+	+	+	+	+	+	+	–	–	–	–	ns
Hordeum brevisubulatum (Trin.) Link ssp. *violaceum* (Boiss & Hohen.) Tzvelev	**H**	PI 401374	Iran	10	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum brachyantherum Nevski ssp. *californicum* (Covas & Stebbins) Bothmer, Jacobsen & Seberg	**H**	H 1942	U.S.A.	11	+	+	+	+	+	+	+	–	–	–	–	ns
Hordeum intercedens Nevski	**H**	H 3251	U.S.A.	12	+	+	+	+	+	+	+	–	–	–	–	ns
Hordeum pusillum Nutt.	**H**	H 2043	U.S.A.	13	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum chilense Roem. & Schult.	**H**	PI 283376	Chile	14	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum cordobense Bothmer, Jacobsen & Nicora	**H**	H 6460	Argentina	15	+	+	+	+	+	+	–	–	–	ns	–	ns
Hordeum erectifolium Bothmer, Jacobsen & M. Jørg.	**H**	H 1150	Argentina	16	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum euclaston Steud.	**H**	H 1263	Argentina	17	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum flexuosum Nees ex Steud.	**H**	H 2127	Uruguay	18	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum muticum J. Presl	**H**	H 958	Bolivia	19	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum patagonicum (Hauman) Covas ssp. *magellanicum* (Parodi & Nicora) Bothmer, Giles & Jacobsen	**H**	H 6209	Argentina	20	+	+	+	ns	+	+	+	–	ns	–	ns	ns
Hordeum pubiflorum Hook. f.	**H**	H 1236	Argentina	21	+	+	+	+	+	+	+	–	–	ns	–	ns
Hordeum stenostachys Godr.	**H**	H 1780	Argentina	22	+	+	+	+	+	+	+	–	–	–	–	–
Pseudoroegneria stipifolia (Czern. ex Nevski) Á. Löve	**S**^{t}	PI 313960	Former U.S.S.R.	23	–	–	(+)	–	–	(+)	–	–	–	–	–	–

Table 1 (*continued*).

	Genome	Accession	Origin	Identification No.	H-genome bands[a]				Barley bands[b]			N^s-genome bands[c]				
					pHch2	pHch93	pHch1.3	AK60A9	pTIUCD2	pHVUCD16	pHVUCD32	pPJUCD5	pPJUCD6	pPJUCD11	pLmUCD1	pTa71
Agropyron cristatum (L.) Gaertn.	**P**	PI 406450	Former U.S.S.R.	24	–	(+)	–	–	(+)	(+)	–	–	–	–	–	–
Australopyrum retrofractum (Vickery) Á. Löve	**W**	PI 531553	Australia	25	–	–	–	–	(+)	–	–	–	–	–	–	–
Lophopyrum elongatum (Host) Á. Löve	$\mathbf{E^e}$	D1-1-2	Unknown	26	(+)	ns	–	–	(+)	ns	–	–	–	–	–	–
Thinopyrum bessarabicum (Savul. & Rayss.) Á. Löve	$\mathbf{E^b}$	D-3483	Former U.S.S.R.	27	–	–	–	–	–	–	–	–	–	–	–	–
Psathyrostachys juncea (Fisch.) Nevski	$\mathbf{N^s}$	PI 314668	Former U.S.S.R.	28	–	–	–	–	–	–	–	+	+	+	+	+
Psathyrostachys juncea	$\mathbf{N^s}$	H 8721	People's Republic of China	29	–	–	–	–	–	–	–	+	+	+	+	+
Leymus alaicus ssp. *karataviensis* (Roshev.) Tzvelev	$\mathbf{N^sX^m}$	PI 314667	Former U.S.S.R.	30	–	–	–	–	–	–	–	+	+	+	+	+
Leymus multicaulis (Kar. & Kir.) Tzvelev	$\mathbf{N^sX^m}$	PI 440324	Former U.S.S.R.	31	–	–	–	–	–	–	–	+	+	+	+	+
Leymus cinereus (Scrib. & Merr.) Á. Löve	$\mathbf{N^sX^m}$	CS–18–61–70	U.S.A.	32	–	–	–	–	–	–	–	+	+	+	+	+
Elymus erianthus Phil.	$\mathbf{N^sX^mX^m}$?	H 213	Argentina	33	–	–	–	–	–	–	–	+	+	+	+	+
Elymus erianthus	$\mathbf{N^sX^mX^m}$?	Rene	Argentina	34	–	–	–	–	–	–	–	+	+	+	+	+
Elymus mendocinus (Parodi) Á. Löve	$\mathbf{N^sX^mX^mX^m}$?	J 601	Argentina	35	–	–	–	–	–	–	–	+	+	+	+	–
Elymus andinus Trin.	$\mathbf{S^tH}$	J 825	Chile	36	+	+	ns	+	+	+	+	ns	–	–	–	–
Elymus angulatus J. Presl	$\mathbf{S^tH}$	H 6419	Argentina	37	+	+	ns	+	+	+	+	ns	–	–	–	–
Elymus antarticus Hook. f.	$\mathbf{S^tH}$	H 203	Argentina	38	+	+	+	+	+	+	+	–	–	–	–	–
Elymus araucanus (Parodi) Á. Löve	$\mathbf{S^tH}$	J 650	Argentina	39	+	+	+	+	+	+	+	–	–	–	–	–
Elymus breviaristatus (Hitchc.) Á. Löve ssp. *scabrifolius* (Döll) Á. Löve	$\mathbf{S^tH}$	J 699	Argentina	40	+	+	+	+	+	+	+	–	–	ns	–	ns
Elymus canadensis L.	$\mathbf{S^tH}$	PI 232249	U.S.A.	41	+	+	+	+	+	+	+	–	–	–	–	–
Elymus cordilleranus Davidse & Pohl	$\mathbf{S^tH}$	H 6486	Perú	42	+	+	+	+	+	+	+	–	–	–	–	+
Elymus gayanus Desv.	$\mathbf{S^tH}$	J 821	Chile	43	+	+	ns	+	+	+	+	ns	–	–	–	–
Elymus mutabilis (Drobov) Tzvelev	$\mathbf{S^tH}$	D–2708	People's Republic of China	44	+	+	+	+	+	+	+	ns	–	–	–	–
Elymus rigescens Trin.	$\mathbf{S^tH}$	J 630	Argentina	45	+	+	+	+	+	+	+	–	–	–	–	–

Table 1 (*continued*).

