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Nuclear DNA Content of Perennial Grasses of the Triticeae

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ABSTRACT

Nuclear DNA content was determined by flow cytometry for an array of perennial species of the Triticeae (Poaceae) which characterize the tribe and are representative of the genomes of the Triticeae. The mean nuclear DNA content expressed on a diploid basis (DNA pg/2C) for the diploid genomes (in parentheses) were as follows: *Agropyron* (PP) 13.9 pg, *Pseudoroegneria* (StSt) 8.8 pg, *Hordeum* (HH) 9.5 pg, *Psathyrostachys* (NsNs) 16.7 pg, and *Thinopyrum* genomes (E^bE^b) 14.9 pg and (E^eE^e) 12.0 pg. The YY genome in *Elymus* was determined by difference to be 9.3 pg. The unknown or XmXm genome or genomes in *Leymus* could have DNA contents that range from 2.7 to 7.7 pg/2C. There were significant differences in DNA content of species with similar diploid genomes. There were also significant differences in nuclear DNA content among polyploid species with the same genomes. In general, the nuclear DNA content of the polyploid species of the Triticeae were similar to the expected DNA contents on the basis of previous genomic classifications. However, in some allopolyploid genera such as *Thinopyrum* and *Pascopyrum*, the nuclear DNA content of some species was less than expected on the basis of summation of the DNA of constituent genomes. The results indicate that gain or loss of nuclear DNA has occurred during the evolution of the perennial Triticeae and was probably a part of speciation.

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PREVIOUS RESEARCH summarized by Dewey (1984), Wang et al. (1994), and Asay and Jensen (1996a,b), and others has demonstrated that the perennial grasses of the tribe Triticeae are based on the P, St, H, Ns, E, W, Y genomes and an unknown × genome(s). The cytogenetic definition of genome, i.e., the haploid set of chromosomes of a diploid species, will be used in this report. The classification of the Triticeae has been and remains a matter of controversy (Barkworth, 1992; Barkworth and Dewey, 1985; Kellogg, 1994). Barkworth (1992) and Kellogg (1994) agree that developing a classification system for the Triticeae has been and will remain difficult because of the complex evolutionary history of the tribe. For the purposes of this paper, the genomic classification described by Dewey (1984) and Barkworth and Dewey (1985) will be used with the following exceptions. Dewey (1984) indicated that *Leymus* species and *Pascopyrum* contain the J (=E) genome. Subsequent reports (Zhang and Dvorak, 1991; Wang and Jensen, 1994) based on molecular genetic and cytogenetic analyses indicate that the J genome does not occur in *Leymus* or *Pascopyrum*. Asay and Jensen (1996a,b) have designated the unknown genome in *Leymus* and *Pascopyrum* the Xm or unknown genome following the genome nomenclature system for the Triticeae proposed by Wang et al. (1994). Species classified as *Critesion* by Barkworth and Dewey (1985) are listed as *Hordeum* in this report. The genome nomenclature system of Wang et al. (1994) will be used

Abbreviations: 2C, DNA content of a diploid nucleus; Da, daltons.

Table 1. DNA content of species of perennial Triticeae.

