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Ubiquity of the *St* chloroplast genome in *St*-containing Triticeae polyploids

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Abstract: Interspecific hybridization occurs between Triticeae species in the grass family (Poaceae) giving rise to allopolyploid species. To examine bias in cytoplasmic DNA inheritance in these hybridizations, the sequence of the 3' end of the chloroplast *ndhF* gene was compared among 29 allopolyploid Triticeae species containing the *St* nuclear genome in combination with the *H*, *I*, *Ns*, *P*, *W*, *Y*, and *Xm* nuclear genomes. These *ndhF* sequences were also compared with those from diploid or allotetraploid Triticeae species having the *H*, *I*, *Ns*, *P*, *W*, *St*, and *Xm* genomes. The cpDNA sequences were highly similar among diploid, allotetraploid, allohexaploid, and allooctoploid Triticeae accessions containing the *St* nuclear genome, with 0–6-nucleotide (nt) substitutions (0–0.8%) occurring between pairs of species. Neighbor-joining analysis of the sequences showed that the *ndhF* DNA sequences from species containing the *St* nuclear genome formed a strongly supported clade. The data indicated a strong preference for cpDNA inheritance from the *St* nuclear genome-containing parent in hybridizations between Triticeae species. This preference was independent of the presence of the *H*, *I*, *Ns*, *P*, *W*, and *Xm* nuclear genomes, the geographic distribution of the species, and the mode of reproduction. The data suggests that hybridizations having the *St*-containing parent as the female may be more successful.

Key words: interspecies hybridization, cytoplasmic inheritance.

Résumé : Chez les graminées, l'hybridation interspécifique survient entre espèces de hordées et donne naissance à des espèces allopolyploïdes. Afin d'examiner le biais dans la transmission de l'ADN cytoplasmique lors de ces hybridations, la séquence de l'extrémité 3' du gène chloroplastique *ndhF* a été comparée chez 29 espèces de hordées allopolyploïdes contenant le génome nucléaire *St* combiné à des génomes nucléaires *H*, *I*, *Ns*, *P*, *W*, *Y* ou *Xm*. Ces séquences *ndhF* ont également été comparées avec celles provenant d'espèces diploïdes ou tétraploïdes possédant les génomes *H*, *I*, *Ns*, *P*, *W*, *St* ou *Xm*. Les séquences d'ADNcp étaient très semblables entre paires d'espèces (0–6 substitutions, soit 0,8 %) parmi les accessions diploïdes, allotétraploïdes, allohexaploïdes et allooctaploïdes contenant le génome nucléaire *St*. Une analyse « neighbor-joining » des séquences a montré que les séquences *ndhF* provenant d'espèces contenant le génome *St* formaient un clade fortement supporté. Les données indiquent une forte préférence pour la transmission de l'ADNcp provenant du parent contribuant le génome nucléaire *St* lors d'hybridations entre espèces de hordées. Cette préférence était inchangée en présence des génomes nucléaires *H*, *I*, *Ns*, *P*, *W* ou *Xm* et s'est avérée indépendante de la distribution géographique des espèces et du mode de reproduction. Les données suggèrent que les hybridations ayant eu le parent à génome *St* comme parent femelle pourraient avoir eu plus de succès.

Mots clés : hybridation interspécifique, hérédité cytoplasmique.

[Traduit par la Rédaction]

Introduction

The Triticeae tribe of the Poaceae includes many of the economically important grain crops, including wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). The group also includes many important temperate forage grass species. Interspecific hybridization is common under natural and artificial conditions in the Triticeae. These events

frequently increase ploidy levels, and the results of hybridizations between Triticeae species can be used for classification in the Triticeae (Dewey 1984). More than 30 cytologically distinct genomes have been identified among the Triticeae, and hybridization between species with distinct genomes gave rise to many common Triticeae grasses (Wang et al. 1994). The most familiar of these is wheat (*T. aestivum*), an allohexaploid grass containing the A, B,

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and D genomes (Dvorák et al. 1993). Plant breeders are attempting to use the ease of interspecific hybridization within the Triticeae to create improved germplasm for revegetation or land restoration efforts. In addition, breeders and geneticists introgress genetic material from noncultivated Triticeae into cultivated species such as wheat, particularly to impart disease resistance (Hoisington et al. 1999). For example, the *Lr19* locus of *Thinopyrum ponticum* (Podp.) Barkw. and D.R. Dewey was incorporated into wheat after hybridization and backcrossing to increase rust resistance in wheat (McIntosh et al. 1998).

Making and analyzing interspecific crosses is a tedious and labor-intensive process, and procedures to ease development of these materials would be useful. In particular, researchers have noticed that crosses made with one of the species as the female parent may be more successful due to differential pre- or post-zygotic barriers to hybridization in the parents (Riesberg and Carney 1998).

Both nuclear and cpDNA sequences have been used to investigate species relationships among the Triticeae (Catalán et al. 1997; Clark et al. 1995; Hsaio et al. 1994, 1995; Kellogg et al. 1996; Mason-Gamer and Kellogg 1996a, 1996b). These studies focused primarily on diploid species, because allopolyploidy complicates molecular analysis of species relationships (Kellogg et al. 1996; Mason-Gamer and Kellogg 1996b). After accounting for poorly supported branches and differential histories for parts of the *T. monococcum* L. and *Aegilops tauschii* Coss. genomes, Kellogg and co-workers (1996) determined that data for three nuclear genes produced congruent trees. Trees based on cpDNA were significantly different than those based on nuclear data. They postulated that the differences may reflect different evolutionary histories for the nuclear and chloroplast genomes among Triticeae.