	Genome	Accession	Origin	Identification No.	H-genome bands[a]				Barley bands[b]			N^s-genome bands[c]				
					pHch2	pHch93	pHch1.3	AK60A9	pTIUCD2	pHVUCD16	pHVUCD32	pPjUCD5	pPjUCD6	pPjUCD11	pLmUCD1	pTa71
Elymus siribicus L.	**S^tH**	PI 315429	Former U.S.S.R.	46	+	+	+	+	+	+	+	ns	–	–	–	–
Elymus sitanion Schult.	**S^tH**	PI 232353	U.S.A.	47	+	+	+	+	+	+	+	ns	–	–	–	ns
Elymus tilcarensis (J.H. Hunz.) Á. Löve	**S^tH**	J 413	Argentina	48	+	+	+	+	+	+	+	–	–	–	–	–
Elymus trachycaulus (Link) Gould ex Shinners	**S^tH**	PI 232156	U.S.A.	49	+	+	+	+	+	+	+	–	–	ns	–	–
Elymus trachycaulus ssp. *trachycaulus* (Link) Gould ex Shinners	**S^tH**	PI 537323	U.S.A.	50	+	+	+	+	+	+	+	ns	–	–	–	ns
Elymus trachycaulus ssp. *subsecundus* (Link) Gould	**S^tH**	PI 236687	Canada	51	+	+	+	+	+	+	+	ns	–	–	–	ns
(*Elymus trachycaulus* ssp. *violaceus* (Hornem.) Á. Löve & D. Löve) *Agropyron spp.*?	**PP**?	PI 276712	Former U.S.S.R.	52	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus patagonicus Speg.	**S^tHH**	PI 297898	Argentina	53	++	++	++	++	++	+	++	–	–	–	–	–
Elymus scabriglumis (Hack.) Á. Löve	**S^tHH**	PI 269646	Argentina	54	++	++	++	++	++	+	++	–	–	–	–	–
Elymus dahuricus Turcz. ex Griseb.	**S^tYH**	PI 499418	People's Republic of China	55	+	ns	+	+	+	+	+	ns	–	–	–	ns
Elymus drobovii (Nevski) Tzvelev	**S^tYH**	PI 314201	Former U.S.S.R.	56	+	ns	+	+	+	+	+	ns	–	–	–	ns
Elymus nutants Griseb.	**S^tYH**	PI 406466	Former U.S.S.R.	57	+	ns	+	+	+	+	+	–	–	–	–	–
Elymus tsukushiensis Honda	**S^tYH**	PI 499622	Japan	58	+	ns	+	+	+	+	+	ns	–	–	–	ns
Elymus tsukushiensis var. transiens (syn. *A. kamoji* (Ohwi) Ohwi)	**S^tYH**	PI 276396	China	59	+	ns	+	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus glaucissimus (M. Pop.) Tzvelev	**S^tS^tY**	PI 564934	Europa	60	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus tschimganicus (Drobov) Tzvelev	**S^tS^tY**	PI 499481	People's Republic of China	61	–	ns	(+)	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus alatavicus (Drobov) Á. Löve	**S^tPY**	PI 499588	China	62	–	ns	ns	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus batalinii (Krasn.) Nevski	**S^tPY**	PI 547361	Kyrgyzstan	63	–	ns	–	ns	+	+	ns	–	–	–	–	–
Elymus grandiglumis (Keng) Á. Löve	**S^tPY**	PI 531614	China	64	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus kengii Tzvelev	**S^tPY**	PI 504457	People's Republic of China	65	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Psathyrostachys tauri (Boiss. & Bal.) Á. Löve	**S^tP**	PI 401329	Iran	66	–	ns	ns	ns	ns	+	ns	ns	ns	–	ns	ns

Table 1 (*concluded*).

	Genome	Accession	Origin	Identification No.	**H**-genome bands[a]				Barley bands[b]			**N**^{s}-genome bands[c]				
					pHch2	pHch93	pHch1.3	AK60A9	pTlUCD2	pHVUCD16	pHVUCD32	pPjUCD5	pPjUCD6	pPjUCD11	pLmUCD1	pTa71
Elymus abolinii (Drobov) Tzvelev	**S^{t}Y**	PI 314617	Former U.S.S.R.	67	–	ns	(+)	ns	ns	+	ns	ns	ns	–	ns	–
Elymus barbicallus (Ohwi) S. L. Chen	**S^{t}Y**	PI 499380	People's Republic of China	68	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus caucasicus (C. Koch) Tzvelev	**S^{t}Y**	PI 531572	Armenia	69	–	ns	(+)	+	ns	+	ns	–	–	–	–	–
Elymus ciliaris (Trin.) Tzvelev	**S^{t}Y**	PI 531575	People's Republic of China	70	–	–	(+)	+	(+)	+	–	–	–	–	–	–
Elymus fedtschenkoi Tzvelev	**S^{t}Y**	PI 531607	Pakistan	71	–	ns	(+)	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus gmelinii (Ledeb.) Tzvelev	**S^{t}Y**	PI 499607	People's Republic of China	72	–	ns	(+)	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus gmelinii ssp. *ugamicus* (Drobov) Á. Löve	**S^{t}Y**	PI 314618	Former U.S.S.R.	73	–	–	–	(+)	(+)	+	–	–	–	–	–	–
Elymus longearistatus (Boiss.) Tzvelev	**S^{t}Y**	PI 401277	Iran	74	–	ns	(+)	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus nipponicus Jaaska	**S^{t}Y**	PI 275776	Japan	75	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus pendulinus (Nevski) Tzvelev	**S^{t}Y**	PI 547309	Former U.S.S.R.	76	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus praeruptus Tzvelev	**S^{t}Y**	PI 531651	Tajikistan	77	–	ns	(+)	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus semicostatus (Nees ex Steud.) Melderis ssp. *foliosus* (Keng) Á. Löve	**S^{t}Y**	PI 531664	People's Republic of China	78	–	ns	–	ns	ns	+	ns	ns	ns	–	ns	ns
Elymus semicostatus ssp. *striatus* (Nees ex Steud.) Á. Löve	**S^{t}Y**	PI 207453	Afghanistan	79	–	ns	+	ns	ns	+	ns	ns	ns	–	ns	ns

Note: +, present; ++, present at relatively higher intensity; (+), present as a faint band; –, absent; ns, not scored.

[a] pHch2 (*Hae*III): **H** genome differential intensity: the intensity of the hybridization signal in species with the **H** genome is at least one order of magnitude higher than in those without the **H** genome (Fig. 1); pHch93 (*Hae*III), pHch1.3 (*Hae*III), and AKM60A9 (*Taq*I): restriction fragments are present in all **H** genome diploid species and absent or faint in other diploid Triticeae.

[b] pTlUCD2 (*Taq*I) and pHvUCD32 (*Taq*I): restriction fragments present in all *Hordeum* (**H**, **I**, **X^a**, and **X^u** genomes) diploid species and absent or faint in other diploid Triticeae; pHvUCD16a (*Hae*III) (Fig. 2): differential hybridization intensity in all *Hordeum* (**H**, **I**, **X^a**, and **X^u** genomes) diploid species, faint hybridization signals in *Agropyron* and *Pseudoroegneria* species, and absent in other diploid Triticeae analyzed.

[c] pPjUCD5 (*Taq*I) (Fig. 3): **N^s** genome tandem repeat (165-bp ladder); pPjUCD6 (*Hae*III) (Fig. 3): **N^s** genome marker bands: one restriction fragment not included in the table is present only in *Leymus* species, *E. erianthus*, and *E. mendocinus*; pPjUCD11 (*Hae*III): four **N^s** genome marker bands: one restriction fragment not included in the table is present only in both accessions of *P. juncea*; pLmUCD1 (*Taq*I) (Fig. 3): **N^s** genome marker band; pTa71 (*Eco*RI) (Fig. 4): additional *Eco*RI restriction site in the intergenic spacer of the rDNA repeat unit.

Table 2. Clones, source of genomic libraries, insert size, vector, and cloning site.

