

**Meiotic Stability of Interspecific Hybrids of Snake River ×
Thickspike Wheatgrasses**

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ABSTRACT

Based on genomic makeup and partial hybrid fertility, Snake River wheatgrass (proposed name *Elymus lanceolatus* ssp. *wawawaiensis*) has been recognized since 1986 as a subspecies of *E. lanceolatus* (Scribn. & J.G. Smith) Gould. Thus it is conspecific with thickspike wheatgrass [*E. lanceolatus* ssp. *lanceolatus* (Scribn. & J.G. Smith) Gould]. However, these two wheatgrasses display distinct morphological features, physiological traits, geographical distributions, and habitat preferences. Hybridization of the two subspecies generates valuable germplasm with considerable heterosis, but the hybrids are partially male sterile. Our objective was to determine the degree and nature of hybrid male sterility. Mean chromosome pairing (and pollen stainability) was 0.11 I + 13.90 II + 0.02 IV (84%) for 'Secar' Snake River wheatgrass and 0.10 I + 13.83 II + 0.01 III + 0.05 IV (78%) for 'Bannock' thickspike wheatgrass, the parents in the original cross, 0.60 I + 13.61 II + 0.01 III + 0.04 IV (55%) for three F₁ hybrid populations, and 0.09 I + 13.92 II + 0.01 III + 0.03 IV (80%) and 0.44 I + 13.66 II + 0.01 III + 0.05 IV (64%) for backcross populations to Bannock thickspike wheatgrass and BBR-syn Snake River wheatgrass, respectively. The low frequency of multivalents in the hybrids indicated that male sterility resulted from some degree of genetic incompatibility rather than a major chromosomal rearrangement. Because hybrid and backcross populations possessed reasonable levels of pollen stainability and chromosome abnormalities were absent, they should respond readily to selection for high pollen stainability.

SINCE 1986, Snake River wheatgrass has been recognized as a distinct taxonomic entity (Carlson, 1986). Prior to this time, this grass was confused with the more common and widespread bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Löve], which it resembles morphologically. These two grasses were initially differentiated on their genomic makeup. Snake River wheatgrass ($2n = 28$) is an SSHH allotetraploid (Carlson, 1987), while bluebunch wheatgrass ($2n = 14, 28$) is a diploid-autotetraploid series of the 'S' genome (Dewey, 1984). Fertility in hybrids between Snake River and thickspike wheatgrass, also an SSHH allotetraploid (Dewey, 1984), suggests that these two allogamous grasses are closely related (Jones et al., 1991). For this reason, J.R. Carlson proposed the trinomial *E. lanceolatus* ssp. *wawawaiensis* for Snake River wheatgrass, making it conspecific with thickspike wheatgrass.

The geographical distribution of Snake River wheatgrass is limited. To date, 50 accessions have been assembled, but these represent only 10 Pacific Northwest counties (Fig. 1). This grass is commonly found growing on steep canyon slopes (Carlson, 1986). In contrast,

Hitchcock (1951) describes thickspike wheatgrass habitat as plains and sandy shores from Michigan to British Columbia, south to Illinois, Nebraska, Colorado, Nevada, and Oregon. The distinct habitat preferences of the two grasses may explain why naturally occurring hybrid populations have not been reported.

The two grasses are distinguished by visual examination of spikes and several vegetative characters (Jones et al., 1991). Snake River wheatgrass is always awned, while thickspike wheatgrass is usually awnless (Fig. 2). Snake River wheatgrass is strictly caespitose, while thickspike wheatgrass is highly rhizomatous. Immature leaves of Snake River wheatgrass are covered with dense pubescence but lack marginal barbs, while those of thickspike wheatgrass are considerably more glabrous and exhibit barbs.