Species	Genome†	2*n*	Strain or accession‡	DNA pg/2C§ Mean	SD	Confidence interval (95%)
Agropyron Gaertner						
A. cristatum (L.) Gaertner	PP	14	PI 297870	13.46	0.06	13.37–13.55
A. cristatum	PP	14	PI 314600	14.25	0.43	13.57–14.93
A. cristatum	PP	14	cv Ruff	13.19	0.16	12.94–13.44
A. mongolicum Keng	PP	14	PI 499391	15.57	0.14	15.35–15.58
A. mongolicum	PP	14	PI 499392	15.16	0.58	14.24–16.08
A. desertorum (Fischer ex Link) Shultes	PPPP	28	PI 249143	26.39	0.55	25.51–27.27
A. desertorum	PPPP	28	cv Nordan	25.45	0.49	24.67–26.23
Pseudoroegneria (Nevski) Á. Löve						
P. strigosa subsp. *aegilopoides* (Drobov.) Á. Löve	StSt	14	PI 531755	9.45	0.41	8.79–10.11
P. strigosa (M. Bieb)	StSt	14	D3778	9.72	0.43	9.03–10.41
P. libanotica (Hackel) D.R. Dewey	StSt	14	PI 380644	7.96	0.42	7.29–8.64
P. libanotica	StSt	14	PI 380652	7.85	0.29	7.38–8.31
P. spicata (Pursh) Á. Löve subsp. *spicata*	StSt	14	PI 232127	9.43	0.16	9.16–9.69
P. spicata	StSt	14	PI 232134	9.09	0.49	8.31–9.87
P. stipifolia (Czern ex Nevski)	StSt	14	PI 440000	8.00	0.07	7.88–8.11
P. geniculata (Trin.) Á. Löve	StStStSt	28	DJ3875	16.96	0.27	16.53–17.39
P. geniculata	StStStSt	28	DJ3882	17.48	0.24	17.11–17.86
Hordeum L.						
H. bogdanii Wilensky	HH	14	PI 440413	9.47	0.14	9.25–9.69
H. brachyantherum Nevski	HH	14	D3571	9.67	0.05	9.58–9.75
H. brevisubulatum-violaceum (Boise & Hofenacker) Tzvelev	HH	14	PI 401374	9.93	0.20	9.61–10.24
H. bulbosum L.	HH	14	PI 318649	9.16	0.13	8.96–9.36
H. bulbosum	HHHH	28	PI 343189	17.74	0.24	17.36–18.13
H. californicum Covas & Stebbins	HH	14	PI 531778	9.52	0.06	9.43–9.61
H. californicum	HH	14	PI 531799	9.52	0.25	9.12–9.92
H. chilense Roemer & Schultes	HH	14	PI 531781	9.90	0.45	9.18–10.62
H. comosum K. Presl	HH	14	D2742	9.27	0.05	9.19–9.35
H. flexuosum Nees	HH	14	cv Castelar 730	8.86	0.16	8.61–9.11
H. haplophilum Griseb.	HH	14	D2749	8.85	0.32	8.34–9.37
H. roshevitzii Bowden	HH	14	PI 499504	10.12	0.42	9.45–10.79
H. stenostachys Godron	HH	14	PI 531791	10.04	0.20	9.72–10.35
H. stenostachys	HH	14	PI 531792	10.05	0.26	9.63–10.46
Psathyrostachys Nevski						
P. fragilis (Boise) Nevski	NsNs	14	PI 343190	16.79	0.32	16.29–17.30
P. juncea (Fisher) Nevski	NsNs	14	PI 406468	15.60	0.15	15.37–15.83
P. juncea	NsNs	14	PI 531824	15.53	0.22	15.18–15.88
P. stoloniformis C. Baden	NsNs	14	D2562	17.85	0.74	16.67–19.02
P. stoloniformis	NsNs	14	D3376	17.91	0.14	17.69–18.12
Thinipyrum Á. Löve						
T. bessarabicum (Savul & Rayass) Á. Löve	E^bE^b	14	PI 431711	14.87	0.25	14.47–15.27
T. bessarabicum	E^bE^b	14	AJC305	14.96	0.42	14.30–15.62
T. elongatum (Host) D.R. Dewey	E^eE^e	14	PI 531719	11.74	0.14	11.51–11.97
T. elongatum	E^eE^e	14	D3610	12.20	0.23	11.84–12.56
T. junceiforme (Löve & Löve) Á. Löve	EEEE	28	PI 297873	25.97	0.40	25.33–26.60
T. junceiforme	EEEE	28	D3463	23.62	0.32	23.12–24.13
T. caespitosum Liu & Wang	EEStSt	28	PI 531716	19.88	1.12	18.09–21.67
T. intermedium subsp. *intermedium* (Host) Barkw. & D.R. Dewey	EEEEStSt	42	cv Slate	26.25	0.62	25.25–27.24
T. intermedium subsp. *barbulatum* (Shur) Barkw. & D.R. Dewey	EEEEStSt	42	cv Manska	25.92	0.47	25.17–26.68
T. ponticum (Podp.) Barkw. & D.R. Dewey	EEEEEEStStStSt	70	cv Platte	45.26	1.49	42.89–47.64

Table 1 continued next page.

in this report; hence, the J genome will be subsequently referred to as the E genome.

