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CYTOLOGICAL EVIDENCE FOR RECIPROCAL INTROGRESSION IN
AGROPYRON TRACHYCAULUM AND AGROPYRON SPICATUM

by

LAVAL M. PITTS

A THESIS SUBMITTED TO THE DEPARTMENT OF BOTANY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

BRIGHAM YOUNG UNIVERSITY

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This thesis, by LaVal M. Pitts, is accepted in its present form by the Department of Botany of Brigham Young University as satisfying the thesis requirement for the degree of Master of Science.

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TABLE OF CONTENTS

| | Page |
|---|------|
| ACKNOWLEDGEMENTS. | .iii |
| INTRODUCTION AND REVIEW OF LITERATURE | 1 |
| MATERIALS AND METHODS | 7 |
| RESULTS | 11 |
| DISCUSSION. | 20 |
| SUMMARY | 26 |
| REFERENCES. | 27 |

INTRODUCTION AND REVIEW OF LITERATURE

Extensive hybridization between species and even genera of the grass family, particularly in the tribe of Hordeae, has been reported in recent years (Boyle, 1963; Dewey, 1962; Dunford, 1958; Stebbins, 1956b; and others). Hybridization might be expected in grasses more often than in many other plants because closely related species often grow sympatrically in large numbers (Cugnac, 1937). Because grasses have light, wind borne pollen, it can be carried long distances. Many species are self-incompatible. Furthermore, many grass species are long-lived perennials and have efficient methods of vegetative reproduction so that even highly sterile hybrids can persist (Stebbins, 1956a).

Anderson (1945) and others have suggested that genes from one species may flow into another by backcrossing of the F_1 hybrid to one or both parents. Backcrossing produces descendants scarcely distinguishable from members of the parental types, and ultimately such backcrosses bring into the population system genes from the other species. This flow of genes provides new variation which may have evolutionary significance.

In nature, hybrids may be detected on the basis of pollen abortion, absence of viable seeds, or by intermediate morphological characters between two species growing in the same area (Snyder, 1950).

Johnson (1943, 1945) pointed out that identification of natural hybrids can be made with considerable certainty by means of careful field studies and even by means of examination of herbarium specimens. Introgressants, however, may be detected in the field only after keen observation and detailed studies (Benson, 1962).

Because most grass hybrids are highly sterile, reports of introgression in grasses have not been common. Peto (1930) reported complete sterility in hybrids of Agropyron trachycaulum and A. subsecundum, and Boyle (1963) reported no seeds in more than 2,500 florets of hybrids between Agropyron trachycaulum and Sitanion hystrix. Johnson (1963) reported complete sterility in Oryzopsis hymenoides X Stipa spp. hybrids. Stebbins (1946a) reported natural F₁ hybrids between Elymus glaucus X Sitanion jubatum, Agropyron trachycaulum X S. jubatum, A. parishii X S. jubatum, A. trachycaulum X E. glaucus, and A. trachycaulum X Hordeum jubatum to be completely sterile.

However, good gametes are produced occasionally in some grass hybrids making it possible for introgression to occur. There are many morphological evidences for introgression. Dewey (1962) reported that F₁ hybrids of Agropyron repens X A. desertorum produced on the average, about two seeds per spike. Shumway (1961) showed that introgression is common between Agropyron trachycaulum and A. subsecundum. Brown (1960) reported introgression in the genus Elymus. Dunford (1958) showed morphological evidence for introgression of A. spicatum into A. trachycaulum.

In addition to morphological evidence for introgression, Agropyron trachycaulum (Link) Malte and Agropyron spicatum Pursh, having different levels of chromosome numbers, provides an opportunity for cytological verification for introgression. Since A. trachycaulum has twenty-eight chromosomes in its somatic tissue and A. spicatum has fourteen chromosomes, it is possible to identify hybrids and introgressants by chromosome numbers alone.

Slender wheat grass (Agropyron trachycaulum) is a perennial bunchgrass found growing from Labrador to Alaska, south to the mountains of West Virginia, west to New Mexico and California including northwestern Mexico. It grows at nearly all altitudes but alpine plants are usually shorter with a denser spike which is commonly purplish in color (Hitchcock, 1950). A. trachycaulum grows 20 to 100 cm. tall with lax leaves which are 3 to 5 mm. wide. The spike ranges from thick and dense to long and slender, 6 to 25 cm. long. The awnless glume is 6 to 12 mm. long and almost covers the entire spikelet. The lemmas range in length from 8 to 12 mm. and are either awnless or awn-tipped. The small anthers are 1 to 1.5 mm. long. As noted above, A. trachycaulum has fourteen pairs of chromosomes (Peto, 1930; Hartung, 1946; Stebbins, 1950; Tateoka, 1956).

Slender wheatgrass was one of the first native species to be cultivated in the northwestern states, and one of the few native grasses to become of commercial importance to seedsmen in that region. Although it is found on all types of soil, A. trachycaulum grows best

in light, sandy-loam soils. It begins its growth early in the spring producing an abundance of palatable forage for all classes of livestock. The forage cures well in the field and furnishes nutritious feed for winter grazing. If cut while still young, it yields a high-quality hay. When planted in rows and cultivated, seed production is from 200 to 300 pounds per acre with 140,000 to 160,000 seeds per pound weighing 18 pounds per bushel (Hoover, 1948).

