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Intergeneric hybridization in crosses involving barley and its wild relatives

presented by

Rye-Ho Huang

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of the requirements for

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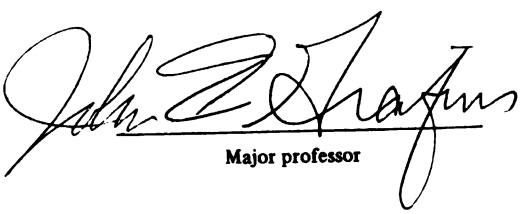

Major professor

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INTERGENERIC HYBRIDIZATION IN CROSSES INVOLVING BARLEY AND ITS WILD RELATIVES

by

Rye-Ho Huang

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ABSTRACT

INTERGENERIC HYBRIDIZATION IN CROSSES INVOLVING BARLEY AND ITS WILD RELATIVES

by

Rye-Ho Huang

Karyotype analysis of Agropyron trachycaulum, Hordeum jubatum, Hordeum vulgare and their hybrid series (AHV442, AHV444, and VH-35) were investigated for chromosome exchange and potential transfer genes from either Agropyron trachycaulum or Hordeum jubatum into Hordeum vulgare. Cytological techniques and light microscopic observations were unsatisfactory in detecting chromosome exchange. This was partly due to the similarity of karyotypes of the genomes involved.

Since the amphidiploid of Agropyron trachycaulum and Hordeum jubatum as female was crossed with diploid and tetraploid Hordeum vulgare, the first backcross was achieved using one of the restored AHV-35 derivatives as female and diploid Hordeum vulgare as the pollen source. Chromosome association observed in the pollen mother cells of the backcross showed a significant decrease in univalents. Based on comparative cytological observation in AHV442, AHV444 and AHV-B_1, a hypothesis of genetic control on genome interaction was derived to elucidate the phenomenon of preferential chromosome elimination. The first backcross, by genome

formula was composed of at least two dosages of Hordeum vulgare genome, therefore genome balance between Hordeum vulgare and the others was achieved by adding one Hordeum vulgare genome at a time to the other combined genome of Hordeum jubatum and Agropyron trachycaulum. The significance of introgressive hybridization between cultivated barley and its wild relatives was discussed. Since some cultivated germplasm is vulnerable to epidemics and limited in adaptation due to man's disturbance, the highlight of this study was emphasizing the reconstruction and replenishing of the cultivated germplasm through hybridization to its wild relatives.

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INTRODUCTION

For several major crops, genetic vulnerability in terms of a lack of genetic variability for stress adaptation has been called to the attention of plant breeders. Germplasm improvement of crops can be achieved more easily by introgression through hybridization than by induced mutation of genetically exhausted cultivars. The domain of introgressive hybridization includes wide crosses between similar species and backcrosses to cultivated progenitors. Triticale is an example of a crop synthesized by wide hybridization between Triticum spp. and Secale cereale L.

The gene pools of wheat and corn can be and have been replenished by backcrossing to their respective progenitors. In the genus Hordeum wild species have been considered a useful source of genes carrying specific attributes for possible transfer to the cultivated forms. A list of hybridizations of barley with its wild relatives has been summarized (Table 1). Genetic transferring has not been satisfactory since pollen incompatibility, hybrid lethality, hybrid sterility and infrequent recombination block successful hybridization on a practical scale.

The basic task of introgression in barley depends largely on the strategy of genetic manipulation at the genome level. This study presents a case for using the complex genome derived from H. jubatum, A. trachycaulum and H. vulgare. The hybrids have created the potential for a wide scale genetic association between H. jubatum and H. vulgare or A. trachycaulum and H. vulgare. The impact of a complementary influence of a third genome is significant. A series of intermediate hybrids has been synthesized during the course of a backcross to H. vulgare. Karyotypic analysis of plants and their hybrids might lead to an understanding of their relationship in terms of genome compatibility and chromosome association. The possible mechanism of the phenomenon of chromosome elimination was investigated for its significance in genetic transfer.

LITERATURE REVIEW

Interspecific and Intergeneric Hybridization

Unconventional hybridization of economic crops is an ideologic means for plant breeders to marshall a new array of germplasm capable of increasing adaptation as well as production. The feature of wild hybridization occasionally advocated and substantiated in some cases has intrigued both plant and animal breeders.

Gordon and Raw (1932) crossed wheat and barley and obtained a wheat-like fertile plant. No barley-type progenies appeared in the subsequent F_2 to F_4. They postulated the cause as parthenogenic stimulation and an accompanying doubling of the maternal chromosome complements. More likely, the progeny was the result of an accidental self-pollination.

The idea of using chemical agents to circumvent crossing barriers between wheat and barley was proposed by Bates (1974), but the results failed to produce a true hybrid from the immunosuppressant treatment.

H. vulgare crossed with Secale yielded somewhat similar results. Quincke (1940) reported that fertilization evidently took place in the hybridization between Hordeum

vulgare hexastichum x Secale cereale but the seed ceased to develop in early stages. In addition, Thompson and Johnson (1945) further proved that fertilization took place in 90 percent of the ovaries. Embryos developed normally in the early stages but disintegrated following endosperm breakdown. Cytological study showed an abnormal size and number of nuclei in the endosperm. Similarly, the phenomenon was observed in certain other species by Huskins (1948); Thompson (1962); Bammi (1965), and Rizzoni et al (1974). They suggested that the cause of separation and grouping of chromosomes was related to genome origin. The genetic make-up and the development of the endosperm are complex features because of the double fertilization involved. The relationship between embryo and endosperm in the course of seed development is not understood. At the present time, embryo culture has been developed to serve the practical need of rescuing the embryo before it aborts. Brink and Cooper (1947) used embryo culture to obtain a hybrid between Secale and Hordeum species. They indicated that mitotic irregularities were the result of malfunctioning of the antipodals. Chromosome abnormalities were prevalent in the form of lagging chromosomes, split univalents, bridges, micronuclei etc.; therefore, the hybrid was sterile and no further backcross was obtainable.

Fedak (1977) reported obtaining a haploid barley in the cross between barley and Secale with no clue to the treatment during the course of hybridization. Selective

chromosome elimination was given as the cause for producing haploid barley.

Hybridization between Hordeum vulgare and Elymus spp. has been unexpectedly successful. Although Smith (1942) and Reznicuk (1939) failed in their attempt to produce a hybrid between Elymus spp. and Hordeum vulgare, Bakhteiev and Darevskaya (1945) were able to hybridize Hordeum vulgare with E. arenarius and E. giganteus. Morphologically, this hybrid had intermediate characteristics and 21 somatic chromosomes. Bakhteiev and Darevskaya (1975) also reported a cross between Hordeum spontaneum C. Koch em. Bacht. (2n=14) and Elymus arenarius L. (2n=56). Unfortunately, that hybrid failed to reach complete development. Ahokas (1973) specified evidence of cytoplasmic influence in a barley and Elymus hybrid. When using Hordeum vulgare as female, fourteen viable hybrids were obtained, but none survived when Elymus was the female. Subsequently, the barley chromosomes were eliminated resulting in monoploid E. arenarius in Hordeum vulgare cytoplasm and the plant finally produced a fertile tiller. Earlier, Pissarev (1945) even obtained a haploid barley instead of a haploid Elymus from a barley and Elymus hybridization. Korablin (1937) reached the point of obtaining 107 seeds from a barley-Elymus cross. Schooler* obtained promising winter-type barley derived from a barley (Dicktoo 2x) X Elymus (4x) hybridization.

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*Personal communication with Dr. J. E. Grafius

Hybridization of barley with wild species was extended to Agropyron spp. by Smith (1942). Since then no success has been reported. But natural hybridization between Agropyron and Hordeum has frequently been discovered, such as North American Agrohordeum G. Camus ex A. Camus, (Stebbins et al 1946); Elymus Macounii complex of Agropyron trachycaulum (Link) Malte and foxtail barley; Hordeum jubatum by Keller (1948); Booher and Tryon (1948); Forsberg (1953), Agropyron pilosilemma x Hordeum jubatum by Mitchell and Hodgson (1965a); Agropyron, Oschense. Roshev., and Hordeum turkestanicum by Nevski (1934).

Eventually, a few cases fulfilled the breeding purpose of transferring agronomic traits from wild Hordeum spp. into cultivated barley. Two successful genetic transfers for disease resistance are from crosses between H. vulgare and H. leporinum by Hamilton et al (1955), and from H. leporinum by Hamilton et al (1955), and from H. bulbosum by Schooler (1964).

TABLE 1

A SUMMARY OF BARLEY INTERGENERIC AND INTERSPECIFIC HYBRIDIZATION

Interspecific

Crosses	Results	Literature
(H. brachyantherum x H. bogdanii (6x)) x H. vulgare (4x Traill)	Hybrid & progeny	Schooler (1976)
H. vulgare x H. Californicum	Hybrid died before maturity*	Davies (1956, 1960)
H. vulgare (4x) x H. bulbosum	Hybrid	Davies (1958) Schooler (1964)
H. vulgare x H. brachyantherum	Hybrid	Morrison et al (1959)
H. vulgare x H. depressum	Hybrid	Morrison (1959)
H. vulgare x H. jubatum	Hybrid	Morrison & Rahathy (1959) Steidl (1976)
H. vulgare x H. leporinum	Hybrid*	Hamilton, Symko and Morrison (1955)
H. vulgare x H. murinum	Hybrids died in the seedling* Parthenocarpic seed Hybrid died before maturity	Malloch (1921) Forlani (1950) Morrison et al (1959)
H. vulgare (4x) x H. bulbosum (4x)	Haploid H. vulgare*	Lange (1969, 1971a) Kao & Kasha (1970 1969) Konzak et al (1951)
H. Lechleri (6x) x vulgare (2x)	Hybrid and haploid* H. lechleri	D. H. B. Sparrow (1974)
(H. jubatum x H. compressum (6x)) x H. vulgare (4x)	Hybrid and haploid (3x)	Steidl (1976) Chung Lee (1970) Schooler (1964)

* The phenomenon of chromosome elimination involved

Table 1-Continued

Intergeneric

Crosses	Results	Literature
H. vulgare x S. cereale	Two hybrid plants and a barley hybrid*	Thompson, W. P. D. Johnston (1945)
H. vulgare x H. jubatum	Hybrid but no chromosome association	Steidl (1976)
H. vulgare x S. cereale	Haploid barley*	Fedak (1977)
H. vulgare x T. vulgare	Unconfirm hybrid*	Bates (1973) Fedak (1977)
H. vulgare x Elymus (2x)	Hybrid and progeny	Schooler (1976)
H. vulgare x E. arenarius	Haploid Elymus in H. vulgare cytoplasm	Ahokas (1970, 1973)

* The phenomenon of chromosome elimination involved

Chromosome Elimination

The phenomenon of chromosome elimination is ubiquitous in unconventional hybridization in both plant and animal cells. The effort to unfold the mechanism of chromosome elimination has practical and theoretical value.

Chromosome elimination refers to the cause of selective loss of chromosomes of one genome. It is characterized by a complex interaction involving genetic and physiological control. Earlier, insect researchers encountered the phenomenon in the family Diptera. The phenomenon was first described by Kahle (1908).

Later on, attempts to interpret the phenomenon of chromosome elimination were made by Huettner (1934), Kraczkiewicz (1936) and Metcalfe (1935). Chromosome elimination occurs immediately after fertilization and continues through embryo development. Generally, a set of daughter chromosomes was excluded from the major group without reconstitution in the daughter nuclei. Kraczkiewicz (1936) extended this explanation to include the state of cytoplasm reaction and specified the function of the spindle while in mitotic cell division. A similar assumption was suggested by Du Bois (1933) for Sciara Coproplila. On the other hand, Geyer-Duszynska (1959), working with Wachtliella persicariae embryos, pinpointed errors in the functioning of the centromeres of germ line chromosomes as the direct cause of their elimination from somatic nuclei. Bantock (1970) even detected chromosome elimination occurring at the

fifth division of Mayetiola destructor embryos. Camenzind (1974) concluded that the predictable loss of specific chromosomes during embryological development was due to complex genetic and physiological controls. Chromosome elimination prevailing in the families (Diptera); the Ceciodmyidae, the Sciaridae, and the Chironomidae was attributed to the heterogeneous genetic makeup and diverse origin of family.

In the plant kingdom, the phenomenon of chromosome elimination is assumed to play a unique role in evolution as well as speciation because it acts as a screening mechanism for compatible genomes in natural hybridization. It was a frequent event in wide hybridization under controlled hybridization between certain species and genera which puzzled plant breeders. Nevertheless as a mechanism per se it has been neglected by plant breeders due to its complexity.

Intensive study of chromosome elimination has been characterized by interspecific hybridization in the genus Hordeum (Hamilton et al 1955, Cauderon and Cauderon 1956) and in the genus Nicotiana (Gupta and Gupta 1973). In corn, Rhoades and Dempsey (1972) reported that B chromosomes might cause the elimination of knob bearing members of the regular chromosomal complement at the second microspore mitosis. The hypothesis is derived from the regulatory gene system extended to the difference in DNA replication in the heterochromatic segment of knobbed chromosomes.

Alternatively, Kasha and Kao (1970) hypothesized that

specific chromosomes in H. vulgare might carry factors controlling genome balance and stability in the hybrid with H. bulbosum L. Further investigation suggested that chromosomes 2 and 3 of H. vulgare contain major genetic factors which are critical to the chromosome balance and stability in interspecific hybrids between H. vulgare and H. bulbosum (Ho 1975). These factors function only in the condition that both are dominant and present in sufficient dosage to overpower the opposite factors in H. bulbosum. In other words, chromosome elimination is preferential as well as directional. In plants, evidence of chromosome elimination can be obtained indirectly by morphological observation; Gupta and Gupta (1973) demonstrated that directional chromosome elimination has a high visual correlation with a phenotypic shift from hybrid intermediacy in the direction of the parent whose chromosomes remain. In addition, Starks (1976) and Steidl (1976) observed a concomitant loss of phenotypic characters associated with H. vulgare in a complex plant; H. jubatum x H. compressum amphiploid crossed with H. vulgare.

Karyotype Analysis

A karyotype is defined as the particular chromosome complement of an individual or a related group of individuals in terms of the number and morphology of the chromosomes in mitotic metaphase. Karyotypes of different species and genera may differ with respect to their basic chromosome number, shape and relative size as well as the number and

size of constrictions. Recently, chromosome banding patterns of hetero and euchromatic chromosomal segments have been used as a technique for karyotypic identification.

Karyotypes of barley chromosomes were first described by Tjio and Hagberg (1951). They assigned Roman numerals to each of the chromosome pairs, I to V designating the non-satellited chromosomes arranged in order of decreasing total length, VI the chromosomes with the large satellite and VII the chromosomes with the small satellite. The accompanying linkage groups in barley were elucidated by a group of barley cytogeneticists and this system has been further summarized by Ramage et al (1951) and by Nilan (1964). Barley may be one of the most genetically well-studied crop plants due to its special chromosome behavior and its economic value.

Studies such as induced mutation, linkage map construction, genome relation and chromosome association in barley have been facilitated by the series of translocation stocks in cultivated species. But karyotypic study is the cornerstone for all related studies. Kunzel and Nicoloff (1975) called for a necessary revision of the barley karyogram. They indicated that the interchangeable chromosomes for revealing the linkage groups are associated with different chromosomes. Although the banding system for chromosome identification is under intensive study in certain plant species, so far the results are still inconsistent (Sharma et al 1974, and Gill and Kingber 1974, Murry 1975).

Tuleen (1973) suggested that chromosome I might not

be the longest chromosome of the standard barley karyotype. This view was later agreed to by Kunzel and Nicoloff (1975) who studied a multiple translocation line. By employing the C-banding Giemsa staining technique, Nada and Kasha (1976) came to a similar conclusion that, namely, the karyotype identified from a trisomic line was different from that of the standard karyotype.

Karyotypes have also served as a key to classify taxa as a supplement to systematic classification based on gross morphology of plants. Chromosomes are the physical bases of heredity, their karyotype characteristics of species comparatively unique from one another. Richards (1972) reported that extensive karyotypic variation was formed in the interspecific taxa of Taraxacum. He developed the karyotypic similarity between taxa. Richards and Booth (1977) employed this comparison formula within taxa of the Hordeum murinum group and indicated that mitotic chromosomes in this group showed a continuous variation in length from four to nine microns. The karyotype transformed into a parameter might reflect similarity as well as compatibility between species. In a practical approach, Smith et al (1972) proposed screening the genus Secale for taxa with chromosome karyotypes more like those of wheat. Then the use of wheat-like Secale taxa as a parent in crossing with wheat might increase chromosome association in Triticale combined genomes.

Superficially, the conventional chromosome association in meiosis could also reflect on karyotypic similarity in

the hybrid. Wagenaar (1959, 1960) perceived the chromosome size difference of Secale from Hordeum jubatum in the form of univalents at Metaphase I.

Hordeum jubatum chromosome morphology was well described by Morrison (1959). Covas (1949) and Hitchcock (1950) made chromosome counts in terms of taxonomical classification. The relationship of Hordeum jubatum within the genus Hordeum (by karyotypic study), especially to the cultivated form of barley, implied both practical and evolutionary significance. Karyotypic analysis has even served as a basic tool in describing the relationships within the genus Hordeum (Rajhathy and Morrison 1959, 1961). By means of karyotype analysis Schulz-Schaeffer (1960) were able to demonstrate specific relationships in Bromus using satellite chromosomes as indicators. In the same manner, Rajhathy and Morrison (1961) specified the satellite chromosome of Hordeum jubatum in a tetraploid form. In addition to understanding species relations, through karyotype analysis, Morrison (1959) reported that the diploid species Hordeum marinum, H. maritimum, H. hystrix and H. gussoneanum possess a similar karyotype and are probably conspecific. Similarly, Rajhathy and Morrison (1961) also indicated by means of karyotypic analysis that Hordeum jubatum and Hordeum brachyantherum are conspecific. With respect to breeding, Elkington et al (1976) coincidently reported that the range of banding styles is correlated with well marked breeding barriers between most species in the genus Allium (Liliaceae).

MATERIALS AND METHODS

1. Plant Material

The stocks of Agropyron trachycaulum, Hordeum jubatum and the spontaneous hybrid used in this study were collected in Alaska with the help of Dr. W. W. Mitchell and R. Taylor, Alaska experiment station staff. The amphiploid was obtained with colchicine treatment by Dr. R. P. Steidl.