The base DNA content of the genomes of the perennial Triticeae has previously not been determined, except for initial reports on the H and E genomes (Bennett and Smith, 1976). Bennett and Smith (1991) described the utility of nuclear DNA content values in phlyogenetic and evolutionary studies. Applications include determining if DNA content increases in proportion to ploidy levels and if the DNA content of allopolyploids equals the sum of the DNA content of genomes of donor species. To avoid confusion with chromosome number, DNA amounts are expressed in picograms as "C" values (Bennett and Leitch, 1995; Bennett and Smith, 1976). The letter C stands for "constant" or the amount of DNA in a haploid nucleus or haploid genome; 2C values, which are reported in this paper, represent the DNA content of a diploid somatic nucleus. DNA amounts in picograms can be approximately converted to daltons or nucleotide pairs by the formulas: 1 nucleotide pair = 660 Da; 1 pg = 0.965×10^9 nucleotide pairs (Bennett and Smith, 1976). For the few species of the perennial Triticeae determined to date, the DNA content as determined by in situ microphotodensitometry of somatic root tips was as follows: *Hordeum bulbosum*, 2*n* = 14, 11.0 pg/2C (H genome); *Hordeum bulbosum*, 2*n* = 28, 22.1 pg/2C; and *Thinopyrum elongatum* (E^e genome), 2*n* = 14, 11.2 pg/2C (Bennett and Smith, 1976).

Determination of DNA content by microphotodensitometry is laborious and time consuming (Michaelson

Table 1. continued.

Species	Genome†	2*n*	Strain or accession‡	DNA pg/2C§ Mean	SD	Confidence interval (95%)
Elymus L.						
E. abolinii (Drob.) Tzvelev	StStYY	28	PI 531554	18.70	0.30	18.22–19.17
E. ciliaris (Trin.) Tzvelev	StStYY	28	PI 531576	17.33	0.44	16.63–18.02
E. canadensis L.	StStHH	28	PI 531565	21.11	1.01	19.49–22.72
E. caninus L.	StStHH	28	PI 253290	17.09	0.44	16.39–17.80
E. caninus	StStHH	28	PI 439906	17.12	0.17	16.85–17.39
E. glaucus (Buckley)	StStHH	28	D3261	18.62	0.15	18.38–18.86
E. glaucus	StStHH	28	D3268	18.33	0.50	17.54–19.12
E. lanceolatus (Schribner & Smith) Gould	StStHH		D3626	16.57	0.30	16.09–17.05
E. lanceolatus	StStHH	28	D3627	16.85	0.18	16.56–17.15
E. mutabilis (Drob.) Tzvelev	StStHH	28	PI 499449	16.50	0.92	15.03–17.97
E. mutabilis	StStHH	28	PI 499589	17.42	0.42	16.75–18.09
E. sibiricus L.	StStHH		PI 499613	16.62	0.31	16.13–17.12
E. sibiricus	StStHH		PI 499616	16.59	0.25	16.19–16.99
E. trachycaulus (Link) Gould ex Shinners	StStHH	28	PI 232168	19.13	0.31	18.63–19.62
E. trachycaulus	StStHH	28	PI 276711	17.48	0.65	16.45–18.51
E. trachycaulus	StStHH	28	PI 315368	17.80	0.51	16.99–18.62
E. alatavicus (Drob.) À. Löve	StStYYPP	42	PI 499475	30.31	1.07	28.61–32.02
E. dahuricus Turez ex Griseb.	StStHHYY	42	PI 499592	26.43	0.45	25.72–27.14
E. dahuricus	StStHHYY	42	PI 499593	25.16	0.37	24.57–25.74
Leymus Hochst.						
L. akmolinensis (Drob.) Tzvelev	NsNsXmXm	28	PI 440306	22.60	0.25	22.21–23.00
L. ambiguus (Vasey & Schribn.) D.R. Dewey	NsNsXmXm		KJ59	22.27	0.71	21.13–23.40
L. chinensis (Trin.) Tzvelev	NsNsXmXm	28	PI 499515	19.42	0.16	19.16–19.68
L. chinensis	NsNsXmXm	28	PI 499518	19.70	0.63	18.70–20.69
L. flavens (Scribner & Smith) Pilger	NsNsXmXm	28	Has & Har	24.44	0.48	23.68–25.50
L. racemosus (Lam.) Tzvelev	NsNsXmXm	28	PI 313965	21.93	0.14	21.70–22.16
L. racemosus	NsNsXmXm	28	PI 531812	22.78	0.40	22.15–23.42
L. cf. *racemosus*	NsNsXmXm	28	DJ3801	21.16	1.08	19.44–22.88
L. cf. *ramosus* (Trin.) Tzvelev	NsNsXmXm	28	PI 499654	20.31	0.20	20.00–20.63
L. sabulosus (M. Bieb.) Tzvelev	NsNsXmXm	28	PI 531813	22.98	0.19	22.68–23.28
L. sabulosus	NsNsXmXm	28	PI 531814	22.71	0.09	22.56–22.85
L. secalinus (Georgi) Tzvelev	NsNsXmXm	28	PI 499524	21.36	0.33	20.83–21.89
L. secalinus	NsNsXmXm	28	PI 499528	21.57	0.16	21.31–21.84
L. triticoides (Buckl) Pilger	NsNsXmXm	28	PI 531822	22.41	0.29	21.94–22.87
L. tritcoides	NsNsXmXm	28	D2950	22.39	0.95	20.87–23.90
L. tritcoides	NsNsXmXm	28	Asay M-9	21.61	0.89	20.19–23.04
L. tritcoides	NsNsXmXm	28	cv. Shoshone	21.08	0.25	20.68–21.48
Pascopyrum À. Löve						
P. smithii (Rydb.) À. Löve	StStHHNsNsXmXm	56	cv. Flintlock	35.39	0.88	34.00–36.78
P. smithii	StStHHNsNsXmXm	56	cv. Barton	33.53	0.70	32.41–34.65
P. smithii	StStHHNsNsXmXm	56	cv. Rodan	34.08	0.46	33.35–34.81