Slender wheatgrass has been reported to have hybridized with many grasses. Stebbins (1946a) and Stebbins and Singh (1950) found it to hybridize in nature with Elymus glaucus, Hordeum jubatum, Agropyron parishii, Sitanion hystrix, and S. jubatum. Stebbins (1946b) and Boyle (1955) have suggested that Elymus macounii is a sterile hybrid of A. trachycaulum and Hordeum jubatum. Stebbins (1946b) and Boyle (1963) have suggested that Agropyron saundersii is a hybrid of A. trachycaulum and Sitanion hystrix.

Agropyron spicatum (bluebunch wheatgrass) is a drought resistant perennial found growing on dry open areas from northern Michigan to Alaska, south to western South Dakota, New Mexico and California (Hitchcock, 1950). It is normally a bunchgrass but according to Dunford (1958) has active rhizomes when growing in some areas. Its growth habit is from 20 to 100 cm. tall with leaves 1 to 4 mm., ranging from flat to loosely involute. The spike is 5 to 15 cm. long, having a completely awnless glume 5 to 8 mm. long. The lemmas are 6 to 8 mm. long bearing awns 5 to 20 mm. long that bend more than

90° to the rachis when mature. The large anthers are 4 to 6 mm. long. It has seven pairs of chromosomes originally described by Peto (1930) and later confirmed by Hartung (1946) Stebbins and Pun (1953) and Tateoka (1956).

Agropyron inerme is an awnless variant of A. spicatum (Hartung, 1946) differing from it by only a few genes (Dunford, 1958). A tetraploid form of A. spicatum, apparently endemic to a local area in northern Idaho and eastern Washington, has been reported by Hartung (1946) and Jurgen (1962).

Being a cool season grass, A. spicatum starts its growth in early spring producing an abundance of basal leaves that remain palatable and nutritious throughout late summer and winter months. Depending upon moisture conditions in the springtime, seed production as high as 250 pounds per acre have been reported with about 95,000 seeds per pound (Hoover, 1948).

Agropyron spicatum has been reported by Stebbins (1950) to hybridize in nature with Elymus glaucus, A. trachycaulum, and Sitanion hystrix. Dunford (1958) and Shumway (1961) also found A. spicatum to hybridize with A. trachycaulum.

Artificial hybrids of A. trachycaulum X A. spicatum made by the author have awn and anther lengths somewhat intermediate between the two parents. On these bases above, natural hybrids of A. trachycaulum and A. spicatum can usually be detected in nature particularly when growing sympatrically. High sterility is also

common to both synthetic and natural hybrids.

In nature, natural hybrids are often associated with plants which, because of their sterility and morphological variation, appear to be backcross segregants. When the parents differ in chromosome numbers, as do A. trachycaulum and A. spicatum, hybrids and introgressants should be verifiable on cytological evidence alone. A natural population of Agropyron trachycaulum and Agropyron spicatum along with apparent F_1 hybrids and introgressants was selected for such cytological analysis.

Thistle Flat was chosen as the study site because well defined putative introgressants with both parents and F_1 hybrids are growing sympatrically. Thistle Flat is a subalpine meadow in the Wasatch Plateau located about ten miles east of the Great Basin Experiment Station, Sanpete County, Utah. It lies at about 9,000 feet near the divide between the Colorado River and Great Basin drainage systems. It slopes to the southeast at about 5 to 30°, covering about ten acres of rich clay-loam soil densely populated with a variety of forbs plus native and introduced grasses. At this study site barriers against hybridization between A. trachycaulum and A. spicatum appear to be unusually weak inasmuch as the hybrids and apparent backcross progeny make-up a major portion of the population.

MATERIALS AND METHODS

Artificial hybrids between Agropyron trachycaulum and A. spicatum were made in the greenhouse during the early spring of 1962. The parent plants, used in the crosses, were exposed to winter temperatures until February 1962. Being vernalized, they were brought into the greenhouse under twelve hours of light which caused them to flower early. In April, five spikes of Agropyron trachycaulum were hand-emasculated and placed in a position to receive an abundance of pollen from A. spicatum. To prevent contamination from foreign pollen both flowering culms were bagged together with a paper towel. After allowing a sufficient amount of time for fertilization to occur, the towel was removed and the seeds were permitted to mature before being harvested. From the crosses made sixteen seeds were harvested in early May and planted later the same month. Only eight seedlings of the thirteen germinated seeds survived to maturity. Five have flowered and have been scored morphologically and cytologically. Since the other three had not yet flowered at the time this was written, they were studied cytologically only.

During the summer of 1962, a careful study of apparent hybrids and their putative parents (A. trachycaulum and A. spicatum) was made at Thistle Flat. On the basis of variation in awn and anther lengths fifteen plants including both parental types were selected for study. A wooden stake bearing a culture number was driven into the ground at

the base of each predetermined plant for subsequent reference. Clonal samples of each culture were transplanted to the greenhouse at Brigham Young University for detailed analysis.

Seed fertility was determined for each culture by hand threshing each of the mature spikes. In order to identify any seed which might have been missed in the threshing, all the chaff was planted in sterilized soil.

Morphological characters of each culture from Thistle Flat were scored from herbarium specimens. The average awn lengths were obtained from measurements of several awns near the middle region of the spike. Anther size was determined from measurements of ten anthers from the lower florets of spikelets attached near the middle of the spike. Pollen fertility was obtained from a count of the turgid pollen grains which were fully stained when treated with cotton-blue stain.

Additional morphological characters were scored from both artificial hybrids and clonal samples of the natural population growing in the greenhouse. Microscopic examination of stomata and guard cells on the lower leaf epidermis showed all cultures to be very similar. Microscopic examination of leaf surface appendages, and lodicules also proved to be of little diagnostic value as they were found to be very similar in all cultures studied.