Clone	Source	Insert size (bp)	Vector	Reference
pTa71	*Triticum aestivum*	9000	pUC19 (*Eco*RI)	Gerlach and Bedbrook 1979
pTa250.15	*Triticum aestivum*	900	pUC19 (*Hha*I)	Appels and Dvořák 1982
pTlUCD2	*Triticum longissimum*	450	pUC18 (*Bam*HI)	Zhang and Dvořák 1992
pHch1.3	*Hordeum chilense*	430	pUC19 (*Bam*HI)	Hueros et al. 1993
pHch2	*Hordeum chilense*	2100	pUC19 (*Bam*HI)	Hueros et al. 1990
pHch3	*Hordeum chilense*	500	pUC19 (*Bam*HI)	Hueros et al. 1990
pHch4	*Hordeum chilense*	2600	pUC19 (*Bam*HI)	Hueros et al. 1990
pHch5	*Hordeum chilense*	2000	pUC19 (*Bam*HI)	Hueros et al. 1990
pHch93	*Hordeum chilense*	1800	pUC19 (*Bam*HI)	E. Ferrer, unpublished data
pHch950	*Hordeum chilense*	560	BSB +	Hueros et al. 1993
pHvUCD16	*Hordeum vulgare*	465	pUC19 (*Bam*HI)	J. Dvořák, unpublished data
pHvUCD19	*Hordeum vulgare*	503	pUC19 (*Bam*HI)	J. Dvořák, unpublished data
pHvUCD24	*Hordeum vulgare*	465	pUC19 (*Bam*HI)	J. Dvořák, unpublished data
pHvUCD32	*Hordeum vulgare*	361	pUC19 (*Bam*HI)	J. Dvořák, unpublished data
pHvUCD41	*Hordeum vulgare*	351	pUC19 (*Bam*HI)	J. Dvořák, unpublished data
pHvUCD49	*Hordeum vulgare*	586	pUC19 (*Bam*HI)	J. Dvořák, unpublished data
AKM60A4	*Hordeum vulgare*	424	BSIIKS + (*Bam*HI)	A. Kilian, unpublished data
AKM60A6	*Hordeum vulgare*	400	BSIIKS + (*Bam*HI)	A. Kilian, unpublished data
AKM60A9	*Hordeum vulgare*	347	BSIIKS + (*Bam*HI)	A. Kilian, unpublished data
AKM60A15	*Hordeum vulgare*	391	BSIIKS + (*Bam*HI)	A. Kilian, unpublished data
AKM60A16	*Hordeum vulgare*	314	BSIIKS + (*Bam*HI)	A. Kilian, unpublished data
pPjUCD3	*Psathyrostachys juncea*	370	pUC18 (*Bam*HI)	Zhang and Dvorak 1991
pPjUCD4	*Psathyrostachys juncea*	310	pUC18 (*Bam*HI)	Zhang and Dvorak 1991
pPjUCD5	*Psathyrostachys juncea*	240	pUC18 (*Bam*HI)	Zhang and Dvorak 1991
pPjUCD6	*Psathyrostachys juncea*	340	pUC18 (*Bam*HI)	Zhang and Dvorak 1991
pPjUCD8	*Psathyrostachys juncea*	450	pUC18 (*Bam*HI)	Zhang and Dvorak 1991
pPjUCD11	*Psathyrostachys juncea*	480	pUC18 (*Bam*HI)	Zhang and Dvorak 1991
pPjUCD12	*Psathyrostachys juncea*	445	pUC18 (*Bam*HI)	J. Dvořák, unpublished data
pPjUCD13	*Psathyrostachys juncea*	240	pUC18 (*Bam*HI)	J. Dvořák, unpublished data
pPjUCD14	*Psathyrostachys juncea*	600	pUC18 (*Bam*HI)	J. Dvořák, unpublished data
pLmUCD1	*Leymus multicaulis*	270	pUC18 (*Bam*HI)	Zhang and Dvorak 1991
pLeUCD1	*Lophopyrum elongatum*	230	pUC18 (*Bam*HI)	Zhang and Dvorak 1991

plus 0.5% SDS for 30 min, at 65°C. Inserted DNA fragments were amplified using PCR with M13/pUC sequencing primer (-20) 17-mer and M13/pUC reverse sequencing primer (-48) 24-mer (New England Biolabs, Beverly, Mass., U.S.A.). PCR products were purified with the Magic PCR purification kit (Promega, Madison, Wis., U.S.A.). The amount of probe hybridization in each lane of the autoradiographs was integrated with a Molecular Imager GS-250 (Bio-Rad) and then adjusted with the estimates of DNA loading that were calculated from the measures of ethidium bromide fluorescence in the gel.

Results

Identification of an H genome specific repeated nucleotide sequence

Thirty-two clones of repeated nucleotide sequences (RNSs) from different Triticeae species were screened for differential hybridization with the **H** genome (Table 2). Clone pHch2 (Fig. 1) produced a hybridization signal with all **H**-genome *Hordeum* species that was at least one order of magnitude greater than those observed for other *Hordeum* species (**I**, $\mathbf{X^a}$, and $\mathbf{X^u}$ genomes) or for the species of *Pseudoroegneria* ($\mathbf{S^t}$ genome), *Agropyron* (**P** genome), *Australopyrum* (**W** genome), *Thinopyrum* ($\mathbf{E^b}$ genome), or *Psathyrostachys* ($\mathbf{N^s}$ genome) (Table 1). The hybridization signal of pHch2 with *Lophopyrum elongatum* ($\mathbf{E^e}$ genome) was only threefold lower than that detected in the **H**-genome species.

The amount of probe hybridization in each lane in Fig. 1A was integrated with a Molecular Imager Analyzer (Fig. 1B) and adjusted by measures of DNA content that were estimated by ethidium bromide fluorescence in the gel. In Fig. 1B, corrected values are displayed as a histogram imposed over the curve. Hybridization signal was at least one order of magnitude greater in *Elymus* species with genome formulas (Lu 1993) that included the **H** genome ($\mathbf{S^tH}$, $\mathbf{S^tHH}$, and $\mathbf{S^tHY}$) than in those without it ($\mathbf{S^tY}$, $\mathbf{S^tS^tY}$, and $\mathbf{S^tPY}$) (Fig. 1A). The only exception was accession PI 276712 from the former U.S.S.R. that was identified as *Elymus trachycaulus* ssp. *violaceum*. Though this accession, PI 276712, had the expected chromosome number ($2n = 28$), the closely imbricate small spikelets and relatively large anthers (2.5 mm) indicated that it had been misclassified and it is likely a tetraploid *Agropyron* species. Inclusion of this accession in *Agropyron* was also supported by the similar hybridization patterns found for the tetraploid accession PI 276712 and the diploid *Agropyron cristatum*, when hybridized with clones pPjUCD11, pHch2 (Fig. 1, Nos. 52 and 24), and pLeUCD1 (data not shown). The two other *E. trachycaulus* subspecies included in this study (*E. trachycaulus* ssp. *trachycaulus* and *E. trachycaulus* ssp. *subsecundus*) showed the hybridization intensity expected for *Elymus* species with $\mathbf{S^tH}$ genomes (Fig. 1, Nos. 50 and 51).