Although these studies indicate that care must be taken when interpreting phylogenies generated from cpDNA among closely related taxa, comparison of cpDNA sequences may be useful in identifying the female parent in interspecies hybridization (Mason-Gamer and Kellogg 1996a). Indeed, cpDNA sequence analysis was used in two studies to determine origin of the cpDNA in hybridizations between *Elymus* and *Leymus* (Jensen et al. 1999; Jones et al. 2000). Sequences for the chloroplast *ndhF* gene of allotetraploid *Elymus* (**StH**) and allooctoploid *Pascopyrum* (**StHNSXm**) were more closely related to those of *Pseudoroegneria* (**St**) than to those from Triticeae containing **H**, **Ns**, or **Ns** plus **Xm** nuclear genomes (Jones et al. 2000). In addition, allooctoploid grasses arising from recent naturally occurring hybridizations between *E. elymoides* (**StH**) and *L. salinus* (**NsXm**) all had *ndhF* gene sequences identical with those obtained from *Elymus* plants from the same locale, and were significantly different from *Leymus* plants collected at the same site (Jensen et al. 1999). These limited observations suggest the broader hypothesis that cpDNA inheritance among Triticeae is biased if one of the parents in interspecies hybridization contains the **St** nuclear genome. To test the hypothesis, we selected 31 *Elymus*, *Pascopyrum*, and *Pseudoroegneria* species that contained the **St** nuclear genome alone (*Pseudoroegneria spicata* and *P. strigosa*) or in allopolyploid combination with the **H**, **I**, **Ns**, **P**, **W**, **Xm**, or **Y** nuclear genomes. We compared cpDNA sequences

from these species with those of 7 diploid and allotetraploid non-**St** Triticeae and 3 poaceous outgroups.

Materials and methods

Plant materials

Seeds were obtained from the Western Regional Plant Introduction Center or the collection of Dr. K.B. Jensen (USDA-ARS, Forage and Range Research Laboratory, Logan, Utah) (Table 1). Seeds were germinated on absorbent paper in Petri dishes. Germinated seeds were transplanted to a sand-peat mixture, then maintained in a greenhouse.

DNA isolation and amplification

Two plants were analyzed independently for each accession. Leaves were frozen in liquid nitrogen, and total DNA was immediately isolated using a modification of the CTAB (cetyltrimethylammonium bromide) extraction method (Lassner et al. 1989; Williams et al. 1993). The 3' end of the chloroplast *ndhF* gene was amplified from 40 ng total DNA using the 1318F and 2110R primers (Olmstead and Sweere 1994) according to the protocol of Jones et al. (2000). Amplified DNAs were purified with Promega Wizard™ PCR Preps DNA Purification System, and DNA concentrations were determined prior to sequence analysis using a Hoefer TKO 100 mini-fluorometer and Hoescht 33258 according to the manufacturer's instructions (Hoefer, San Francisco, Calif.).

DNA sequence analysis

DNA sequence analysis was performed at the Utah State University Biotechnology Center's sequencing facility on an ABI 373 autosequencer using AmpliTaq or AmpliTaq FS polymerase and dye-terminators according to the manufacturer's protocols. Both strands of each DNA fragment were sequenced using the 1318F and 2110R and 3 additional primers as described by Jones et al. (2000). Previously determined sequences were included in the analyses as indicated (Table 2). DNA sequences were aligned using the Lineup and Pileup functions of the Wisconsin Sequence Analysis Package, v. 8 (Genetics Computer Group, Madison, Wis.). Sequence statistics, bootstrap values, and phylogenetic analyses were carried out using MEGA (Kumar et al. 1993).

Results and discussion

To investigate interspecies hybridization patterns among allopolyploid Triticeae grasses that contain the **St** genome, the DNA sequence of the 3' end of the chloroplast *ndhF* gene was determined for 31 diploid, allotetraploid, allohexaploid, and allooctoploid Triticeae species (Table 1). Also, previously determined sequences for 9 additional Triticeae species (Gaut et al. 1997; Jones et al. 2000) were used in the comparisons (Table 2). Species chosen for this study contained the **St** nuclear genome in allopolyploid combinations with the **H**, **Ns**, **P**, **W**, **Xm**, and **Y** nuclear genomes. The accessions were distributed pancontinentally, and were a mixture of autogamous, allogamous, and apomictic species. Diploid species containing the **H** (*Hordeum bogdanii*, *H. violaceum*), **I** (*H. vulgare*), **Ns** (*Psathyrostachys juncea*), **P** (*Agropyron mongolicum*), **St** (*Pseudoroegneria spicata* and *P. strigosa*), and **W** (*Australopyrum retrofractum*) nuclear genomes were also selected. No diploid species containing **Y** has been identified (Wang et al. 1994). Similarly, a diploid species having the **Xm** genome has not been identified. However, the characteristics of the *ndhF* gene associated with the **Xm**

Table 1. Species for which the DNA sequence of the *ndhF* gene was determined.