† Superscript on genome letters refers to a subset of that genome.
‡ PI numbers are accession numbers of the USDA Plant Germplasm system; other strain numbers are from the *Triticeae* collection at Logan, UT (K. Jensen).
§ Strain means and standard deviations (SD) based on four plants per accession; 1000 nuclei were scanned per plant.

et al., 1991). The recent development of methods to determine nuclear DNA content of plants by flow cytometry facilitates the determination of DNA content of a large number of plants (Galbraith et al., 1983; Rayburn et al., 1989; Michaelson et al., 1991; Arumuganathan and Earle, 1991). The purpose of this study was to use flow cytometry analyses to determine the base DNA content of the genomes of the perennial Triticeae. Diploid, autopolyploid, and allopolyploid species that are characteristic of the tribe were used to relate DNA content to previously reported genomic relationships.

MATERIALS AND METHODS

The grasses used in this study were primarily plant introductions obtained from the U.S. Department of Agriculture's National Plant Germplasm System via the USDA-ARS Forage and Range Research Laboratory at Utah State University, Logan, UT, (K. Jensen) where they were identified. Authorities for genera and species are listed with genus and species names in Table 1. These accessions have a PI (for plant introduction) numerical designation and can be obtained from the USDA Plant Germplasm System. Chromosome numbers of these accessions were determined by the USDA-ARS Forage and Range Laboratory at Logan. The other strains used in this study are experimental breeding lines maintained by USDA-ARS Laboratory at Logan or were released cultivars (designated cv.) obtained by the USDA-ARS grass breeding program at Lincoln, NE (K.Vogel). To simplify terminology, the cultivars, experimental breeding lines, and plant germplasm accessions will be referred to as strains in this report. The strains used in the study (Table 1) were chosen to represent the autoploid and alloploid combinations that exist within the perennial Triticeae. Many of the species were represented by two strains. *Australopyrum* species that have the W genome were not available for use in this study.

Plants were grown in a greenhouse of the USDA Forage Research Laboratory at Lincoln. The grasses were planted in super-cell cone-tainers (Steuwe and Sons, Corvallis, OR) or mini-pots in the greenhouse. A plot consisted of two rows with 7 cells per row for a total of 14 seedlings per strain. Four

Table 2. Nuclear DNA content of the genomes of the perennial Triticae.

Species	Basic genome	2n	DNA pg/2C Mean†	DNA pg/2C SD‡	Confidence interval§	Estimated DNA content Mean¶	Estimated DNA content Confidence interval#
Agropyron	PP	14	13.93	1.08	12.46–14.46		
Pseudoroegeneria	StSt	14	8.75	0.71	8.22–9.28		
Hordeum	HH	14	9.48	0.47	9.21–9.75		
Psathyrostachys	NsNs	14	16.73	1.16	15.29–18.18		
Thinopyrum	E^bE^b	14	14.91	0.07	14.29–15.54		
	E^eE^e	14	11.97	0.33	9.05–14.89		
	EEEE	28	24.80	1.66††	9.93–39.66	26.88	22.61–31.15
T. caespitosum	EEStSt	28	19.83‡‡	1.12†††	18.09–21.67	20.72	18.46–22.98
T. intermedium	EEEEStSt	42	26.08	0.23††	24.05–28.11	31.80	30.08–33.58
T. ponticum	EEEEEEStStStSt	70	45.26‡‡	1.49†††	42.89–47.64	45.91	44.24–47.58
Elymus	StStYY	28	18.01	0.97	9.37–26.65		
(calculated)	YY	14	9.26				
	StStHH	28	17.66	1.29	16.90–18.42	18.23	17.40–19.06
	StStYYPP	42	30.31	1.07	28.61–32.02	31.94	29.94–33.94
	StStHHYY	42	25.79	0.90	17.71–33.87	27.49	24.22–30.76
Leymus	NsNsXmXm	28	21.81	1.26			
(calculated)	XmXm	14	2.7 to 7.7				
Pascopyrum	StStHHNsNsXmXm	56	34.33	0.96	31.97–36.70	39.47	38.95–39.99