A highly intense hybridization signal was detected for all South American tetraploids analyzed (Table 1), suggesting the presence of an **H** genome in all these species. The hybridization signal found in hexaploid species *E. patagonicus* and *E. scabriglumis* was more intense than those found in most tetraploid species and probably resulted from the double dose of **H** genome in these species (Fig. 1B, Nos. 53 and 54). Restriction fragments present in the hybridization profiles of all diploid **H** genome species (clones pHch93, pHch1.3, and AKM60A9; Table 1) were also present in the hybridization profiles of all South American tetraploid *Elymus* species and in the hexaploid species *E. patagonicus* and *E. scabriglumis*. These **H** genome specific restriction fragments had more intense hybridization signals in the hexaploid species (Table 1).

Genome analysis of *E. erianthus* and *E. mendocinus*

The **H** genome specific clone pHch2 showed no hybridization signal when hybridized with *E. erianthus* and *E. mendocinus* (Figs. 1A and 1B, Nos. 33 and 35). Restriction fragments absent in the hybridization profiles of diploids with the $\mathbf{N^s}$ genome, but present in the hybridization profiles of all diploid **H** genome species (clones pHch93, pHch1.3, and AKM60A9) or all diploid barley species (genomes **I**, $\mathbf{X^a}$, $\mathbf{X^u}$, and **H**; clones pTlUCD2 and pHvUCD32), were also absent in the hybridization profiles of *E. erianthus* and *E. mendocinus* (Table 1). These restriction fragments were present in all the $\mathbf{S^tH}$-, $\mathbf{S^tHH}$-, and $\mathbf{S^tYH}$-genome *Elymus* species analyzed (Table 1).

The hybridization profiles of *E. erianthus* and *E. mendocinus* differed not only from the *Elymus* species with the **H** genome ($\mathbf{S^tH}$, $\mathbf{S^tHH}$, and $\mathbf{S^tYH}$) but also from the *Elymus* species with the $\mathbf{S^tY}$ genome (Fig. 2). Clone pHvUCD16 from *Hordeum vulgare* revealed a strong hybridization signal in the hybridization profiles of all the diploid *Hordeum* species and also in the hybridization profiles of the $\mathbf{S^tH}$- and $\mathbf{S^tY}$-genome *Elymus* species analyzed (Fig. 2). This clone showed a relatively lower hybridization signal with the $\mathbf{S^t}$- and **P**-genome diploids but no signal at all with *E. erianthus*, *E. mendocinus* (Fig. 2, Nos. 33 and 35), *Psathyrostachys juncea*, and the three *Leymus* species analyzed (Table 1, Nos. 28–32). Clone pLeUCD1 from *L. elongatum* showed a very strong hybridization signal with DNAs from *Lophopyrum* ($\mathbf{E^e}$ genome) and *Thinopyrum* ($\mathbf{E^b}$ genome) (data not shown) and a lower hybridization signal with DNAs from *Pseudoroegneria* ($\mathbf{S^t}$ genome), *Agropyron* (**P** genome), and all the *Elymus* species analyzed (Fig. 2). The hybridization signal of clone pLeUCD1 with DNAs from *E. erianthus*, *E. mendocinus* (Fig. 2, Nos. 33 and 35), *P. juncea*, and *Leymus* (data not shown) was either zero (Fig. 2) or one order of magnitude lower than that observed with DNAs from the *Elymus* species when autoradiographs were overexposed (data not shown).

RNSs cloned from *Psathyrostachys* or *Leymus* that hybridized with $\mathbf{N^s}$ genome specific bands (marker bands) (Zhang and Dvořák 1991) revealed a complementary hybridization pattern to that previously described for the *Hordeum* genome specific marker bands (Fig. 3). Hybridization with clone pPjUCD5 (Fig. 3A) revealed a ladder pattern typical of a tandem repeated sequence family with a monomeric unit of 165 bp. This characteristic ladder was observed only in the hybridization profiles of *P. juncea*, *Leymus*, *E. erianthus*, and *E. mendocinus* (Fig. 3A, Nos. 28–35). Almost no hybridization signal was detected in *Elymus* ($\mathbf{S^tPY}$-, $\mathbf{S^tYH}$-, $\mathbf{S^tY}$-, or $\mathbf{S^tH}$-genome combinations) or in the other diploid Triticeae analyzed (Fig. 3A; Table 1). Clones pPjUCD6 and pLmUCD1 did not show ladder patterns, suggesting interspersion of the copies of the repeated nucleotide family. Both clones revealed marker bands for the $\mathbf{N^s}$ genome that were present in the hybridization profiles of *Leymus*, *E. erianthus*, and *E. mendocinus* but absent from *Elymus* or other diploid Triticeae analyzed (Figs. 3B and 3C; Table 1). Clone pPjUCD6 revealed bands that were present in all three

Fig. 1. (A) Hybridization of *Hae*III-digested DNAs with pHch2. DNA loading per lane was adjusted by ploidy level (diploids, 2 μg; tetraploids, 4 μg; hexaploids, 6 μg; octoploid, 8 μg). (B) Integration of the amount of probe hybridization per lane in A. Rectangles correspond to the integrated values further adjusted for variations in DNA loading independent of ploidy level. Numbers correspond to accession identification as given in Table 1 and capital letters to genome formulas (*eri*, *E. erianthus*; *men*, *E. mendocinus*).