PI No.	Species	Nuc. gen.	RM	Source	Origin	Set No.	GenBank Acc. No.
PI 531543	<i>Agropyron mongolicum</i> Keng.	P		WRPIS	Nei Mongolia, China		AF267661
PI 547363	<i>Australopyrum retrofractum</i> (Vickery) A. Löve	W		WRPIS	Cowangerong, N.S.W., Aust.		AF267662
PI 565001	<i>Elymus alatavicus</i> (Drobow) A. Löve	StYP	⊗	WRPIS	Lake Issyk Kul, Kazakhstan	U	AF267664
PI 565002	<i>Elymus batalinii</i> (Krasn.) A. Löve	StYP	⊗	WRPIS	Lake Issyk Kul, Kazakhstan	1	AF267665
PI 564915	<i>Elymus caninus</i> (L.) L.	StH	⊗	WRPIS	Gorno Altay A.O., Russian Fed.	1	AF267666
PI 531573	<i>Elymus caucasicus</i> (K. Koch) Tzvelev	StY	⊗	WRPIS	Dilidjan, Armenia	U	AF267667
PI 564917	<i>Elymus ciliaris</i> (Trin.) Tzvelev	StY	⊗	WRPIS	Vladivostock, Russian Fed.	1	AF267668
PI 574531	<i>Elymus dahuricus</i> Turcz. ex Griseb.	StHY	⊗	WRPIS	China	1	AF267669
PI 531606	<i>Elymus elymoides</i> (Raf.) Swezey	StH	⊗	WRPIS	Central Ferry, Wash. U.S.A.	1	AF267670
PI 564934	<i>Elymus glaucissimus</i> (Popov) Tzvelev	StStY	⊗	WRPIS	Alma Ata, Kazakhstan	2	AF267671
PI 504456	<i>Elymus grandiglumis</i> (Keng) A. Löve	StYP	X	WRPIS	Menyuan, Qinghai, China	3	AF267672
PI 531620	<i>Elymus kengii</i> Tzvelev	StYP	X	WRPIS	China	3	AF267673
PI 564954	<i>Elymus mutabilis</i> (Drobow) Tzvelev	StH	⊗	WRPIS	Lake Issyk Kul, Kazakhstan	1	AF267676
PI 564925	<i>Elymus nevskii</i> Tzvelev	StY	⊗	WRPIS	Novosibirsk, Russian Fed.	1	AF267677
PI 531646	<i>Elymus panormitanus</i> (Parl.) Tzvelev	StY	⊗	WRPIS	Sarsang, Iraq	1	AF267678
PI 378052	<i>Elymus patagonicus</i> Speg.	StHH		WRPIS	Chile	U	AF267679
PI 564961	<i>Elymus pendulinus</i> (Nevski) Tzvelev	StY	X	WRPIS	Gorno Altay A.O. Russian Fed.	1	AF267680
PI 557454	<i>Elymus rectisetus</i> (Nees) A. Löve & Connor	StYW	a	WRPIS	Launceston, Victoria, Aust.	1	AF267681
PI 565005	<i>Elymus repens</i> (L.) Gould	StStH	X	WRPIS	Dikorastuscij, Ukraine	U	AF267682
PI 533232	<i>Elymus scabrus</i> (R. Br) A. Löve	StYW	⊗	WRPIS	Warialda, N.S.W., Aust.	1	AF267683
PI 531663	<i>Elymus semicostatus</i> (Nees ex Steud.) Melderis	StY	⊗	WRPIS	Hinapin, Gilgit, Pakistan	1	AF267684
PI 531683	<i>Elymus strictus</i> (Keng) A. Löve	StY	⊗	WRPIS	Ma-er-kang, Sichuan, China	1	AF267685
PI 432403	<i>Elymus trachycaulus</i> (Link) Gould ex Shinners	StH	X	WRPIS	Mississippi, U.S.A.	4	AF267686
PI 547372	<i>Elymus transhyrcanus</i> (Nevski) Tzvelev	StStH		WRPIS	Poland	4	AF267687
PI 564998	<i>Elymus tschimganicus</i> (Drobov) Tzvelev	StStY	⊗	WRPIS	Lake Issyk Kul, Kazakhstan	2	AF267688
PI 531700	<i>Elymus tsukuchiensis</i> Honda	StHY	⊗	WRPIS	Hua-Shan, Shanxi, China	U	AF267689
PI 531761	<i>Hordeum bogdani</i> Wilensky	H	⊗	KBJ	Qilian Shan, China		AF267690
PI 531775	<i>Hordeum violaceum</i> (Boiss. & Hohen.) Tzvelev	H	⊗	KBJ	Nei Mongolia, China		AF267691
PI 502264	<i>Pseudoroegneria deweyi</i> Jensen, Hatch & Wipff	StP	X	KBJ	Russian Federation	U	AF267708
PI 401328	<i>Pseudoroegneria pertenuis</i> (C.A. Mey.) A. Löve	StP	X	KBJ	Tabriz, Iran	U	AF267709
PI 380645	<i>Pseudoroegneria pertenuis</i> (C.A. Mey.) A. Löve	StP	X	KBJ	Tabriz, Iran	1	AF267710
PI 565802	<i>Pseudoroegneria strigosa</i> (Drobov) A. Löve	St	X	WRPIS	Tian Lake, Xinjiang, China	1	AF267712