† Means of species with same genomes in Table 1.
‡ Standard deviation (SD) of means of species with same genomes in Table 1.
§ Confidence interval (95%) of mean of species with same genomes.
¶ Estimated mean obtained by summing mean values of constituent genomes of probable diploid or polyploid progenitors.
# Estimated confidence intervals obtained by using variances of estimated means. Variance of estimated means obtained by summing variances of constituent genomes.
†† DNA content of strains with same genomes differed significantly resulting in large SD and confidence interval.
‡‡ Standard deviation of plants from the single accession with this genome configuration.

seedlings of each strain were sampled as individuals. One seedling was sampled twice with "a" or "b" added to the plant number. By taking two samples from the same seedling, the laboratory standard error for the flow cytometry procedure was determined.

Flow cytometry procedures were those of Arumuganathan and Earle (1991). Approximately 50 mg of fresh, green tissue from a collared leaf of a Triticeae seedling was excised and placed on ice in a sterile 35- by 10-mm plastic petri dish. About 20 mg of barley (*Hordeum vulgare* L. cv. Stark) or hexaploid wheat (*Triticium aestivum* L. cv. Chinese Spring or cv. Arapahoe) leaf tissue from seedling leaves was added as a standard. The tissue was chopped into 0.25- to 1.0-mm segments in 1 mL of solution A [24 mL $MgSO_4$ buffer (ice-cold); 25 mg dithiothreitol; 500 μL propidium iodide stock (5.0 mg propidium iodide in 1.0 mL double distilled H_2O); 625 μL Triton X-100 stock (1.0 g Triton X-100 in 10 mL ddH_2O)]. The homogenate was filtered through a 30-μm nylon mesh into a microcentrifuge tube and centrifuged at high speed (13 000 RPM) for 20 s. The supernatant was discarded, the pellet was resuspended in 400 μL of solution B [7.5 mL solution A; 17.5 μL RNAse (DNAse free)] and incubated for 15 min at 37°C before flow cytometric analyses.

The prepared material was analyzed in the University of Nebraska Flow Cytometry Core Research Facilities on a standard FACScan flow cytometer (Becton Dickinson Immunocytometry System, San Jose, CA). For each measurement, propidium iodide fluorescence area signals (FL2-A) from 1000 nuclei were collected by CellQuest software (Becton Dickinson Immunocytometry System, San Jose, CA). A live gate was set with the FL2-2 and FSC parameters allowing the fluorescence measurement from nuclei to generate a histogram of FL2-A. Mean position of G0/G1 (nuclei) peak of the sample and internal standard were determined by analyzing the data by CellQuest software. The mean DNA content per plant was based on the 1000 scanned nuclei. The standards used for comparison were Stark diploid barley (10.68pg/2C) or hexaploid wheats (34.68pg/2C) for which the DNA content was known. Both barley and wheat were used as standards because of the large range in DNA content of the strains analyzed. The DNA content of Stark barley was determined with chicken erythrocytes (2.33 pg/2C; Galbraith et al., 1993) as the standard. Chinese Spring and Arapahoe wheat had the same nuclear DNA content (34.68 pg/2C) as determined with Stark barley as the standard. The nuclear DNA content of the hexaploid wheats used in this study as standards is equivalent to the DNA content (34.63 pg/2C) listed for Chinese Spring by Bennett and Leitch (1995). Because of the large number of plants analyzed in this study, numerous trays of the standards had to be planted. Arapahoe is a widely grown winter wheat and seed is more readily available than seed of Chinese Spring. The formula used for converting fluorescence values to DNA content was:

$$\text{Nuclear DNA content} = (\text{mean position of unknown peak})/(\text{mean position of known}) \times \text{DNA content of known standard.}$$

In Table 1, the strain or accession means are based on the four seedlings analyzed per strain. In Table 2, the means are based on the means of accessions with the same base genome. Autotetraploid mean values were divided by 2 to obtain a mean diploid value for calculating the mean genome values in Table 2. A simple statistical procedure using confidence intervals was used to compare mean DNA content of the strains (Steel and Torrie, 1960). A confidence interval was calculated for each mean by the following equation:

$$P(\bar{x}_1 - t_{0.05}s_{\bar{x}} < \mu < \bar{x}_1 + t_{0.05}s_{\bar{x}}) = 0.95,$$

where $t_{0.05}$ is the "t" statistic and $s_{\bar{x}} = s/n^{1/2}$ where n is the number of plants analyzed for a strain and s is their standard deviation. Accession means with overlapping confidence intervals were assumed to be similar. This is equivalent to conducting a simple t test to compare specific means (Steel and Torrie, 1960).