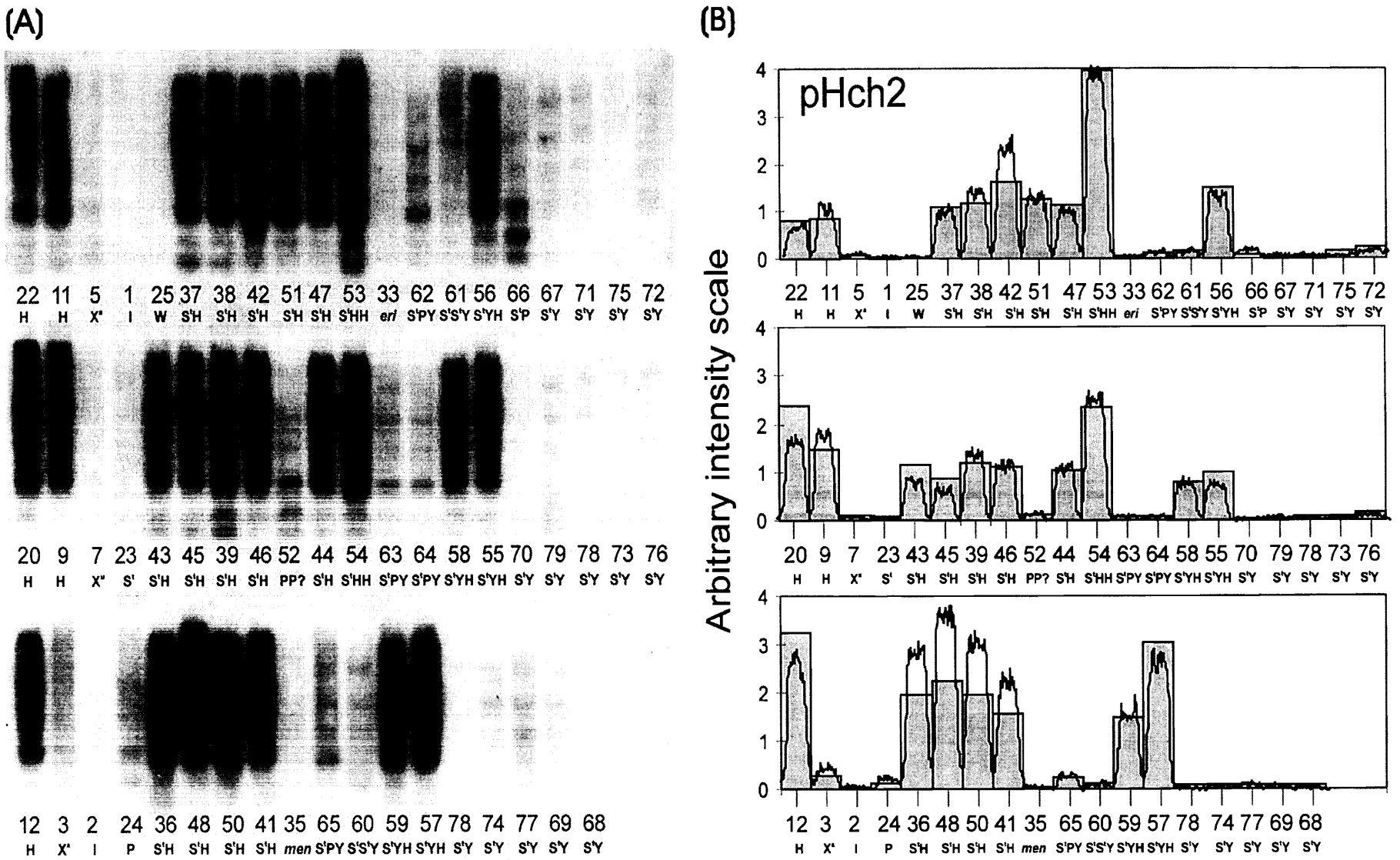
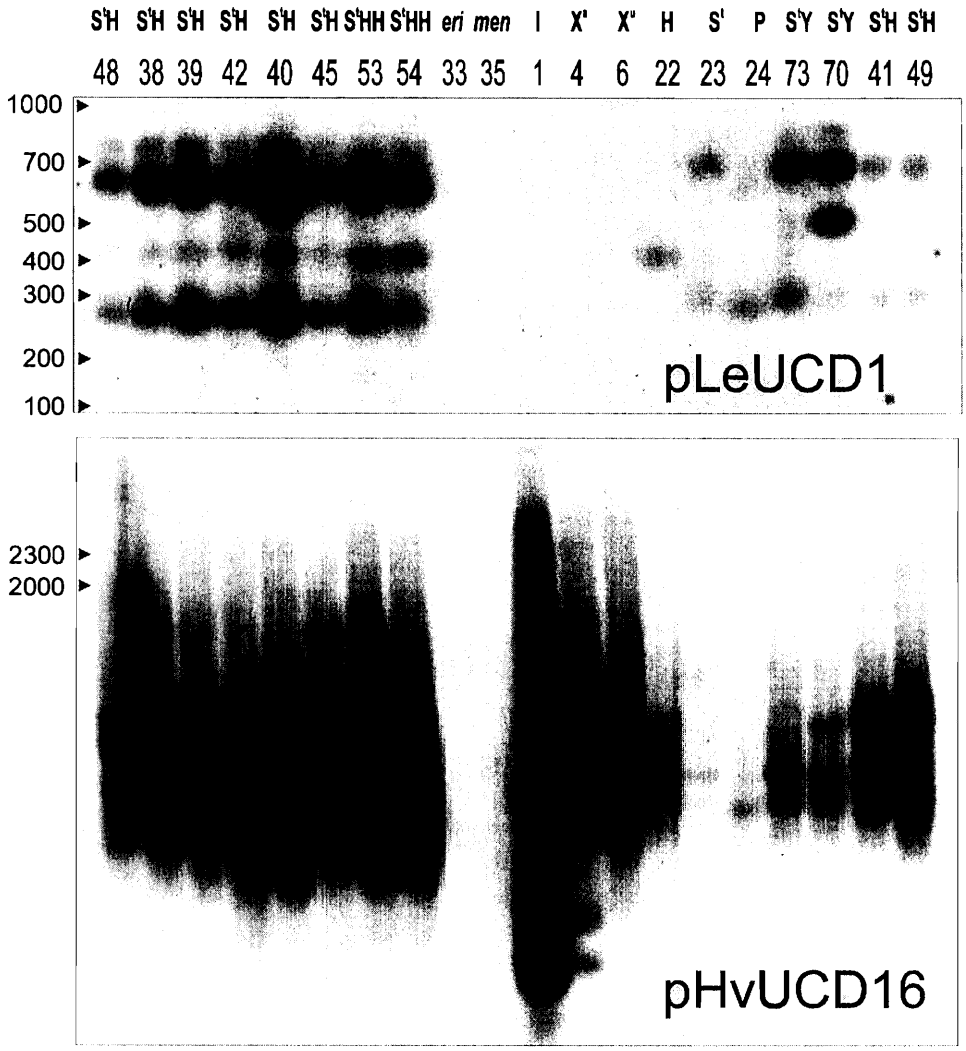


Fig. 2. Hybridization of *Hae*III-digested DNAs with RNSs pHvUCD16 (A) and pLeUCD1 (B). DNA loading per lane was adjusted by ploidy level (diploids, 2 μg; tetraploids, 4 μg; hexaploids, 6 μg; octoploid, 8 μg). Numbers correspond to accession identification as given in Table 1 and capital letters to genome formulas (*eri*, *E. erianthus*; *men*, *E. mendocinus*). Molecular marker size (at the left) is given in basepairs.



Leymus species, *E. erianthus*, and *E. mendocinus* (Fig. 3B, Nos. 30–35) but absent in *P. juncea* (Fig. 3B, Nos. 28 and 29) and all other species analyzed. These bands were also present in *Leymus racemosus* (= *giganteus*) (Lam.) Tzvelev (D2949) and absent in *Psathyrostachys fragilis* (Boiss.) Nevski (PI 401397) (H.-B. Zhang, personal communication).