Diploid H. vulgare; Larker (CI 10648), Coho (CI 13852) Dicktoo (CI 5529) and one tetraploid H. vulgare from Dr. A. B. Schooler were used to cross with the amphiploid and the first backcross. (Table 2)

2. Methods of Hybridization

In the winter, plants were grown in the greenhouse with daytime temperatures between 15-25°C while in the summer, stock was kept in a growth chamber. Frequent repotting and regular fertilizing were necessary in maintaining plant vigor and healthy heads. During the pollination period, a relatively low night temperature was necessary to prolong pollen dehiscence. The crosses were made by the approach method (Curtis and Croy 1958) and better results were achieved when the plants were pollinated with massive amounts of pollen for two to three consecutive days. It was generally

observed that the lodicule ceased to open the lemma and palea after fertilization had taken place. The pollinated head was covered loosely with an aluminum foil envelope and was unwrapped and exposed to light for 20-30 minutes every day.

3. Embryo Culture

Embryo culture was necessary to rescue the hybrid from degenerated seed. Transfer to media was essential for the immature embryo to be germinated before reaching full development.

Two kinds of media were generally used in this study; Norstog's medium II (Norstog 1973) and wick medium (see appendix for the content of media). Obtaining hybrids from proembryos grown in either media was unsuccessful. Well developed embryos did best in wick media since the liquid later surrounds the scutellum which functions as an absorbing appendate of the embryo. The poorly developed embryo might have suffocated from the liquid layer in the wick medium and therefore a solid medium was preferred.

The procedure of excising the embryo and transferring it to the media was as follows:

a. Spike and seeds were sterilized for one minute with commercial clorox diluted with an equal part of distilled water and then rinsed three times with distilled water.

b. The transfer was made under the transfer hood and dissecting microscope. The immature

caryopsis was held by pointed tweezers and the embryo taken out by dissecting needles.

3. The excised embryo was then transferred to the culture medium with careful orientation of the embryo with the scutellum facing the media.
4. The embryos were grown in the dark in an incubator at 24°C. After 7-10 days some embryos started developing into tiny plantlets and were then given artificial light until they reached the two-leaf stage. The seedlings were then transferred to sterile soil under high humidity conditions until they were established.

4. Tissue Culture

Tissue culture techniques were employed to induce a meristem point in the callus from which development of the plant takes place. The appropriate media (B-5) was experimentally selected by Dr. Carlson's laboratory. B-5 medium developed at the Prairie Regional laboratory for growing soybean tissue culture has also been used successfully to grow cells of a large variety of plant tissue. B-5 medium refers to the basic medium with no growth hormone or organic supplements. In this study we used 2-B5 medium, referring to the basic medium plus 2 ppm of 2,4-D. Kao and Kasha (1969) used B-5 medium (omitting 2,4-D) for barley embryo culture. X42 media is a modified B-5 media with no 2,4-D

which was employed to regenerate a plantlet from callus. The procedure for tissue culture is as follows:

a. The immature spike wrapped deeply in the boot was a suitable tissue to be induced to produce callus.

b. Boots with the spike inside were sterilized by 95 percent alcohol for 30 seconds and then transferred to a petri dish for dissecting.

c. Using the tweezer held at one end of the boot, the boots were opened by dissecting needles to dissect out an immature spike. The spike was laid on a scratch media surface. The petri dish was sealed with parafilm.

d. The culture was incubated in the growth cabinet in the dark at 28°C. The callus grew rapidly in fresh media and was regularly subcultured once a week.

e. When the callus reached a certain size, it was transferred to X42 regeneration media and was exposed to light. A clone of plantlets emerged from small meristem domes. Colchicine (.05%) was incorporated in the media (B5 and X42) and subcultured for a range of two days to one week.

f. Plantlets removed from the media were washed off the agar and transferred to sterile soil under high humidity conditions before they were established.

5. Cytological Techniques

For determining the chromosome numbers, plants were put in the cold room around 2°C overnight and then treated in .05% colchicine for 10 minutes at room temperature. In some cases plants with root systems were pretreated in .05% colchicine solution in the cold room (2°C) overnight and then fixed in Newcomer's solution (Newcomer, 1953). The root-tips were then rinsed in distilled water, hydrolysed in 1N HCL at 60°C for 20 minutes. Hydrolysis is essential to subsequent Feulgen staining; therefore, a range of time from 10 to 30 minutes was used for hydrolysis of the small root-tip samples to find the optimum time.

Slides were prepared by a squash technique. Photomicrographs recorded by a Zeiss photomicroscope II with a built-in 35 mm camera and Linholf Technika 4x5 attached to the photomicroscope II using Kodak panatomic-X film (FX402) and contrast process ortho film, respectively. This system was sophisticatedly developed in Dr. Tai's laboratory.

Karyotypic chromosomes were prepared from a photograph print with well-spread metaphase. Karyotypic treatments as chromosome length, ratio and index were referred to the nomenclature from Levan et al (1965).

6. Feulgen-Aceto Carmine Differential Banding Technique

Aceto-carmin solution was prepared as a standard solution by the formula in the Darlington and La Cour book (1966).

The Feulgen preparation is in the appendix and the staining procedures were as follows:

a. For arresting mitotic cell division at metaphase I stage, intact root tips from the whole plant were put in cold jars with ice in the cold room for overnight and then the root tips were cut to 2-3 cm lengths.

b. The root tips were fixed with Carnoy's solution and rinsed with distilled water and placed in 1N HCL at 60°C for an average of eight minutes (a range of time is necessary to cover the optimum hydrolysis).

c. The root tips were transferred to Feulgen solution and placed in a dark place during the staining process. In order to get maximum or overstaining by Feulgen stain, the root tip must remain in Feulgen solution for at least one hour.

d. The root tips were sampled to assure the maximum Feulgen staining which shows dark, shining (florescent-like) chromosomes.

e. The root tips were squashed in a drop of aceto-carmine and the aceto-carmine was added to the cover slip while gradually heating the slide. This process will allow the aceto-carmine to stain differentially on top of Feulgen stain.

f. The slide was sealed with wax, stored overnight and then rinsed with 45% acetic acid.

g. The slide was exposed under the light so the Feulgen stain would fade out. The slide was checked every day to choose the best differential banding pattern showing on the chromosome.

h. Pictures were taken before the slide was permanently mounted.

Table 2

ORIGIN AND DESIGNATION OF RESEARCH MATERIALS

Plant	2n	Genome Assignment	Designation	Origin
H. jubatum	28	$A_1A_1A_2A_2$	HJ	Seed from W. W. Mitchell and R. Taylor, Palmer, Alaska
A. trachycaulum	28	A_3A_3BB	AT	Seed from W. W. Mitchell and R. Taylor, Palmer, Alaska
H. vulgare	14	VV	HV	Coho CI 13852 Larker CI 10648 Dicktoo CI 5529
H. vulgare	28	VVVV	HV	Seed from A. B. Schoolor, North Dakota State U. Fargo, North Dakota
A. trachycaulum x *H. jubatum* (natural hybrid	28	$A_1A_2A_3B$	AT x HJ	Clone from W. W. Mitchell, Palmer, Alaska
Amphiploid-(AT x HJ)	56	$A_1A_1A_2A_2A_3A_3BB$	(AT x HJ) (6x)	Colchine treated by R. Steidl, Department of Crop and Soil Sciences, Michigan State University

RESULTS

I. Karyotype analysis

Karyotypes of *H. vulgare*, *H. jubatum*, *A. trachycaulum*, *H. vulgare* x *H. jubatum*, (*A. trachycaulum* x *H. jubatum*) (8X) x *H. vulgare* (2X), and *H. vulgare* (4X) x (*A. trachycaulum* x *H. jubatum*) were obtained.

H. vulgare (Fig. 1)

Chromosome types and karyotypes. Generally, three chromosome types were recognized in the somatic metaphase stage: (1) Chromosomes without satellites (chromosomes 1, 2, 3, 4, and 5). Within this group, chromosomes 1 and 2 were difficult to distinguish from each other by conventional cytological techniques such as relative length and arm ratio. The only difference is that chromosome 2 is slightly longer in the long arm. Chromosomes 3, 4, and 5 are relatively shorter in both arms. (2) Chromosome 6 has a large satellite and (3) Chromosome 7 has a short satellite. The arm difference (difference between long arm "l" and short arm "s"; d=l-s) ranged from 1 to 8, the arm ratio ($r=\frac{l}{s}$) from 1.1 to 2.1, and the index ($i=\frac{s}{l+s} \times 100$) from 32 to 62. Chromosomes 1 and 4 were median, chromosomes 2, 3, 5, and 6 were submedian, and chromosome 7 was subterminal.

Fig. 1 The karyotype of H. vulgare with 2n=14. The chromosomes are arranged in order of decreasing length from 1 to 5. Chromosome 6 has a large satellite chromosome and chromosome 7 has a small satellite.

d: arm difference

r: arm ratio

i: chromosome index

Hordeum vulgare (2N = 14)

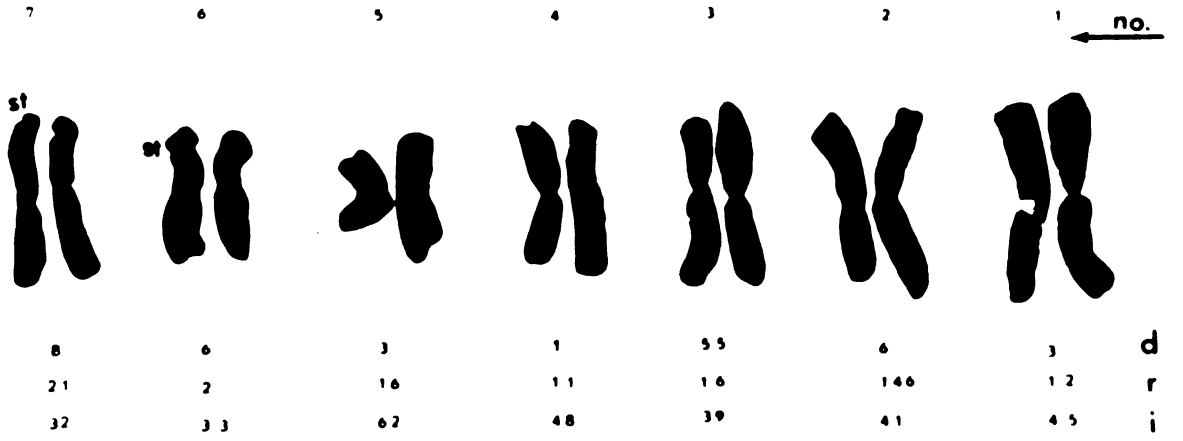

Figure 1

H. jubatum (Fig. 2)

The chromosome morphology of H. jubatum has been described by Morrison (1959), and Rajhathy and Morrison (1961). There are three types of chromosomes in Figure 2:

(1) Large satellites with a constriction cutting the long arm (chromosome 1).

(2) Small satellites on a short arm (chromosomes 5 and 10).

(3) Subterminal chromosomes (chromosomes 4 and 13).

(4) Submedian chromosomes.

The chromosomes are not stained uniformly by the Feulgen-Carmine double staining technique. A dicentric chromosome and a plant with 42 chromosomes were reported by Morrison (1959). In this study, H. jubatum has been confirmed as a segmental allotetraploid with a chromosome number of 28. It has been used as a major entry for interspecific and intergeneric hybridization in the genus Hordeum.

A. trachycaulum (Fig. 3)

The karyotype of A. trachycaulum has not yet been fully described in the literature. The chromosomes are arranged according to length, and in this study two pairs of satellited chromosomes were found: one pair of chromosomes had a very small satellite (chromosome No. 2); the other (chromosome No. 7) is relatively larger. One pair of the subterminal chromosomes, and the rest of the pairs, are median and submedian chromosomes. The classical

Fig. 2 The karyotype of H. jubatum with $2n=28$. The chromosomes are arranged in order of decreasing length.

Hordeum jubatum (2N= 28)

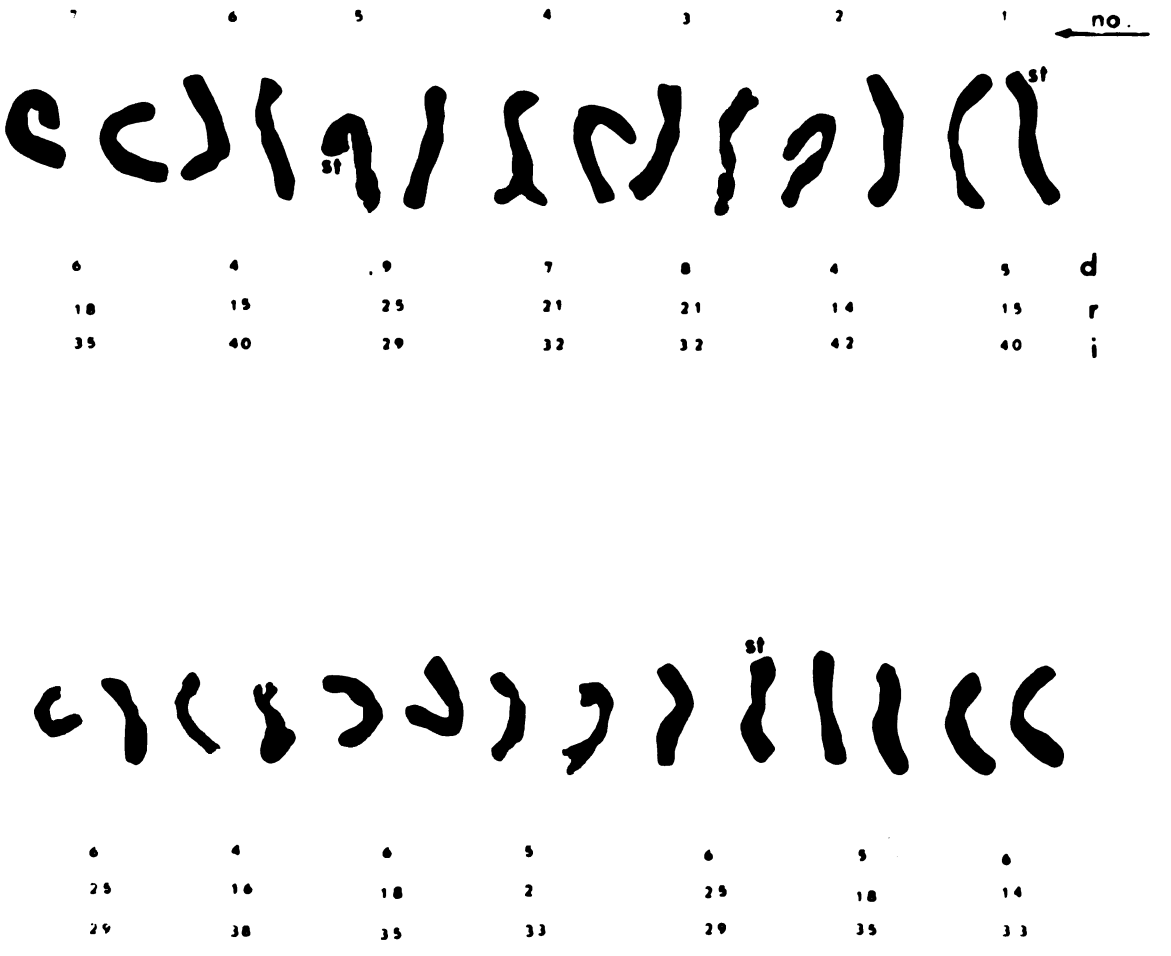

Figure 2

Fig. 3. The karyotype of A. trachycaulum with 2n=28. The chromosomes are arranged in order of decreasing length.

Agropyron trachycaulum (2N = 28)

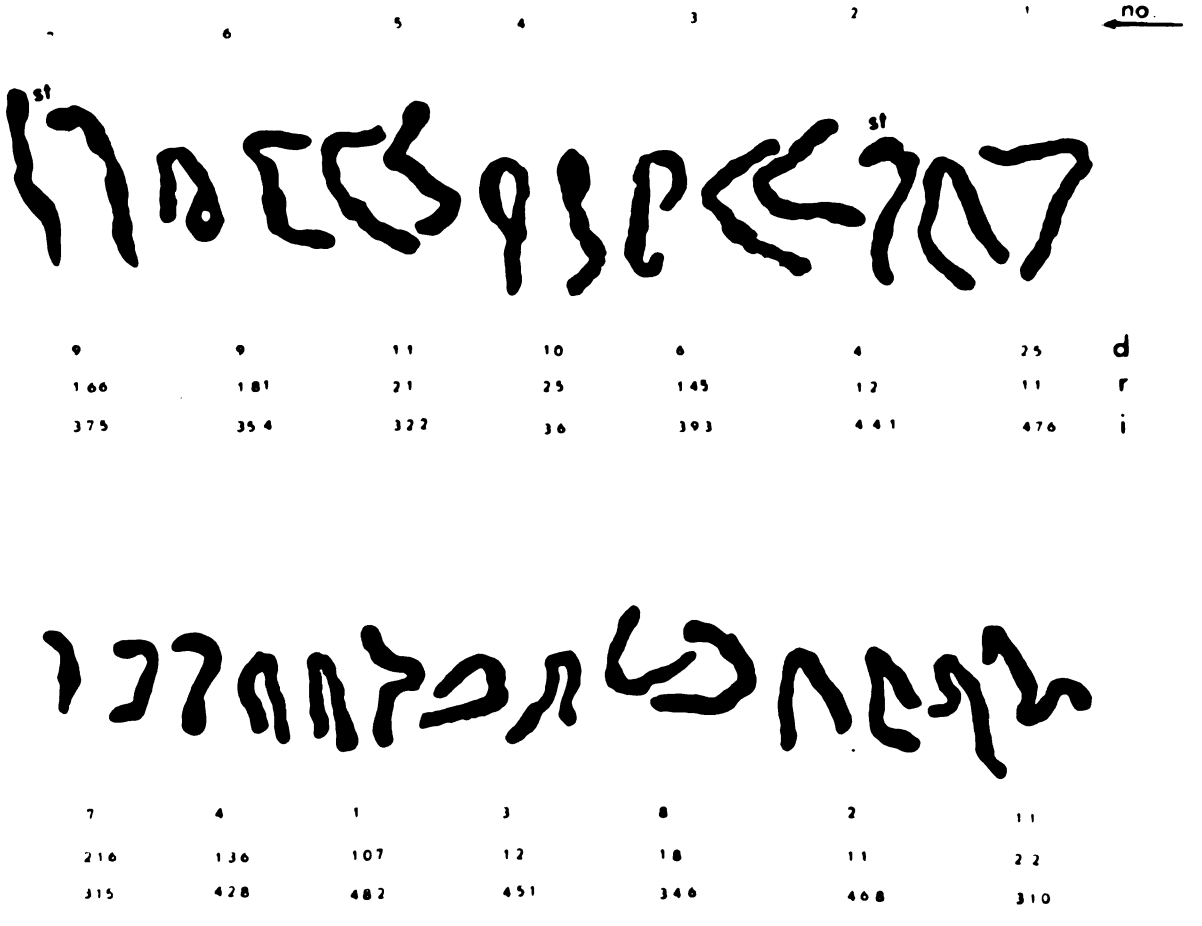

Figure 3

descriptions of fourteen pairs are as follows (Table 3): The tip and proximal regions of chromosomes were stained darker and the region between the tip and centromer was stained lighter comparatively. In relation to stain reaction, the chromosome of the darker stained region was more compressed, and the region where they were of a puffy texture was stained lighter.