Note: The Plant Introduction (PI) number, taxon, and authorities are given. The nuclear genome (Nuc. gen.) designations are according to Wang et al. (1994). The primary mode of reproduction (RM) of the species is indicated as allogamous (X), autogamous (⊗) or apomictic (a). The source (WRPIS, Western Regional Plant Introduction Station; KBJ, K.B. Jensen collection) and origin of the seeds are given. Sequences found to be identical were grouped into the same Set No. (1–4). A U indicates a unique sequence.

genome were deduced from comparison of the allotetraploid *Leymus* (**NsXm**) and the diploid *Psathyrostachys juncea* (**Ns**), because the *ndhF* sequence of the allotetraploid was significantly different from that of the **Ns**-containing diploid (Jones et al. 2000).

We compared sequences for 762 nucleotides (nt) of the 796-bp DNA amplified from the 3' end of the chloroplast *ndhF* gene from each accession (Tables 1 and 2). In each case, identical DNA sequences were obtained from independent analyses of two individuals of a given accession. This result suggested that heterogeneity of cpDNA within the grass populations was not a problem in this study, and that no point mutations were introduced during amplification. For two accessions of *Pseudoroegneria pertenuis* from the same locale, the sequences differed by one nt (Table 3), and these two accessions were treated as separate sequences in the analysis. In contrast, two accessions of *P. deweyi* had identical sequences, and were treated as one sequence in the analysis (data not shown). Among the species examined in this study, no insertions or deletions were found in this region of the *ndhF* gene, as might be expected for the coding region of a gene in closely related subfamilies. However,

length differences were found among *ndhF* genes among more distantly related Pooideae (e.g., *Oryza sativa* L., Clark et al. 1995) and dicots (e.g., *Nicotiana tabacum* L., Olmstead and Sweere 1994).

Partial DNA sequence analysis for this region of the *ndhF* gene was previously presented for several of the species in Tables 1 and 2 by Catalán et al. (1997). For three species, *Australopyrum retrofractum*, *Elymus repens*, and *Psathyrostachys juncea*, the available sequence was essentially identical to that presented here. However, Catalán et al. (1997) identified a 15-bp insertion in the *Pseudoroegneria spicata ndhF* DNA sequence that was not found in the *P. spicata* P-5 sequence determined by Jones et al. (2000). Because the P-5 sequence was complete and no insertions were found for this gene in two other *Pseudoroegneria* species in this study, the P-5 was used in sequence comparisons presented below.

The *ndhF* DNA sequences were highly similar among Triticeae grasses containing the **St** nuclear genome, with 0–6 nt substitutions (0–0.8%) occurring between pairs of species (Table 3). There were five sets of accessions that had identical sequences among the **St**-genome-containing grasses (Tables 1

Table 2. Previously sequenced *ndhF* sequences included in this analysis.

GenBank Acc. No.	Taxon	Nuclear genome	RM	Origin	Set No.	Ref.*
U22000	<i>Avena sativa</i>		⊗			a
AF056166	<i>Elymus lanceolatus</i> cv. Critana	StH	X	Havre, Mont.	4	b
AF056168	<i>Elymus wawawaiensis</i> cv. Secar	StH	X	Lewiston, Idaho	4	b
AF056181	<i>Hordeum bogdanii</i>	H	⊗	Xinjiang, China		b
U22003	<i>Hordeum vulgare</i>	I	⊗			a
AF056179	<i>Leymus cinereus</i> cv. Trailhead	NsXm	X	Roundup, Mont.		b
AF056165	<i>Leymus triticooides</i> Acc. 642	NsXm	X	Jamieson, Oreg.		b
AF056172	<i>Pascopyrum smithii</i> cv. Arriba	StHNSXm	X	Flagler, Co.	5	b
AF056173	<i>Pascopyrum smithii</i> Atkins-172	StHNSXm	X	Utah	5	b
AF056178	<i>Pascopyrum smithii</i> Atkins-142	StHNSXm	X	Ariz.	4	b
AF056174	<i>Pascopyrum smithii</i> cv. Barton	StHNSXm	X	Barton Co., Kans.	5	b
AF056176	<i>Pascopyrum smithii</i> EPC-8	StHNSXm	X	Vernal, Utah.	U	b
AF056169	<i>Pascopyrum smithii</i> cv. Flintlock	StHNSXm	X	Nebr.-Kans.	5	b
AF056171	<i>Pascopyrum smithii</i> R-9-1-5	StHNSXm	X	Green River, Wyo.	5	b
AF056175	<i>Pascopyrum smithii</i> cv. Rosana	StHNSXm	X	Forsyth, Mont.	4	b
AF056170	<i>Pascopyrum smithii</i> cv. Rodan	StHNSXm	X	Morton Co. Mont.	5	b
AF056177	<i>Pascopyrum smithii</i> cv. Walsh	StHNSXm	X	Alta.-Sask.	5	b
U21924	<i>Piptatherum racemosum</i>					a
U21980	<i>Poa pratensis</i>		a			a
AF056167	<i>Psathyrostachys juncea</i> cv. Bozoisky-Select	Ns	X	Kazakhstan		b
AF056180	<i>Pseudoroegneria spicata</i> P-5	St	X	Unknown	1	b

Note: The GenBank accession number, nuclear genome composition, primary mode of reproduction (RM; X = allogamous, ⊗ = autogamous, a = apomycitic), and origin (if known) are given. Sequences found to be identical were grouped into the same Set No. (1, 4, and 5). A U indicates a unique sequence.