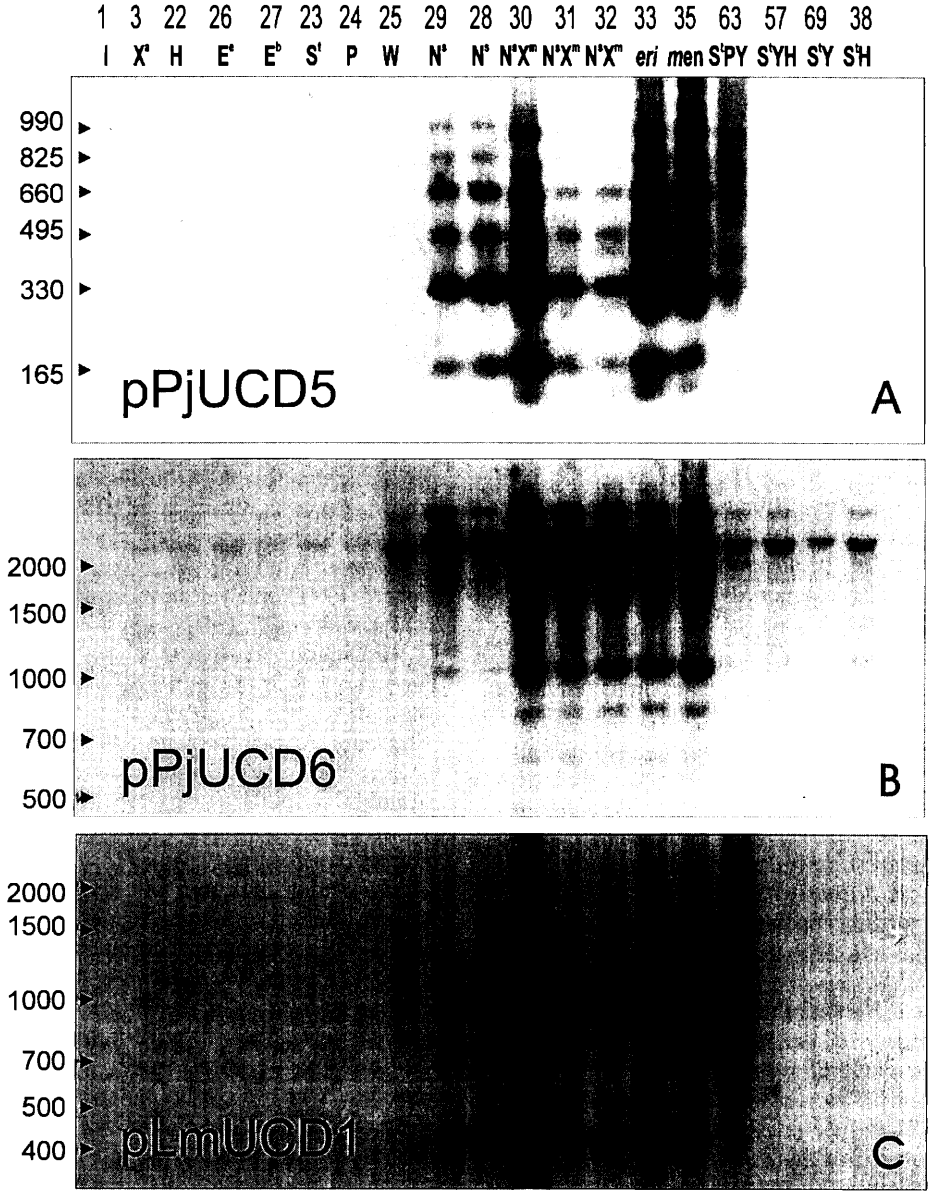
In spite of the similar hybridization patterns in *E. erianthus* and *E. mendocinus* revealed by many $\mathbf{N}^{s}$ genome specific clones (Fig. 3, Nos. 33 and 35), numerous differences were observed when DNAs from these two species were hybridized with clones pTa250.15, pTlUCD2, pHch1.3, pHch950, pHVUCD41, AKM60A4, pPjUCD4, and pPjUCD13, indicating a large differentiation between some of the genomes of these two polyploid species. Differences among the *Leymus* accessions analyzed were detected with clones pLmUCD1, pPjUCD4, pPjUCD8, and pTa71. Restriction fragments present in all three *Leymus* species analyzed but absent in *E. erianthus*, *E. mendocinus*, and *P. juncea* were detected by hybridization with clones pHvUCD32 and pHch93 (data not shown).

Restriction site variation in the rDNA repeat unit

Restriction maps of the rDNA repeat unit were constructed using restriction enzymes *Eco*RI, *Bam*HI, and *Sst*I (single-enzyme digests), and *Eco*RI–*Sst*I and *Eco*RI–*Bam*HI (double digests) (Fig. 4). All accessions of *Psathyrostachys*, *Leymus*, and *E. erianthus* analyzed exhibit a second *Eco*RI site located in the IGS at 2.7, 2.55, and 2.4 kb from the 18S *Sst*I site, respectively (Fig. 4). No additional *Eco*RI site was found in *E. mendocinus*, other diploid Triticeae species, or any of the *Elymus* species analyzed, with the exception of *Elymus cordilleranus*. However, in the last species the additional *Eco*RI restriction site is approximately 600 bp closer to the 18S *Sst*I site (Fig. 4).

Additional *Sst*I restriction sites were found in *Australopyrum retrofractum* and *Leymus alaicus* ssp. *karataviensis*,

Fig. 3. (A) *Taq*I-digested DNAs hybridized with clone pPjUCD5. (B) *Hae*III-digested DNAs hybridized with clone pPjUCD6.(C) *Taq*I-digested DNAs hybridized with clone pLmUCD1. DNA loading per lane was adjusted by ploidy level (diploids, 2 μg; tetraploids, 4 μg; hexaploids, 6 μg; octoploid, 8 μg), but accessions 30 and 63 were overloaded in A and C. Numbers correspond to accession identification as given in Table 1 and capital letters to genome formulas (*eri*, *E. erianthus*; *men*, *E. mendocinus*). Molecular marker size (at the left) is given in basepairs.



but in different regions of the repetitive unit (Fig. 4). An additional IGS *Bam*HI site was found in *P. juncea* (Fig. 4).

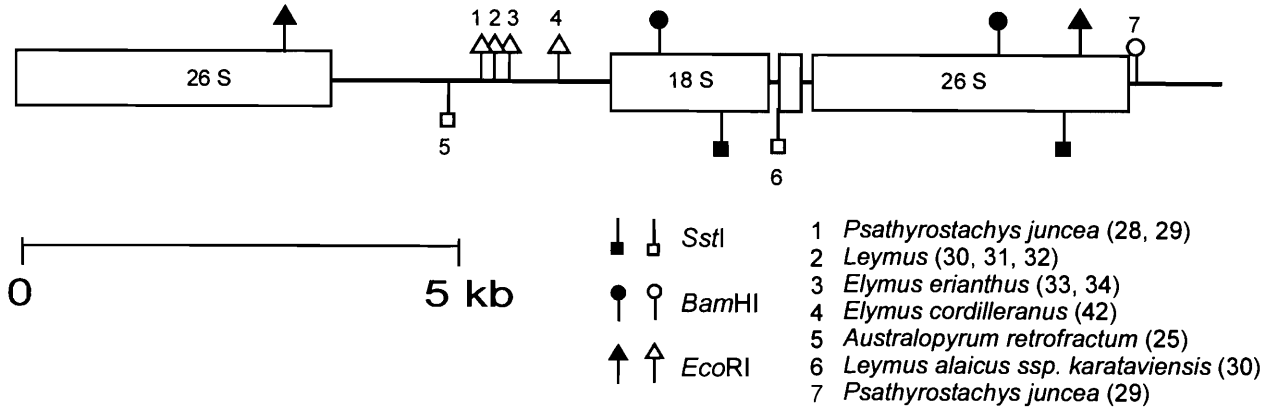
Discussion

Genome specific RNSs

At the level of entire families, variation in the RNSs assayed by Southern blot hybridization is remarkably low within species and is a powerful tool for scrutinizing the origin of allopolyploid taxa (Dvořák and Dubcovsky 1996). Homogeneity within species is maintained through homogenization and recombination of RNS families (Dover 1982; Strachan et al. 1985).