Hybridizing thickspike wheatgrass with Snake River wheatgrass may improve the latter's grazing tolerance and remove its awn. Thickspike wheatgrass is more tolerant of clipping than Snake River wheatgrass, particularly when plants are under stress (Jones and Nielson, 1993). Damage sustained by spring clipping of the hybrid population D38 was intermediate to that of Secar Snake River wheatgrass and Bannock thickspike wheatgrass. Analysis of segregating populations indicated that inheritance of the awn is primarily controlled by a single major recessive gene (Jones et al., 1991). Intersubspecific hybrid populations exhibit heterosis and offer considerable opportunity for rangeland seedings if low fertility can be overcome (Jones et al., 1991). Meiotic chromosome behavior of hybrids has not been investigated and the degree of male sterility in hybrids is unknown. Our objectives were to compare the genomes of the two grasses, to determine if any major chromosomal rearrangements separate the taxa, to assess the male sterility of hybrid populations, and to determine if backcross populations show improved male fertility.

MATERIALS AND METHODS

Seven populations were analyzed in this study. Parent populations were Secar Snake River wheatgrass (Morrison and Kelley, 1981) and Bannock thickspike wheatgrass. These populations trace to collections made at Lewiston, ID, and The Dalles, OR, respectively. All three F₁ populations were generated from crosses between individual plants of the two parent populations. Thus, they were related, but distinct. Backcrosses were made to BBR-syn parents, plants from several Snake River wheatgrass populations selected for resistance to bluegrass billbug (*Sphenophorus parvulus* Gyllenhal), and to Bannock. Both cytoplasms were represented within all F₁ and backcross populations, but cytological samples were taken without regard to cytoplasm. Therefore, the two cytoplasms could not be compared. Spikes were fixed in Carnoy's (6 parts 95% ethanol : 3 parts chloroform : 1 part glacial acetic acid) solution and the pollen mother cells were stained with 1% acetocarmine. All population means were based on measurements recorded on 16 to 18 plants per population. Plant means

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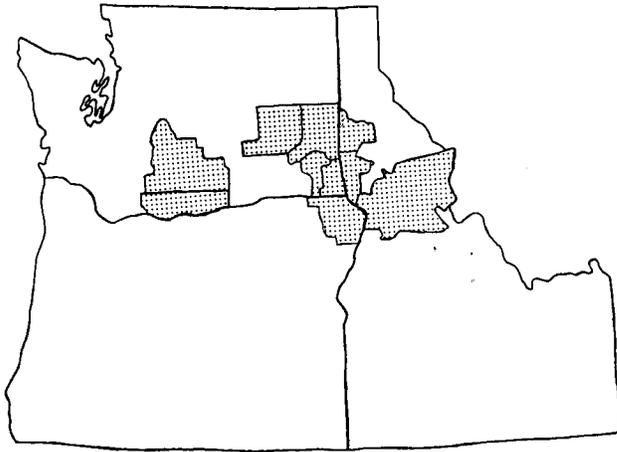


Fig. 1. Ten Pacific Northwest counties where Snake River wheatgrass has been collected.

of metaphase I configurations were based on 25 pollen mother cells per plant. Plant means of number of micronuclei per quartet were based on 100 quartets per plant. Pollen viability was estimated by stainability in I_2 -KI of 200 pollen grains per plant. Pollen stainability means were bounded by a 95% confidence interval. Samples for meiotic analyses and pollen stainability were taken from the same plants.

RESULTS AND DISCUSSION

Analysis of metaphase I cells indicated normal chromosome pairing in Secar Snake River and Bannock thickspike wheatgrasses (Table 1). The number of total bivalents approached 14; 93% were ring bivalents. Fourteen ring bivalents were observed in at least one cell of all 17 Secar plants and 17 of 18 Bannock plants. Univalents, multivalents, and micronuclei were consistently present in parent populations in low frequencies. Pairing in the three hybrid populations was only slightly lower than in the parents. The total number of bivalents in hybrids was similar to that of the parents, but the hybrids averaged about one less ring bivalent and one more rod bivalent (Table 1). There were slightly more univalents and micronuclei in the hybrids than in the parents. Fourteen ring bivalents were observed in at least one cell of 17 of 18 F_1 -1 plants, 17 of 18 F_1 -2 plants, and 16 of 17 F_1 -3 plants, indicating complete chromosome homology of the two grasses. The hybrids exhibited infrequent chromosome abnormalities, including bridges at telophase I (Fig. 3) and II. The backcross F_1 × Bannock thickspike population had numbers of ring and rod biva-