Estimated DNA content of the allopolyploid genera was determined by summing the means of the two probable con-

stituent diploid or polyploid progenitors. For example, the estimated mean DNA content of *Pascopyrum* (StStHHNs NsXmXm) was obtained by summing the mean DNA content values for *Elymus* (StStHH) and *Leymus* (NsNsXmXm) as per Dewey (1975). The variance of the estimated mean DNA content, which was calculated by summing the variances of the constituent genomes, was used to calculate a confidence interval for each estimated mean (Snedecor and Cochran, 1967).

RESULTS

The standard error of difference of the duplicate samples analyzed for each strain was 0.04 pg/nucleus indicating a high degree of precision for the flow cytometry procedure used in this study. In addition, low standard deviation values were obtained for each of the accessions analyzed (Table 1). Flow cytometry results for *Hordeum bulbosum*, both diploid and tetraploid, are smaller than those reported previously by Bennett and Smith (1976) but are larger than the previous report for *Thinopyrum elongatum* (Table 1). Our flow cytometry results are based on different strains and on a larger number of cells.

Agropyron

In the classification system of Barkworth and Dewey (1985), the genus *Agropyron* is restricted to the group of species with the P genome known as the crested wheatgrasses. *Agropyron* contains about 10 species and includes diploids, autotetraploids, and autohexaploids. We did not analyze the hexaploids in this study. Among the diploid *Agropyron* species, there are significant differences in DNA content. The DNA content of the *A. cristatum* strains as typified by the cultivar Ruff and PI 297870 is less than the DNA content of the *A. mongolicum* strains (Table 1). Hsiaso et al. (1986) indicated that the genome length of *A. cristatum* based on measurement of chromosome lengths was slightly shorter than that of *A. mongolicum*. One *A. cristatum* strain, PI 314600, was intermediate in DNA content between the *A. mongolicum* strains and the cultivar Ruff. The DNA content of the two tetraploid *Agropyron* strains, PI 249143 and the cultivar Nordan, suggest that they are based on the smaller P genome typified by the cultivar Ruff. If they were based on the larger P genomes, the expected DNA content value per nuclei would exceed the confidence interval obtained for the tetraploid *Agropyron* species(Table 1). Satellite or B chromosomes have been reported in *Agropyron* (McCoy and Law, 1965). Small differences in DNA content within *Agropyron* species may be due to the presence or absence of satellite chromosomes, but the large differences in genome size between *A. cristatum* and *A. mongolicum* exceeds the probable DNA content of satellite chromosomes since the average DNA content of an *Agropyron* chromosome is about 1 pg.

Pseudoroegneria

Pseudoroegneria is based on the St genome and contains about 15 species, but only one, *P. spicata*, is native to North America (Dewey and Barkworth 1985). The genus contains diploid and autotetraploid species (Table 1). The DNA content of *P. libanotica* and *P. stipifolia* accessions was significantly smaller than the DNA content of *P. aegilopoides*, *P. strigosa*, and one of the *P. spicata* strains (PI 232127). The other *P. spicata* strain (PI 232134) was intermediate in DNA content to the previously described small and large St genomes. On the basis of DNA content and the 95% confidence interval range for the autotetraploid *P. geniculata* strains, it appears that this autotetraploid species contains a small and a large St genome.

Hordeum

The perennial species in the genus *Hordeum* are based on the H genome (Dewey, 1984). The nuclear DNA content of diploid species of this genus also varied significantly ranging from 8.9 pg DNA/2C for *H. flexuosum* and *H. haplophilum* to 10.0 pg DNA/2C for the two strains of *H. stenostachys* (Table 1). Only one autotetraploid, a *H. bulbosum* strain (PI 343189), was analyzed. Its DNA content was consistent with the expected DNA content considering the mean DNA content and confidence interval of the diploid *H. bulbosum* strain (PI 318649). The H genome is one of constituent genomes of many of the *Elymus* species.