At the interspecific level, divergence and amplification or deletions of sequences eventually result in an emergence of RNS families that can be detected by hybridization in only a single species or a group of closely related species (Prosnyak et al. 1985; Rayburn and Gill 1986; Dvořák et al. 1988; Zhao et al. 1989; Hueros et al. 1990; Zhang and Dvořák 1990; Crowhurst and Gardner 1991; Iwabuchi et al. 1991; Raz et al. 1991; Talbert et al. 1991; Baldauf et al. 1992; Zentgraf et al. 1992; Talbert et al. 1993). Since numerous species can be analyzed simultaneously in the same Southern blot (Fig. 1), these genome specific RNS families can be very useful for screening large germplasm collections. *Elymus* species with genome formulas not including the **H** genome are not always easy to

Fig. 4. Restriction map of the repeated unit of the rDNA. Restriction sites indicated with black symbols are constant and those indicated with white symbols are variable within the set of species analyzed. Numbers after the species correspond to accession identification as given in Table 1.



differentiate morphologically from *Elymus* species with the **H** genome (Salomon and Lu 1992). The **H** genome specific RNS clone pHch2 is a new tool for differentiating these groups. Hybridization with clone pHch2 detected the *Agropyron* accession PI 276712 that had been erroneously labeled at the germplasm collection as *E. trachycaulus* ssp. *violaceus*.

South American *Elymus*

All South American tetraploid *Elymus* species analyzed using pHch2 showed a strong hybridization signal indicating the presence of an **H** genome. This result is in accordance with those previously obtained by chromosome pairing in interspecific hybrids, including *Elymus agropyroides*, *Elymus tilcarensis*, and *E. magellanicus* (Dewey 1970*b*, 1977; Jensen 1993). Particularly interesting is the demonstration that *E. breviaristatus* ssp. *scabrifolius* has an **H** genome, as this species shows very low chromosome pairing in interspecific hybrids with other South American tetraploid species (Hunziker and Ferrari 1986).

RNSs that hybridize differentially to single genomes, such as pHch2, are not easy to isolate. Fortunately, phylogenetic information can also be obtained from the interspecific variation in the hybridization patterns of RNSs that readily cross-hybridize among different species. Restriction fragments in the Southern hybridization profiles that are unique for a diploid species or group of species, designated marker bands, can also be used to investigate the origin of polyploid taxa (Talbert et al. 1991; Zhang and Dvořák 1991, 1992; Zhang et al. 1992; Dvořák et al. 1993; Dubcovsky and Dvořák 1994, 1995; Dvořák and Dubcovsky 1996).

The **H** genome in *Hordeum* represents a phylogenetic lineage differentiated from the **I**, $\mathbf{X^a}$, and $\mathbf{X^u}$ genomes and, consequently, shared marker bands among **H**-genome *Hordeum* species were readily found in this study and by other authors (Bothmer et al. 1986; Svitashev et al. 1994; Vershinin et al. 1994). The presence of these marker bands in South American tetraploid *Elymus* species further confirmed the presence of the **H** genome in these species. Hexaploids *E. patagonicus* and *E. scabriglumis* generally showed stronger hybridization intensity than $\mathbf{S^tH}$ tetraploids when hybridized with clone pHch2 (Fig. 1, Nos. 53 and 54). Stronger hybridization intensity was also found in the **H** genome specific marker bands. These results confirm the $\mathbf{S^tHH}$-genome formula previously assigned to these species by studies of chromosome pairing in interspecific hybrids (Hunziker 1953, 1966; Dewey 1972*c*; Seberg 1991) and variation in the *Bam*HI sites of the rDNA (Dubcovsky et al. 1992).

Restriction fragments characteristic of the **H**-genome *Hordeum* species from the New World but absent in the three diploid species from the Old World (*Hordeum bogdanii*, *Hordeum roshevitzii*, and *Hordeum brevisubulatum*) were detected by hybridization with clones pHch3 (*Hae*III), pHch950 (*Hae*III), and pHch1.3 (*Alu*I, but different from pHch1.3 *Hae*III; Table 1). Differentiation of the New World **H** genome *Hordeum* species from the Old World diploid species was also indicated by meiotic analysis of interspecific hybrids within and between these regions (Bothmer et al. 1991). These restriction fragments specific to the American **H** genome species were also found in all the South American *Elymus* species with the **H** genome, with the exception of *E. tilcarensis*. These restriction fragments were not detected in the few *Elymus* species with $\mathbf{S^tH}$ or $\mathbf{S^tHY}$ genomes from Europe, Asia, or North America that were analyzed. It is tempting to speculate that the presence of American **H** genome specific bands in a large group of South American tetraploid *Elymus* species indicates an independent origin for this group. However, at present there is insufficient data to confirm the polyphyletic origin of *Elymus*.

South American *Leymus*

Hexaploid *E. erianthus* and octoploid *E. mendocinus* differ from all other *Elymus* species in the hybridization patterns detected with clone pHch2. The RNS family detected with pHch2 is strongly amplified in the **H** genome but seems to be absent, or present in a very low copy number, in *E. erianthus* and *E. mendocinus*. Restriction fragments present in all barley species or in all **H**-genome *Hordeum* species are also present in *Elymus* species with $\mathbf{S^tH}$, $\mathbf{S^tHH}$, or $\mathbf{S^tHY}$ genomes, but are absent in *E. erianthus* and *E. mendocinus*. These results, together with failure of the chromosome N-banding technique

to produce bands in *E. erianthus* (Seberg and Linde-Laursen 1996) and the lack of hybridization with a GAA satellite abundant in the **H** genome (Pedersen et al. 1996), confirm the absence of the **H** genome in *E. erianthus*.

Differences between *E. erianthus* and the other South American *Elymus* species were also suggested by their particular morphological characteristics (Nicora 1978; Seberg and Linde-Laursen 1996), unique restriction sites in the rDNA repeat unit (Dubcovsky et al. 1992), and sequence variation in the chloroplast gene *rbc*L (Seberg and Linde-Laursen 1996). Both *E. erianthus* and *E. mendocinus* also differ from the South American *Elymus* species in their karyotype parameters (Lewis et al. 1996). Values of interchromosomal asymmetry in the karyotype of *E. erianthus* and *E. mendocinus* were significantly higher than in the karyotypes of other South American *Elymus* species. This suggests relatively large differences in chromosome size between the genomes of the progenitors of these two species. Although all these differences clearly segregate *E. erianthus* and *E. mendocinus* from the $\mathbf{S^tH}$- and $\mathbf{S^tHH}$-genome South American *Elymus* species, they do not rule out a relationship between *E. erianthus* and *E. mendocinus* and the Asiatic *Elymus* species that do not have an **H** genome ($\mathbf{S^tPY}$, $\mathbf{S^tS^tY}$, or $\mathbf{S^tY}$). Hybridization with clone pLeUCD1 differentiates *E. erianthus* and *E. mendocinus* from the $\mathbf{S^tPY}$-, $\mathbf{S^tS^tY}$-, or $\mathbf{S^tY}$-genome Asiatic *Elymus* species and suggests the absence of the $\mathbf{S^t}$ genome from these two South American polyploids.