Root-tips from clonal samples were prepared for chromosome counts by pre-fixing in 0.2% colchicine for a period of four hours at room temperature, and then fixing overnight in a mixture of three parts absolute ethyl alcohol and one part glacial acetic acid (Setterfield et al, 1954). After washing in 70% ethyl alcohol, the

root-tips were stored in fresh 70% ethyl alcohol until it was convenient to examine them microscopically.

In order to soften the middle lamella of the cell walls so that the individual cells would separate, the root-tips were transferred to one part 95% ethyl alcohol and one part concentrated hydrochloric acid for one minute at 60° C. After washing in two changes of distilled water for two minutes each, the root-tips were transferred to leuco-basic fuchsin stain (Stowell, 1945) for one hour. Staining time varied from fifteen minutes to overnight. To further soften the tissue, the roots were transferred into 5% pectinase prepared according to Stowell's (1945) directions for a period of four to thirty-six hours.

Slides were prepared by placing the dark stained portion of root-tips near the center of glass slides and cutting free and removing everything except the meristematic region of the roots. In a drop of aceto-carmine stain (Wolff, 1956) root-tips were macerated by teasing the cells apart with a needle and squashing the larger portions of the tissue with the blunt end of a metal-handled scalpel. After complete separation of the cells, small glass cover slips were placed over the macerated material. The slides were heated by passing them slowly through the flame of an alcohol lamp three or four times (being cautious not to allow the material beneath the cover slips to boil), and squashed between two paper towels. It was essential that the pressure be applied perpendicular to the slides, thus preventing any horizontal movement of the cover slips. By adding a drop of 45%

acetic acid to the edge of the cover slips the temporary slides remained sufficiently moist for examination.

Slides were made permanent according to a modified method of Bradley (1948). All the excess aceto-carmine stain around the cover slip was first washed off with 45% acetic acid, and then allowed to slowly dehydrate in a vapor chamber of 95% ethyl alcohol. After a few days each slide was removed from the vapor chamber and a drop of absolute ethyl alcohol was immediately applied to the edge of the cover slip. While the slides were saturated with alcohol, the cover slips were "ringed" with diaphane. Being soluble in alcohol, the diaphane diffused under the cover slip and around the macerated material. After returning the slides to the vapor chamber for three or four days to allow for complete and uniform diffusion of the diaphane, the lid of the vapor chamber was partially removed to permit slow evaporation of the alcohol. Before storing the slides, they were dried at room temperature for three to four weeks, and the excess diaphane was cleaned off with 70% alcohol.

Photomicrographs of chromosomes were taken with an American Optical Orthophot mount through a Bausch and Lomb microscope with 97X objective and 10X eye piece, using 4 x 5 inch Kodak Contrast Process Ortho film. Photographs of the vegetative structures were taken with a 4 x 5 Graflex press-camera using Kodak Panatomic-X film.

RESULTS

Of all the morphological characters examined, awn length and anther size proved to be the most useful in distinguishing Agropyron spicatum from A. trachycaulum. At maturity A. spicatum has long bent awns; A. trachycaulum is awnless. The anthers of A. spicatum are about four times longer than A. trachycaulum. A. inerme, as shown by Dunford (1958) to be an awnless form of A. spicatum, also has large anthers.

F₁ hybrids of these two taxa are easily recognized because they are intermediate in awn and anther lengths as shown in Plate I.

Plate II shows the variation in awn lengths and anther size found in the natural population at Thistle Flat. After Anderson (1949) hybrid index scores were obtained for each plant by assigning numerical values to awn length, anther length, and chromosome numbers as shown in Table 1. The index number for each culture was obtained by rating spicatum-type plants 0 (zero) and trachycaulum-type plants 15. Figure 4 shows the hybrid index number plotted against the number of plants. The five synthetic hybrids scored and used for comparison are from sixteen seeds harvested from artificial crosses of A. trachycaulum and A. spicatum.

