Table I. In vitro mutagenic evaluation of 5TDG and gossypol in the *Salmonella*-microsome assay

Contraceptive	Mean number of revertants per plate							
	TA 98		TA 100		TA 1538		TA 1535	
	−S−9	+S−9	−S−9	+S−9	−S−9	+S−9	−S−9	+S−9
Spontaneous (control)	26	44	137	163	18	30	14	16
DMSO (control)	25	38	145	162	17	21	13	25
5-thio:								
250 μg/plate	28	41	109	135	23	39	13	16
500 μg/plate	23	48	129	116	23	43	14	18
1000 μg/plate	20	46	170	164	17	34	12	15
Gossypol:								
50 μg/plate	28	39	151	110	24	22	17	16
100 μg/plate	30	30	137	129	22	58	16	17
250 μg/plate	35	54	109	122	30	25	20	15
500 μg/plate	—	—	—	—	—	—	—	—

-, indicates no background mat

obtained from these concentrations in the presence or absence of liver homogenates, when compared to the spontaneous and DMSO controls, showed a similar trend in formation of the revertant colonies from histidine$^-$ to histidine$^+$ in all four tester strains.

Four concentrations of gossypol were tested in the *Salmonella*-microsome assay. Like 5TDG, gossypol, at 50 μg, 100 μg, or 250 μg/plate did not significantly increase the revertant numbers over the control level. At 500 μg/plate, gossypol exerted toxic effects on all four strains. The toxicity was determined by the absence of a background mat.

In conclusion, the results seem to indicate that both 5TDG and gossypol are not mutagenic at the tested concentrations. Previous studies with 5TDG revealed its nonmutagenic nature in the sex-linked recessive lethal test on *Drosophilia melanogaster* and on mouse chromosomes[8]. In a preliminary study, gossypol was not shown to be mutagenic in the Ames test[13]. Although no genotoxic properties of either contraceptive could be detected in the present testing method, it should be noted that both 5TDG and gossypol are known to induce abnormalities in the spermatogenic cells and to damage sperm. In addition, the long-term effects of the chemicals are not known and their weak mutagenic properties cannot be excluded. Because of their reversible male antifertility action and the promise they hold as potential "male pills," it is essential that additional sensitive and careful tests be made before any definite conclusions are made about safety in terms of mutagenicity of 5-thio-D-glucose and gossypol acetic acid.

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Cytogenetics of hybrids of *Elymus canadensis* × *Hordeum vulgare*

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ABSTRACT: The cytogenetics of intergeneric hybrids between *Elymus canadensis* ($2n = 4x = 28$, SSHH) and *Hordeum vulgare* cv. Manker ($2n = 14$, HH) is described. The hybrids had a somatic complement of $2n = 3x = 21$, SSHH, with a mean chromosomal relationship of 19.51 univalents, 0.49 rod bivalents, 0.04 ring bivalents, 0.12 trivalents, 0.01 quadrivalent, and a low chiasmata frequency of 0.81 per cell, suggesting lack of genome homology in the SHH hybrids. The hybrids are male- and female-sterile. No backcross seed or amphiploid seed has yet been obtained. Hybrids from the reciprocal combination are reported, together with a discussion involving genome homology between *H. vulgare* and *Elymus* species.

INTERGENERIC HYBRIDIZATION research at the International Maize and Wheat Improvement Center (CIMMYT) was initiated in 1973, with early emphasis on obtaining hybrid combinations between *Hordeum*, *Triticum*, *Agropyron*, and *Secale* species. In 1976 *Elymus* species were included in the intergeneric hybridization program. Although components such as drought, salt, or disease were not tested, knowledge of plant habitat and morphology was adequate to include some *Elymus* species as germ plasm donors. Hybrids have since been obtained for *Triticum aestivum* × *E. giganteus*, and *Hordeum vulgare* × *E. patagonicus*[9,10].

This paper reports the morphocytogenetics of a few *E. canadensis* ($2n = 4x = 28$, SSHH) *H. vulgare* ($2n = 4$, HH) hybrids. It also elucidates the chromosome pairing homology between the genomes of *E. canadensis* and *H. vulgare*.