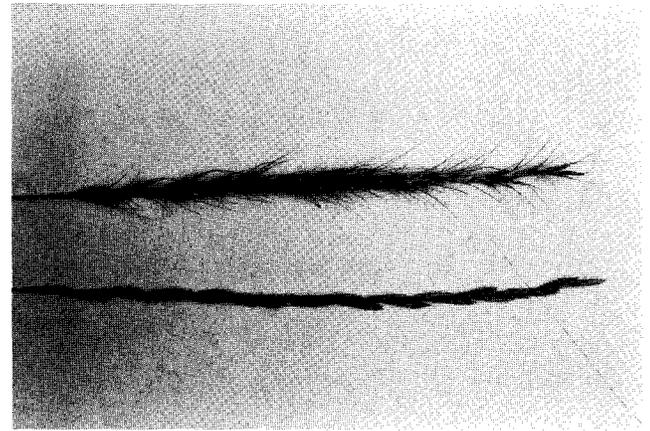


Fig. 2. Comparative spike morphology of Snake River wheatgrass (top) and thickspike wheatgrass (bottom).

lents similar to those seen in the Secar and Bannock parents, but the backcross F_1 × BBR-syn Snake River wheatgrass population was no better than the F_1 hybrids. c -Values were above 0.90 for all seven populations, another indication of high chromosome homology between Snake River and thickspike wheatgrass chromosomes.

Multivalents were found in all seven parents, F_1 hybrids, and backcross populations, indicating that a low frequency of pairing between homoeologous S and H chromosomes is a normal feature in these grasses. The presence of a chain hexavalent in each of two cells of a single Bannock plant was unexpected (Fig. 3). Hexavalents were not found in any other plants of Bannock or in any other population. This particular Bannock plant had only 42% pollen stainability, more than 35% lower than the Bannock population mean. The simplest explanation for the chain hexavalent is a combination of a balanced reciprocal translocation and a duplication-deletion chromosomal rearrangement involving one of the chromosomes of the translocation.

Pollen stainability was about 81% in the parent populations and about 55% in the F_1 populations. Pollen stainability of the backcross population to Bannock thickspike wheatgrass was significantly greater than that of the three hybrid populations (Table 1). Pollen stainability across the seven *Elymus* populations was negatively correlated with micronucleus ($r = -0.71$; $P < 0.10$) and univalent ($r = -0.79$; $P < 0.05$) frequencies and positively correlated with c -value ($r = 0.90$; $P < 0.01$).

Table 1. Meiotic chromosome pairing, micronuclei per quartet, and pollen stainability (%) in 'Secar' *Elymus lanceolatus* ssp. *wawawaiensis*, Bannock *E. lanceolatus* ssp. *lanceolatus*, their F_1 hybrids, and backcross populations (X BBR-syn, Bannock).

Population	No. plants	Metaphase I chromosome configurations								c -Value	Micronuclei per quartet	Pollen stainability (%)
		I	Rod II	Ring II	Total II	III	Chain IV	Ring IV	Total IV			
Secar	17	0.11	0.91	12.99	13.90	—	0.01	0.01	0.02	0.96	0.01	84.1 ± 4.2
Bannock	18	0.10	1.03	12.80	13.83	0.01	0.01	0.04	0.05	0.96	0.01	77.7 ± 6.6
F_1 -1	18	0.20	1.70	12.15	13.85	—	0.02	0.00	0.02	0.93	0.04	55.9 ± 5.5
F_1 -2	18	0.81	1.84	11.70	13.54	0.01	0.01	0.01	0.02	0.90	0.19	57.1 ± 7.3
F_1 -3	17	0.79	1.95	11.49	13.44	0.01	0.02	0.06	0.08	0.90	0.13	51.9 ± 7.5
F_1 × BBR-syn	17	0.44	2.07	11.59	13.66	0.01	0.02	0.03	0.05	0.91	0.17	63.9 ± 6.2
F_1 × Bannock	16	0.09	1.30	12.62	13.92	0.01	0.01	0.02	0.02	0.95	0.02	80.2 ± 4.1