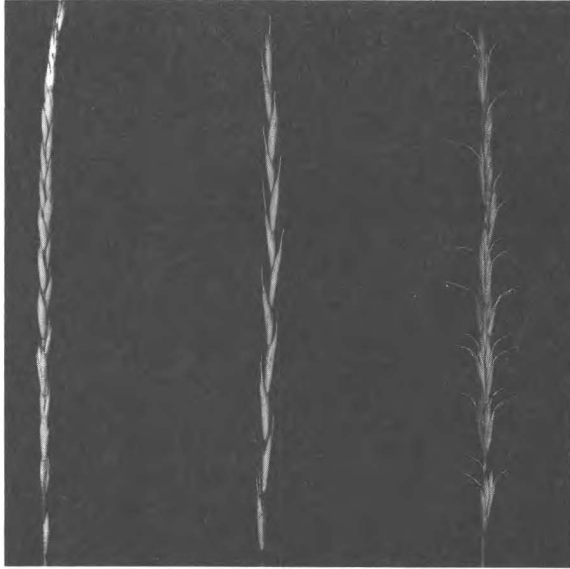


Figure 1

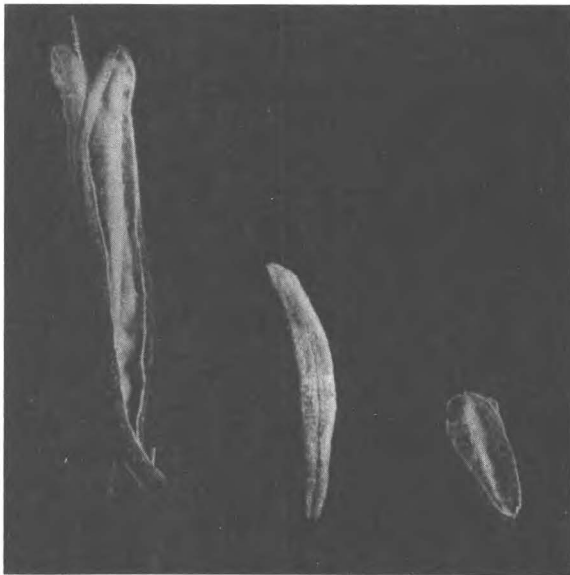


Figure 2

PLATE I

Spikes and anthers of Agropyron trachycaulum, A. spicatum, and the F_1 hybrid between them. Figure 1 shows spikes of A. trachycaulum on the left, A. spicatum on the right, and artificial F_1 hybrid in the middle. \bar{X} ca $3/4$. Figure 2 shows anthers of A. spicatum on the left, A. trachycaulum on the right and artificial F_1 hybrids in the middle. \bar{X} ca 12.

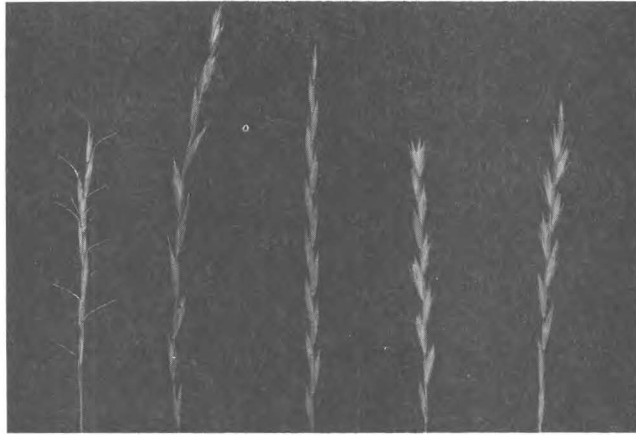


Figure 3

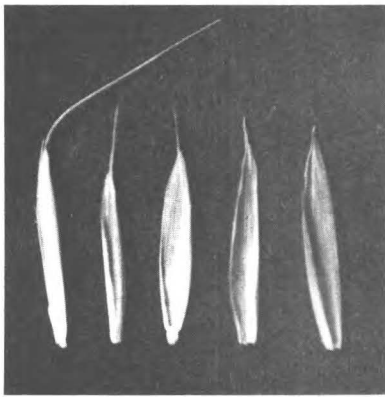


Figure 4

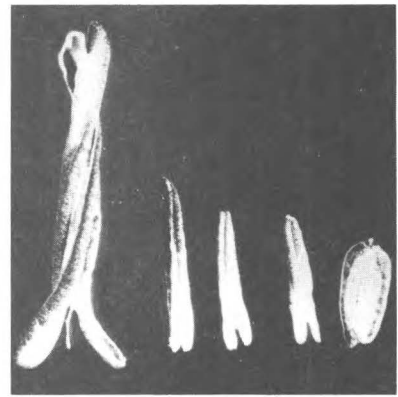


Figure 5

PLATE II

Spikes, lemmas, and anthers from a natural population at Thistle Flat showing variation of morphological characters. In each case A. spicatum is on the left, A. trachycaulum is on the right with three putative hybrids in the middle. Figure 3 shows spikes. X ca $\frac{1}{2}$. Figure 4 shows lemmas. X ca 3. Figure 5 shows anthers. X ca 9.

TABLE 1

Awn lengths, anther lengths, chromosome numbers, and hybrid index scores for F₁ hybrids and selected plants from the natural population at Thistle Flat.

| Culture Number | Anther Length | Awn Length | Chrom. No. (2n) | Hybrid Index Score | Limits | Hybrid Index Number |
|-------------------|---------------|------------|-----------------|--------------------|-----------|---------------------|
| 2530 ^a | 4.0 | 10.0 | 14 | 2.0 | 1.5- 2.4 | 2 |
| 2531 ^a | 5.0 | 14.0 | 14 | 1.0 | 0.5- 1.4 | 1 |
| 2532 ^b | 1.5 | 1.0 | 28 | 14.0 | 13.5-14.4 | 14 |
| 2533 ^c | 1.7 | 3.0 | 21 | 10.3 | 9.5-10.4 | 10 |
| 2534 ^c | 1.9 | 3.0 | 21 | 10.3 | 9.5-10.4 | 10 |
| 2535 ^c | 2.0 | 3.0 | 21 | 10.1 | 9.5-10.4 | 10 |
| 2536 ^c | 1.7 | 5.0 | 21 | 9.0 | 9.5- 9.4 | 9 |
| 2537 ^c | 1.4 | 0.0 | 21 | 11.8 | 11.5-12.4 | 12 |
| 2538 ^c | 1.3 | 1.0 | 28 | 14.1 | 13.5-14.4 | 14 |
| 2539 ^b | 1.5 | 1.0 | 28 | 14.2 | 13.5-14.4 | 14 |
| 2540 ^b | 1.4 | 2.0 | 21 | 11.0 | 10.5-11.4 | 11 |
| 2541 ^c | 1.7 | 1.0 | 28 | 14.1 | 13.5-14.4 | 14 |
| 2542 ^b | 1.8 | 2.5 | 21 | 10.5 | 10.5-11.4 | 11 |
| 2543 ^c | 1.7 | 1.5 | 21 | 11.0 | 10.5-11.4 | 11 |
| 2544 ^c | 1.5 | 2.5 | 21 | 10.5 | 10.5-11.4 | 11 |
| LP21 ^d | 1.5 | 3.0 | 21 | 9.7 | 9.5-10.4 | 10 |
| LP22 ^d | 1.5 | 5.0 | 21 | 8.9 | 8.5- 9.4 | 9 |
| LP23 ^d | 1.6 | 4.0 | 21 | 9.5 | 9.5-10.4 | 10 |
| LP24 ^d | 1.8 | 5.0 | 21 | 9.0 | 8.5- 9.4 | 9 |
| LP25 ^d | | 5.0 | 21 | 8.8 | 8.5- 9.4 | 9 |