Materials and Methods

Elymus canadensis ($2n = 4x = 28$, SSHH) and *Hordeum vulgare* L. cv. Manker (M16), $2n = 14$, HH, plants were grown in pots in a greenhouse maintained at 26.6°C/15.5°C (14/10 hours day/night) and approximately 45 percent relative humidity. *E. canadensis* florets were clipped, emasculated, pollinated with *H. vulgare* cv. Manker pollen after two days, and allowed to develop for approximately 20 days.

The embryos were excised and cultured on Taira's media for small embryos (T. Taira, pers. comm.). Upon differentiation, the plantlets were transferred to jiffy-7 peat pots,

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kept in the greenhouse for one month before growing seedlings were transferred to a soil media in pots. These were maintained in the same greenhouse conditions as the parental plants.

Root tips were periodically sampled and processed according to the procedure of Mujeeb et al.[13]. From each of the six hybrids, three spikes were sampled for meiotic analysis. Metaphases were observed for chromosome relationships using 2 percent propionic-orcein staining. Mean chromosome relationships were expressed over 79 pollen-mother-cells (PMC's).

Results and Discussion

E. canadensis L., Canadian wildrye, is an allotetraploid with a genomic composition of SSHH. The SS denotes the *Agropyron spicatum* genome, and HH represents a genome from *Hordeum*. The species is a strict allotetraploid with virtually no capacity for autosyndetic pairing[4,5]. Natural *E. canadensis* and *H. jubatum* hybrids were reported by Bowden[2] and *H. bogdanii* × *E. canadensis* hybrids were obtained by Dewey[3]. The overall pairing in this hybrid suggested that about half of the *H. bogdanii* genome chromosomes were fully homologous with *E. canadensis*, and the other half were partially homologous. The hybrids were sterile, although up to 7 univalents + 7 bivalents were observed in metaphase I chromosomes.

The mean meiotic relationships in the six *E. canadensis* × *H. vulgare* cv. Manker hybrids (Table I) over 79 PMC's were 19.51 univalents, 0.49 rod bivalents, 0.04 ring bivalents, 0.12 trivalents 0.01, quadrivalents. Consequently, all hybrids deviated remarkably from the H genome homology, as compared to Dewey's[3] results from *H. bogdanii* × *E. canadensis* crosses. The seven univalents were expected from the S genome *A. spicatum* derivation, but the lack of observed closed bivalents, other than those present in meiocytes with 19 univalents, + 1 ring bivalent (2.5 percent) and 15 univalents + 2 bivalents + 1 ring bivalent (1.3 percent), leaves in doubt the H genome homology in *E. canadensis* × *H. vulgare*. Dewey[3] raised the question of whether *H. bogdanii* was the only source of the H genome. We may draw another conclusion from the present chromosomal relationship in *E. canadensis* × *H. vulgare*, making it interesting to assess the interspecific pairing between *H. vulgare* and *H. bogdanii* as a preliminary step. If normal homology prevails it would be highly probable that the H genome of *E. canadensis* did indeed undergo modifications, incorporating contributions to its complement from other Asian and North American diploid *Hordeum* species as suggested by Dewey[3]. Several *H. vulgare* × *H. bogdanii* interspecific hybrids have been produced. The embryos were embryo-cultured despite the presence of endosperm. Validity of the cross has yet to be determined through meiotic analysis and F_2 segregation.

All *E. canadensis* × *H. vulgare* hybrids were sterile, and attempts to induce amphiploidy so far have been unsuccessful. Most meiocytes reflect the meiotic stability of the hybrids (Figure 1*A–C*) with 21 univalents, one heteromorphic bivalent, and one rod bivalent. It was only in a low frequency of PMC's that instability was detected as in Figure 1*D* with 25 univalents. No somatic instability was observed, and cells had $2n = 3x = 21$ chromosomes (Figure 2). The meiotic variation observed is commonly seen in intergeneric hybrids within the Triticeae[6,7,9–14]. Obtaining backcross-1 (BC_1) progeny in *H. vulgare* crosses with *T. turgidum*, *T. aestivum*, or *T. aestivum* with *E. giganteus*, *Agropyron junceum*, or *T. turgidum* with *A. junceum*, was significantly aided by the meiotic nonreduction phenomenon, where (except for *T. aestivum* × *A. junceum*) difficulties of inducing amphiploidy otherwise would have hampered the advancement of intergeneric programs[10–12]. It has not been possible to obtain amphiploids or BC_1 progeny from *H. vulgare* × *E. patagonicus* hybrids (unpub. data), and a similar level of difficulty exists for hybrids of *E. canadensis* × *H. vulgare*. Post-pollination treatments with gibberrellic acid (GA_3, 75 ppm), which gave successful results in other crosses[1,6–11] has not been successful in these hybrids. We have recently obtained 4 reciprocal *H. vulgare* × *E. canadensis* hybrids and are continuing efforts to obtain BC_1 seed by