*The sequences were originally published in work by (a) Gaut et al. (1997) or (b) Jones et al. (2000).

Table 3. Number of nucleotide differences in *ndhF* gene sequence.

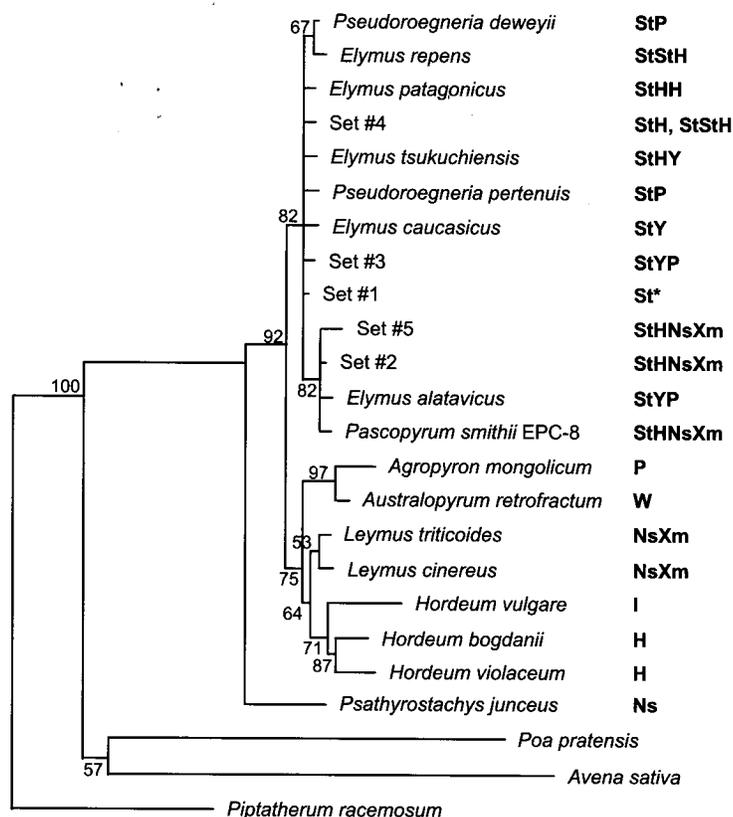
OTUs	Hv	Am	Ar	S1	S2	Ec	Pp	Pd	Et	S3	Ea	S4	Er	Ep	S5	Ps	Lt	Lc	Pj	Hvu	Ppr	As	Pr
Hb	5	11	9	8	10	9	9	9	9	9	11	9	10	9	12	11	6	5	18	9	62	65	47
Hv		12	10	9	11	10	10	10	10	9	12	10	11	10	13	12	7	6	19	10	63	66	49
Am			4	9	11	10	10	10	10	10	12	10	11	10	11	12	7	8	19	14	63	64	49
Ar				7	9	8	8	8	8	8	10	8	9	8	9	10	5	6	17	12	61	64	47
S1					2	1	1	1	1	1	3	1	2	1	4	3	4	5	12	11	56	61	44
S2						3	3	3	3	3	1	3	4	3	2	1	6	7	14	13	58	63	46
Ec							2	2	2	2	4	2	3	2	5	4	5	6	13	12	57	62	45
Pp								2	2	2	4	2	3	2	5	4	5	6	13	12	57	62	45
Pd									2	2	4	2	1	2	5	4	5	6	13	12	57	62	45
Et										2	4	2	3	2	5	4	5	6	13	12	57	62	45
S3											4	2	3	2	5	4	5	6	13	12	57	62	45
Ea												4	5	4	3	2	7	8	15	14	59	64	47
S4													3	2	5	4	5	6	13	12	57	62	45
Er														3	6	5	6	7	14	13	58	63	46
Ep															5	4	5	6	13	12	57	62	45
S5																3	8	9	16	15	60	65	48
Ps																	7	8	15	14	59	64	47
Lt																		1	14	9	58	61	44
Lc																			15	8	59	62	45
Pj																				20	59	61	46
Hvu																					64	67	44
Ppr																						73	58
As																							67

Note: The number of substitutions in the 753-bp sequence for each operational taxonomic unit (OTU) was determined using MEGA (Kumar et al. 1993). Identical sequences are grouped into Sets 1–5 (S1, S2, S3, S4, and S5) as indicated in Tables 1 and 2. Hb, *Hordeum bogdanii*; Hv, *Hordeum violaceum*; Am, *Agropyron mongolicum*; Ar, *Australopyrum retrofractum*; Ec, *Elymus caucasicus*; Pp, *Pseudoroegneria pertenuis*; Pd, *Pseudoroegneria deweyii*; Et, *Elymus tsukushiensis*; Ea, *Elymus alatavicus*; Er, *Elymus repens*; Ep, *Elymus patagonicus*; Ps, *Pascopyron smithii* EPC-8; Lt, *Leymus triticooides*; Lc, *Leymus cinereus*; Pj, *Psathyrostachys juncea*; Hvu, *Hordeum vulgare*; Ppr, *Poa pratensis*; As, *Avena sativa*; Pr, *Piptatherum racemosum*.