Hybridization with RNSs specific for the $\mathbf{N^s}$ genome of *Psathyrostachys* also differentiates *E. erianthus* and *E. mendocinus* from Asiatic *Elymus* species with the $\mathbf{S^tPY}$, $\mathbf{S^tS^tY}$, or $\mathbf{S^tY}$ genomes. All the RNSs that hybridized with $\mathbf{N^s}$ genome specific restriction fragments (Zhang and Dvořák 1991) also hybridized with *E. erianthus* and *E. mendocinus*, but showed no hybridization signal with any of the other *Elymus* species analyzed. An $\mathbf{N^s}$ genome in *E. erianthus* was also suggested by the presence of a second *Eco*RI restriction site in the IGS of all repeating units of the rDNA genes from *E. erianthus*, *P. juncea*, and the three *Leymus* species analyzed. Orgaard and Heslop-Harrison (1994) have also reported the presence of a second *Eco*RI site in the IGS of all the repeated units in *P. stoloniformis*. This additional site is absent in *E. mendocinus* and in some of the repeated units of the dodecaploid *Leymus angustus* (Trin.) Pilg. (Orgaard and Heslop-Harrison 1994). Almost all other Triticeae species analyzed in this study and by Orgaard and Heslop Harrison (1994) have a single *Eco*RI restriction site in the 26S rDNA. The only exception is *E. cordilleranus* (= *Elymus attenuatus*), which has an IGS *Eco*RI site but at a shorter distance from the 18S *Sst*I restriction site (Fig. 4).

The absence of the **H** and $\mathbf{S^t}$ genomes and the presence of the $\mathbf{N^s}$ genome in *E. erianthus* and *E. mendocinus* clearly indicate that these two species do not belong to the genus *Elymus* and suggest their inclusion in the genus *Leymus*. The presence of an $\mathbf{N^s}$ genome in polyploid species of *Leymus* has been repeatedly demonstrated by meiotic analysis in interspecific hybrids (Dewey 1970*a*, 1972*a*, 1972*b*, 1976; Wang and Hsiao 1984; Sun et al. 1995) and by variation in RNSs (Zhang and Dvořák 1991; Wang and Jensen 1994; Mason-Gamer and Kellog 1996). Though the presence of an $\mathbf{N^s}$ genome in *Leymus* is well established, the identification of the other genome in tetraploid *Leymus* is a matter of controversy. Löve (1984) suggested, on morphological grounds, the presence of an $\mathbf{E^b}$ genome in tetraploid *Leymus* species. However, recent studies based on RNSs have shown the absence of the $\mathbf{E^b}$ genome from this genus (Zhang and Dvořák 1991; Wang and Jensen 1994). Based on the hybridization intensity of $\mathbf{N^s}$-genome marker bands, Zhang and Dvořák (1991) suggested that the second pair of *Leymus* genomes was also from *Psathyrostachys*, and designated their genomes $\mathbf{N^s{}_1N^s{}_1N^s{}_2N^s{}_2}$. Other authors preferred to designate *Leymus* genomes $\mathbf{N^sX^m}$, based on meiotic analysis of triploid hybrids (Wang and Jensen 1994; Sun et al. 1995; Wang et al. 1994).

A close relationship between *E. erianthus* and *E. mendocinus* with *Leymus* was further indicated by the presence of marker bands in all three *Leymus* species, *E. erianthus*, and *E. mendocinus* that were absent in *P. juncea* and all other species analyzed. Heterogeneity within *Leymus* species in the observed restriction patterns was evident even in the few species analyzed here. Numerous differences were also observed between *E. erianthus* and *E. mendocinus*, suggesting a large genome differentiation within *Leymus* species.

Seberg and Linde-Laursen (1996) pointed out that the satellite (SAT) chromosome types of *E. erianthus* are similar to those present in species of the genus *Psathyrostachys* and *Leymus*. However, they found differences between *E. erianthus* and *Psathyrostachys* in the distribution of the C-bands. Results from the same authors on sequence variation in the chloroplast gene *rbc*L did not exclude the possibility that *E. erianthus* could be related to *Leymus* (Seberg and Linde-Laursen 1996).

Taxonomic treatment

Leymus is a segregate genus of the Triticeae that can be recognized morphologically and genomically and is now accepted by most European, Russian, Chinese, and American taxonomists (Pilger 1954; Keng 1965; Tzvelev 1976; Melderis 1980; Barkworth and Atkins 1984; Dewey 1984; Löve 1984). *Elymus erianthus* and *E. mendocinus* share with *Leymus* not only similar genomes, as revealed by hybridization patterns with RNSs, but also ecological and morphological characteristics. Both South American species are found in dry habitats (stony slopes and dry steppes) similar to those inhabited by many Northern Hemisphere *Leymus* species (Barkworth and Atkins 1984; Dewey 1984). *Elymus erianthus* and *E. mendocinus* share with *Leymus* species their stiff and glaucous leaf blades, narrow 1–3 nerved glumes, shortly awned lemmas, and long anthers (*E. erianthus*: 3–4.5 mm; *E. mendocinus*: 5–6 mm) (Parodi 1940; Barkworth and Atkins 1984; Barkworth and Dewey 1985; Clayton and Renvoize 1986). Both South American species differ from most species of *Leymus* in that they lack long rhizomes. However, long rhizomes are also absent in North American *Leymus* species that grow in rocky habitats, for example, *Leymus ambiguus* (Scrib. & Merr.) D.R. Dewey and *Leymus salinus* (M.E. Jones) Á. Löve (Hitchcock 1951; Barkworth and Atkins 1984).

Similar ecological and morphological characteristics combined with similar hybridization patterns with RNSs in *Psathyrostachys*, *Leymus*, and *E. erianthus* suggest that the creation of the new monotypic genus *Eremium* (Seberg and Linde-Laursen 1996) is not justified. Therefore, we transfer *E. erianthus* and *E. mendocinus* to the genus *Leymus*.

Leymus erianthus (Phil.) Dubcovsky comb.nov.
Basionym: *Elymus erianthus* Phil., An. Mus. Nac. Santiago Chile. 2 Secc. Bot. 13. 1892. TYPE: Argentina, Mendoza, Las Heras, in andibus uspallatensis ad thermas Baños del Inca, invenit 1866, A. Borchers (clastotypus, BAA!).
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Cryptochloris spathacea auct. non Benth. 1882; Speg., Rev. Fac. Agron. Vet. La Plata, 3: 584. 1897.
Elymus spegazzinii Kurtz, Bol. Acad. Nac. Cienc. (Argent.), 16: 259.1899. *Nomen nudum.*
Elymus erianthus Phil. *var. aristatus* Hicken, Physis (Buenos Aires), 2: 41. 1915.
Elymus erianthus Phil. *var. spegazzinii* Kurtz ex Hauman, An. Mus. Nac. Hist. Nat. Buenos Aires, 29: 410. 1917.
Eremium erianthus (Phil.) Seberg and Linde-Laursen, Syst. Bot. 21: 1996

Leymus mendocinus (Parodi) Dubcovsky comb.nov.
Basionym: *Agropyron mendocinus* Parodi, Rev. Mus. La Plata Secc. Bot. 3:14. 1940. TYPE: Argentina, Mendoza, San Rafael, Pampa del Plateado, Nevado hill, altitude 2500 m., T. et B. Mácola, n° P.8, 17/1/1926 (isotype SI!)
Elymus mendocinus (Parodi) Á. Löve, Feddes Repert. 95(7–8): 471.1984.
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