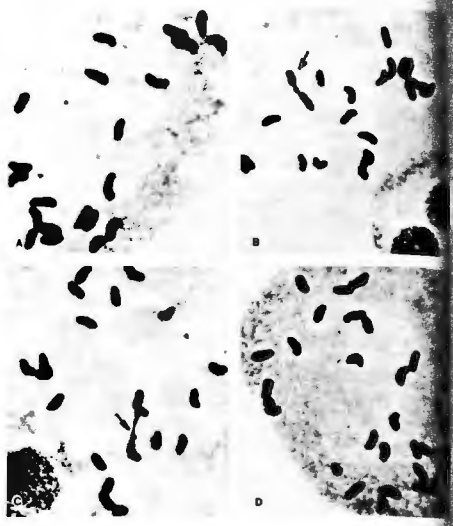

FIGURE 1 Meiotic relationships of *E. canadensis* × *H. vulgare* hybrids. *A*—21 univalents; *B*—19 univalents + 1 bivalent (heteromorphic) arrow; *C*—19 univalents + 1 bivalent arrow; *D*—25 univalents (meiotic instability).

Table I. Mean chromosome associations in *Elymus canadensis* L. ($2n = 4x = 28$, SSHH) × *Hordeum vulgare* L. cv. Manker ($2n = 14$, HH) hybrids, $2n = 3x = 21$, SHH

No. cells	Chromosome associations					% of total	$\bar{X}$ ta mean frequency
	I	II*	II†	III	IV		
48	21					60.8	
18	19	1				22.8	
2	19		1			2.5	
1	18			1		1.3	
3	17	2				3.8	
1	16	1		1		1.3	
1	15	2	1			1.3	
1	14	2		1		1.3	
1	13	2		1	1	1.3	
1	12			3		1.3	
1	10	4		1		1.3	
1	4	4		3		1.3	
79	1541	39	3	10	1		0.81
Mean =	19.51	0.49	0.04	0.12	0.01		

* Rods
† Rings

FIGURE 2 Somatic chromosome complement of *E. canadensis* × *H. vulgare* with $2n = 3x = 21$, SHH.

FIGURE 3 Left to right: *Elymus canadensis*; *Elymus canadensis* × *Hordeum vulgare* hybrid; *Hordeum vulgare* cv. Manker (awns clipped on the central axis for the photograph).

introducing, in addition to the postpollination GA_3 treatment, a 2,4-dichloro phenoxyacetic acid treatment as a pre-pollination application, as Kruse[8] used effectively for hard-to-cross *T. aestivum* × *H. vulgare*.

The *E. canadensis* × *H. vulgare* hybrids phenotypically resemble the maternal parent more than *H. vulgare* (Figure 3). The tetraploid status of *E. canadensis* is evidently masking the diploid *H. vulgare* phenotype. This expression is consistent with the expression of an intergeneric hybrid phenotype between barley and wheat[11]. The reciprocal hybrids are still vegetative, but the foliar characteristics seem to resemble *E. canadensis* more than *H. vulgare*.

Intergeneric hybridization of *Elymus* was initiated because of the plant type expressed by the genus, together with its habitat that suits practical agriculture. Had the genomic relationships of the H genomes been consistent with the expectation inferred from Dewey's[3] observations, the *Elymus* × *Hordeum* hybrids would have served as an excellent bridge between *Triticum* and *Hordeum*. Also, it would have aided in *Hordeum* improvement where the *Elymus* × *Hordeum* hybrids undoubtedly would carry the genetic transfer potential expected from the H genome homology in *E. canadensis* × *H. vulgare* hybrids, or *H. vulgare* × *E. patagonicus* hybrids.

However, the low chiasmata frequency of 0.81 for the former (Table I), and only 4.95 for the latter (unpub. data) has changed our direction such that we need to examine more minutely the evolution of the H genome in tetraploid and hexaploid *Elymus*. To what degree intergeneric hybridization has contributed to the evolutionary changes from the present findings is merely speculation at this point.

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