^aTypical Agropyron spicatum.

^bTypical Agropyron trachycaulum.

^cPutative hybrids between A. trachycaulum and A. spicatum.

^dArtificial F₁ hybrids between A. trachycaulum and A. spicatum.

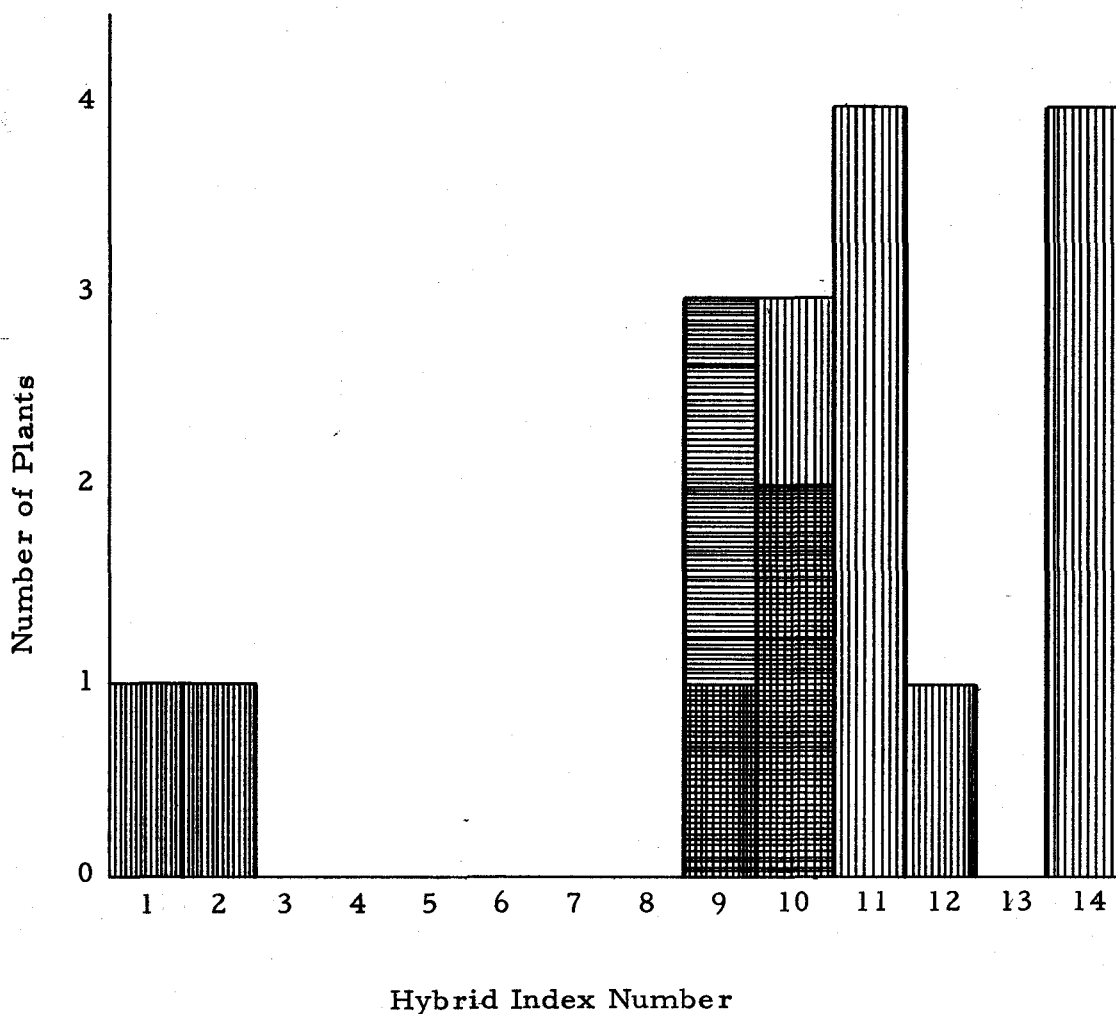




FIGURE 4

A Hybrid Index Graph representing 15 plants collected at Thistle Flat, and 5 synthetic hybrids.

-  Represents plants from the natural population at Thistle Flat.
-  Represents artificial F₁ hybrids.

Fertility

The number of seeds set compared with the total number of florets produced were obtained for each selected plant from the natural population at Thistle Flat. As shown in Table 2, considerable variation in fertility is expressed between the individual plants. Of the two A. spicatum plants studied, one produced 12 seeds per 103 florets and the other had no seeds in the 183 florets produced giving an average of 4.2% seed set. From A. trachycaulum plants, 157 seeds were produced from 356 florets giving an average of 53.7% fertility, with a range from 3.1% to 94.0%. From the putative hybrid plants five seeds were obtained from 28,870 florets. Four of these seeds were produced by a single plant having 183 florets giving 2.2% fertility. The other seed was produced by a plant having 500 florets, giving 0.2% fertility. No seeds were obtained from the 28,187 florets of the other seven plants.