Psathyrostachys

Psathyrostachys contains about 10 diploid species (Barkworth and Dewey, 1985) and they all have the Ns genome. The *Psthyrostachys* strains analyzed in this study had the largest DNA contents of the diploid species analyzed in this study (Tables 1 and 2). Diploid Russian wildrye (*P. juncea*), which is used extensively as a pasture and rangeland grass in the Northern Plains of the USA and Canada, had significantly smaller DNA content than the DNA content of *P. fragilis* and *P. stoloniformis*. The Ns genome is one of the base genomes of the *Leymus* species. Since DNA content variation exists among the diploid *Psathyrostachys* species, it would be expected that variation in DNA content would exist among *Leymus* species.

Thinopyrum

Thinopyrum contains diploid, autotetraploid, and allotetraploid species and is based on the E (formerly J) and St genomes. It has recently been demonstrated that there are two forms of the E genome, E^b and E^e, as indicated by a genome specific RAPD (random amplified polymorphic DNA) marker (Zhang and Wang, 1996). The polyploid species that contain the E genome may have one or both genome types (Zhang and Wang,1996; Zhang et al., 1996). The diploid E^bE^b genome as typified by two *T. bessarabicum* strains had 14.9 pg DNA/2C (Tables 1 and 2). The diploid E^eE^e genome as typified by *T. elongatum* had 12.0 pg DNA/2C. The E^b and E^e genomes differed significantly in DNA content (Table 1). *Thinopyrum junceiforme* is an allotetraploid based on the E genome. The two strains

of *T. junceiforme* analyzed differed significantly in DNA content (Table 1). Our results indicate that *T. junceiforme* strain D3463 may have an $E^eE^eE^eE^e$ genomic constitution but that *T. junceiforme* strain PI 297873 probably has an $E^eE^eE^bE^b$ genomic constitution. Because the two stains of *T. junceiforme* differed in size, the 95% confidence interval for the genomic combination EEEE is large (Table 2). The expected DNA content/nuclei of a plant with the genomic configuration EEEE based on a summation of the DNA content of the base genomes lies within this confidence interval.

Thinopyrum caespitosum is an allotetraploid that contains the E and St genomes (Liu and Wang, 1993). The expected DNA content/nuclei of a plant with the genomic configuration EEStSt, based on a summation of the DNA content of the base genomes, lies within the confidence interval for DNA content measurements made on *T. caespitosum* plants (Table 2). On the basis of DNA content size, *T. caespitosum* probably contains the E^e genome. The intermediate wheatgrass cultivars that were analyzed typify the two types of intermediate wheatgrass (Table 1). The cultivar Slate (*Thinopyrum intermedium* subsp. *intermedium*) represents the "Intermediate" wheatgrass type; the cultivar Manska (*Thinopyrum intermedium* subsp. *barbulatum*) represents the "Pubescent" wheatgrass type. The DNA content/nuclei of the two cultivars are similar (Table 1). Genome-specific RAPD markers were recently used to determine genome components of *T. intermedium* and *T. ponticum* species (Zhang and Wang,1996; Zhang et al., 1996). Their results indicate that *T. intermedium* had E^b and/or E^e and St genomes in its allohexaploid genome, whereas *T. ponticum* had three E genomes (probably 2 E^b and 1 E^e) and two St genomes in its haplome. If the assumption is made that *T. intermedium* developed by the hybridization of plants with the E^eE^e and E^eE^eStSt genomes with subsequent doubling of the chromosomes of the hybrid F_1 plants, the expected DNA content/nuclei of an intermediate wheatgrass plant with the genomic configuration $E^eE^eE^eE^eStSt$ is 31.8 pg/2C, which is larger than the upper limit of 95% confidence interval for the measured DNA content of the intermediate wheatgrasses (Table 2). Assuming hybridization of plants with the EEStSt and EEEEStSt genomes, the estimated mean DNA content for *T. ponticum*, which has the EEEEEEStStStSt genomic configuration, is 45.91 pg, which is within the 95% confidence interval of the measured DNA content.