Canderon (1962) found three pairs of satellited chromosomes and the rest were median and submedian in A. junceum karyotypes.

AHV 442 (2n=35) (Fig. 4)

This plant was obtained from a cross between amphiploid-(A.t. x H.j.) and diploid H. vulgare. By the genome formula, it is composed of $A_1A_2A_3BV$ (2n=35); fourteen chromosomes as A_1A_2 from H. jubatum, fourteen chromosomes as A_3B from A. trachycaulum and seven chromosomes as V from H. vulgare.

Chromosome types and karyotype. The chromosomes of AHV 442 do not occur in identifiable pairs. The karyotypes were made by sequence of their relative length. By observation, the chromosomes from H. vulgare might be identified under the microscope by their staining texture. A preliminary result of Feulgen-Carmine staining shows differential staining similar to banding. Further studies are needed to use the chromosome banding technique as a tool to classify chromosome in Hordeum.

Table 3

THE ARM RATIO, DIFFERENCE AND DESCRIPTION OF A. TRACHYCAULUM KARYOTYPE

No.	r	d	Designation
1	1.1	2.5	median
2	1.2	4	subtelocentric with large satellite
3	1.45	6	submedian
4	2.5	10	submedian
5	2.1	11	subtelocentric
6	1.81	9	submedian
7	1.66	9	median with a small satellite
8	2.2	11	submedian
9	1.1	2	median
10	1.8	8	submedian
11	1.2	3	submedian
12	1.07	1	median
13	1.36	4	submedian
14	2.16	7	submedian

As can be seen in Fig. 3, it is impossible to classify all 14 pairs on the basis of chromosome morphology. However, using the arm ratio (4) and designation of centromer position plus the presence or absence of satellite it is possible to distinguish some.

Fig. 4 The karyotype of AHV 442 with 2n=35. There are 14 chromosomes from H. jubatum, 14 from A. trachycaulum and 7 from H. vulgare.

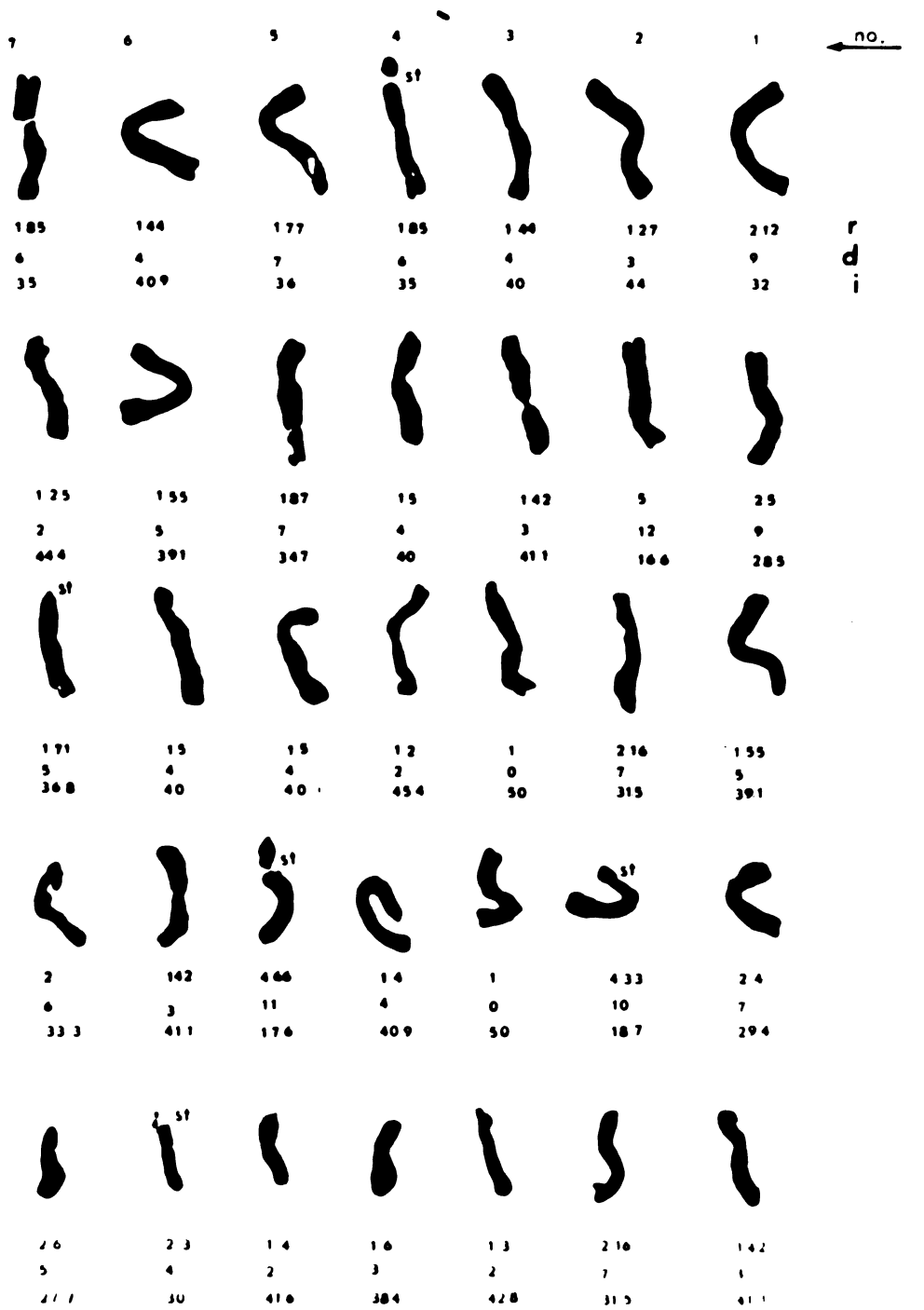

Figure 4

AHV 444 (2n=42) (Fig. 5)

This plant was produced by using the amphiploid (A. t x H.J.) as female and tetraploid H. vulgare as the pollen parent. It was composed of $A_1A_2A_3BVV$ (2n=42) with A_1A_2 from H. jubatum, A_3B from A. trachycaulum and VV from H. vulgare. No identifiable pairs could be found with confidence. Satellited chromosomes might selectively be assigned with confidence to each parent. By their origin, there should be 14 satellite chromosomes in this hybrid, only 10 satellites were recognized. Based on morphology and satellites chromosomes 13 and 15 might be from H. vulgare, chromosomes 5 and 21 from H. jubatum, and chromosomes 6 and 26 from A. trachycaulum. Median and submedian chromosomes were prevalent in nonsatellited chromosomes. One chromosome was comparatively small; it might be similar to a B chromosome.

VH-35 (Fig. 6)

This plant was produced by using a winter type male sterile H. vulgare (2n-14) as female and H. jubatum as the pollen parent. The hybrid was somatically unstable since various chromosome numbers were counted in the root tip, pollen mother cell and root tip from callus regenerated plants. A karyotype was made from a callus regenerated plant with chromosome 2n=35 which might be a partial chromosome doubled plant. At a glance, chromosome configuration and staining texture showed different patterns in comparison to either H. jubatum or H. vulgare. Originally, they

Fig. 5 The karyotype of AHV444 with 2n=42. There are 14 chromosomes from H. jubatum, 14 from H. vulgare and 14 from A. trachycaulum.

AHV 444 (2N=42)

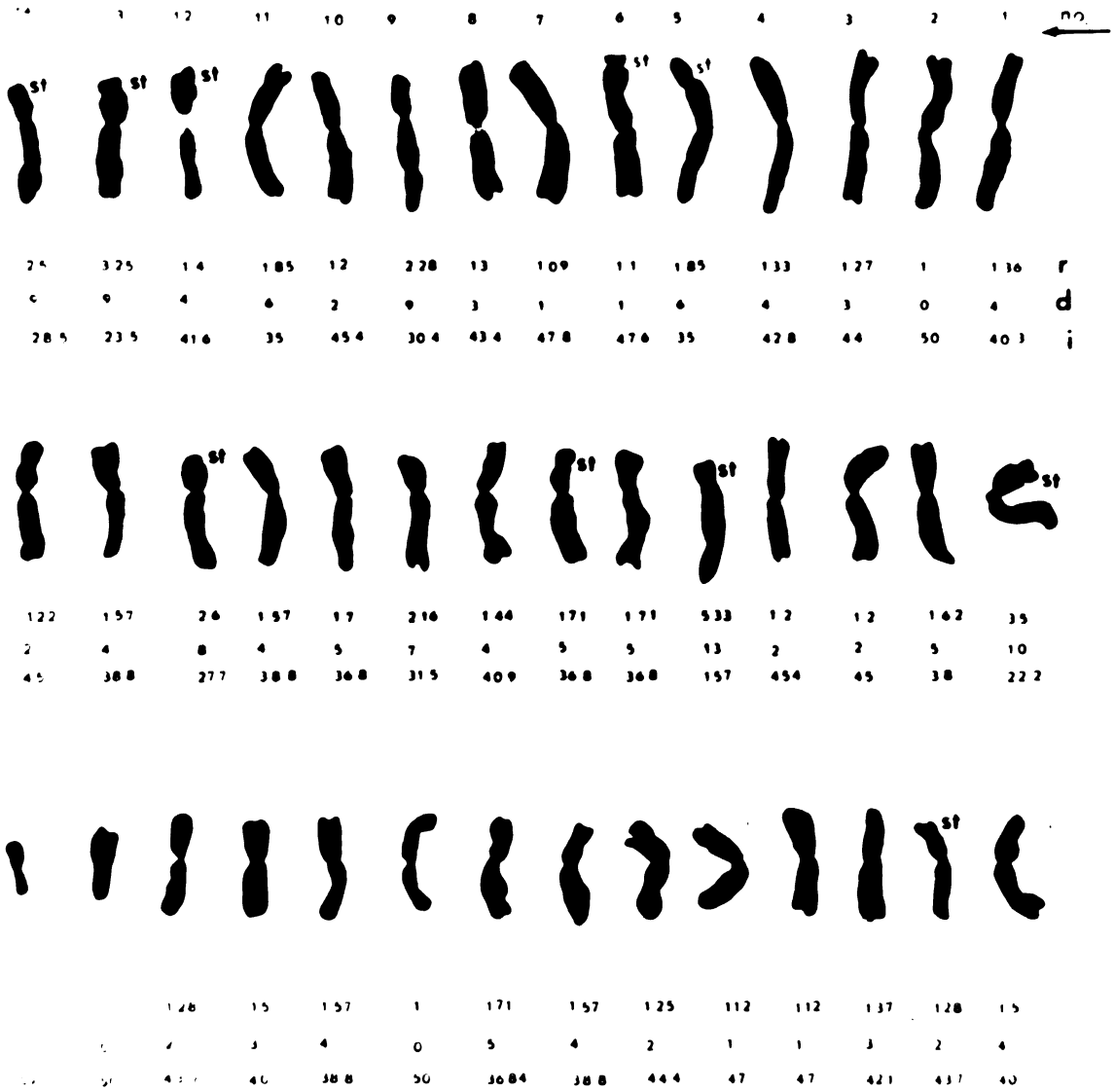

Figure 5

Fig. 6 The karyotype of VH-35 with 2_n=35. There are mixed ploidy of both H. vulgare and H. jubatum.

VH 35

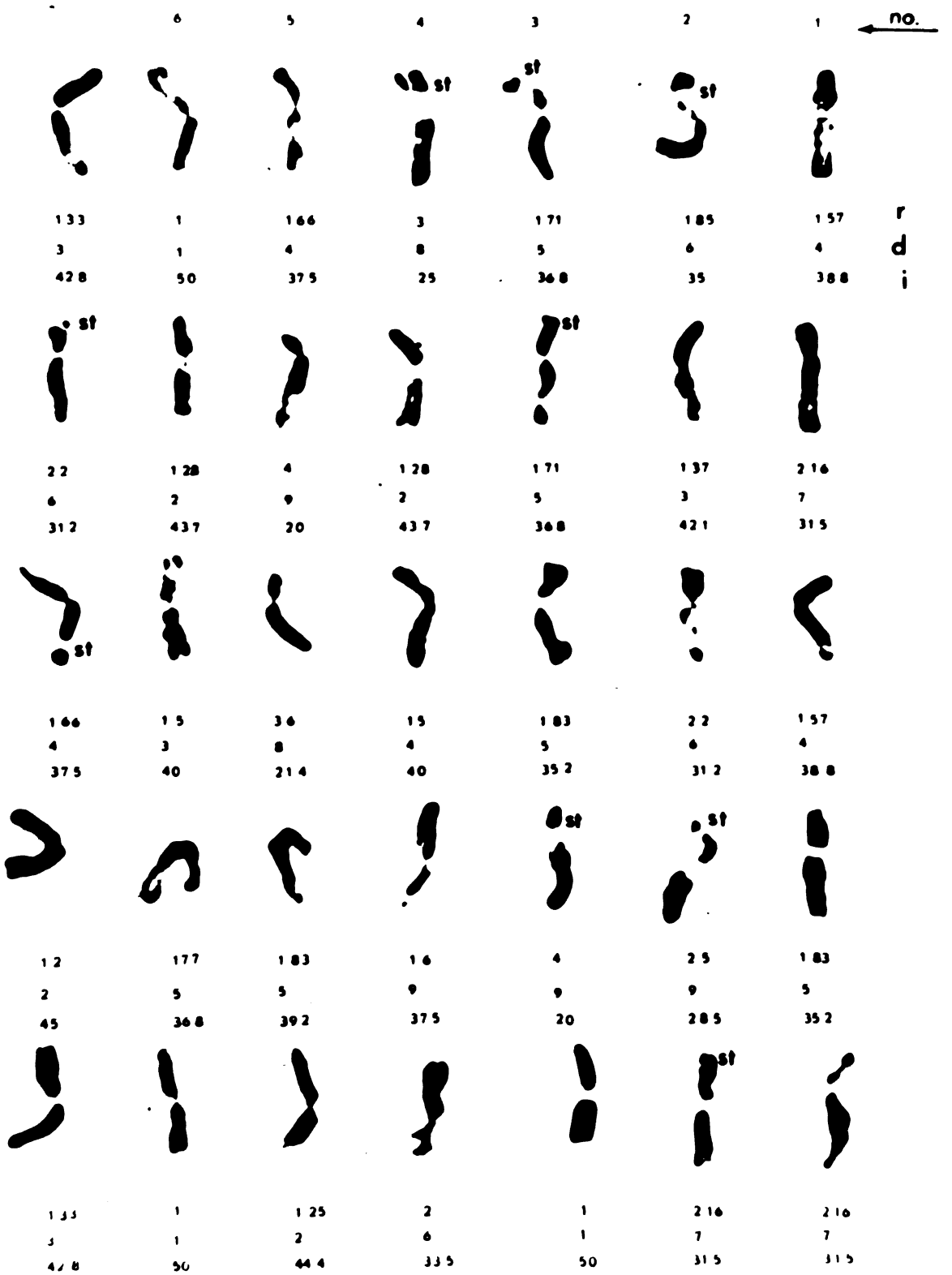

Figure 6

were 7 chromosomes from H. vulgare and 14 chromosomes from H. jubatum at the zygotic stage. Chromosome conformation and ploidy were severely changed through callus cell differentiation. This karyotype showed at least 8 satellited chromosomes, only one of them (chromosome 21) can be assumed to be similar to H. vulgare. Also, no identifiable pairs could be selected. Differential staining of Feulgen-Carmine was recorded in some of the chromosomes. Generally there is one arm stained darker than the other arm. Banding only shows in four chromosomes.

Chromosome Index Similarity

Faced with a situation of great karyotypic complexity in parental entries and series of hybrids, a simple numerical method of comparing karyotypic similarity is strongly needed for certain reasons:

1) There is no single karyotypic character which absolutely differentiates the two species.
2) Karyotype of species can be stratified on their similarity.
3) No karyotypic relation was ever established between genera.

Based on the ploidy and chromosome index, karyotypic similarity of A. trachycaulum, H. jubatum, and H. vulgare shows certain differences (Fig. 7):

1) A. trachycaulum has a broader chromosome index which covers the range of both H. jubatum and

Fig. 7 A. trachycaulum shows broader chromosome index ranging from 17-52 with a peak at 37. The range covers both H. jubatum and H. vulgare but the peak at 37 has fallen next to H. jubatum.