All five seeds from the "hybrids" at Thistle Flat germinated, but have grown much more slowly than either A. trachycaulum or A. spicatum seedlings of the same age.

For comparison two additional populations were checked for seed fertility. A population of A. spicatum from northeast Provo produced about 76 seeds in approximately 4500 florets giving an average of about two per cent seed set. A population of A. trachycaulum from southeast Provo produced approximately 3000 seeds

per 3200 florets giving an average of about ninety percent seed fertility.

As shown in Table 2, plants of A. spicatum which were examined had a very high pollen fertility, but only one of the four A. trachycaulum plants had a high pollen fertility. Of the three A. trachycaulum plants having reduced pollen fertility, the lowest plant had 35.6%, another one had 61.3% and the third plant had 64.4%. The natural hybrid plants have, as expected, a very low pollen fertility ranging from zero to about four per cent.

Cytology

Only three levels of chromosome numbers were found in plants from the natural population at Thistle Flat. (See Plate III). As shown in Table 1, plants having 28 chromosomes are morphologically, typical A. trachycaulum, those having 14 chromosomes are typical A. spicatum, and plants having 21 chromosomes show considerable morphological variation.

All five progeny from the highly sterile, 21 chromosome plants have only 14 chromosomes. These seedlings express some degree of morphological variation in the vegetative stage and have grown more slowly than either A. trachycaulum or A. spicatum seedlings the same age.

The chromosomes of A. spicatum and A. trachycaulum are so similar that they can not be distinguished in hybrids. Each chromosome is large, metacentric without any visible satellites. (See Plate III.)

TABLE 2

Seed and pollen fertility of plants from the natural population at Thistle Flat.

| Culture Number | Seeds Produced | Florets Produced | Seed Fertility Percentage | Viable Pollen | Aborted Pollen | Pollen Fertility Percentage |
|-------------------|----------------|------------------|---------------------------|---------------|----------------|-----------------------------|
| 2530 ^a | 12 | 103 | 11.5 | 480 | 5 | 99.8 |
| 2531 ^a | 0 | 183 | 0.0 | 519 | 11 | 97.8 |
| 2532 ^b | 170 | 182 | 94.5 | 384 | 24 | 94.0 |
| 2533 ^c | 0 | 1200 | 0.0 | 9 | 327 | 2.6 |
| 2534 ^c | 0 | 102 | 0.0 | 0 | 194 | 0.0 |
| 2535 ^c | 0 | 306 | 0.0 | ... | ... | |
| 2536 ^c | 0 | 445 | 0.0 | 8 | 430 | 1.8 |
| 2537 ^c | 0 | 146 | 0.0 | 0 | 543 | 0.0 |
| 2538 ^b | 4 | 130 | 3.1 | 369 | 234 | 61.3 |
| 2539 ^b | 83 | 121 | 68.6 | 212 | 386 | 35.6 |
| 2540 ^c | 0 | 525 | 0.0 | 0 | 350 | 0.0 |
| 2541 ^b | 99 | 230 | 43.4 | 332 | 182 | 64.4 |
| 2542 ^c | 4 | 183 | 2.2 | 3 | 525 | 0.6 |
| 2543 ^c | 1 | 500 | 0.2 | 7 | 516 | 1.3 |
| 2544 ^c | 0 | 560 | 0.0 | 2 | 505 | 3.9 |

^a Typical Agropyron spicatum.

^b Typical Agropyron trachycaulum.

^c Putative hybrids between A. trachycaulum and A. spicatum.