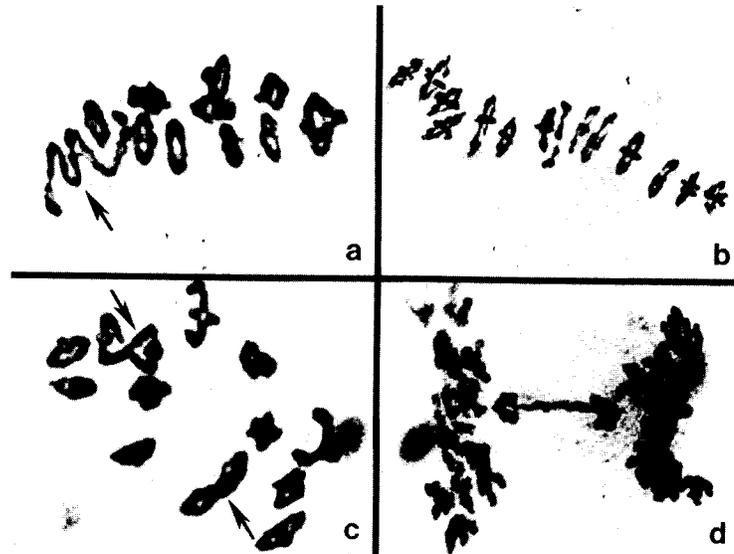


Fig. 3. a. Hexavalent (arrow) in Bannock thickspike wheatgrass. b. 14 bivalents (13 rings + 1 rod) in a hybrid. c. 10 bivalents (8 rings + 2 rods) + 2 quadrivalents (arrows) in a hybrid. d. Bridge at Telophase I in a hybrid.

Like numbers of bivalents, pollen stainability of the backcross population to BBR-syn Snake River wheatgrass was not significantly higher than the hybrids. The backcross to Bannock thickspike wheatgrass exhibited a higher *c*-value and pollen stainability and lower frequencies of micronuclei and univalents than the backcross to BBR-syn Snake River wheatgrass. Perhaps backcrossing the F_1 hybrids to the original Bannock thickspike wheatgrass parent provided greater restoration of genetic balance, while backcrossing to a different Snake River wheatgrass parent, i.e., BBR-syn instead of Secar, provided less restoration of genetic balance.

Based on the paucity of abnormal meiotic configurations in the hybrids, we conclude that the versions of the S and H genomes in the two grasses are similar. The low multivalent frequency of the hybrids provided no evidence that the two taxa were distinguished by a major chromosomal rearrangement, such as a translocation. In a study with similar objectives, the multivalent pattern in *Agropyron mongolicum* \times *A. cristatum* hybrids indicated that these two species differed by a reciprocal translocation between a long and a short chromosomal segment (Hsiao et al., 1989). In this instance, where the two *Agropyron* parents differed by a major chromosomal rearrangement, pollen stainability was 35% in the hybrid compared with 74 and 87% in the *A. mongolicum* and *A. cristatum* parents, respectively. Thus, the pollen stainability of these *Agropyron* parents was similar to that of our *Elymus* parents, but the *Agropyron* hybrid had about 20% lower pollen stainability than our *Elymus* hybrid.

In conclusion, even though pollen stainability declined in F_1 hybrids relative to the parents, it was still reasonably high. A single backcross to Bannock thickspike wheatgrass restored pollen stainability to the parental level. The F_1 and backcross populations should respond to selection for high pollen stainability because this trait was not determined by chromosomal rearrangement.

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