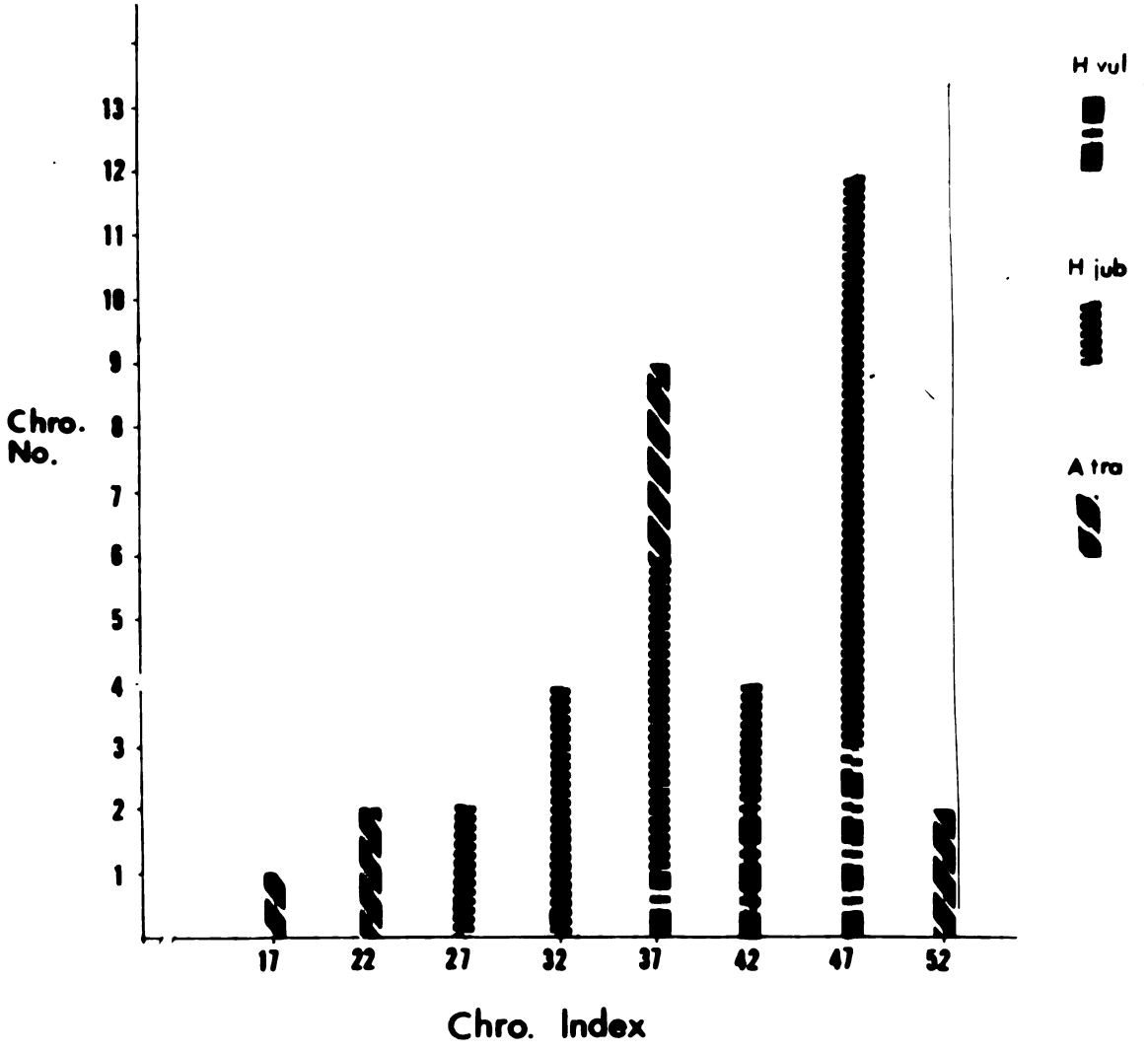
H vul
H jub
A tra
Chro. No.
13
12
11
10
9
8
7
6
5
4
3
2
1
17
22
27
32
37
42
47
52
Chro. Index

Figure 7

H. vulgare. H. jubatum has a wider chromosome index than H. vulgare.

2) A. trachycaulum chromosome index has a peak at 37 which is second to H. jubatum. H. vulgare has the same peak as H. jubatum.

3) Based on the chromosome index comparison, H. vulgare has a higher karyotypic similarity with H. jubatum than the similarity with A. trachycaulum.

In the hybrid series, the genomes and chromosomes are derived from A. trachycaulum, H. jubatum and H. vulgare. The karyotype of each hybrid is a temporary set. Alteration of ploidy and chromosome will follow the subsequent cross and genetic instability. Therefore, a comparison between hybrids and their progenitors would not be a solid assessment for a genetic relationship. Plotting the ploidy and chromosome index of hybrids, AHV444, AHV442 and VH 35 shows (Fig. 8):

1) Generally, hybrid groups have chromosome index peaks between 37 to 42. This is in accord with the parental group.

2) AHV444 with a high, ploidy nature has a wider range of karyotypic index.

3) VH-35 increases the variation of chromosome index in comparison to the scope of its parents.

Fig. 8 The chromosome index peaks in hybrid group VH-35 show co-center between 37-42 which is in accord with their parental group. Chromosome rearrangement in terms of translocation might be the cause of index change.

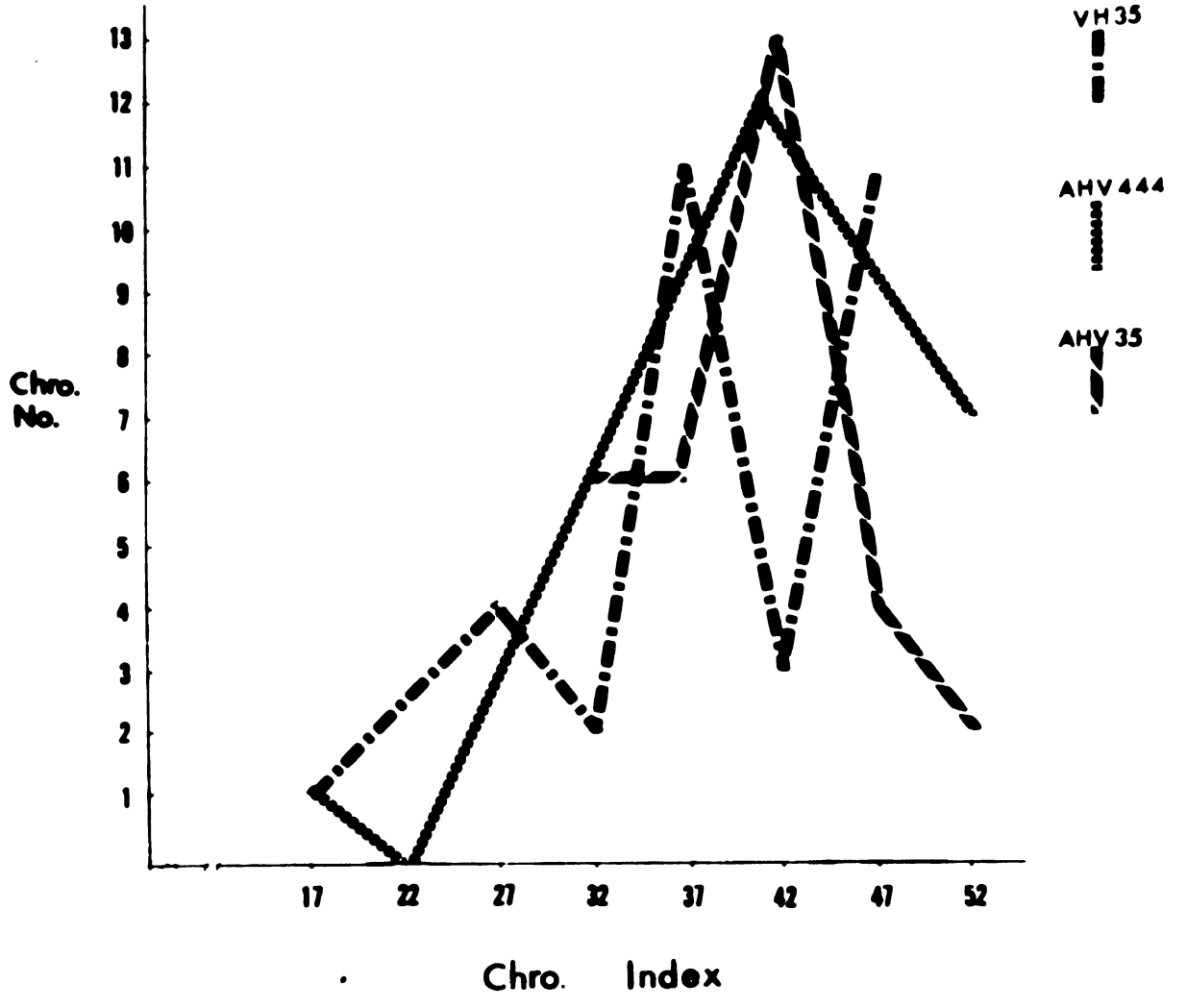
VH35
AHV444
AHV35
Chro. No.
13
12
11
10
9
8
7
6
5
4
3
2
1
17
22
27
32
37
42
47
52
Chro. Index

Figure 8

II. Results of Hybridization and Cytological Studies

Interspecific and Intergeneric Hybridization

The crosses and hybrids obtained are shown in Table 4. Their spike morphology is shown in Plate B, Figure 1, 2, 4, 5, 6. Embryo culture was used to rescue the premature embryo before abortion and to stimulate embryo germination in order to establish the hybrid development (Plate B, Figure 3.

In the first backcross, AHV-B, the embryos developed without endosperms. This indicated that a certain independence between embryo and endosperm development was initiated during the course of early zygotic differentiation. The seedling of AHV-B, was vigorous and morphologically related to H. vulgare, concomitant to increasing H. vulgare genomes due to the backcross (Plate B 4, 5, 6). Generally, the characteristics of AHV-B_1 could be described as follows:

1) Three spikelets per node.
2) More than two florets per spikelet
3) Disarticulated below the glume
4) Facultative pubescence on the leaf
5) A shortened spike of a similar size to cultivated form.

Certain characters could not be defined within the general morphology of the genus Hordeum since the hybrid still contained portions of the Agropyron genome and was maintained in the Agropyron cytoplasm (Plate B, Figure 1, 2.)

The meiotic chromosome association of plants was observed in the pollen mother cell. These data are compared with those of other plants in Table 5 and Plate A, Fig 2, 3, 4. In AHV-B, univalents averaged 4.49, bivalents averaged 14.41 and trivalents averaged 16. The univalents were significantly decreased in comparison to other plants studied, while the multivalents were slightly increased.

III. Results of Genome Interaction and Chromosome Elimination in AHV444

A systematic microscopic observation of microsporogenesis was carried out to investigate a possible cause of chromosome elimination in plant AHV444.

A. Prophase. In prophase stages, chromosomes could not be traced back to their genome of origin by staining and morphology. As prophase is a progressive sequence, it is hard to discern these stages due to the condition of abnormal condensation associated with the sticky formation of chromosomes. Superficially, all chromosome complements seem to congregate within the nucleus in a major group. When the mucleus envelope is disrupted, small chromosome groups gradually segregate from the major group during the condensing process. The univalents segregated randomly throughout the spindle in most of the plates before reaching metaphase I. Uncoiled chromosomes expelled from the major chromosome group in early prophase were occasionally observed.

Table 4

DESIGNATION OF ALL CROSSES ATTEMPTED

Cross Designation	2n	Genome Designation	Plants Obtained	Remark
AT x HJ	28	$A_1A_2A_3B$	18	
HJ x AT	28	$A_1A_2A_3B$	1	
AHV442	35	$A_1A_2A_3V$	7	
AHV444	42	$A_1A_2A_3VV$	1	
mix-octoploid	56	$A_1A_1A_2A_2A_3A_3VV$	1	
AHV-B_1	35	A_xA_yVVV	10	Octoploid was used as female to cross tetraploid H. vulgare
VAH444	42	$A_1A_2A_3VV$	2	
VAH442	35	$A_1A_2A_3V$	5	Winter type male sterile H. vulgare was used as female in crosses with the amphiploid (A.t.XH.j.)
Amphiploid-(At x Hj) X Amphiploid-(Hj x Hc)			20	
VH	35		8	Hybrid made by R. P. Steidl

Table 5

THE CHROMOSOME ASSOCIATION OF PLANTS

Plants		Chromosome Association I	II	III	IV	No. of cells
A. trachycaulum (2n=28)	Range:	0	14	0	0	100
	Mean :	0	14	0	0	
H. jubatum (2n=28)	Range:	0	14	0	0	100
	Mean :	0	14	0	0	
(A.t. x H.j.) Natural (2n=28)	Range:	7-24	2-8	0-2	0	50
	Mean :	16.38	5.44	0.14	0	
(A.t. x H.j.)*** Artificial (2n=28)	Range:	7-18	5-10	0-3	0	31
	Mean :	11.51	7.22	0.67	0	
(H.j. x A.t.)*** Artificial (2n=28)	Range:	7-22	3-7	0-2	0-1	96
	Mean :	15.80	5.60	0.32	0.11	
Amphiploid-$(A.t. \times H.j.)^2$ (2n=56)	Range:	0-6	22-28	0-2	0-1	71
	Mean :	2.36	25.63	0.71	0.06	
AHV 442* (2n-35)	Range:	1-14	9-17	0-1	0	66
	Mean :	6.53	13.98	0.16	0	
AHV 444** (2n=42)	Range:	5-15	12-17	0-1	0	112
	Mean :	7.89	15.92	0.86	0	
AHV-B (2n=35)	Range:	1-7	11-17	1-2		50
	Mean :	4.49	14.41	0.56		
Amphiploid - (A.t. x H.j.) x Amphiploid - (J.j. x H.c.)	Range:	2-8	10-17	1-3	0-1	30
	Mean :	6.64	15.56	2.76	1	

* Amphiploid - (A.t. x H.j.) x H. vulgare (2n=14)
** Amphiploid - (A.t. x H.j.) x H. vulgare (2n=28)
*** Reciprocal crosses

Plate A

Fig. 1 Metaphase I in amphiploid - (A.t. x H.j.) with 2n=56

Fig. 2 Metaphase I in AHV 442 with 13 I + 8 II (3 ring) + 2 III (arrow) (x915)

Fig. 3 Metaphase I in AHV 444 with 13 I + 13 II (7 ring by arrow) + 1 III (x 915)

Fig. 4 Metaphase I in AHV-B_1 with 3 I + 13 II + 2 III (arrow)

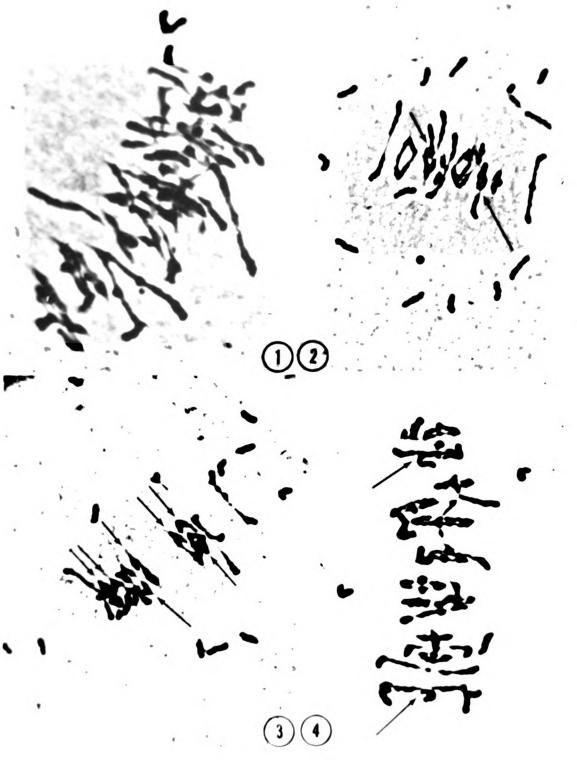

Plate A

Plate B

Fig. 1 Spike of A. trachycaulum (right) hybrid (center) and H. jubatum (left)

Fig. 2 Spike of Amphiploid-(A.t. x H.j.)

Fig. 3 Young seedling of hybrid plant grows on paper towel in liquid media

Fig. 4 Spike of AHV 442

Fig. 5 Spike of AHV 444

Fig. 6 Spike of AHV-B_1 (left) and H. vulgare (right)

Plate B

These cells seemed to be aborted before reaching metaphase I since no reduced chromosome number was recorded at metaphase I.

B. Metaphase I. Selected metaphase I was the main stage used to record chromosome associations in the form of their pairing patterns. The chromosome number was in accord with the expected complements of 2n=42 in most cases. Multivalents were observed in spite of a confusing sticky association which usually involved more than three chromosomes.

At metaphase I, the chromosomes of H. vulgare presumably in form of rings seem to be larger and stained deeper than the chromosomes of either H. jubatum or A. trachycaulum (Plate A, Fig. 3). But such a difference was not clearly discernible and consistent in all the cells.

C. Anaphase I. An anaphase bridge was frequently observed; this might be the result of belated separation of chromosome association due to sticky association or to a deletion and a translocation. The stickiness of the chromosome in conjunction with spindle abnormality might be a physical mechanism of chromosome differentiation such as the centri fission-fusion[1] process which was significantly seen in metaphase II.

---

[1]Gross chromosomal rearrangement involving in centromer region

D. Interphase I. Incursion chromosomes, or chromosome fragments in the process of uncoiling, were observed in interphase I. (Plate D, Fig. 7). The impact of chromosome association resulting in chromosome differentiation after first division was significantly revealed through abnormality in chromosome number and behavior after first division. The process in two daughter cells was not synchronized since they were independent, with different genetic make-up.

E. Metaphase II. A variety of chromosome numbers in metaphase II were commonly observed. The majority of chromosome complements in metaphase II were under 21, the expected number under the normal reduction process of the first meiotic division. Mainly the chromosome number after reduction ranged from 20-15. Due to the lack of a technique for individual chromosome identification, this selective reduction process could not be specified by different genome of origin. Secondary chromosome association in the form of a sticky connection at the terminal and centric region between chromosome was a common phenomenon. Obviously, chromosome fragments derived from centric fission can be identified (Plate C, Fig. 1, 2, 3, 4, Plate D, Fig. 5). Unequal segregation in proceeding anaphase II was recorded (Plate D, Fig. 6). Chromosome enclosed in microspore cells by abnormal furrowing separation from

the major cytoplasm was an alternative to individual chromosome degredation (Plate D, Fig. 8).

F. Photomicrographs of microspore with different chromosome number are shown in Plate E, Fig. 9, 10, 11, 12; Plate F, Fig. 13, 14, 16; Plate G, Fig. 19, 20. The exine fragments of the microspore wall are washed off during the process of slide preparation. The chromosomes in microspores were stained darker than usual. As general view, chromosomes seemed to be grouped rather than randomly dispersed, but the chromosomes did not orient themselves on the metaphase plate. A range of chromosomes of 13 to 23 with a peak around 17 (Table 6). Preferential chromosome viewing in the microspore is evidently associated with the phonomenon of chromosome elimination. Two hypotheses are advanced to explain the distribution of chromosome number in the microspore based on the assumption that chromosome elimination results from genome interaction then the data in Table 6 could support the hypotheses by the microspore having a full set of 7V plus unknown number from either Agropyrom and H. jubatum versus 7A (Agropyrom) + 7j (H. jubatum) with unknown number from H. vulgare. Microspore failure to reorganize the nucleolus, and the disoriented separation of chromosomes in the microspore, were observed (Plate G , Fig. 15, Plate G, Fig. 17, 18). Thus the presence or absence of some factor could result in the elimination

of certain combinations of chromosomes.