and 2). Set 1 contained 13 *Elymus* and 3 *Pseudoroegneria* species. The sequences of *E. glaucissimus* and *E. tschimganicus* were identical (Set 2), as were those of *E. grandiglumis* and

E. kengii (Set 3). Set 4 contained 2 *Elymus* species and two accessions of *Pascopyron smithii* (Atkins-142 and Rosana). Set 5 contained 7 accessions and cultivars of *P. smithii*

Fig. 1. Phylogenetic tree for the *ndhF* DNA sequences. Jukes–Cantor distances were calculated and used to generate a neighbor-joining tree with MEGA (Kumar et al. 1993). *Piptatherum racemosum* was the selected outgroup. The bootstrap confidence levels for 500 replications are indicated at the branch points, and the branch lengths are proportional to the Jukes–Cantor distance. Species having identical sequences were grouped into Sets 1–5 as outlined (Tables 1 and 2). For Set 1 (**St***), species contained the **St**, **StH**, **StHY**, **StP**, **StY**, **StYP**, or **StYW** nuclear genomes.



(Jones et al. 2000). Among the tested species containing the **St** nuclear genome, 7 had unique sequences for this region of the *ndhF* gene (U, Tables 1 and 2).

Higher levels of variation in the *ndhF* sequence occurred among all Triticeae species, with 0–19 nt changes (0–2.5%) in the 762-bp sequence (Table 3). As expected, the non-Triticeae grasses were less similar. There were 56–64 nt differences between *Poa* and all other species (7.4–8.5%), 61–73 nt differences between *Avena* and all other species (8.1–9.7%), and 44–67 nt differences between *Piptatherum* and all other species (5.8–8.9%). This level of sequence variability was consistent with that previously reported for the *ndhF* gene in grasses (Catalán et al. 1997; Clark et al. 1995; Jones et al. 2000).

There was a high degree of sequence similarity among species that were from geographically diverse origins. Set 1 species were collected from Asia, the Middle East, North America, and Australia. In addition, while 3 of the species in Set 4 were from the U.S.A., *E. transhyrcanus* was collected in Europe. Conversely, 3 different sequences were found among 4 accessions collected from Lake Issyk Kul, Kazakhstan. These data indicated that specific *ndhF* sequences were not associated with particular geographic locations. Similarly, the accessions in Set 1 included species with allogamous, autogamous, and apomictic modes of reproduction, indicating that this *ndhF* sequence was not associated with a specific reproductive mode.

To examine the relationships for the *ndhF* gene among the species, the distances between the aligned sequences were determined using the Jukes–Cantor algorithm and used to generate a phylogenetic tree by the neighbor-joining method (Fig. 1). The Jukes–Cantor distance was chosen because of the small number of substitutions, the low ratio of transitions to transversions (<2, data not shown), and the similar number of nonsynonymous and synonymous substitutions. The neighbor-joining method for tree construction was most likely to produce the correct topology for these data (Kumar et al. 1993). However, phylogenies determined using the UPGMA (unweighted pair group method with arithmetic means) or maximum parsimony method had essentially the same topology as the neighbor-joining tree (data not shown).

In the phylogenetic analysis, the Triticeae grasses were grouped together with a high bootstrap confidence level (BCL) of 93. The *ndhF* DNA sequences of species containing the **St** nuclear genome, alone or in allopolyploid combination with other genomes, formed a strongly supported subgroup within the Triticeae (BCL = 82). The *ndhF* sequences of diploid Triticeae species containing the **P**, **W**, **I**, and **H** genomes were grouped with those of species having the **Ns** and **Xm** nuclear genomes, and separately from the **St**-containing species. However, the support for these clades, as indicated by the BCL, was somewhat weaker. The sequences of the allotetraploid *Leymus* (**NsXm**) species grouped with the *Hordeum* species, and separately from the

diploid *Psathyrostachys* (Ns). These phylogenetic relationships were consistent with previous analyses using the chloroplast *ndhF* and *rbcL* sequences (Catalán et al. 1997; Clark et al. 1995; Gaut et al. 1997; Jones et al. 2000; Mason-Gamer and Kellogg 1996a).

Thus, species containing the *St* nuclear genome had cpDNA sequences that placed them in a single clade separated from a sister clade containing the diploid *P*-, *W*-, *H*-, and *I*-containing species and the allotetraploid *Leymus* (NsXm) species. More distantly related, but still within a single Triticeae clade was *Psathyrostachys* (Ns). Because it was not possible to analyze cpDNA from species containing the *Y* genome in the absence of the *St* genome, it was not possible to distinguish whether cpDNA was inherited from the *St*-containing progenitor or whether there was no selection for inheritance of two highly related cpDNAs. However, there was no grouping of *Y*-genome-containing species within the *St* clade. This is in contrast to phylogenies based on repetitive DNA sequences from *Elymus* species that indicated polyphyletic origins for the species (Svitashev et al. 1996).