Elymus

The genus *Elymus* contains allopolyploid species that have the StStYY, StStYYPP, StStHH, and StSt genomic configuration. The diploid origin of the Y genome is unknown (Dewey, 1984). The two species that have the StStYY tetraploid configuration, *E. abolinii* and *E. ciliaris*, differed in mean nuclear DNA content (Table 1). The difference in nuclear DNA content between these two species could be due to differences in size of the constituent St or Y genome size. The estimated size of the YY diploid genome, as determined by difference, was approximately 9.3 pg/2C. The nuclei DNA content of *E. alatavicus* (StStYYPP) was 30.3 pg/nucleus (Table 1). The expected DNA content/nuclei of a plant with the same genomic configuration based on a summation of the DNA content of the base genomes StStYY and PP lies within the 95% confidence interval for *E. alatavicus* (Table 2). Several species of *Elymus* have the genomic configuration StStHH including *E. canadensis* and *E. lanceolatus*. The genomes of these two species differ significantly in DNA content (Table 1). Gabraith et al. (1983) used flow cytometry to determine nuclear DNA content of an array of species. They reported that *E. canadensis* had 21.6 pg/2C DNA which is equivalent to the value obtained in this study. There also is variation in DNA content among the other *Elymus* species with the allotetraploid configuration StStHH. Variation in DNA content among these species would be expected since variation in DNA content was found among species of their diploid progenitors. On the basis of component genomes (Table 2), the mean DNA content/nuclei of some *Elymus* species with the StStHH genomic configuration were outside the confidence interval range for all species with this genomic configuration and outside the estimated confidence interval range.

Leymus

Leymus species are allotetraploids that have the NsNsXmXm genomic configuration. The Xm genome previously was believed to be the J (= E) genome (Dewey, 1984) but as indicated previously Zhang and Dvorak (1991) and Wang and Jensen (1994) using molecular and cytogenetic analyses, respectively, determined that the E genome is not found in *Leymus*. The diploid XmXm genome has an estimated DNA content of 2.7 to 7.7 pg/2C (Table 2) which would make it significantly smaller than the diploid E^eE^e genome which has an average of 12.0 pg/2C. The results of this study strongly support the research that indicates that the E genome is not found in *Leymus*. There are significant differences in DNA content among the *Leymus* species (Table 1).

Pascopyrum

Pascopyrum contains a single species, *Pascopyrum smithii* or western wheatgrass, and is believed to have originated as the product of hybridization between beardless wildrye, *Leymus triticoides* (Buckl.) and thickspike wheatgrass (*Elymus lanceolatus*) or closely related species (Dewey, 1984). The expected DNA content of a western wheatgrass plant produced by the hybridization of plants with the genomic configuration described by Dewey (1975) is 39.47 pg/2C, which is larger than the upper limit of the 95% confidence interval for the DNA content of the three western wheatgrass strains analyzed in this study (Table 2).

This report presents the first information on DNA content for most of the species that were evaluated. In a previous report, Hsiao et al. (1986) determined relative genome size of diploid species reprenting the P, E^e, E^b, St, Ns, and H genomes by summing the length of chromosomes for each species. Thirteen species evaluated by Hsiao et al. (1986) were included in this study. The correlation between genome size as determined by

genome length (micrometers) and DNA content (pg/2C) was $r = 0.84$ (significant at the 95% level of probability).

DISCUSSION

DNA content of diploid species of plants can change via an array of genetic processes including deletion or insertion of chromosomal fragments during meiosis (Schultz-Shafer, 1980). Changes in DNA content also can be due to differences in repeated DNA sequences. Alteration in DNA content and the resulting change in gene expression is part of the speciation process. Our results indicate that genomes of the perennial Triticeae differ significantly in DNA content as measured by flow cytometry. DNA content also varies among the diploid species within each of the following genera: *Agropryon*, *Pseudoroegneria*, *Hordeum*,*Psathyrostachys*, and *Thinopyrum*. These genera are defined on the genomic system of classification which uses the degree of normal pairing of homologous chromosomes of interspecific and intergeneric hybrids during meiosis as a primary classification criterion. Our results indicate that related subsets of genomes can vary in size as measured by DNA content and on the basis of previous research that was extensively reviewed by Dewey (1984) still have homologous chromosome pairing during meiosis in interspecific and intergenomic hybrids. The processes that enable this to occur are not known. In *Agropyron*, *Pseudoroegneria*, *Hordeum*, *Psathyrostachys*, and *Thinopyrum*, genomic mapping studies will be needed to explain the genetic basis for the differences in genome size within the P, St, H, Ns, and E genomes.

Since significant differences in genome size were found to exist among and within the base genomes of the perennial Triticeae as determined by analysis of diploid species, it is not surprising that significant differences were found among auto- and allopolyploids that have the same genomic configuration in *Thinopyrum*, *Elymus*, and *Leymus*. The DNA content of the alloploid species, *T. intermedium* and *Pascopyrum smithii*, was significantly smaller than expected on the basis of DNA content of their constituent genomes. These results clearly indicate that DNA was lost, probably as chromosome fragments, in the evolutionary development of these allohexaploid wheatgrasses from their diploid progenitors. In summary, our results clearly demonstrate that gain or loss of nuclear DNA occurred during the evolution of the perennial Triticeae and was a part of the species development process.

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