Table 6

CHROMOSOME NUMBER AND ITS VARIATION IN MICROSPORE IN AHV444

Chromosome Number	Microspore Counted	Percent	Expected Form
13	8	3.23	a
14	14	5.66	a or b
15	29	11.74	a or b
16	48	19.43	a or b
17	44	17.81	a or b
18	35	14.17	a or b
19	33	13.36	a or b
20	20	9.71	a or b
21	8	3.23	a or b
22	7	2.83	b
23	1	0.40	b
	247		

a $xA + yj = 7V$

b $7A + 7j + xV$

Plate C

Fig. 1. Metaphase II in AHV 444 with group separation and two chromosome fragments (arrow)

Fig. 2. Metaphase II in AHV 444 with two chromosome fragments (arrow)

Fig. 3. Metaphase II in AHV 444 with one chromosome fragment and sticky association

Fig. 4. Metaphase II in AHV 444 with centromer transverse breakage (arrow)

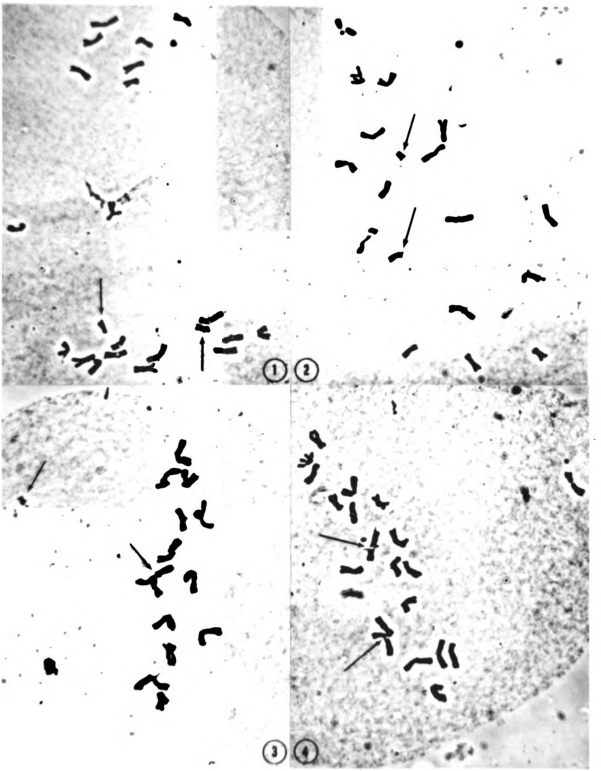

Plate C

Plate D

Fig. 5. Metaphase II with a chromosome fragment and a centromere transverse breakage (arrow)

Fig. 6. Telophase II with an unequal chromosome

Fig. 7. A tetrad with a some chromosome excluded from major group

Fig. 8. A chromosome was eliminated by means of budding cell in Metaphase II

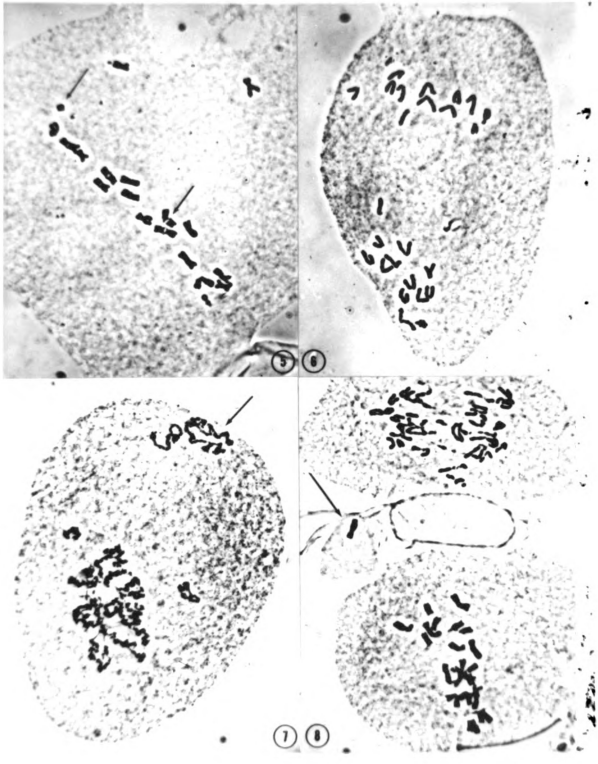

Plate D

Plate E

Fig. 9. Pollen mitosis with 21 chromosomes (678x)

Fig. 10. Pollen mitosis with 14 chromosomes (678x)

Fig. 11. Pollen mitosis with 16 chromosomes (678x)

Fig. 12. Pollen mitosis with 15 chromosomes (678x)

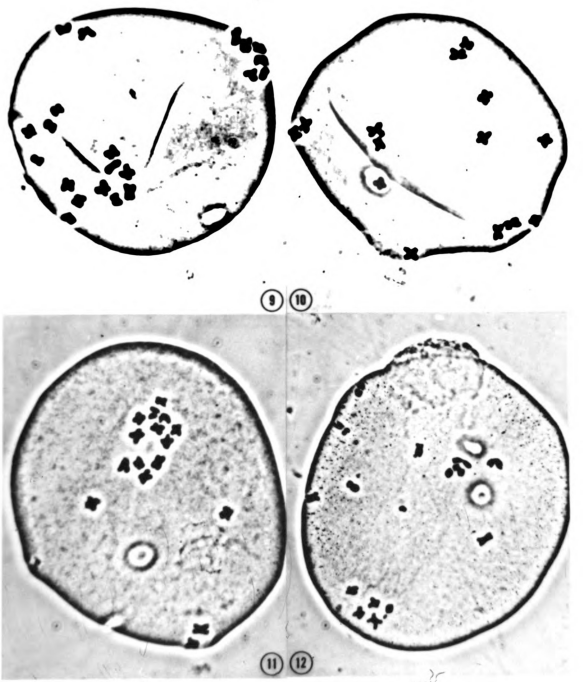

Plate E

Plate F

Fig. 13. Pollen mitosis with 19 chromosomes (678x)

Fig. 14. Pollen mitosis with 18 chromosomes (678x)

Fig. 15. Pollen mitosis with disoriented Anaphase (678x)

Fig. 16. Pollen mitosis with 16 chromosomes (678x)

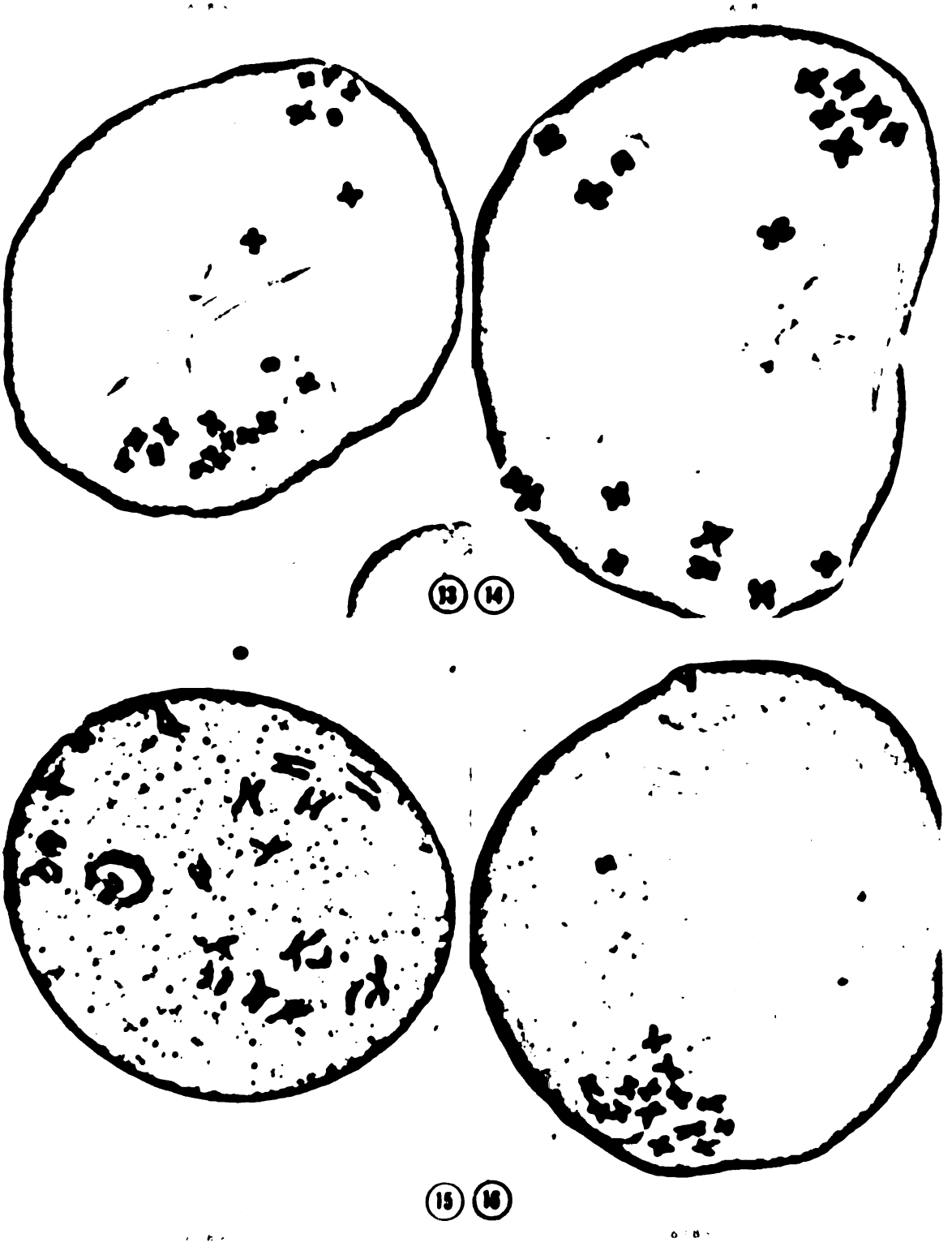

Plate F

Plate G

Fig. 17. Two pollen cell with unorganized nucleolus (590x)

Fig. 18. Two synchronized pollen Anaphase (590x)

Fig. 19 and 20 Synchronized pollen mitosis cells (678x)

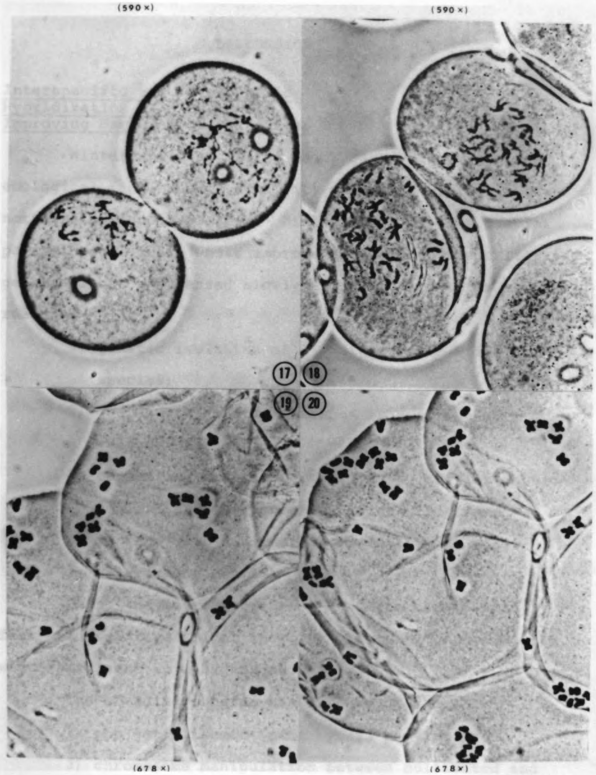

Plate G

DISCUSSION

Interspecific and Intergeneric Hybridization as a Means of Improving Barley Winterhardiness

Winterhardiness is the major factor limiting both ecological range and productivity of winter barley in the northern agricultural area. Winterhardiness is a complex physiological trait whose improvement through the cultivated gene pool has progressed slowly in recent years for several reasons:

1) genetic isolation of H. vulgare from wild species;
2) inadequate genetic variance in existing H. vulgare gene pool;
3) concealing genetic variability by complex physiological and environmental interaction, and
4) linkage with disease susceptibility or other deleterious traits.

Thus, the introgression of an alien gene source from wild relatives might increase the genes governing the winterhardiness to a higher ceiling.

The breeding program has five major steps:

1) the establishment of a hybrid population;
2) chromosome manipulation between cultivated and wild genomes in hybrids (Fig. 22)

3) metabolic identification and genetic modification of differentiated and undifferentiated cell populations;
4) fertility restoration, and
5) gene accumulation through random breeding and selection using male sterile populations.

A hypothetical outline of a possible attack on the problem of gene introgression from wild species is given in Figure 21 of this section. In conjunction with previous studies, the partially fertile octoploid was obtained by colchicine treatment on AHV442. The genetic formula of this octoploid is presumed to be $A_1A_1A_2A_2A_3A_3VV$ with a chromosome number 2n=56. It is difficult to determine chromosome associations in metaphase I since many multiple associations are involved. The B genome from AHV442 is assumed to have been lost in the process of chromosome doubling because the B genome seems most different but we have no way of knowing that is so. The double dosage of VV might cause preferential elimination of the less compatible B genome. The dosage effect of VV genomes has been fully discussed in previous studies. Chromosome or genome substitution is prevalent in an intergeneric hybrid; Knott et al (1977) reported a stem rust-resistant wheat line derived from a wheat-Agropyron hybrid. The resistance proved to be carried on an Agropyron chromosome which had replaced the wheat chromosome 7D. Apparently, Agropyron chromosomes compensate well for 7D in both vegetative and reproductive gene systems in substituted lines.

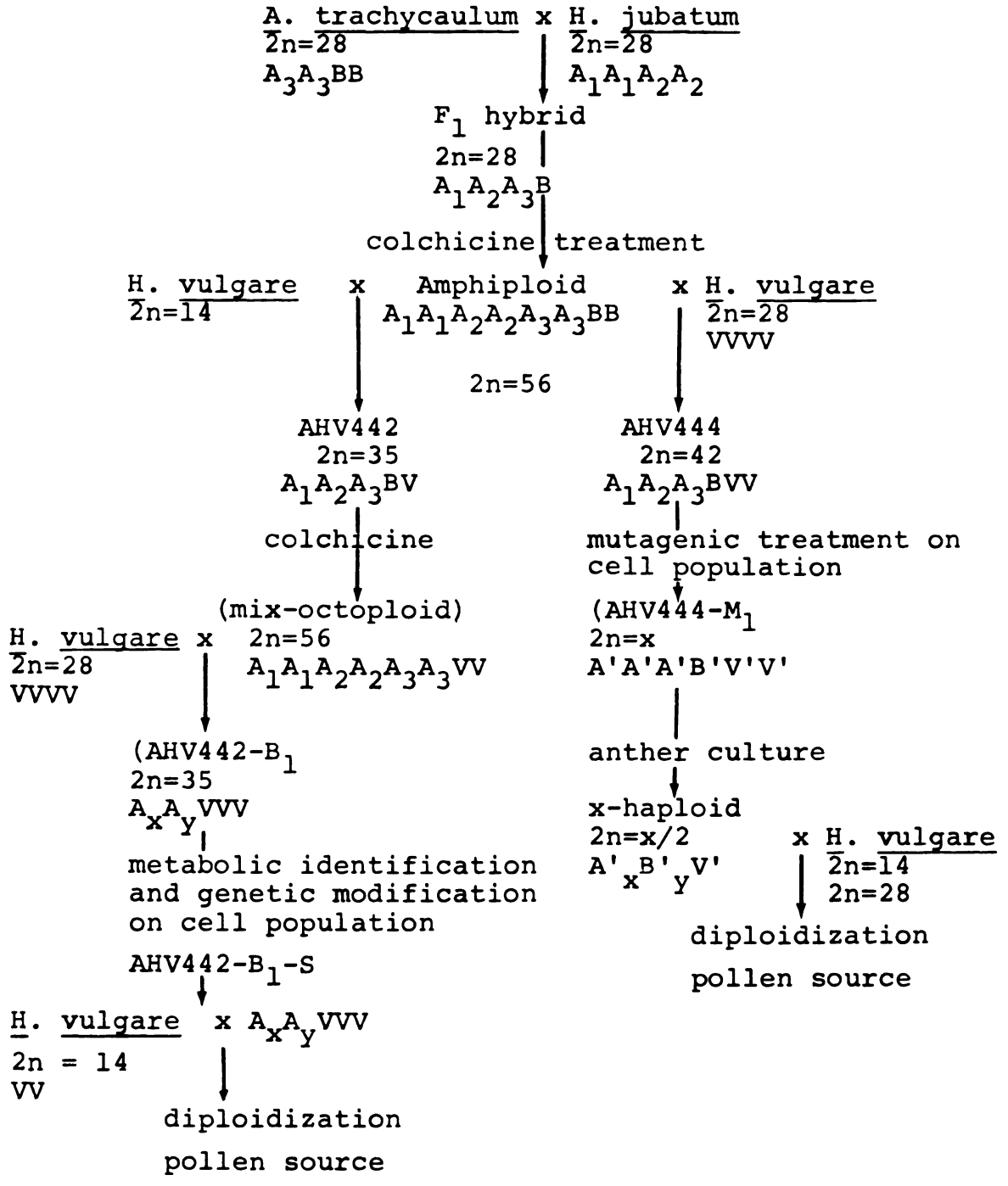

Figure 21. Diagramatic outline of the steps and procedures in introgressing agronomic gene source from wild germplasm into cultivated barley