The data were consistent with previous studies showing that cpDNA sequences of the allooctoploid *StHNsXm* grasses were similar to that of the *St* diploid and *StH* allotetraploid progenitors (Jensen et al. 1999; Jones et al. 2000). The data were also consistent with RFLP analyses that suggested that cpDNA was inherited from the *St*-genome-containing progenitor in two allotetraploid *Elymus* species and in allohexaploid *E. repens* (Mason-Gamer and Kellogg 1996a). Although the biological reason for this bias remains unknown, a similar selection for cpDNA inheritance from progenitors containing a specific nuclear genome was noted among some *Triticum* and *Aegilops* species (Wang et al. 1997). In this case, however, chloroplast genome dominance was not always complete, and some species, particularly those with the *U* and *C* genomes, appeared to inherit cpDNA from either progenitor.

Differences between phylogenies based on nuclear and cpDNA were previously described for the Triticeae (Kellogg et al. 1996; Mason-Gamer and Kellogg 1996b). The results of this study also suggested some differences between the evolutionary histories of the nuclear and chloroplast genomes. For both nuclear and chloroplast genes, *Pseudoroegneria* (*St*) was found in a sister clade to *Agropyron* (*P* genome) and *Australopyrum* (*W* genome). However, in contrast to cpDNA, nuclear gene sequences placed *Psathyrostachys* (Ns) in a clade with *Pseudoroegneria*, *Agropyron*, and *Australopyrum*, whereas the Hordeum group (*H* and *I*) was more distantly related. These discrepancies between chloroplast and nuclear gene evolution were unexpected, and may reflect different histories for the chloroplast and nuclear genomes (Mason-Gamer and Kellogg 1996b). Because analysis of nuclear gene inheritance of allopolyploid genomes is complicated by the presence of analogous sequences in each genome, allopolyploids have generally been excluded from this type of analysis. It is known that the genomes are relatively intact and identifiable in allopolyploids; however, inequalities in gene expression from different nuclear genomes have been identified (Pikaard 1999). There was nucleolar dominance associated with transcription of rRNA genes from specific nuclear

genomes in *Brassica* (Chen and Pikaard 1997). The rRNA genes of only one parent in the progeny of interspecific hybridizations were expressed, and the genes expressed were associated with specific nuclear genomes. Among Triticeae, it was shown that rRNA genes from wheat were normally dominant over rye rRNA genes in Triticale, and that suppression of the rye genes required several unlinked rye genes (Neves et al. 1997; Viera et al. 1990). It is not known whether the bias in rRNA gene expression in allopolyploids is correlated with inheritance of the chloroplast genome. The high degree of cpDNA relatedness among Triticeae grasses containing the *St* nuclear genome indicated a strong bias toward the *St*-containing parent serving as the cytoplasmic DNA donor in interspecific hybridizations. Because cpDNA is most frequently inherited from the maternal parent in grasses, the data suggested that the *St*-genome-containing parent served as the maternal parent in all hybridizations. However, nonmaternal inheritance of cytoplasmic DNA in plants has been demonstrated (Soltis and Kuzoff 1995), and mixed cpDNA inheritance was found for backcrossed hybrids of *Festuca pratensis* and *Lolium perenne* (Kiang et al. 1994). Unfortunately, cpDNA inheritance patterns have not been investigated for Triticeae hybrids. If some character of the cpDNA associated with the *St* nuclear genome of hybrid progenitors conferred increased fitness on the hybrids, then it did not matter whether the genome was inherited from the male or female parent. On the other hand, if cpDNA was inherited from the maternal parent in these Triticeae hybridizations, then increased fitness in hybrids could be associated with either the chloroplast genome or the *St* nuclear genome. More work is needed in this area to distinguish between these possibilities.

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References

- Catalán, P., Kellogg, E.A., and Olmstead, R.G. 1997. Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndhF* gene sequences. *Mol. Phylogenet. Evol.* **8**: 150-166.
- Chen, Z.C., and Pikaard, C.S. 1997. Transcriptional analysis of nucleolar dominance in polyploid plants: Biased expression silencing of progenitor rRNA genes is developmentally regulated in *Brassica*. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 3442-3447.
- Clark, L.G., Zhang, W., and Wendel, J.F. 1995. A phylogeny of the grass family (Poaceae) based on *ndhF* sequence data. *Syst. Bot.* **20**: 436-460.
- Dewey, D.R. 1984. The genomic system of classification as a guide to intergeneric hybridization with perennial Triticeae. *In* Gene Manipulation in Plant Improvement. Edited by J.P. Gustafsson. Plenum, New York. pp. 209-279.