Figure 6

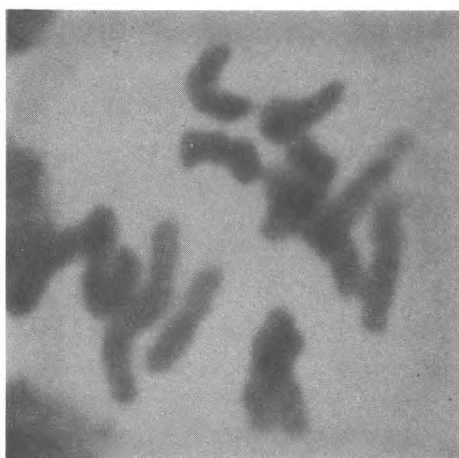


Figure 7

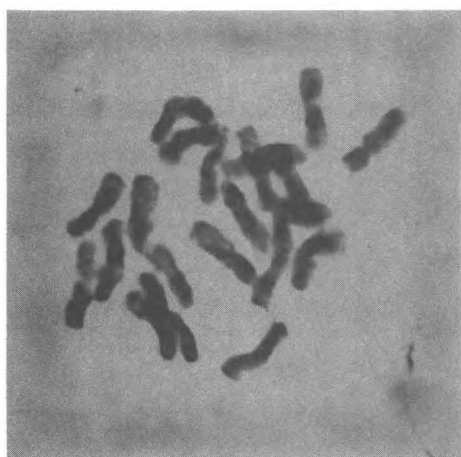


Figure 8

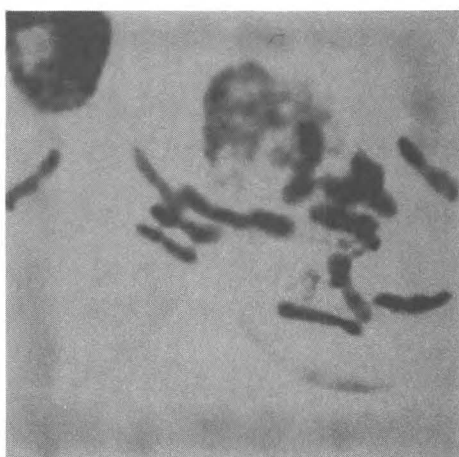


Figure 9

PLATE III

Chromosomes from root-tip smears of parents, hybrids, and backcross progeny from a natural population at Thistle Flat. X ca 1250. Figure 6 shows the 28 chromosomes of a typical *Agropyron trachycaulum*. Figure 7 shows the 14 chromosomes of *A. spicatum*. Figure 8 shows the 21 chromosomes of a natural hybrid between *A. trachycaulum* and *A. spicatum*. Figure 9 shows the 14 chromosomes of a backcross **segregant**.

DISCUSSION

Although Agropyron spicatum and A. trachycaulum are considered by most botanists to be separate species, the only two characters which can be used to consistently separate them in the field are anther size and the presence or absence of awns. Since A. inerme has been shown to be essentially just an awnless form of A. spicatum (Dunford, 1958), anther size alone is the only key morphological character which can be used to separate these two taxa. However, cytologically they are distinct. A. trachycaulum has 28 chromosomes and A. spicatum has 14 chromosomes in their somatic cells.

Since these two species are so morphologically similar, they are apparently very closely related. Furthermore, as shown by Stebbins (1956), F_1 hybrids between A. trachycaulum and A. spicatum have seven bivalents and seven univalents at meiosis, which suggests that A. trachycaulum is an allotetraploid having one genome in common with A. spicatum. If these chromosome pairs are the result of autosyndesis, however, A. trachycaulum may be an autotetraploid derivative of A. spicatum. Diploidization of the ancestral autotetraploid could have subsequently yielded the prevalent fertile 28-chromosome plants.

But there is good evidence that the pairing found in F_1 hybrids of A. trachycaulum and A. spicatum is allosyndetic and hence

that A. trachycaulum is an allotetraploid derivative of A. spicatum. Lack of pairing which has been noted during meiosis of other hybrids involving A. trachycaulum infer, little if any homology among A. trachycaulum chromosomes. For instance from pairing relationships of A. trachycaulum and Elymus glaucus chromosomes with each other and with other species, Stebbins (1946b) concluded that pairing was always allosyndetic in hybrids involving A. trachycaulum. He reasoned that since Elymus glaucus X Sitanion jubatum hybrids have complete pairing and yet neither parent, at meiosis, shows quadrivalents that this pairing must be allosyndetic. And therefore, the pairing observed in A. trachycaulum X Elymus glaucus and A. trachycaulum X Sitanion jubatum hybrids must also be allosyndetic. Therefore, the pairing observed in the F₁ hybrid of A. trachycaulum and A. spicatum is probably also allosyndetic.

But probably the best evidence for allosyndesis in the A. trachycaulum X A. spicatum hybrids is the five progeny derived from highly sterile 21-chromosome plants from Thistle Flat. Because each of the segregant plants has 14 chromosomes, they must have resulted from the union of two 7-chromosome gametes. Since the fertility is very low in these 21-chromosome plants and since each of the seedlings which were produced had only 14 chromosomes, it suggests that only 7-chromosome gametes are viable. These fertile gametes probably resulted from normal segregation of the seven pairs of chromosomes

accompanied by a concurrent loss of the randomly segregating univalents. Since only plants having 14, 21, or 28 chromosomes were found in samples from Thistle Flat, apparently all aneuploids are aborted.

A further deduction made possible by the 14 chromosome progeny of the hybrid plants is that the male parent in each case must have been A. spicatum. This may have been because A. spicatum was in a better position to shower pollen on the 21-chromosome female parent, or because of its larger anthers, A. spicatum may have furnished the majority of the available pollen. In any case the 14-chromosome plants indicates that A. spicatum was the male parent.

Since production of gametes is apparently rare in the 21-chromosome plants from Thistle Flat, intercrossing between them is probably also rare. As indicated above less than 4% of the male gametes are fertile among the 21-chromosome plants at Thistle Flat, and since there are several thousand male spores produced for each female gamete, the number of female gametes per plant would be even less. Therefore, because of the extremely low fertility in both male and female gametes, seeds would probably never be produced by hybrids alone. Progeny from F_1 hybrids are, therefore, probably always backcross segregants.

Since A. trachycaulum is tetraploid with 14 pairs of chromosomes and A. spicatum is diploid with 7 pairs, the F_1 hybrid between them is triploid. As shown in Jimson weed (Blakeslee, 1937) Cotton, (Stephens, 1947) and wheat (Riley, 1960), triploids often

segregate aneuploids when backcrossed to their parents. However, the triploid hybrid between A. trachycaulum and A. spicatum apparently yields chromosomes on the diploid or haploid level only. As noted above, every plant studied at Thistle Flat had either 14, 21, or 28 chromosomes.

In spite of this rather strict chromosome balance required by viable gametes of A. trachycaulum and A. spicatum, considerable morphological segregation is evident in the natural population at Thistle Flat. With strict chromosome restrictions, such abundant segregation demands either whole chromosome substitution or abundant crossing-over. In either case it verifies the close genetic affinity between A. trachycaulum and A. spicatum. This close affinity is further indicated by the ease with which they hybridize. These hybrids, even though differing in chromosome numbers, are vigorous and produce occasional offspring. The weak barriers to hybridization between A. trachycaulum and A. spicatum growing at Thistle Flat may be unique to this population, but nevertheless is in harmony with all other evidence for close relationship between these two species.