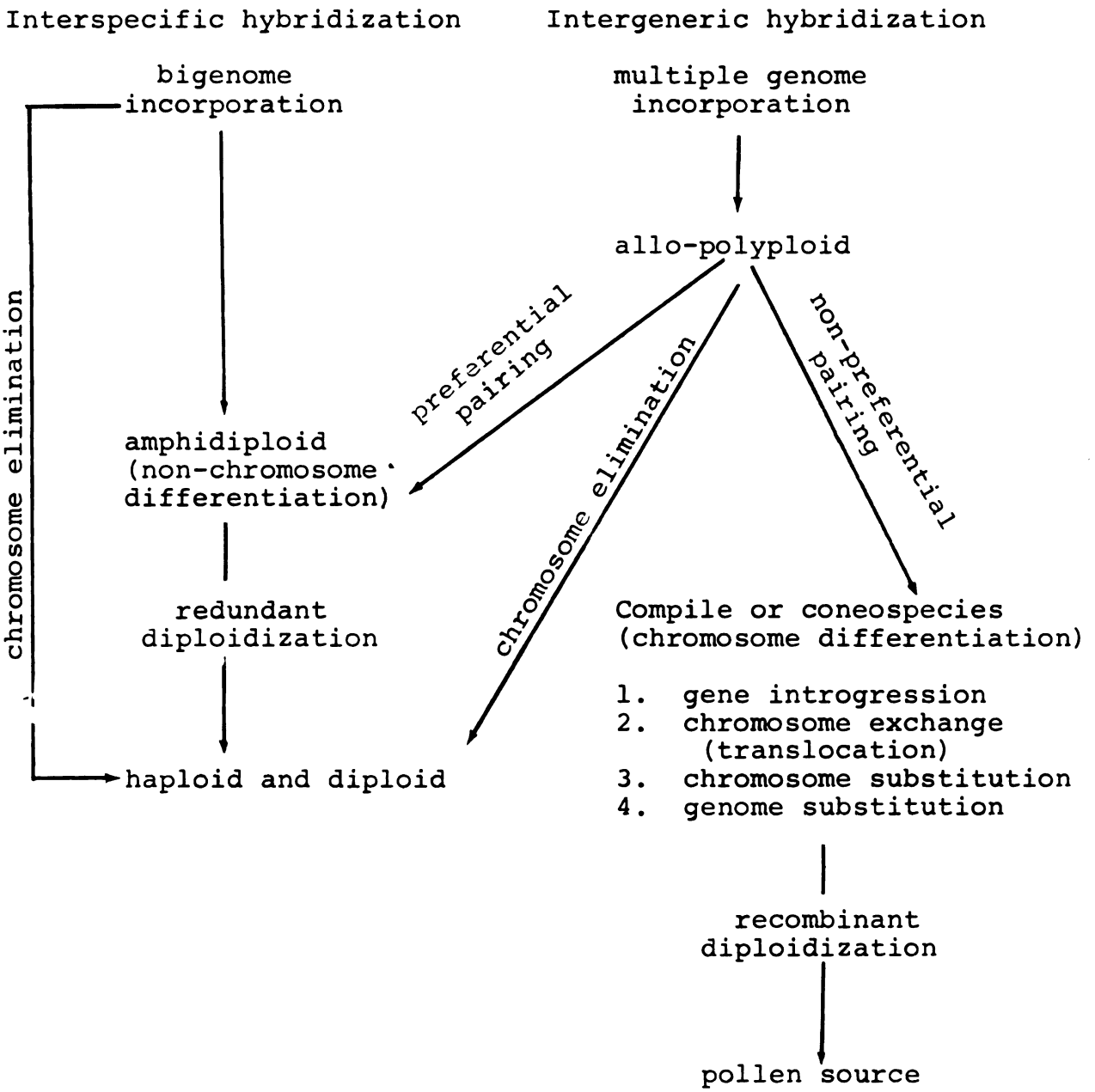

Figure 22. Scheme of Chromosome Manipulation

Similarly, Thomas and Thomas (1974) reported that mildew resistance of Avena barbata has been successfully incorporated into the cultivated oat by replacing a pair of chromosomes. The use of colchicine to induce chromosome doubling in many plant species has been well documented since 1939. There has been speculation that colchicine disturbs spindle formation in mitotic division yielding to a doubling of the chromosome number. The consequence of this doubling is to stabilize the genome in the form of diploidization. With heterogeneous genome constitution of AHV442, selective genome doubling in order to reach somatic stability is one alternative. On the other hand, stability as well as genome balance can be achieved by a regular disjunction of quadrivalents. Multivalents were prevalent in this octoploid plant. Thomas (1974) reported that meiotic stability and fertility were even accomplished by quadrivalents instead of bivalents in an allotetraploid form of Lolium multiflorum.

With respect to gene introgression in the form of non-preferential bivalents, it was unique in the genetic control over chromosome pairing by a BL genes system between an intergeneric hybrid of wheat and Agropyron (Larson, 1970; Sears, 1973; Wienhues, 1973; Cauderon and Ryan 1974; Dvorak and Knott, 1974; Sinigovets, 1974; Knott, D. R. et al, 1977) and by a Pb gene system between wheat and Aegilops (Riley et al., 1968; Athwal and Kimbar, 1972; Maan and Sasakuma, 1977). The knowledge of the occurrence of genetic factors

affecting pairing, compels us to treat the assessment of genetic relationship, based on the pairing behaviour of chromosomes to a certain degree of extension. If an octoploid has been reached under certain degrees of chromosome differentiation through non-preferential multivalents, then the probability of an ovum with a balanced chromosome complement of A_1A_2V may be higher than 1 to 2^{21} as postulated on a strictly random basis.

Since the fertility of the octoploid has been partially restored, the first backcross to the tetraploid H. vulgare (2n=28) was achieved with a chromosome number 2n=35. The genome formula was estimated to be A_1A_2VVV. The frequency of univalents was significantly decreased in the first backcross. This is a promising step toward obtaining a further progressive backcross to H. vulgare and leading to gene transgression. In the literature, the number of univalents has been considered a more precise indicator than multivalents of meiotic stability and fertility. The attempt at a progressive backcross is under way.

Chromosome association as an indicator of the course of chromosome elimination

Irregularity of cell division and chromosome behavior following Metaphase I might indicate the possible cause and mechanism of chromosome elimination in the intergeneric and interspecific hybrid. Somatic chromosome elimination following fertilization would have a similar pattern in a certain stage after Metaphase I but in zygotic differentiation it

would involve both genetic and physiological interation.

Plant AHV444 had an equal dosage of three different genome origins; A_1A_2 from H. jubatum, A_3B from A. trachycaulum and V V from H. vulgare. In fact, mosaic tissue and reduced ploidy tillering are commonly produced from the cloned population. The somatic unstability induced by V V dosage from H. vulgare was a common phenomenon in most of the intergeneric and interspecific hybrids. In addition, the somatic instability in the form of chromosome elimination can be enhanced by a chemical agent such as gibberellic acid during zygotic development, or colchicine at mitosis of meristematic differentiation. Based on systematic observation from Metaphase I of pollen mitosis on this somatic unstable plant, the phenomenon of chromosome elimination was speculated as a result of genome interaction. Specifically, homoeologous association of $A_1A_2A_3$ was suppressed by VV homologous genomes. Consequently, the suppressed genome of A_1A_2 and A_3 were misgrouped and disoriented in conjunction with spindle irregularity. The microcells containing one or two exiled chromosomes were frequently observed through furrowing division in certain stages after Metaphase I.

A constant 7-ring bivalent was formulated as from VV genomes while A_1A_2 were under loosely terminal association, A_3B as free associated univalents, and eventually only the VV genome proceeded further to pollen development while the chromosome of A_1A_2 and A_3B genomes were randomly eliminated along the course of microsporogenesis. In comparison with

plant AHV442 which lacked a V genome and was somatically more stable since the single V dosage cannot function complementarily to inhibit other genomes. Therefore an homoeologous association of A_1A_2 and A_3B were indirectly enhanced by the V. genome. In AHV442, the V genome was assumed to be the source of the seven univalents which were observed scattered around the metaphase plate. When the number of bivalents approaches fourteen, it only comes from enhanced non-homologous pairing between A_3B. On the other hand, when VV was present in a double dosage, it inhibited the homoeologous pairing of A_1A_2. Bivalents were then seen as ring shaped from VV genome and it would seem, therefore, that directional chromosome elimination can be predicted whenever a homoeologous association is inhibited. The impact of homoeologous-homologous interaction and its consequences for directional chromosome elimination were traced from Metaphase I to pollen mitosis. The patterns of unequal chromosome number of Metaphase II ranged from 14-23. The variation in chromosome number could be explained by nondisjunction, but in this case with such a wide range of variation and a specific interval between 16-17 indicated a preferential elimination between genomes and then, a random chromosome is lost within the eliminated tenome. (Table 6) On the other hand, nondisjunction due to gene disturbance would not reach such a wide spectrum, nevertheless the cause of nondisjunction in this complex hybrid might also link incompatibility of chromosome association and genome unbalance. In accordance

with the chromosome number in the pollen, two possible formulas can be derived: 7V+xA+yJ versus 7A+7JxV. In the first form, 7V chromosome resulted from reduced homologous pairing of VV genomes and a random number within 7 from each of A_1 and A_2. In the 7A+7J+V form, a constant of 14 chromosomes were derived from $A_1A_2A_3$ and B but excluded V. The formula of 7V+7A+xJ with randomly eliminated A_3 and B would fit rather in the range 14-21 with a peak at 16-17. In fact, the H. vulgare type of the somatic sector and tiller were observed. The directional chromosome elimination was in favor of H. vulgare in the AHV 444 plant but inferior to the other genome in the AHV 442 plant. Using pollen cell culture and their regenerated plantlet might further reveal the details of the directional chromosome elimination. This approach would be more direct than an embryo squash as well as morphological speculation, which is difficult to insure that normal fertilization takes place before elimination proceeds.

Kasha (1974) systematized three basic mechanistic categories as:

1) Spindle or centromere abnormalities

2) Asynchrony in parental mitotic cycles

3) A host modification-restriction enzymatic system.

Based on these categories, chromosome elimination was assumed to be a functionally gradual process of individual chromosome degeneration over the period of many division cycles. Data from a series of early embryo squashes has not yet materialized to back up this proposal. None of the final

chromosome elimination products of eight chromosome progenies ever existed in the cross between the diploid trisomic H. vulgare with tetraploid H. bulbosum.

A more dramatic somatic reduction would cause a decrease in chromosome number. The process is a spindle oriented abnormality. Handmaker (1971) proposed the spindle abnormality as the mechanical cause of chromosome elimination, based on the study of somatic hybridization of the mammalian cell. Groups of chromosomes are not included on the spindle of Chinese hamster x mouse somatic hybrid and consequently the grouping chromosome was excluded from the daughter nuclei. The asynchronous biogenic process due to differences in parental cell cycle also is a special form leading to the possible cause of chromosome elimination. Gupta (1973) observed a high frequency of anaphase bridges concomitant with chromosome elimination. Asynchronous cell cycles leading to insufficient time to complete replication was allotted to the slower constituent before condensation was induced. The chromosome bridge and the loss of a fragment was seen as the form of chromosome elimination of the interspecific hybrid in the genus Nicotiana. The various forms of chromosome elimination in mitotic cell division was similar to the form after Metaphase I, therefore, the common cause derived from the result of chromosome association which takes place naturally in meiotic genome behavior can also be in mitotic association in a zygote if the normal fertilization process takes place before chromosome elimination following somatic genome association.

Ho and Kasha'a (1975) hybrid population constituted IV:2B derived from crosses between trisomic diploid H. vulgare with tetraploid H. bulbosum from their data, univalents with the mean around 7 and bivalents around 7 are observed. The stability was based on homologous pairing with a B genome as B_1-B_2; the univalent should come from the H. vulgare genome, a case quite similar to AHV442 which has 7 univalents from H. vulgare and 14 bivalents from the homoeologous pairing of A_1A_2 and the non-homologous pairing A_3B. Chromosome elimination occurred dramatically when tetraploid H. vulgare was crossed with tetraploid H. bulbosum since homologous pairing was undertaken by the VV genome and eventually inhibited the B_1B_2 homoeologous pairing.

As long as chromosome elimination was directional and preferential, the strategy to bring it under control has a significant meaning in evolution and is more important in practical value to plant breeding. In this study, AHV442 was repeatedly treated with colchicine. As a result, genome interactions were reconciled even doubling of the V genome along with compatible genomes from $A_1A_2A_3$. The genome balance therefore reached a higher level with a total chromosome number 2N=56 and sterility was partially restored. Further backcrosses were subsequently achieved. The chromosome number in the first backcross was 35. Chromosome number in the association significantly decreased in the univalents with a mean around 4.49 and multivalents increased to .56 (trivalents). The constitution of this hybrid was assumed

to be A_xA_yVVV. Phenotypically the first backcross resembled H. vulgare in dosage. The hybrid again balanced non-preferential association of either A_xA_y and VVV genome; A_xA_y might form a 7-ring bivalent since they went through 5 mitotic and 2 meiotic cycles of chromosome association and VVV under unbalanced homologous pairing to form trivalents. Therefore, no chromosome elimination would result in this first backcross. The conclusion reached is that the phenomenon of chromosome elimination involving the genus Hordeum could be alleviated by 1) neutralizing the genome interaction to H. vulgare by introducing single dosage of H. vulgare genomes at first cross; 2) chemical disturbance of normal spindle function on specific genomes; 3) maintain a wild cytoplasm which would prevent cyto-nuclei harmony for a specific genome (Figure 22).

In an attempt to have artificial control of directional chromosome elimination, Sagar and Ramanis (1967, 1973) used UV irradiation to block the chloroplast DNA elimination mechanism and subsequently produced large numbers of biparental zygotes for genetic analysis in Chlamydomonas. Pontecorvo (1971) observed that X-ray or gamma irradiation of one parent before somatic cell fusion of mouse and Chinese hamster cell lines would arrest those chromosomes which were directionally lost under no treatment.

Karyotype analysis

Agropyron trachycaulum was quite distinct morphologically from both H. jubatum and H. vulgare. But in the

hybrid of AHV442 and AHV444, A. trachycaulum chromosomes could not be distinguished from either H. jubatum or H. vulgare chromosomes in either meiotic or mitotic chromosome configurations. Their relationship can only be speculated on the basis of the genome formula and meiotic chromosome associations. Karyotypic analysis has been employed to typify the relationship in terms of evolutionary speciation between species by karyotypic similarity. At the present stage, karyotypic analysis was unable to define the relationships of karyotypic similarity between A. trachycaulum, H. jubatum, and H. vulgare. But it established the potentiality of detecting chromosome differentiation leading to gene introgression in comparison to subsequent hybrids.

Individually, the karyotype of H. jubatum has three pairs of satellite chromosomes and two pairs of telocentric chromosomes which are quite different karyotypically from H. vulgare which has two pairs of satellite chromosomes and the rest are median and submedian chromosomes. Compatibility between genomes of H. jubatum and H. vulgare might depend on their physical structure. Therefore, there is quite a gap for these two genomes to match up physically in meiotic synapsis. In fact, the asynaptic behavior between genomes of H. vulgare (2X) and H. jubatum (4X) has been observed (Murry, 1975). The karyotypic analysis is in accord with the chromosome association as a compatible indicator between genomes of H. vulgare and H. jubatum.

In the genus Hordeum, cultivated H. vulgare is not the only species evolved to become incompatible with other wild forms. For instance, H. jubatum was significantly different in karyotype to H. lechleri, H. californicum and H. pusillum. As a result, persistent 7-14 univalents were observed in the hybrid of H. jubatum when it was crossed with any of the three species. In addition, there was a significant number of univalents among combinations of 7 species in the genus Hordeum (Rajhathy, 1961). Chromosome exchange, as well as gene transgression, was limited in most of these hybrids. Karyotypic variation, accompanied by uncompatibility within the genus Hordeum, of heterogeneous genome origin, and, subsequently, genome differentiation were assumed within the genus Hordeum. Based on hybridization within the genus Hordeum, H. jubatum has been described as a segmental allotetraploid.

In addition, spontaneous hybrids between Hordeum spp, with other genera such as Agropyron, Elymus, Secale, and Haynaldia were well documented. On the other hand, a gene pool overlap among genera could be reflected in their karyotypic ambiguity within the tribe Triticeae. In this study, a plot of chromosome index range and frequency of H. jubatum shared the co-center with those of A. trachycaulum and H. vulgare (see Fig. 7). A similar pattern was also found among the hybrids of AHV444, AHV442, and VH-35.

The genome of A. trachycaulum has been speculated to play the role of a buffer or balancer between genomes of

H. jubatum and H. vulgare in the hybrid series of AHV444, AHV442 mix octoploid and AHV-B_1. Dosage effect of a specific genome has been well documented for the genome interaction link with the phonomenon of chromosome elimination in certain interspecific and intergeneric hybrids. Phenotypically, Agropyron characteristics are still contained in all hybrids in spite of a combined genome of H. jubatum and H. vulgare. This might show that A. trachycaulum has a wider range of chromosome index than either H. jubatum or H. vulgare in addition hybrids are maintained in A. trachycaulum cytoplasm. Regarding evolution, the genus Agropyron has a broader germplasm which has been used in replenishing the gene pool of cultivated wheat. Cauderon (1962) reported allopolyploidy, segmental allopolypoidy, and complex allopolyploidy among eight species in the genus Agropyron. In addition, spontaneous and artificial hybrids occurred frequently between Agropyron spp. with other genera such as Triticum, Aegilops, Secale, Haynaldia, Hordeum, and Elymus. These hybrids are also unique in maintaining Agropyron cytoplasm. In a previous study it was illustrated that most hybrid series were derived from an Agropyron cytoplasm hybrid. Considering the cytoplasmic effect, karyotypic similarity of entries involved in hybridization is just one factor in a complex evolutionary system.

SUMMARY

Comparative karyotype studies among AHV442, AHV444 and VH-35 were unable to detect chromosome exchange by traditional cytological techniques due to the similarity of the original karyotypes of A. trachycaulum, Hordeum jubatum and Hordeum vulgare.

H. vulgare genome was expressed morphologically in hybrids and in the first backcross in proportion to the genome dosage involved. The factors of chromosome elimination facautated in the H. vulgare genome by the dosage ratio to the combined genome of A. trachycaulum and H. jubatum. The chromosome elimination was circumvented by introducing a single dosage of H. vulgare genome at beginning and then doubling the dosage by colchicine before adding more dosage in subsequent backcrosses to H. vulgare.

The potential of gene transfer from germplasm of either A. trachycaulum or H. jubatum into H. vulgare was discussed. Results have typified the concept of reconstruction and replenishing barley germplasm for improving its environmental stress, disease and insect resistance through hybridization with its wild relatives.

APPENDIX

APPENDIX

Chromosome Association in Hybrids Involving *Agropyron trachycaulum*, *Hordeum jubatum* and *Hordeum vulgare*[1]

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ABSTRACT

A natural cross between *Agropyron trachycaulum* (Link), Malte (4X), and *Hordeum jubatum* L. (4X) collected in Alaska was duplicated by making reciprocal crosses in the greenhouse. Comparison of the progeny of these crosses with the natural hybrid showed that *Agropyron trachycaulum* was the female in the natural hybrid. The natural hybrid was doubled by colchicine and crossed with diploid and tetraploid *Hordeum vulgare* L. emend. Lam.

The cytology of the natural hybrid, synthetic hybrids, amphiploid, and hybrids of the amphiploid with *Hordeum vulgare* was studied and genome formulas for the above were suggested. The results suggest that the chromosome association of *Hordeum vulgare* with its wild relatives could be enhanced with

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[2]The work of the first author was done in partial fulfillment for the Ph.D. degree at Michigan State University.

appropriate parental combination which contributes to genome balance. The impact of Agropyron and Hordeum jubatum genomes on Hordeum vulgare was discussed.

Agropyron is one of the most variable genera in the Gramineae. A. trachycaulum is a tetraploid with a chromosome number 2n=28 (Myers, 1947). The genome formula for A. trachycaulum (2n=28) was given as SSXX in which "S" denotes the genome of A. spicatum (Pursh) Scrib and Smith and "X" is a genome of unknown origin. (Dewey, 1968, 1969). Boyle and Holmgren (1954) suggested that H. jubatum and A. trachycaulum (Link) Malte share a common genome as AACC for H. jubatum and AABB for A. trachycaulum. This conclusion has been supported by Bowden (1960) and Gross (1960). Gross's studies produced two different partially fertile octoploid plants (2n=56) by colchicine treatment of sterile tetraploids X Agrohordeum macounii. A similar hybrid was reported by Mitchell and Hodgson (1965) and classified as X Agrohordeum pilosilemma. Recent cytological studies on the genome of H. jubatum with other related species show that H. jubatum is a segmental allotetraploid. (Wagenaar, 1959, 1960; Rajhathy et al., 1964; Starks and Tai, 1974).

Starks and Tai (1974) also suggested that the chromosome association in H. jubatum is genetically controlled with a dosage effect: when there is a single dose from each genome of H. jubatum, homoeologous pairing occurs. However, if each genome appears twice, homologous pairing prevails. They suggested the genome formula AAA'A' for H. jubatum.

Riley and Kempanna (1963), Feldman (1966) observed genetic control of chromosome pairing in Triticum aestivum. They suggested the genes promoting dissociation between homoeologous chromosomes act at the time of premeiotic somatic chromosome pairing.

The genus Agropyron has been well documented as a source for desirable agronomic traits in cultivated wheat. (Elliott, 1957; Rillo et al., 1970; Sears, 1973; Sinigovets, 1974; Knott et al., 1977). Species of Agropyron have hardly been thought of as useful germplasm for improving H. vulgare. The intergeneric hybrid between wild species of Agropyron and Hordeum can be obtained spontaneously and synthetically.

Hybridization between distant species and genera have intrigued plant breeders as a way to broaden and replenish the germplasm of economic plants. The factors involved in using wild relatives to improve cultivated barley were evaluated by Steidl (1976), but the attempts at using some wild species within the genus Hordeum to improve cultivated barley have had unexpected results. For example, crosses between H. bulbosum L. (2X) and H. vulgare (2X) abruptly produced haploid H. vulgare due to a phenomenon of chromosome elimination (Kao and Kasha, 1969; Kash and Sadasivaiah, 1971; Subrahmanyam and Kasha, 1973; and Ho and Kasha, 1975). Transfer of disease resistance from H. leporinum Link to cultivated barley was reported by Hamilton et al. (1955) and from H. bulbosum by Schooler (1964). The first and second backcrosses of [H. jubatum X H. compressum (6X)] to H. vulgare produced weak and sterile H. vulgare type plants due to

H. jubatum cytoplasm (Steidl, 1976). The phenomenon of chromosome elimination is reported in the hybridization between H. vulgare (2X) and Secale (2X) (Fedak, 1977).

The purpose of this study is to further elucidate the genomic nature of A. trachycaulum, H. jubatum and H. vulgare by comparative studies among natural, synthetic and their amphidiploid cross with H. vulgare. Attempts were also made to use H. jubatum and A. trachycaulum as sources of germplasm in barley breeding programs.

The stocks of Agropyron trachycaulum, Hordeum jubatum and the spontaneous hybrid of these two species used in this study were collected in Alaska with the help of Drs. W. W. Mitchell and R. Taylor. The amphiploid was obtained from natural hybrid with colchicine treatment by Dr. R. P. Steidl.

Diploid H. vulgare "Larker" (CI 10648), "Coho" (CI 13852) and one tetraploid H. vulgare from Dr. A. B. Schooler were used to cross with the amphiploid. The plants were grown in a greenhouse at 18-20°C. Spikes used as the female were emasculated and covered with aluminum foil one or two days before pollination. Fresh pollen was shed onto the stigma by tapping the designated male spike. Pollination was repeated on two consecutive days. Embryo culture was employed to obtain the hybrid in crosses between the amphiploid and H. vulgare. Immature seeds were sterilized for five minutes with commercial Clorox diluted with an equal part of distilled water. The embryos were dissected from the underdeveloped seeds and transferred to Norstog media (1973).

For cytological studies, root tips were collected after the plant had been subjected to cold treatment (0-2°C) overnight.

Farmer's solution was used for fixation of the root tip and spike. The aceto-carmine smear technique was used for cytological slide preparation.

RESULTS AND DISCUSSION

The crosses and hybrids obtained are shown in Table 1. The morphology of the hybrids was generally intermediate between the parents. A. trachycaulum when used as female has a relatively higher seed set than H. jubatum in reciprocal crosses. Fertility was partially restored in the amphiploid which was subsequently used as the female to cross with H. vulgare.

The meiotic chromosome association of plants was observed in the pollen mother cells and is summarized in Table 2.

Root tip chromosome counts showed that the natural hybrid had 2n=28; amphiploid, 2n=56; amphiploid crossed with diploid H. vulgare, 2n=35 (Figure 1); and crossed with tetraploid H. vulgare had 2n=42 (Figure 2).

Metaphase I was the main stage used for interpretation of chromosome association. Meiotic chromosome behavior of the synthetic hybrid of reciprocal crosses was similar to that of the spontaneous hybrid. Generally, they all had a minimum of seven univalents, an average of less than seven bivalents, and a few trivalents. Various numbers of micronuclei were observed in quartets. The pollen grains were of different

Table 1A

Number of Seeds and Plants Obtained in Hybridization

Crosses		No. spikes pollinated	Total seeds set	No. of hybrid plants
A. trachycaulum (2n=28)	H. jubatum (2n=28)	20	19	18
H. jubatum (2n=28)	A. trachycaulum (2n=28)	15	25	1
Amphiploid-(AT x HJ)^2 (2n=56)	H. vulgare (2n=28)	34	13	1*
Amphiploid-(AT x HJ)^2 (2n=56)	H. vulgare (2n=14) (Coho)	32	3	1*
Amphiploid-(AT x HJ)^2 (2n=56)	H. vulgare (2n=14) (Larker)	21	31	8*
H. vulgare (2n=28)	Amphiploid-(AT x HJ)^2 (2n=56)	58	6	1*
H. vulgare (2n=14) (winter type)	Amphiploid-(AT x HJ)^2 (2n=56)	105	50	8*

* embryo culture needed

Table 2 A

The Chromosome Association of Plants

Plants		Chromosome association I	II	III	IV	No. of cells
A. trachycaulum (2n=28)	Range:	0	14	0	0	100
	Mean :	0	14.0	0	0	
H. jubatum (2n=28)	Range:	0	14	0	0	100
	Mean :	0	14.0	0	0	
Natural-(AT x HJ) (2n=28)	Range:	7-24	2-8	0-2	0	50
	Mean :	16.38	5.44	0.14	0	
Artificial-(AT x HJ) (2n=28)	Range:	7-18	5-10	0-3	0	31
	Mean :	11.51	7.22	0.67	0	
Artificial-(HJ x AT) (2n-28)	Range:	7-22	3-7	0-2	0-1	96
	Mean :	15.80	5.60	0.32	0.11	
Amphiploid-(AT x HJ)^2 (2n=56)	Range:	0-6	22-28	0-2	0-1	71
	Mean :	2.36	25.63	0.71	0.06	
AHV442* (2n=35)	Range:	1-14	9-17	0-1	0	66
	Mean :	6.53	13.98	0.16	0	
AHV444** (2n=42)	Range:	5-15	12-17	0-1	0	112
	Mean :	7.89	15.92	0.86	0	

* amphiploid - (At x HJ) x H. vulgare (2x)

** amphiploid - (At x HJ) x H. vulgare (4x)

Figure 1A

(1) Somatic metaphase in AHV442 with 2n=35 (X1360)

(2) Somatic metaphase in AHV444 with 2n=42 (X880)

(3) Metaphase 1 in AHV442 with 13I + 8II (3 ring by arrow) + 2 III (X915)

(4) Metaphase 1 in AHV444 with 13I + 13II (7 ring by arrow) + 1 III (X915)

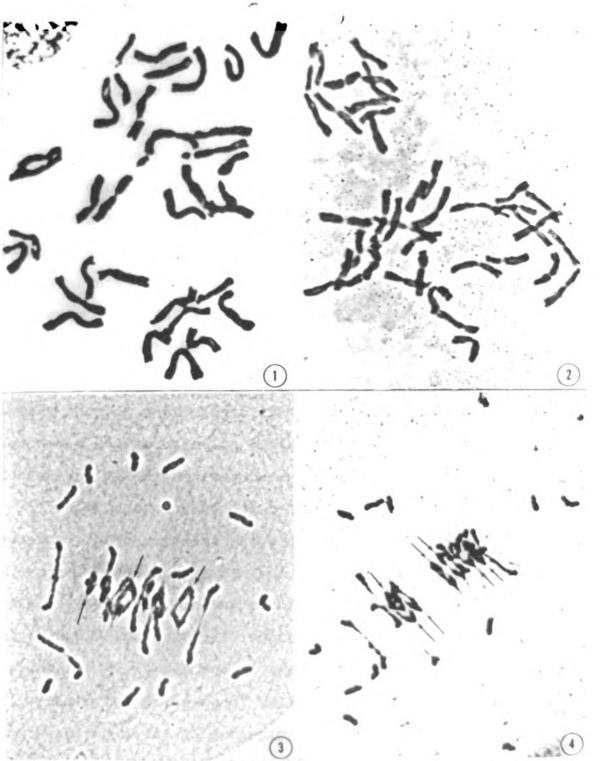

Figure 1A

sizes; none stained with I_2-KI. The anthers were shrunken and the plants were completely sterile.

The amphiploid from the spontaneous hybrid had very few multivalents but the number of univalents was substantially decreased. The average number of bivalents was 25 with a range from 22 to 28.

The progenies from the amphiploid as female crossed with the diploid or the tetraploid H. vulgare are coded as AHV442 and AHV444, respectively. The chromosome number of 2n=35 is expected in AHV442, likewise 2n=42 was obtained in AHV444. Univalents averaged 6.53 in AHV442 and 7.09 in AHV444. Bivalents in AHV442 averaged 13.93 and 15.92 in AHV444. Only a few multivalents were observed in these hybrids. Morphologically, these hybrids have significant H. vulgare characteristics such as a prominent auricle and spike style. Some agronomic characteristics of the wild species such as pubescent leaves and mildew resistance were perceivable in these hybrids.

With a common genome between A. trachycaulum and H. jubatum, the chromosome association in the hybrid should average 7 bivalents, a minimum of 14 univalents, and no multivalents. However, in the combined data of reciprocal crosses, Boyle and Holmgren (1954) reported the presence of multivalents in 23.55 percent of the cells observed. It is hard to explain the presence of multivalents if both parents of the amphiploid are allopolyploids. We observed trivalent associations in the synthetic hybrids from reciprocal crosses. In the synthetic hybrids trivalents occurred in 22.22 percent

of the cells when the female parent was A. trachycaulum. When H. jubatum was the female parent, the frequency increased to 28.72 percent.

Since both parents were tetraploids, the hybrid received two genomes from each parent. The two genomes from A. trachycaulum are non-homologous with each other, but the chromosomes from H. jubatum are homoeologous with each other. Thus, in the hybrid a single set of chromosomes from each of the two genomes of H. jubatum is present and the chromosomes should pair autosyndetically, A-A. With one genome of A. trachycaulum in common with one of the two genomes of H. jubatum, any trivalent occurring in the hybrid should have a chromosome contributed by A. trachycaulum and two chromosomes contributed by H. jubatum, A-A'-A.

In the synthetic hybrids, 6-7 chromosomes often behaved in a manner quite different from the rest of the chromosomes and were often excluded from the main group. We have tentatively concluded that these are the chromosomes belonging to the B genome from A. trachycaulum. Separation and grouping of chromosomes based on genome originality was suggested by Huskins and Chouinard (1950), Thompson (1962), Bammi (1965), Tai (1970), and Rizzoni et al. (1974). It has been demonstrated that some homology exists among the two genomes from H. jubatum and one genome from A. trachycaulum. The chromosomes of the fourth group, the B genome, were comparatively separated and excluded from the main group.

The chromosome association observed in the amphiploid showed a mean of 2.36 univalents, 25.63 bivalents, 0.71 trivalents, and 0.06 quadrivalents. Since few multivalents occurred in the amphiploid, obviously there was no pairing between homoeologous genomes from H. jubatum. However, the frequency of trivalents and quadrivalents was lower than expected with a combined total of 0.76 multivalents per cell.

The dosage theory of Starks and Tai (1974) is further confirmed by the chromosome behavior in the amphiploid. The lack of multivalents in our amphiploid can be explained on the same basis. We suggest that the gene or genes controlling chromosome pairing in the amphiploid also promotes dissociation between less like chromosomes (the homoeologous chromosomes) and that genes affect pairing among the one genome, A', from A. trachycaulum and the two genomes, AA, from H. jubatum. In the hybrid, AAA'B, allosyndetic pairing of H. jubatum chromosomes would occur to form about seven bivalents (AA') with a maximum of seven trivalents (AAA'). The chromosome number doubled in the amphiploid; however, the number of bivalents observed in the hybrid did not become the number of quadrivalents in the amphiploid. The relationship between the one genome of A. trachycaulum in common with one genome of H. jubatum is not as close as a regular homologous set.

Therefore, preferential compatibility among genomes existed. We suggest changing the genome formula of the plants to: A. trachycaulum, A_3A_3BB; H. jubatum, $A_1A_1A_2A_2$;

the hybrid, $A_1A_2A_3B$; and the amphiploid, $A_1A_1A_2A_2A_3A_3BB$.

The amphiploid has been successfully crossed with diploid and tetraploid cultivated barley, H. vulgare. The hybrid between the amphiploid and the diploid H. vulgare is expected to be a pentaploid with 2n=35 chromosomes; coded AHV442.

By the formula, the genome constitution of AHV442 plant would be as $A_1A_2A_3BV$, V is the designation for the H. vulgare genome. It has the same genome constitution as the natural hybrid in addition to an extra V genome from H. vulgare. Therefore, the association should be seven bivalents of allosyndetic pairing between A_1A_2 or 1-3 trivalents from $A_1A_2A_3$ plus at least 14 univalents each from the B and V genomes.

By observation, it was shown that there was an average of 6.53 univalents and 13.98 bivalents (Figure 3). Multivalents were very rare. There has been no documentation of the fact that H. vulgare genome would associate with genomes of its wild relatives. Murry (1975) observed no chromosome association in hybrids between H. jubatum and H. vulgare. Therefore, seven univalents in this hybrid have been speculated to be from the V genome rather than the B. Consequently, fourteen bivalents should come from allosyndetic pairing of the A genome with B under the influence of the V genome. The hybrid derived from the cross between the amphiploid with tetraploid H. vulgare had a genome constitution of $A_1A_2A_3BVV$ with a chromosome number 2n=42. The univalents averaged

7.92, the bivalents 15.92 and the trivalents increased from 0.16 in AHV442 to 0.86 in AHV444 (Figure 4). Under microscopic observation, seven large ring bivalents apparently from VV genomes could be seen that were different from other genomes which prevailed in a form of a rod bivalent. The seven univalents probably belonged to the B genome as an association pattern of $A_1A_2A_3B$ genomes in a natural hybrid without the influence of two dosages of the V genome. A concomitant increase of H. vulgare characters in the plant was observed comparing two doses of V in AHV444 with only one dose of V in AHV442. Subrahmanyam and Kasha (1973 and Ho and Kasha (1975) reported that rate and degree of chromosome elimination was dependent on a well-defined ratio of genomes in the hybrid of H. vulgare X H. bulbosum. In addition, they demonstrated that the process was genetically controlled and is essential to H. vulgare genomes ratio to other genomes. Manipulation on the cell level had the advantage of reducing the gene load in single cells rather than in the whole plant. Tissue culture plus the appropriate treatment might genenerate the plant population with a variable chromosome make-up.

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LITERATURE CITED

Bammi, R. K. 1965. "Complement Fractionation" in a natural hybrid between Rubus procerus Mu 11 and R. laciniatus Wild. Nature 208:608.

Bowden, W. M. 1960. The typification of Elymus macounii Vasey. Bull. Torrey Bot. Club 87:205-208.

Boyle, W. S. and A. H. Holmgren. 1954. A cytogenetic study of natural and controlled hybrids between Agropyron trachycaulum and Hordeum jubatum. Genetics 40:539-545.

Dewey, D. R. 1968. Synthetic Agropyron-Elymus hybrids: III. Elymus Canadensis x Agropyron Caninum, A. trachycaulum and A. striatum. Amer. J. Bot. 55:1133-1139.

Dewey, D. R. 1969. Trispecies hybrids of Agropyron, Elymus and Sitanion. Bot. Gaz. 130:203-213.

Elliott, F. C. 1957. X-4ay-induced translocation of Agropyron stem rust resistance to common wheat. J. Heredity 48:77-81.

Fedak, G. 1977. Haploids from Barley x Rye crosses. Can. J. Genet. Cytol. 19:15-19.

Feldman, M. 1966. The effect of chromosomes 5B, 5D, and 5A on chromosomal pairing in Triticum aestivum. Proc. Nat. Acad. Sci. 55:1447-1453.

Gross, A. T. H. 1960. Distribution and cytology of Elymus macounii Vasey. Can. J. Bot. 38:63-67.

Hamilton, D. G., S. Symko and J. W. Morrison. 1955. An anomalous cross between Hordeum leporinum and Hordeum vulgare. Can. J. of Agric. Sci. 35:287-293.

Ho, K. M. and K. J. Kasha. 1975. Genetic control of chromosome elimination during haploid formation in barley. Genetics 81:263-275.

Huskins, C. L. and L. Chouinard. 1950. Somatic reduction: diploid and triploid roots and a diploid shoot from a tetraploid Rhoeo. Genetics 35:115.

Kao, K. N. and K. J. Kasha. 1969. Haploidy from interspecific crosses with tetraploid barley. In Proc. Second Intern. Barley Genetics Symp. July 6-11, 1969.

Kasha, K. J. and R. S. Sadasivaiah. 1971. Genome relationships between Hordeum vulgare L. and H. bulbosum L. Chromosoma (Berl.) 35:264-287.

Knott, D. R., J. Dvorak and J. S. Nanda. 1977. The transfer to wheat and homoeology of an agropyron elongatum chromosome carrying resistance to stem rust. Can. J. Genet. Cytol. 19:75-79.

Mitchell, W. W. and H. J. Hodgson. 1965a. A new x Agrohordeum from Alaska. Bull. Torrey Bot. Club 92: 403-407.

Murry, L. E. 1975. A styogenetic investigation of x Agrohordeum philosilemma. Ph.D. Thesis, Michigan State University, 96 pages.

Myers, W. M. 1947. Cytology and genetics of forage grasses. Botan. Rev. 13:319-421.

Norstog, K. 1973a. New synthetic medium for the culture of premature barley embryos. In Vitro 8:307-308.

Rajhathy, T., J. W. Morrison, and S. Symko. 1964. Interspecific and intergeneric hybrids in Hordeum. In: Barley Genetics I. Proceedings of the First International Barley Genetics Symp. Wageningen, pp. 195-212.

Riley, R. and C. Kempanna. 1963. The homoeologous nature of the non-homologous meiotic pairing in Triticum aestivum deficient for chromosome v (5B). Heredity 18:287-306.

Rillo, A. O., R. M. Caldwell, and D. V. Glover. 1970. Cytogenetics of resistance to wheat leaf blotch (Septoria tritici) in backcross derivatives of an Agrotriticum line. Crop. Sci. 10:223-227.

Rizzoni, M., F. Palitti, and P. Perticone. 1974. Euploid segregation through multipolar mitosis in mammalian cell cultures. Chromosoma (Ber. l) 45:151-162.

Schooler, A. B. 1964. Wild barley crosses show disease resistance. North Dakota Farm Research 23:13-15.

Sears, E. R. 1973. Agropyron-wheat transfers induced by homoeologous pairing. Proc. Fourth Int. Wheat Genet. Symp., Columbia Missouri. pp. 191-199.

Sinigovets, M. Ye. 1974. Inheritance of resistance to stinking smut (Tilletia caries Tul.) in Triticum-Agropyron hybrids. Genetika 10:25-31.

Starks, G. D. and W. Tai. 1974. Genome analysis of Hordeum jubatum and H. compressum. Can. J. Genet. Cytol. 16: 663-668.

Steidl, R. P. 1976. Hybridization of barley (Hordeum vulgare L. Emend. Lam.) with its wild relatives. Ph.D. Thesis, Michigan State Univ. 100 pp.

Subrahmanyan, N. C. and K. J. Kasha. 1973. Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. Chromosoma 42:111-125.

Tai, W. 1970. Multipolar meiosis in diploid crested wheatgrass. Agropyron cristatum. Amer. J. Bot. 57:1160-1169.

Thompson, M. M. 1962. Cytogenetics of Rubus IV. Meiotic instability in some hybrid polyploids. Amer. J. Bot. 49:575-582.

Wagenaar, E. B. 1959. Intergeneric hybrids between Hordeum jubatum L. and Secale cereale L. J. Heredity 50: 194-202.

Wagenaar, E. B. 1960. The cytology of three hybrids involving Hordeum jubatum L.: The chiasma distributions and occurrence of pseudo ring-bivalents in genetically induced asynapsis. Can. J. Bot. 38:69-85.

BIBLIOGRAPHY

BIBLIOGRAPHY

Ahokas, H. 1973. Evidence of cytoplasmic evolution from mating between barley and elymus. Barley Genetics Newsletter Vol. 3.

_____. 1970. Ann. Bot. Fenn. 7:182-192.

Athwal, R. S. and G. Kimber. 1972. The pairing of an alien chromosome with homoeologous chromosomes of wheat. Can. J. Genet. Cytol. 14:325-333.

Bakhteiev, F. and Darevskaya, E. M. 1945. An intergeneric hybrid between barley and elymus. Compt. Rend. (Dok.) Acad. Sci. URRS 47:300.

_____. 1975. An intergeneric hybrid between barley and elymus. Barley Genetics Newsletter Vol. 5.

Bates, L. S., A Campos, V., R. Rodrigous R. and R. G. Anderson. 1974. Progress toward novel cereal grains. Cereal Science Today 19:283-286.

Bates, L. S. 1976. Chemical manipulation of crossability barriers. Barley Genetics 3:271-273.

Bantock, C. R. 1970. Experiments on chromosome elimination in the Gall Midge, Mayetiola destructor. J. Emb. Exptl. Med. 24:257-286.

Bammi, R. K., 1965. Complement fractionation in a natural hybrid between Rubus procerus muell. and R. laciniatus wild. Nature 208:608.

Booher, L. E. and Tryon, R. M., Jr. 1948. A study of Elymus in Minnesota. Rhodora 28:80-91.

Brink and Cooker. 1947. The endosperm in seed development. Bot. Rev. 13:423-541.

Camenzind, Rene. 1974. Chromosome elimination in Heteropeza pignaea. Chromosona 49:87-98.

Cauderon, Y. and Ryan, G. 1974. Aegiolps speltiodes promotion of homoeologous pairing in one Triticum x Agropyron intermedium derivative wheat inf. Serv. 39:1-5.

Cauderon, Y. 1962. Etude cytogenetigue du genre Agropyron Rev. Cytol. Biol. Veg. 25:287-301.

Cauderon, Y., and A. Cauderon. 1956. Study of the F_1 hybrid between Hordeum bulbosum L and H. secalinum. Schreb. Anals Amel. Pl. 6:307-317.

Cevan, A., Freolga, K. and Sandberg, A. A. 1964. Momenchatune for contromeric position on chromosomes. Hereditas 52:201-220.

Covas, G. 1949. Taxonomic observations on the North American species of Hordeum. Madrono 10:1-21.

Curtis, B. C. and Croy, L. J. 1958. The approach method of making crosses in small grains. Agron. J. 51:49-51.

Davies, D. R. 1960. The embryo culture of interspecific hybrids of Hordeum. New Phytologist 59:9-14.

DuBois, A. M. 1933. Chromosome behavior during cleavage in the eggs of Sciara coprophila (Diptera) in relation to the problem of sex determination. Z. Zellforsch. Mikrosk. Anat. 19:595-614.

Dvorak, J. 1975. Meiotic pairing between single chromosomes of diploid A. elongatum in Triticum aestivum.

Dvorak, J. and Knott, D. R. 1974. Disonic and ditelosomic additions of diploid Agropyron elongatum chromosomes to Triticum aestivum. Can. J. Genet. Cytol. 16:399-417.

Dvorak, J. 1972b. Homoeologous relationships between chromosomes of Agropyron elongatum (Host.) P. B. (2n=14) and those of Triticum aestivum. Ph.D. Thesis. University Saskatchewan Saskatoon. pp.142.

Dvorak, J. and Knott, D. R. 1972. Homoeologous pairing of specific Agropyron elongatum (2n=70) chromosome with wheat chromosomes. Wheat Inf. Serv. 33-34:35-37.

Fedak, George. 1977. Haploids from barley x rye crosses. Can. J. Genet. Cytol. 19:15-19.

Forsberg, D. E. 1953. The response of various forage crops and saline siols. Can. J. Agr. Sci. 33:542-549.

Geyer-Duszynska, J. 1959. Experimental research on chromosome elimination in Cecidomyidae (Diptera). J. Expt. Zool. 141:391-448.

Gill, B. C. and Kimber, G. 1974. Giemsa c-banding and the evolution of wheat. Proc. Nat. Acad. Sci. U.S.A. 71:4086-4090.

Gordon, G. S. and Raw, A. R. 1932. Wheat-barley mating. J. Agr. Victoria, Australia. 30:138-144.

Gupta, S. B. and Gupta, Pratibha. 1973. Selective elimination of Nicotiana glutinesa chromosomes in the F_1 hybrids of N. suaveolens and N. glutinosa. Genetics 73:605-612.

Hamilton, D. G., Symko, S. and Morrison, J. W. 1955. An anomalous cross between Hordeum leporinum and H. vulgarie. Can. J. Agric. Sci. 35:287-293.

Hamilton, D. G. 1953. The approach method of barley hybridization. Can. J. Agric. Sci. 33:98.

Handmaker, S. D. 1971. Cytogenetic analysis of Chinese hamster-mouse hybrid cell. Nature (London) 233:416-419.

Hitchock, A. S. and Chase, A. 1950. Manual of grasses of the United States. U. S. Dept. Agri. Misc. Publ. No. 200, 1051p.

Ho, K. M. and Kasha, K. J. 1975. Genetic control of chromosome elimination during haploid formation in barley. Genetics 81:263-275.

Huettner, A. F. 1934. Octopoidy and diploidy in Miastor americana Anat. Rec. 60, Suppl. 80.

Huskins, C. Leonard and Cheng, K. S. 1948. Segregation and reduction in somatic tissues. J. Hered. 39:311-325.

Kahle, W. 1908. Die paedogenesis der Cecidomyiden. Zoologica, Stuttg. 21:1-80.

Kao, K. N. and Kasha, K. J. 1969. Haploidy from interspecific crosses with tetraploid barley pp. 82-88. In: Barley Genetics II. Ed. R. A. Nilan. Washington State University.

Kasha, K. J. 1974. Haploids from somatic cells in: Haploids in higher plants--advances and potential proceedings of the First International Symposium, K. J. Kasha, ed. The University of Guelph. pp.421.

Kasha, K. J. and Kao, K. N. 1970. High frequency haploid production in barley (Hordeum vulgare L.) Nature (London) 225:874-876.

Keller, W. 1948. Wanted: a paragon for the range. GRASS yearbook of Agriculture, U. S. Dept. of Agri. Washington, D. C. pp. 347-351.

Kivi, E. I., Redunen, M. and Varis, E. 1974. Use of induced mutations in plant breeding. International Atomic Energy Agency, Vienna. pp. 187-194.

Konzak, C. F., Randolph, L. F. and Jensen, N. F. 1951. Embryo culture of barley species hybrids. Cytological studies of Hordeum sativum X Hordeum bulbosum. J. Hered. 42:124-134.

Knott, D. R., Dvorak, J. and Nanda, J. S. 1977. The transfer to wheat and homoeology of an Agropyron Elongatum chromosome carrying resistance to stem rust. Can. J. Genet. Cytol. 19:75-79.

Korablin, I. I. 1937. New varieties of barley bred at Omsk. Breeding and Growing 11:57-59. PBA 8:1542.

Kraczkiewicz, Z. 1936. Etudes cytologigues sur loogenese et la diminution de la chromatine dans les larves paedogenetiques de Miastor metraloas (Meinert) (Diptera). Folia Morph., Warszawa 6:1-37.

Kunzel, G. and Nicoloff, H. 1975. Indications for a necessary revision of the barley karyogramme. Barley Genetics Newsletter Vol. 5. pp. 23.

Larson, R. I. and Atkinson, T. G. 1970. Identity of the wheat chromosomes replaced by Agropyron chromosomes in a triple alien chromosome substitution line immune to wheat streak mosaic. Can. J. Genet. Cytol. 12:145-150.

Maan, S. S. and Sasakuma, T. 1977. Genomic homology among the D- and M- genome diploid Aegilops species. Agronomy Abstracts. Annual Meetings Nov. 13-18.

Malloch, W. S. 1921. An F1 species cross between Hordeum vulgare and Hordeum muramium. Am. Nat. 55:281-286.

Metcalfe, M. 1935. The germ cell cycle in phytophaga destructor Say. Quart. J. Micr. Sci. 77:585-603.

Mitchell, W. W. and Hodgson, H. J. 1965a. A new x Agrohordeum from Alaska. Bull. Torrey Bot. Club. 92:403-407.

Morrison, J. W. 1959. Cytogenetic studies in the genus Hordeum. I. Chromosome morphology. Can. J. Bot. 37: 527-538.

Murry, L. E. 1975. A cytogenetic investigation of x Agrohordeum pilosilemma. Ph.D. Thesis. Michigan State Univ. pp. 96.

Nevski, S. A. 1934. Hoadeae Benth. In: V. L. Komorov, Flora UYSR Vol. II. Leningrad. pp. 590-728.

Newcomer, E. H. 1953. A new cytological and histological fixing fluid. Science 118:161.

Nilan, R. A. 1964. The cytology and genetics of barley 1951-62, 278pp. In research studies. Monog. Suppl. 3. Washington State Univ. Pullman.

Noda, K. and Kasha, K. J. 1976. Barley chromosome identification with the C-banding giemsa stain technique. Barley Genetics Newsletter. Vol. 6 pp. 47.

Norslog, K. 1973a. New synthetic medium for the culture of premature barley in vitro. 8:307-308.

Pissarev, V. E. et al. 1945. A haploid barley plant produced by remote hybridization. Compt. Rend. (Dok.) Acad. Sci. URSS 49:372.

Pontecorvo, G. 1971. Induction of directional chromosome elimination in somatic cell hybrids. Nature 230: 367-369.

Quincke, F. L. 1940. Interspecific and intergeneric crosses with Hordeum. Canad. J. Res. C. 18:372-373.

Rajhathy, T. and Morrison, J. W. 1961. Cytogenetic studies in the genus Hordeum v. H. Jubatum and New world species. Can. J. Genet. Cytol. 3:378-390.

_____. 1959. Cytogenetic studies in the genus Hordeum IV. Hybrides of H. jubatum, H. brachyantherum, H. vulgare and a hexaploid Hordeum sp. Can. J. Genet. Cytol. 1:124-132.

Reznicuk, S. P. 1939. (Perennial rye). Sotsialist Zern, Khoz (Socialist Grain Farming). 4:87-90. PBA 9: 1470.

Rhoades, M. M. and Dempsey, E. 1972. On the mechanisms of chromatin loss induced by the B chromosome of maize. Genetics 71:73-96.

Richards, A. J. 1972. The daryology of some taraxacum species from alpine regions of Europa. Bot. J. Linn. Soc. 65:48-59.

Richards, A. J. and Booth, T. A. 1977. Current chromosome research. Edited by K. Jones and P. B. Brandham. Brandham Holland Biomedical Pross. Amsterdam, the Netherlands.

Riley, R., Chapman, V. and Hohnson, R. 1968. Introduction of yellow rust resistance of Aegiolops comosa into wheat by genetically induced homoeologous recombination. Nature 217:383-384.

Riley, R. and Kempanna, C. 1963. The homoeologous nature of the nonhomologous meiotic pairing in Triticum aestivum deficient for chromosome V (5B) Heredity. 18:287-306.

Rizzoni, M., Palitt, F. and Perticone, P. 1974. Euploid segregation through multipolar mitosis in mammalian cell cultures. Chromosoma (Berl.) 45:151-162.

Romage, R. T., Burnham, C. R. and Hagberg, A. 1961. A summary of translocation studies in barley. Crop Sci. L:277-279.

Sager, R. and Ramanis, Z. 1967. Biparental inheritance of nonchromosomal genes induced by ultraviolet irradiation. Proc. Nat. Acad. Sci. (&.S.) 58:931-937.

_____. 1973. The mechanism of maternal inheritance in Chlamydomonas: Biochemical and genetic studies. Theoretical and Applied Genetics. 43:101-108.

Sarma, N. P. and Tenden, S. L. 1974. Banding techniques and plant chromosomes. Current Sci. 43:635-637.

Schooler, A. B. 1964. Wild barley crosses show disease resistance. North Dakota Farm Research 23:13-15.

Schooler, A. B. 1962. Interspecific hybrides of Hordeum. Barley News 6:47-48.

Schooler, A. B. 1976. Personal communication with Dr. J. E. Grafius.

Sears, E. R. 1973. Agropyron-wheat transfers induced by homoeologous pairing. Proc. Fourth Int. Wheat Genet. Symp. pp. 191-199.

Sinigovdts, M. Ye. 1974. Inheritance of resistance to stinking smut (Tilletia caries Tul.) (In Triticum-Agropyron hybrids. Genelika 10:25-31.

Smith, D. F. 1972. Hordeum species in grasslands. Herb. Abstr. 42:213-223.

Smith, D. C. 1942. Intergenetic hybridization of cereals and other grasses. Jour. Agri. Res. 64:33-47.

Smith, D. C. 1943. Intergeneric hybridization of Triticum and other grasses, principally Agropyron. J. Hered. 34:219-224.

Sparrow, D. H. B. 1974. Chromosome elimination in interspecific hybrides: involving H. arizonicum (6X) x H. vulgare (2X) and H. arizonicum (6X) x H. bulbosum (2X). Barley Genetics Newsletter Vol. 4. pp. 36.

Stalker, H. T., Harlan, J. R. and DeWel, J. M. J. 1977. Cytology and morphology of maize-Rripsacum introgression. Crop Science 17. pp. 745.

Starks, G. D. 1976. A cytogenetic investigation of some species of Hordeum (Gramimeae). Ph.D. Thesis, Michigan State University. pp. 89.

Stebbins, G. L., Jr. et al. 1946. Artificial and natural hybrids in the Gramineae tribe Hordeae II Agropyron, Elymus and Hordeum. Am. J. Bot. 33:579-586.

Steidl, R. P. 1976. Hybridization of barley (Hordeum vulgare L. Emend. Lam.) with its wild relatives. Ph.D. Thesis, Michigan State University. 100pp.

Thomas, P. T. and Thomas, H. 1974. Chromosome manipulation and plant breeding polyploidy and induced mutations in plant breeding. International Atomic Energy Agency. Vienna.

Thompson, W. P. and Johnson, D. 1945. The cause of incompatibility between barley and rye. Can. J. Res. C. 23: 1-15.

Tjio, J. H. and Hagberg, A. 1951. Cytological studies of some X-ray mutants of barley. Anales. Estac. Exp. Aula Dei 2:149-167.

Tuleen, N. A. 1973. Karyotype analysis of multiple translocation stocks of barley. Can. J. Genet. Cytol. 15: 267-273.

Wagenaar, E. B. 1959. Intergeneric hybrids between Hordeum jubatum L. and Secale Cereale L. J. Hered. 50:194-202.

_____. 1960. The cytology of three hybrids involving Hordeum jubatum L. The chiasma distributions and occurrence of Pseudo ring-bivalents in genetically induced asynapsis. Can. J. Bot. 38:69-85.

Wienhues A. 1973. Translocations between wheat chromosomes and an Agropyron chromosome conditioning rust resistance. Proc. 4 h Int. Wheat Genet. Symp., Columbia, Missouri. pp. 201-207.