- Dvorák, J., de Terlizzi, P., Zhang, H.-B., and Resta, P. 1993. The evolution of polyploid wheats: Identification of the A genome donor species. *Genome*, **36**: 21–31.
- Gaut, B.S., Clark, L.G., Wendel, J.F., and Muse, S.V. 1997. Comparisons of the molecular evolutionary process at *rbcL* and *ndhF* in the grass family (Poaceae). *Mol. Biol. Evol.* **14**: 769–777.
- Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J.-M., Skovmand, B., Taba, S., and Warburton, M. 1999. Plant genetic resources: What can they contribute toward increased crop productivity? *Proc. Natl. Acad. Sci. U.S.A.* **96**: 5937–5943.
- Hsaio, C., Chatterton, N.J., Asay, K.H., and Jensen, K.B. 1994. Phylogenetic relationships of 10 grass species: An assessment of phylogenetic utility of the internal transcribed spacer region in nuclear ribosomal DNA in monocots. *Genome*, **37**: 112–120.
- Hsaio, C., Chatterton, N.J., Asay, K.H., and Jensen, K.B. 1995. Phylogenetic relationships of the monogenomic species of the wheat tribe, Triticeae (Poaceae), inferred from the nuclear rDNA (internal transcribed spacer) sequences. *Genome*, **38**: 211–223.
- Jensen, K.B., Redinbaugh, M.G., Blood, M., Horton, W.H., and Asay, K.H. 1999. Natural hybrids of *Elymus elymoides* × *Leymus salinus* subsp. *salmonis* (Poaceae: Triticeae). *Crop Sci.* **39**: 976–982.
- Jones, T.A., Redinbaugh, M.G., and Zhang, Y. 2000. Chloroplast inheritance of western wheatgrass from its diploid and tetraploid ancestors. *Crop Sci.* **40**: 43–47.
- Kellogg, E.A., Appels, R., and Mason-Gamer, R.J. 1996. When genes tell different stories: The diploid genera of Triticeae (Gramineae). *Syst. Bot.* **21**: 321–347.
- Kiang, A.S., Connolly, V., McConnell, D.J., and Kavanagh, T.A. 1994. Paternal inheritance of mitochondria and chloroplasts in *Festuca pratensis*-*Lolium perenne* intergeneric hybrids. *Theor. Appl. Genet.* **87**: 681–688.
- Kumar, S., Tamura, K., and Nei, M. 1993. MEGA: Molecular Evolution and Genetic Analysis ver. 1.0. The Pennsylvania State University, University Park, Pa.
- Lassner, M.W., Peterson, P., and Yoder, J.I. 1989. Simultaneous amplification of multiple DNA fragments by polymerase chain reaction in the analysis of transgenic plants and their progeny. *Plant Mol. Biol. Rep.* **7**: 116–128.
- Mason-Gamer, R.J., and Kellogg, E.A. 1996a. Chloroplast DNA analysis of the monogenomic Triticeae: Phylogenetic implications and genome-specific markers. *In* *Methods of Genome Analysis in Plants*. Edited by P.P. Jauhar. CRC Press, New York. pp. 301–324.
- Mason-Gamer, R.J., and Kellogg, E.A. 1996b. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst. Biol.* **45**: 524–545.
- McIntosh, R.A., Hart, G.E., Devos, K.M., Gale, M.D., Rogers, W.J., and Slinkard, A.E. 1998. Proceedings of the 9th International Wheat Genetics Symposium, Vol. 5., held at Saskatoon, Canada, 2–7 Aug. 1998. Edited by A.E. Slinkard. University of Saskatchewan Press, Saskatoon, Canada. p. 236.
- Neves, N., Silva, M., Heslop-Harrison, J.S., and Viegas, W. 1997. Nucleolar dominance in triticales: Control by unlinked genes. *Chromosome Res.* **5**: 125–131.
- Olmstead, R.G., and Sweere, J.A. 1994. Combining data in phylogenetic systematics: An empirical approach using three molecular data sets in the Solanaceae. *Syst. Biol.* **43**: 467–481.
- Pikaard, C.S. 1999. Nucleolar dominance and silencing of transcription. *Trends Plant Sci.* **4**: 478–483.
- Rieseberg, L.H., and Carney, S.E. 1998. Tansley Review No. 102. Plant hybridization. *New Phytol.* **140**: 599–624.
- Svitashev, S., Salomon, B., Bryngelsson, T., and von Bothmer, R. 1996. A study of 28 *Elymus* species using repetitive DNA sequences. *Genome*, **39**: 1093–1101.
- Soltis, D.E., and Kuzoff, R.K. 1995. Discordance between nuclear and chloroplast phylogenies in the *Huechera* group (Saxifragaceae). *Evolution*, **49**: 727–742.
- Viera, R., Mello-Sampayo, T., and Viegas, W. 1990. Genetic control of 1R nucleolus organizer region expression in the presence of wheat genomes. *Genome*, **33**: 713–718.
- Wang, R.R.-C., von Bothmer, R., Dvorák, J., Fedak, G., Lindelaursen, I., and Muramatsu, M. 1994. Genome Symbols in the Triticeae (Poaceae). *In* Proceedings of the 2nd International Triticeae Symposium, held at Logan, Utah, 20–24 June 1994. Edited by R.R.-C. Wang, K.B. Jensen, and C. Jaussi. Utah State University Publication Design and Production, Logan, Utah. pp. 29–34.
- Wang, G.-Z., Miyashita, N.T., and Kiochiro, T. 1997. Plasmon analyses of *Triticum* (wheat) and *Aegilops*: PCR-single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. *Proc. Natl. Acad. Sci. U.S.A.* **94**: 14 570 – 14 577.
- Williams, J.G.K., Hanafey, M.K., Rafalski, J.A., and Tingey, S.V. 1993. Genetic analysis using random amplified polymorphic DNA markers. *Methods Enzymol.* **218**: 704–740.