Since Thistle Flat is located near the divide separating two major drainage systems, previously isolated strains of A. trachycaulum and A. spicatum, may come together at this site. When closely related species that maintain their identity grow sympatrically for many generations, strong genetic barriers may be acquired to prevent any hybridization and gene flow between them. However, if they are

isolated from each other for many generations these barriers may be lowered because of the relaxation of selection for them. Thus A. trachycaulum growing in the Great Basin and also those growing in the Colorado River Basin may have genetic barriers against hybridization with A. spicatum when growing sympatrically, but widely separated populations may have very weak barriers. Since Thistle Flat is located where previously isolated strains can come together, hybridization between them may be common because of the weak barriers already present.

The variable awn lengths and wide range in seed and pollen fertility present in A. trachycaulum at Thistle Flat is indicative of abundant genetic variation. However, in a population of A. trachycaulum from southeast Provo, seed production is high (about 85-95%) which indicates that pure strains of A. trachycaulum are normally highly fertile. Furthermore, most populations of A. trachycaulum are also morphologically uniform. Therefore, the sporadic low fertility in A. trachycaulum and the variable morphological patterns at Thistle Flat must be the result of introgression of A. spicatum into A. trachycaulum.

The two plants of A. spicatum examined from Thistle Flat had a very low seed fertility, but this is typical of other populations of A. spicatum and may not indicate introgression. For instance a population of A. spicatum distinctly isolated from A. trachycaulum and other closely related species, located near the mouth of Provo

canyon, has low seed fertility ranging from zero to five per cent. The low seed production noted in populations of A. spicatum may be due to inbreeding effects in local populations, or it may represent a reaction towards lowered seed fertility permitted by acquiring asexual means of reproduction. Dunford (1958) and others have reported populations of A. spicatum to have active rhizomes. Nevertheless, as noted above, cytological evidence alone indicates introgression of A. trachycaulum into A. spicatum at Thistle Flat.

Since introgression is common between A. trachycaulum and A. spicatum, it is probably beneficial to both species. Introgression may increase variation and be of significant evolutionary importance to each taxon. Increased variation may be useful to A. spicatum in maintaining the variation it needs to inhabit the wide range of ecological niches where it is found growing. The variation may be of even more importance to A. trachycaulum because A. trachycaulum is normally self-fertilized, and introgression could provide it with a constant source of variation which would otherwise be unavailable.

SUMMARY

1. Phylogenetically, Agropyron trachycaulum and Agropyron spicatum are very closely related.
2. Artificial hybrids of Agropyron trachycaulum X Agropyron spicatum are somewhat intermediate, both morphologically and cytologically.
3. Hybrids of A. trachycaulum and A. spicatum often occur in nature when growing sympatrically.
4. Highly sterile hybrids of A. trachycaulum and A. spicatum produce occasional offspring. Five of such progeny were found to be backcrosses onto A. spicatum.
5. A natural population of A. trachycaulum, A. spicatum and apparent hybrids between them expressed considerable morphological variation, but have only three levels of chromosome numbers.
6. Cytological and morphological evidence indicate reciprocal introgression occurring between A. trachycaulum and A. spicatum.
7. Even though there is a difference in chromosome numbers between these two species it is not a strong enough barrier to prevent gene flow between A. trachycaulum and A. spicatum.
8. The flow of genes between these two species of Agropyron may be of considerable evolutionary significance by furnishing variation into each reproductive system.

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CYTOLOGICAL EVIDENCE FOR RECIPROCAL INTROGRESSION
IN AGROPYRON TRACHYCAULUM AND AGROPYRON SPICATUM

An Abstract of a Thesis Submitted to
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by
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ABSTRACT

Agropyron trachycaulum (Link) Malte and Agropyron spicatum Pursh, both native range grasses of the Great Basin, often hybridize in nature. A. trachycaulum, is a tetraploid with 28 chromosomes. It has awnless or awn-tipped lemmas and small anthers. A. spicatum is a diploid with 14 chromosomes. It is characterized by having long awns that bend at maturity, and anthers which are about four times longer than A. trachycaulum.

Artificial hybrids are intermediate between parents in awn and anther lengths and have 21 chromosomes.

A natural population in Utah was found consisting of A. trachycaulum, A. spicatum, plants resembling the artificial hybrids, and intermediates exhibiting the whole spectrum of variation between the two parents. Regardless of the morphological variation expressed by the natural population only three levels of chromosome numbers are found. Plants have either 14, 21, or 28 chromosomes. This means that only gametes on the seven or fourteen chromosome levels are functional.

Only five seeds from 28,870 florets were harvested from the natural hybrids from Thistle Flat. Since these

natural hybrids are highly sterile, interbreeding among the hybrids is probably rare. Because progeny from 21-chromosome plants have only 14 chromosomes, A. spicatum was probably the male parent. The variable morphology of A. trachycaulum accompanied by reduced pollen and seed fertility suggests that it has been introgressed with A. spicatum characters. Therefore, both cytological and morphological evidence indicates reciprocal introgression between A. trachycaulum and A. spicatum.

Even though A. trachycaulum and A. spicatum have different levels of chromosome numbers, this is not a strong enough genetic barrier to prevent genes to flow between them. Because of introgression considerable variation can be maintained which may be of considerable evolutionary importance to both species.

APPROVED BY: