A CYTOGENETIC INVESTIGATION OF X AGROHORDEUM PILOSILEMMA

Dissertation for the Degree of Ph. D.
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LYNN ELLEN MURRY
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This is to certify that the

thesis entitled

A CYTOGENETIC INVESTIGATION OF

X AGROHORDEUM PILOSILEMMA

presented by

Lynn Ellen Murry

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Botany and Plant Pathology

Major professor

Date Nov 24, 1975

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ABSTRACT

A CYTOGENETIC INVESTIGATION OF X AGROHORDEUM PILOSILEMMA

By

Lynn Ellen Murry

The cytology of X Agrohordeum pilosilemma Mitchell & Hodgson was investigated to determine the genome relationships of Agropyron sericeum Hitchc., Hordeum jubatum L., and Hordeum vulgare L. The plants studied were Agropyron sericeum, Hordeum jubatum, X Agrohordeum pilosilemma, its amphiploid, the amphiploid x Agropyron sericeum, the amphiploid x Hordeum vulgare (4x), Hordeum vulgare (2x) and (4x), and Hordeum vulgare (2x) x Hordeum jubatum. Light microscope observations of chromosome behavior included examination of all stages of microsporogenesis and compilation of comparative data for diakinesis-metaphase I, anaphase I, telophase I, and the quartet stage for each plant. Plant fertility was estimated from pollen stainability and seed set.

Analysis of microsporogenesis in Agropyron sericeum, Hordeum jubatum, X Agrohordeum pilosilemma, the amphiploid, and the backcross of the amphiploid to Agropyron sericeum elucidated the genome relationships of Agropyron sericeum and Hordeum jubatum. The tetraploid

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parental species, Agropyron sericeum and Hordeum jubatum, share a partially homologous genome which affects the pairing relationships evidenced in their hydrids. The genome formulae assigned to these plants are: Agropyron sericeum, A"A"BB; Hordeum jubatum, AAA'A'; X Agrohordeum pilosilemma, AA'A"B; the amphiploid, AAA'A'A"A"BB; and the amphiploid x Agropyron sericeum, AA'A"A"BB. Observed pairing configurations were compatible with the expected maximum pairing configurations predicted under the assumption of genetic control of pairing with dosage effects.

Hordeum vulgare x Hordeum jubatum was found to display asynaptic behavior that is believed to represent a physiogenetic incompatibility. The pairing configuration of the amphiploid x Hordeum vulgare was comparable to the pairing seen in X Agrohordeum pilosilemma indicating that the genomes of Hordeum vulgare are effectively isolated from the genomes of both Agropyron sericeum and Hordeum jubatum either by homology or through genes controlling pairing. The genome formulae, AA'V and AA'A"BVV, were tentatively assigned to Hordeum vulgare x Hordeum jubatum and the amphiploid x Hordeum vulgare, respectively.

A CYTOGENETIC INVESTIGATION OF X AGROHORDEUM PILOSILEMMA

By

Lynn Ellen Murry

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1975

DEDICATION

To my Mother and Father

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ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. William Tai, my major professor, and Drs. William G. Fields, John E. Grafius, and Stephen N. Stephenson, my committee members, for their guidance, assistance, and encouragement during the course of this research and for their constructive criticism of the manuscript.

I would also like to thank Michael Christianson, Margaret Mead, Gilbert Starks, Robert Steidl, and Joanne Whallon for their interest and suggestions. The moral support given by my family and many friends truly sustained me through the completion of this work.

The financial support for my final year of study, a Donald F. Jones Predoctoral Scholarship granted by the Research Corporation of New York, is gratefully acknowledged.

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INTRODUCTION

Investigations of North American X Agrohordeum G. Camus ex A. Camus hybrids began with the work of Stebbins, et al. (1946). This cytological and morphological study revealed that the parents of a natural, Pacific Coast hybrid classified as Elymus macounii Vasey were Agropyron pauciflorum (Schwein) Hitchc. and Hordeum nodosum L. Based on their examination of herbarium specimens, Stebbins, et al. (1946) suggested that materials classified as Elymus macounii represented collections of several different, sterile hybrids between Hordeum jubatum L. and various Agropyron species. Subsequently there have been additional investigations of the Elymus macounii complex.

Keller (1948) stated that Elymus macounii was a natural hybrid between slender wheatgrass, Agropyron trachycaulum (Link) Malte, and foxtail barley, Hordeum jubatum. Booher and Tryon (1948) arrived at this same conclusion through their study of herbarium specimens of both the parents and sterile hybrid from Minnesota. A publication on forage crops by Forsberg (1953) indicated the same parentage and reported a fertile amphiploid of this hybrid had been obtained by colchicine-doubling. Lepage (1952, 1953) renamed the taxon, X Agrohordeum macounii (Vasey) Lepage, on the basis of its presumed parentage and on a comparison of the morphological characters of

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In 1955, Boyle and Holmgren published the first cytogenetic investigation of X Agrohordeum macounii. They studied the cytology and morphology of the parental species, Agropyron trachycaulum and Hordeum jubatum; the natural; and the reciprocal, artificial hybrids. Meiosis in the parental species, assumed to be allotetraploids, was normal; fourteen bivalents consistently formed. The natural and artificial hybrids (2n=28) displayed similar chromosome associations. The present author's calculations from their data show averages of 15.45 I, 5.88 II, 0.07 III, and 0.14 IV per cell. Boyle and Holmgren attributed bivalent formation to allosyndesis between the chromosomes of Agropyron trachycaulum and Hordeum jubatum and interpreted the sterility of the hybrids as failure of the two complements to synapse completely during meiosis. Morphologically, the natural and artificial hybrids were more or less intermediate between the parents and indistinguishable from one another. They suggested AABB and AACC as genome formulae for Agropyron trachycaulum and Hordeum jubatum, respectively.

Ashman and Boyle (1955) continued the previous investigation and reported on the meiotic behavior of the fertile, colchicine-doubled amphiploid, X Agrohordeum macounii. They found an average of 1.3 I, 24.6 II, 0.15 III, and 0.7 IV per cell at metaphase I; laggards and precocious dyad division at anaphase I; laggards and, rarely, bridges at anaphase II; an average of 5.8 micronuclei per quartet; 56 % pollen fertility; and 30 % seed set. Ashman and Boyle

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remarked that the only morphological character distinguishing the amphiploid from the F_1 was the presence of caryopses. They advanced the genome formula, AAAABBCC, for the amphiploid.

Bowden (1959) discussed the intergeneric hybrid, X Agrohordeum macounii in his paper on the chromosome numbers and taxonomy of northern grasses. He corroborated the chromosome number of $2n=28$, and the parentage, Agropyron trachycaulum x Hordeum jubatum, reported by Boyle and Holmgren (1955); listed several voucher specimens and their localities; and stated that the hybrid may be expected to occur wherever the two parental species are sympatric. In 1960, Bowden typified Elymus macounii Vasey, the basonym of X Agrohordeum macounii (Vasey) Lepage. The type specimens were selected from materials collected by J. Macoun in Saskatchewan in 1879. Bowden (1960) also clarified the status of the fertile, artificial amphiploids; they assume the same binary name as the natural hybrid and should be regarded as cultivars.

The distribution and cytology of Elymus macounii was restudied by Gross (1960). The hybrids' widespread distribution was determined primarily from examination of herbarium specimens and hypothesized to result from a combination of frequent hybridization between Agropyron trachycaulum and Hordeum jubatum and of the hybrids' tolerance to salinity and flooding. Some differences are evident between the cytological data presented by Gross (1960) and that from the earlier studies by Boyle and Holmgren (1955) and Ashman and Boyle (1955).

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(this author's calculations from the published data) for the sterile hybrid but reported only those amphiploid cells which displayed 28 bivalents.

Further investigations of X Agrohordeum macounii were carried out by Mitchell and Hodgson (1965a) in an attempt to explain the differences in lemma pubescence apparent in Alaskan collections of the hybrid. In 1942, Hulten had mentioned that Alaskan specimens had glumes and lemmas that were pilose whereas the lemmas of the type specimen of Elymus macounii were glabrate. Mitchell and Hodgson (1965a) studied the comparative morphology (length of the first spike internode, glume length, glume epidermal pattern, and lemma pubescence) of the "Alaskan hybrid", X Agrohordeum macounii, and the species, Agropyron latiglume (Scribn. & Smith) Rydb., Agropyron sericeum Hitchc., and Agropyron trachycaulum, which coexist with Hordeum jubatum in Alaska. Their morphological and field observations resulted in the establishment of a new taxon, X Agrohordeum pilosilemma Mitchell & Hodgson, whose parents were identified as Agropyron sericeum and Hordeum jubatum. The original distribution of X Agrohordeum pilosilemma, "from south of the Brooks range, about 66° N. latitude, to southcentral Alaska, about 61° N. latitude" (Mitchell and Hodgson, 1965a) has been extended by Bowden (1967) to include the Yukon and the District of Mackenzie. The work of Mitchell and Hodgson (1965a) reconfirms the contention of Stebbins, et al. (1946) that more than one Agropyron species contributed its genome to the hybrids comprising the Elymus macounii complex.

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The purpose of the present cytogenetic investigation of X Agrohordeum pilosilemma was to determine the genome relationships of the species, Agropyron sericeum, Hordeum jubatum, and Hordeum vulgare L. through observations on chromosome behavior during microsporogenesis of these species and their hybrids.

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MATERIALS AND METHODS

The plants, Agropyron sericeum, Hordeum jubatum, X Agrohordeum pilosilemma, Hordeum vulgare (2X), and Hordeum vulgare (4X), were obtained from Dr. John Grafius, Department of Crop and Soil Sciences, Michigan State University. The origins and designations of the research materials are given in Table 1, and the breeding program appears as Figure 1.

The research materials were cultivated under a combination of field, greenhouse, and growth chamber conditions. Perennial stocks are maintained year-round in the barley nursery at the Department of Botany and Plant Pathology farm. Duplicates are kept in the Plant Science greenhouse under 14 hour light and 21 C temperature conditions from early October to early May. Plants being crossed, coddled, or forced to bloom are grown in a Scherer-Gillett Model Cel 37-14 growth chamber in which sixteen 6' fluorescent tubes and twelve 25 W incandescent bulbs provide a 14 hour-a-day light regime and temperature settings are 21 C days and 13 C nights.

The natural hybrid, X Agrohordeum pilosilemma, was treated with colchicine by Robert Steidl, Department of Crop and Soil Sciences, Michigan State University, in March, 1973. Approximately 300 treated culms were planted in the barley nursery in early June

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of that year. Seeds produced by those plants were harvested in early August, cold-treated at 7 C for five days, and germinated at room temperature in a dark cabinet. Plantlets were transferred to clay pots when the coleoptyle was 1 cm. in length and grown to anthesis in the growth chamber. Both meiotic chromosome counts and relative seed set were considered in selecting the amphiploid used in this investigation.

The procedures employed for crossing the research plants are as follows:

1. Awns of the female spike were clipped with cuticle scissors.
2. Upper and lower immature florets of the spike were removed carefully to avoid injuring the flag leaf.
3. The remaining florets were opened and emasculated with fine-pointed tweezers; anthers were discarded.
4. Florets with feathery, receptive stigmas were individually hand-pollinated by breaking dehiscing anthers from selected male spikes over them. This process was repeated on the following day.
5. The pollinated spike was covered loosely with an aluminum foil envelope and supported by an iron rod.

The florets of the covered spike were examined for ovary development 6-8 days after pollination. Developing seeds were checked daily thereafter for yellowing, an initial sign of endosperm collapse (Brink, et al., 1944).

In the case of both interspecific (Davies, 1960; Konzak, et al.,

TABLE 1

Origin and Designations of the Research Materials

PLANT	DESIGNATION	ORIGIN

TABLE 1

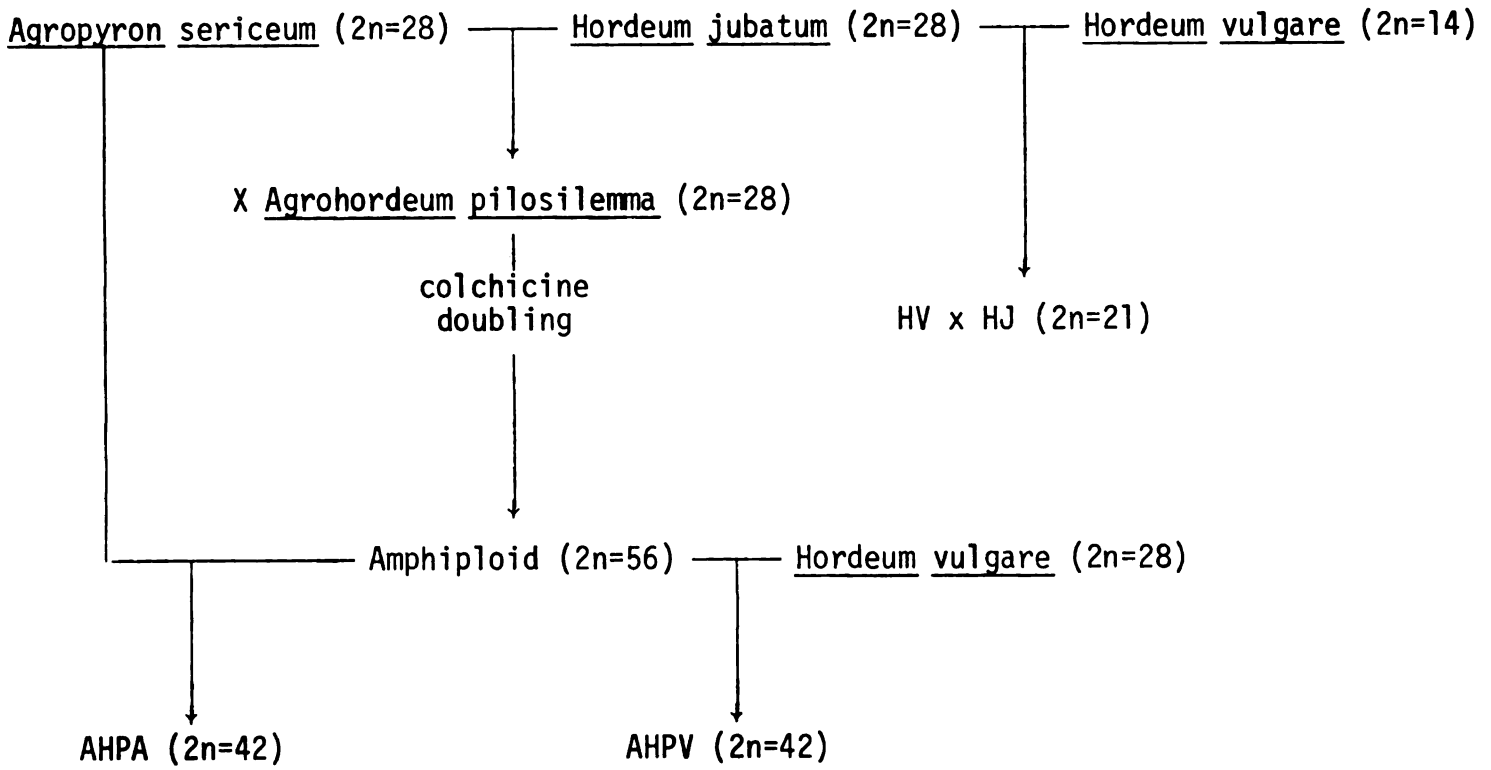
Origin and Designations of the Research Materials

PLANT	DESIGNATION	ORIGIN
Agropyron sericeum		Seeds from W.W. Mitchell and R. Taylor, Palmer Alaska
X Agrohordeum pilosilemma		Clone from W.W. Mitchell, Palmer, Alaska
Hordeum jubatum		Seeds from W.W. Mitchell, Palmer, Alaska
Hordeum vulgare (2x) x H. jubatum	HV x HJ	Crossed and embryo cultured by R. Steidl, Department of Crop and Soil Sciences, Michigan State University
Hordeum vulgare (2x)		Seeds from J.E. Grafius, Department of Crop and Soil Sciences, Michigan State University
Amphiploid		Colchicine treated by R. Steidl, Department of Crop and Soil Sciences, Michigan State University
Amphiploid x A. sericeum	AHPA	Crossed and embryo cultured by author
Amphiploid x H. vulgare (4x)	AHPV	Crossed and embryo cultured by R. Steidl, Department of Crop and Soil Sciences, Michigan State University
Hordeum vulgare (4x)		Seeds from A.B. Schooler, North Dakota State University, Fargo, North Dakota

FIGURE 1

FIGURE 1

X Agrohordeum pilosilemma Breeding Program



1951) and intergeneric (Cooper and Brink, 1944) barley hybrids, endosperm collapse, interpreted as a nutritional incompatibility between the embryo and endosperm, necessitates embryo culture. The method outlined by Morrison, et al. (1959) was adapted for the present study. Embryo culture was carried out in a small darkroom lacking obvious air currents. The immediate transfer area was swabbed with 95 % alcohol and ringed with burning alcohol lamps. Tools, tweezers and needles, were flame-sterilized before each use. The caryopsis was surface-sterilized in 5 % "Clorox" and placed in a petri dish containing sterile distilled water. Excision, complete removal of the endosperm and ovary wall from the embryo, was done under a dissecting microscope (15X-45X). The embryo was placed on the surface of 50 ml. of sterile culture medium (Norstog, 1973) contained in a foam-stoppered, 125 ml. Erlenmeyer flask. The flasks were stored in a room-termperature (21 C), dark cabinet until the embryos germinated. As soon as the embryos showed well-developed roots, the flasks were removed to a light table. After emergence of the second leaf, the plantlets were potted and placed in the growth chamber.

Spikes for cytological studies were fixed (1973-1975) in bottles of Newcomer's solution (Newcomer, 1953) from the field, between 6 and 10 AM in early June; from the greenhouse, between 8 and 11 AM depending on the season; and from the growth chamber, between 9 and 12 AM. These collection times were established to allow harvest during periods of maximum meiotic activity. After

24 hours at room temperature, the fixed materials were stored in a refrigerator until used.

All cytological observations made in this investigation were from microspore mother cells. Temporary slides were prepared according to the technique described by Tai (1967). All stages of meiosis were examined for each plant, and comparative data from at least five spikes was compiled for a minimum total of 30 euploid cells at diakinesis-metaphase I and anaphase I. Minima of 60 telophase I cells (henceforth designated T-I cells) and 200 quartets were scored for micronuclei.

Phase contrast microscopy was accomplished using either a Zeiss Standard WL Research Microscope with an external light source or a Zeiss Photomicroscope II with a built-in light source. Photomicrographs were recorded on Panatomic X film using the planapochromatic, oil immersion, objective lenses (40x/1.0 and 63x/1.4) and the built-in 35 mm. camera on the Photomicroscope II.

Fertility of the parental plants and hybrids was estimated from observations of pollen stainability and seed set. Pollen stainability was tested using I_2KI (Johansen, 1940) on a minimum total of 1000 grains from at least five spikes per plant. Seed set was determined by counting the number of florets which developed seed on a minimum of five mature spikes.

It was originally proposed that the plants included in this research program be karyoptyped using giemsa chromosomal banding techniques. Banding would allow the specific identification of

homologous chromosomes (or chromosomal segments) of the various genomes. Despite almost seven months of experimental effort, attempts to obtain reproducible giemsa bands and karyotype barley chromosomes proved futile. Various techniques available in the literature (Gill and Kimber, 1974; Merritt and Burns, 1974; Verma and Rees, 1974; Doebel, et al., 1973; Schweizer, 1973; Stack and Clark, 1973; and Vosa and Marchi, 1972) have been tried, but faint heterochromatic bands are obtained less than 10 % of the time in Hordeum vulgare Larker seedling, root tip chromosomes. The variables of giemsa banding continue to be elusive since two slides of the same material treated in the same way at the same time may result in only one slide exhibiting chromosomal banding. Attempts at banding with leuco-basic-fuchsin (Feulgen Stain) and with aniline blue also failed.

RESULTS

Agropyron sericeum (Figure 2 A) is a self-fertile (Hodgson, 1964), perennial tetraploid endemic to Alaska, the Yukon, and the District of Mackenzie (Bowden, 1965). Hodgson (1956) first reported its chromosome number, 2n=28, and later published the only account of its cytology (Hodgson, 1964). Microsporogenesis in Agropyron sericeum (Figure 3) was normal with 14 bivalents formed at metaphase I (Figure 3 D). No univalents or multivalents were observed in 92 cells, but the bivalents were often difficult to separate and appeared to have tenuous connections one to another. This characteristic, previously noted by Hodgson (1964), was present regardless of fixative used, growth conditions, or the time of harvest. Chromosome segregation was regular, 14-14 (Figure 3 E) with 27.9% of the 43 anaphase I cells showing chromosome bridges. The T-I cells and quartets reflected this bridge formation and associated fragmentation in that 11.8% of the 187 T-I cells and 7.2 % of the 807 quartets contained micronuclei. Pollen stainability was 84.0% under field conditions, and seed set was 88.6% in the growth chamber. The latter percentage falls within the range of 83-100% seed set reported for Agropyron sericeum by Mitchell and Hodgson (1965 b).

Hordeum jubatum (Figure 2 C) is a highly fertile (Mitchell and

Figure 2. Spike Morphology

A. Agropyron sericeum (0.6x)

B. X Agrohordeum pilosilemma (0.8x)

C. Hordeum jubatum (1.1x)

D. Amphiploid (0.8x)

E. AHPA (0.8x)

F. Hordeum vulgare (0.6x)

G. HV x HJ (1.0x)

H. AHPV (0.8x)

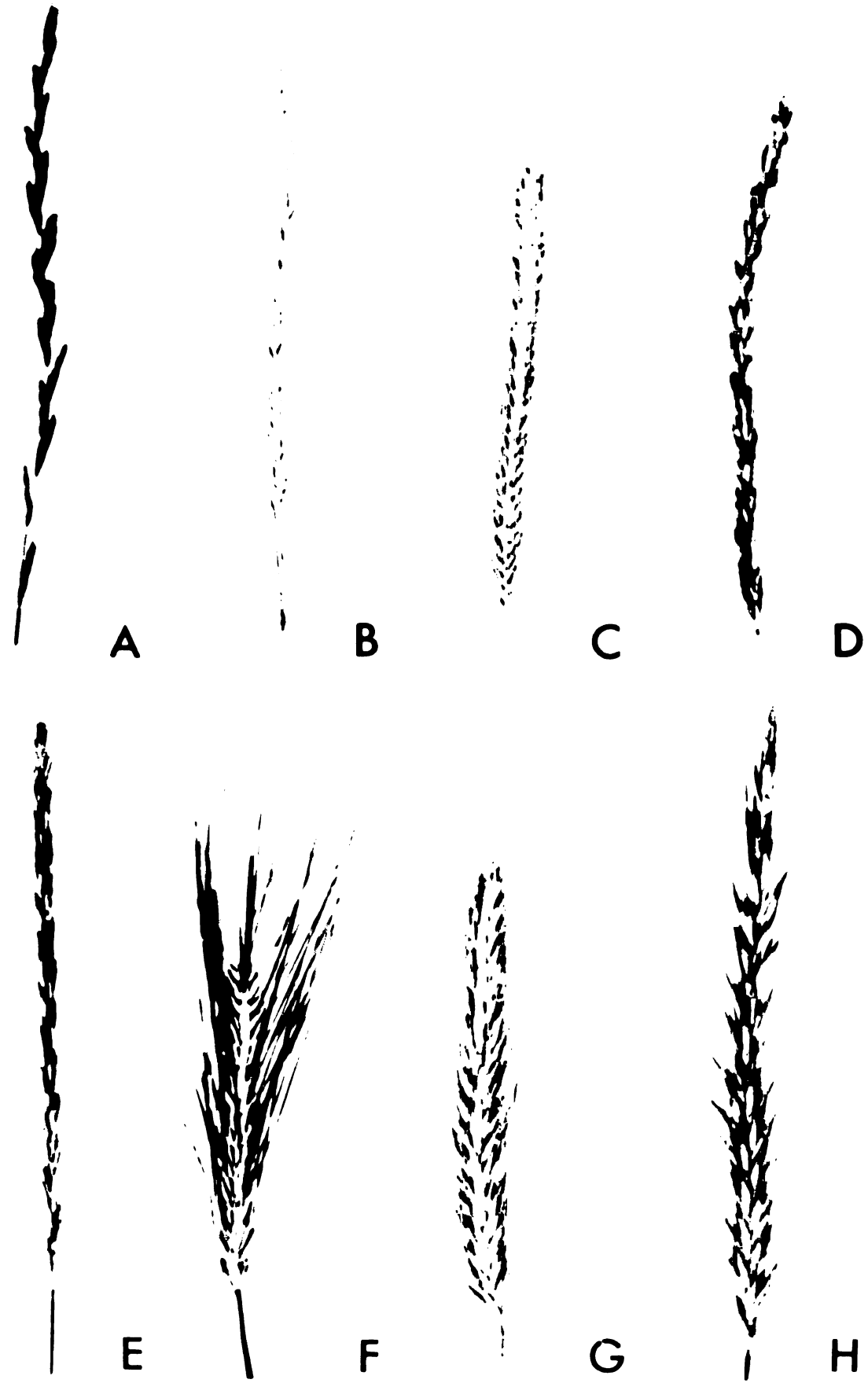


Figure 2

Figure 3. Stages of Microsporogenesis in Agropyron sericeum (2n=28)

A. Zygotene (1450x)

B. Pachytene (1175x)

C. Diplotene (1375x)

D. Metaphase I with 14 bivalents (1975x)

E. Anaphase I with a 14 - 14 distribution and a double bridge involving three dyad chromosomes (1550x)

F. Two daughter cells from the first meiotic division (1550x)

G. Metaphase II (2240x)

H. Telophase II (1060x)

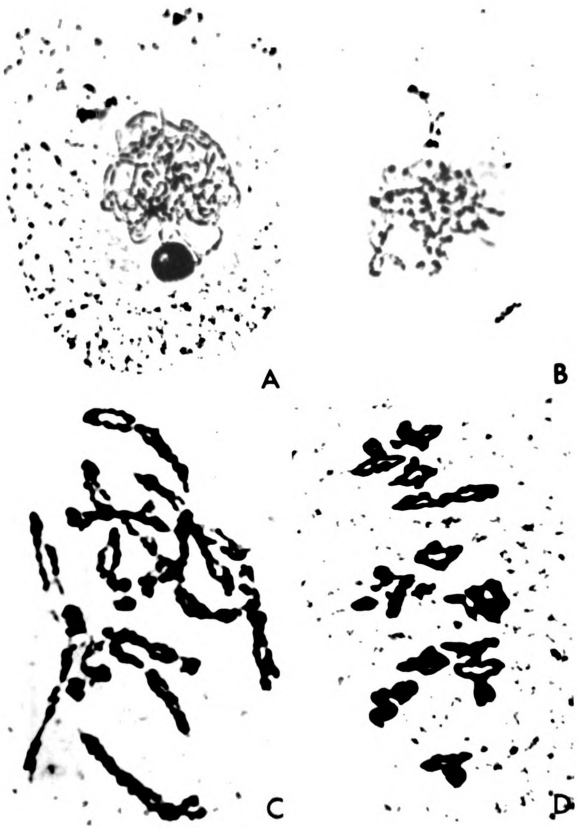


Figure 3

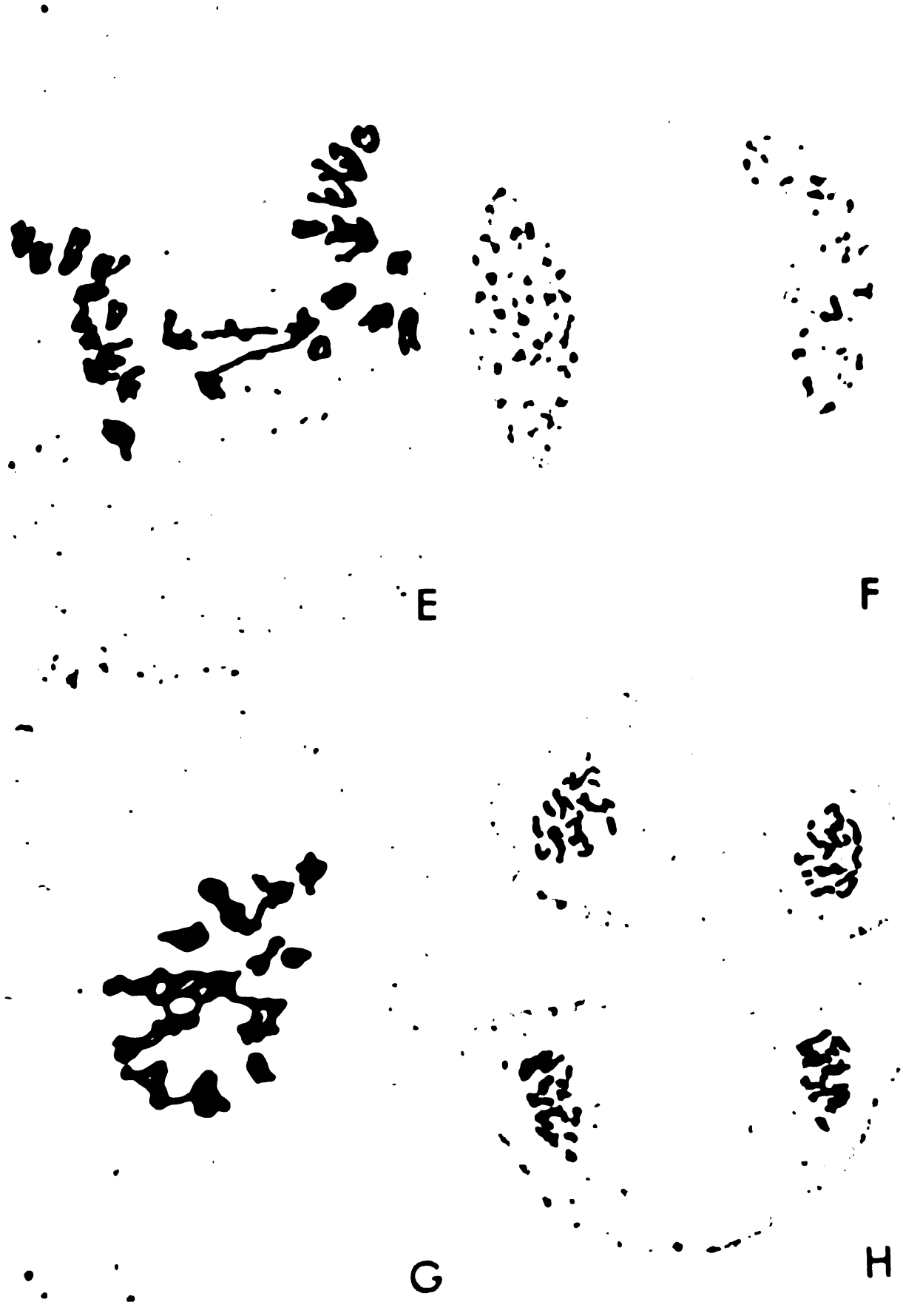


Figure 3 (cont'd.)

Wilton, 1964; Smith, 1944), perennial, segmental allotetraploid (Starks and Tai, 1974; Redmann and Borgaonkar, 1966; Rajhathy, et al., 1964; and Wagenaar, 1959, 1960) with arctic-alpine-temperate distribution in both the Old and New World (Bowden, 1962; Hitchcock and Chase, 1950; Covas, 1948; and Nevski, 1934). From meiotic counts of microspore mother cells, Aase and Powers (1926) published the first determination of chromosome number, 2n=28, for Hordeum jubatum. Microsporogenesis (Figure 4) was completely normal with consistent formation of 14 bivalents in 40 diakinesis-metaphase I cells (Figure 4 B-D). Quadrivalents, observed in Hordeum jubatum by Schooler, et al. (1966) and Rajhathy and Morrison (1961), have never been seen in the Alaskan material used in this investigation (cf. Huang, 1975; Starks and Tai, 1974). Anaphase I segregation (Figure 4 E) was an orderly 14-14 with chromosome bridges found in 10.0 % of the 30 cells. Micronuclei were present in 17.7% of the 96 T-I cells (Figure 4 F) and 11.1% of the 207 quartets (Figure 4 H) counted. Under field conditions, pollen stainability was 84.8% (Starks, 1975), and seed set was 93.7%, higher than the 72% reported by Smith (1944) for greenhouse-grown plants.

Mitchell and Hodgson (1965 a) established the chromosome number, 2n=28, for X Agrohordeum pilosilemma (Figure 2 B), but its cytology has not been studied previous to this investigation. Microsporogenesis of the spontaneous hybrid is represented in Figure 5. Metaphase I configurations (Figure 5 C) averaged 13.32 I, 5.89 II, 0.08 III, and 0.05 IV for 38 cells (Table 2). Secondary association of

Figure 4. Stages of Microsporogenesis in Hordeum jubatum (2n=28)

A. Zygotene (1375x)

B. Diakinesis with 14 bivalents (1880x)

C. Prometaphase I with 14 bivalents (2750x)

D. Metaphase I with 14 bivalents (2075x)

E. Anaphase I with a 14-14 distribution, one fragment (arrow), and one bridge (1625x)

F. Telophase I (1350x)

G. Metaphase II (1175x)

H. Quartet (1075x)

Figure 4

Figure 4 (cont'd.)

Figure 5. Stages of Microsporogenesis in X Agrohordeum pilosilemma (2n=28)

A. Zygotene (1600x)

B. Pachytene (1625x)

C. Metaphase I with 1II, 4II, and (arrows) 3III (1625x)

D. Anaphase I with an 8-9 distribution, 1l laggards, and one bridge (1325x)

E. Telophase I with two micronuclei (1075x)

F. Late telophase I with trailing laggards (1175x)

G. Late telophase I with tripolar segregation (1125x)

H. Prophase II (1450x)

I. Metaphase II (1175x)

J. Anaphase II with a 14-14 distribution and two laggards (2015x)

K. Quartet with six nuclei and six micronuclei (1450x)

L. Linear quartet with eight micronuclei (1200x)

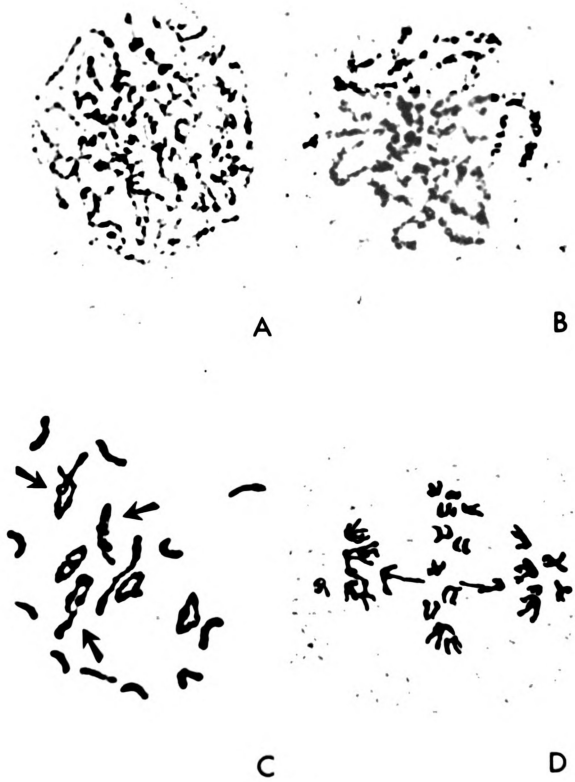


Figure 5

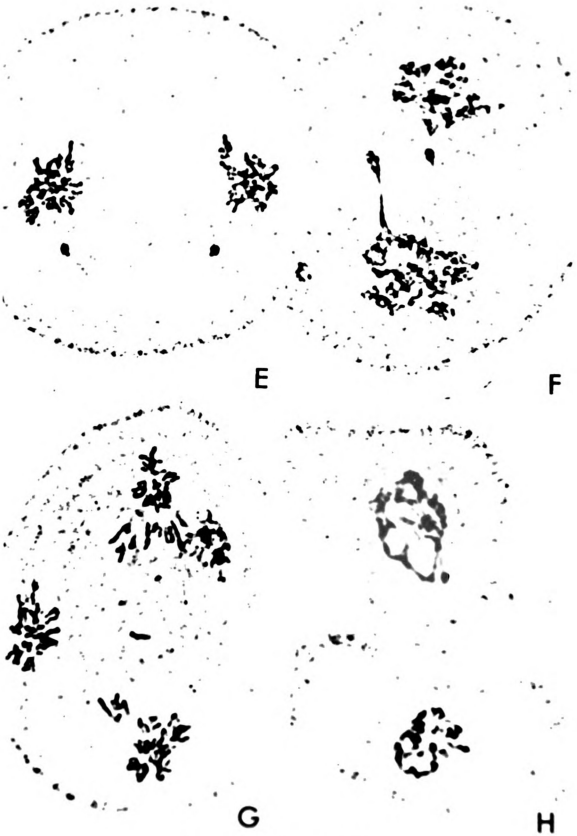


Figure 5 (cont'd.)

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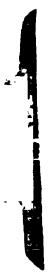
TABLE 2

Diakinesis-Metaphase I Chromosome Association in X Agrohordeum pilosilemma

Chromosome Association				# of cells	%
I	II	III	IV		
9	8	1		1	2.63
10	6	2		1	2.63
11	4	3		2	5.26
11	7	1		1	2.63
12	5	2		4	10.52
12	8			3	7.89
13	3	3		1	2.63
13	6	1		4	10.52
14	4	2		2	5.26
14	5		1	2	5.26
14	7			10	26.31
15	5	1		5	13.15
16	6			1	2.63
18	5			1	2.63
Total 506	224	34	2	38	
Average 13.32	5.89	0.89	0.05		



univalents (Person, 1955; Richardson, 1935; and Percival, 1930) was occasionally noted. The 32 cells counted for anaphase I (Figure 5 D, Table 3) showed an average of 18.0 chromosomes migrating to the poles while the remaining 10.0 were lining up on the metaphase plate. Precocious centromere division (Clayberg, 1959) was observed among the lagging chromosomes, but was not quantified. Figure 5 F may represent either a laggard or a bridge of the latter type trapped during cytokinesis. Tripolar segregation (Figure 5 G) was noted in 5.0% of the 61 T-I cells (Figure 5 E) recorded. Micronuclei were found in 90.2% of the T-I cells and 100% of the 572 quartets (Figure 5 L). The quartet shown in Figure 5 K has two binucleate microspores suggesting previous multipolar segregation. Regardless of growth conditions, both pollen stainability and seed set were zero.

The amphiploid of X Agrohordeum pilosilemma (Figure 2 D) is slightly more robust than the spontaneous hybrid under both greenhouse and growth chamber conditions. Its chromosome number, 2n=56, was stable, and microsporogenesis (Figure 6) was more regular than in the undoubled hybrid. Chromosome association in 30 metaphase I cells (Figure 6 B; Table 4) averaged 2.43 I, 19.80 II, 0.70 III, and 2.97 IV. Anaphase I (Figure 6 C) displayed a range of 1-8 univalents assembling on the metaphase plate after segregation of an average 52.6 synapsed chromosomes of the complement (Table 5). These univalents, which were present in 93.3% of the 30 anaphase I cells observed, invariably underwent precocious centromere division.

TABLE 3

Anaphase I Chromosome Distribution in X Agrohordeum pilosilemma

Groupings			# of Cells	%
Pole	Laggards on Plate	Pole		
6	13	9	1	3.13
6	14	8	1	3.13
7	11	10	1	3.13
7	13	8	2	6.25
7	14	7	3	9.37
8	9	11	1	3.13
8	10	10	3	9.37
8	11	9	5	15.63
8	12	8	1	3.13
9	7	12	2	6.25
9	8	11	2	6.25
9	9	10	1	3.13
9	10	9	3	9.37
10	7	11	1	3.13
10	8	10	3	9.37
12	4	12	2	6.25
Total 270	320	306	32	
Average 8.44	10.00	9.56		

Figure 6. Stages of Microsporogenesis in the Amphiploid (2n=56)

A. Pachytene (1225x)

B. Metaphase I with 2I, 2III, and (arrows) 3IV (1650x)

C. Anaphase I with a 26-26 distribution and 4 laggards undergoing precocious centromere division (1050x)

D. Late telophase I with five micronuclei (1075x)

E. Metaphase II (1075x)

F. Anaphase II with a 26-26 distribution and two micronuclei (1175x)

G. Quartet with eight micronuclei (1350x)

H. T-shaped quartet with two micronuclei (1075x)

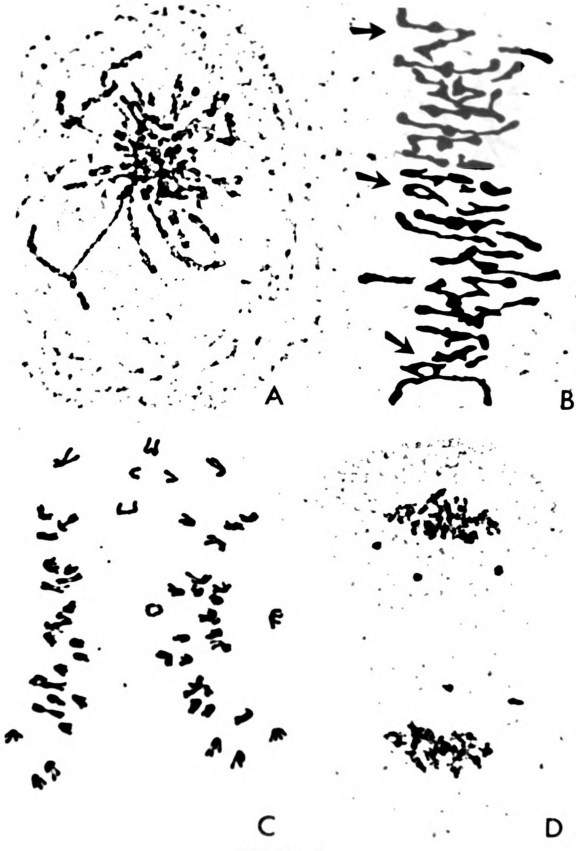


Figure 6

Figure 6 (cont'd.)

TABLE 4

Diakinesis-Metaphase I Chromosome Association in the Amphiploid

Chromosome Association					
I	II	III	IV	# of Cells	%
1	18	1	4	3	10.00
1	22	1	2	3	10.00
2	18	2	3	1	3.33
2	19		4	2	6.67
2	21		3	2	6.67
3	15	1	5	1	3.33
3	17	1	4	1	3.33
3	19	1	3	2	6.67
3	21	1	2	1	3.33
4	20		3	1	3.33
4	22		2	1	3.33
5	18	1	3	3	10.00
5	19	3	1	1	3.33
6	18	2	2	1	3.33
8	18		3	1	3.33
	20		4	2	6.67
	22		3	1	3.33
	24		2	3	10.00
Total 73	594	21	89	30	
Average 2.43	19.80	0.70	2.97		

TABLE 5

Anaphase I Chromosome Distribution in the Amphiploid

	Groupings				
	Pole	Laggards on Plate	Pole	# of Cells	%
	22	8	26	1	3.33
	23	5	28	1	3.33
	23	6	27	1	3.33
	24	5	27	2	6.67
	24	7	25	1	3.33
	25	3	28	2	6.67
	25	5	26	2	6.67
	25	6	25	1	3.33
	26	1	29	2	6.67
	26	2	28	3	10.00
	26	3	27	4	13.32
	26	4	26	4	13.32
	27	2	27	4	13.32
	27		29	1	3.33
	28		28	1	3.33
Total	766	102	812	30	
Average	25.53	3.40	27.07		

Generally, the "chromatids" rapidly migrated to the poles and were included in the Telophase I nuclei, but sometimes they were excluded and became micronuclei (Figure 6 D). The results of precocious centromere division were also evident in anaphase II: the previously included "chromatids" lagged on the metaphase II plate (Figure 5 J) and fragmented. Micronuclei occurred in 91.1% of the 403 T-I cells and 95.7% of the 937 quartets (Figure 6 G,H) scored. Under growth chamber conditions, pollen stainability was 51.8%, and seed set was 54.9%.

The backcross of the amphiploid to Agropyron sericeum, AHPA (Figure 2 E), resembles Agropyron sericeum in both vegetative and floral morphology. Figure 7 depicts microsporogenesis in this stable, 2n=42, hybrid. The metaphase I chromosome association (Figure 7 C) averaged 6.58 I, 9.68 II, 0.97 III, and 3.29 IV for 31 cells (Table 6). Univalents (2-9) showed late alignment and precocious centromere division at anaphase I (Figure 7 D) in 96.8% of the 31 cells examined (Table 7). In AHPA, 92.5% of the 254 T-I cells (Figure 7 E) and 99.4% of the 1018 quartets (Figure 7 H) carried micronuclei. Pollen stainability and seed set were 58.6% and 25.4%, respectively, under growth chamber conditions.

Hordeum vulgare (2x, Figure 2 F) is a self-fertile, annual diploid of worldwide temperate distribution both as a crop and as a weed (Harlan, 1971; Weibe, 1968; Covas, 1948; and Nevski, 1934). Its chromosome number, 2n=14, was first reported by Nakao (1911, cited in Love and Love, 1961), and karyotypes are common in the

Figure 7. Stages of Microsporogenesis in AHPA (2n=42)

A. Cytomixis at zygotene (1105x)

B. Pachytene (1350x)

C. Metaphase I with 5I, 13II, (open arrow) III, and (solid arrows) 2IV (1325x)

D. Anaphase I with a 19-20 distribution and three laggards (1025x)

E. Late telophase I with three micronuclei (1050x)

F. Metaphase II with one micronucleus (1100x)

G. Anaphase II with a 17-19 / 20-20 distribution and three and four laggards, respectively (800x)

H. Quartet with 11 micronuclei (865x)

Figure 7

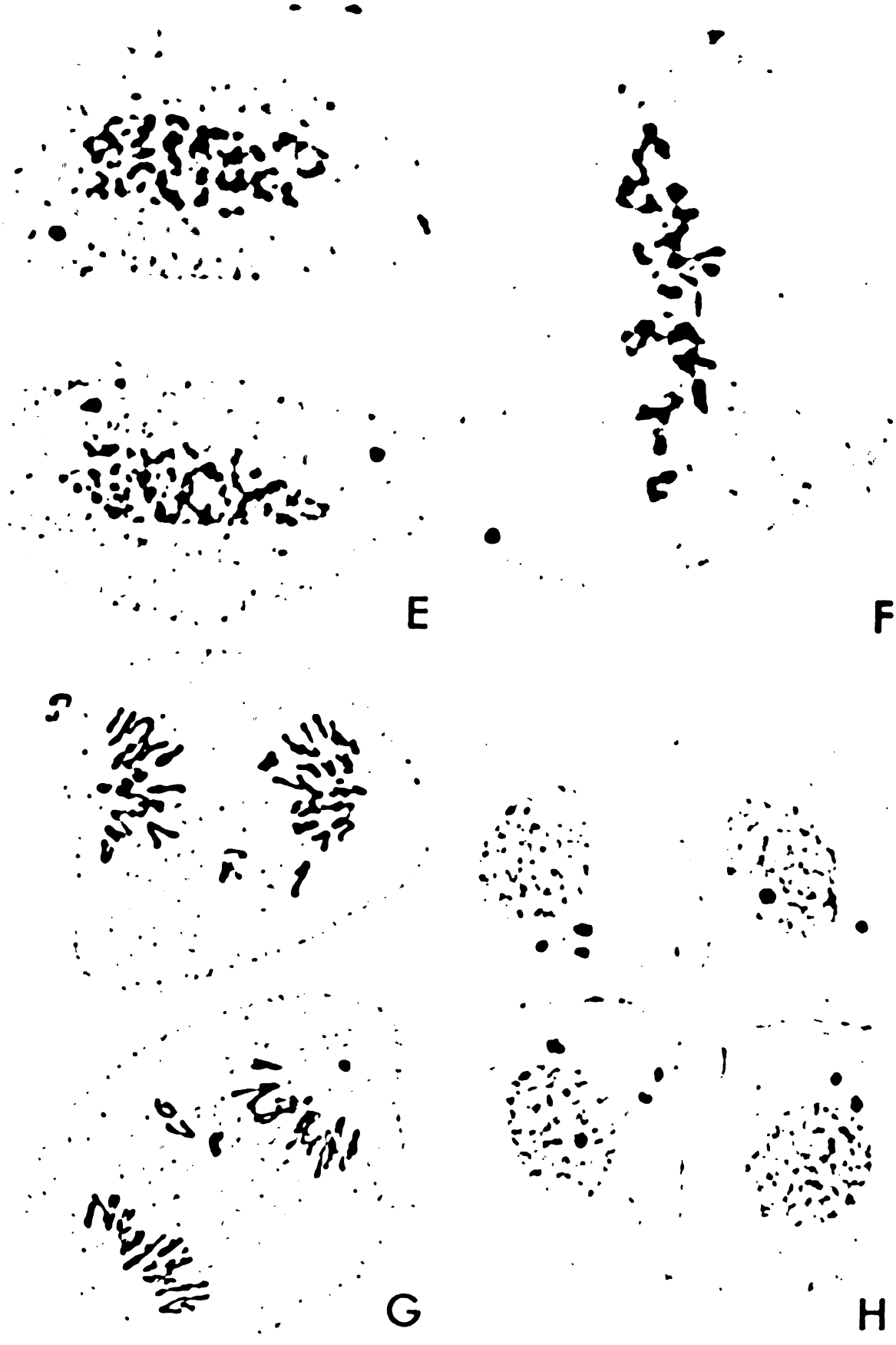


Figure 7 (cont'd.)

TABLE 6

Diakinesis-Metaphase I Chromosome Association in AHPA

	Chromosome Association					
	I	II	III	IV	# of Cells	%
	1	11	1	4	1	3.22
	3	10	1	4	1	3.22
	3	12	1	3	1	3.22
	4	7		6	1	3.22
	4	8	2	4	1	3.22
	4	9		5	1	3.22
	4	10	2	3	2	6.45
	5	9	1	4	1	3.22
	5	11	1	3	2	6.45
	5	13	1	2	1	3.22
	6	6		6	1	3.22
	6	10		4	2	6.45
	6	11	2	2	1	3.22
	6	12		3	3	9.68
	7	10	1	3	1	3.22
	8	8	2	3	1	3.22
	8	10	2	2	1	3.22
	8	11		3	1	3.22
	9	6	3	3	1	3.22
	9	9	1	3	2	6.45
	10	8	4	1	1	3.22
	10	8		4	1	3.22
	12	9		3	1	3.22
	12	11		2	1	3.22
	13	7	1	3	1	3.22
Total	204	300	30	102	31	
Average	6.58	9.68	0.97	3.29		

TABLE 7

Anaphase I Chromosome Distribution in AHPA

	Groupings				
	Pole	Laggards on Plate	Pole	# of Cells	%
	16	7	19	1	3.22
	16	9	17	2	6.45
	17	4	21	1	3.22
	17	5	20	2	6.45
	17	6	19	1	3.22
	17	7	18	1	3.22
	18	4	20	3	9.68
	18	5	19	4	12.90
	19	2	21	2	6.45
	19	3	20	6	19.35
	19	4	19	5	16.13
	20	2	20	2	6.45
	21		21	1	3.22
Total	567	130	605	31	
Average	18.29	4.19	19.52		

literature (Tsuchiya, 1960; Morrison, 1959; Sarvella, et al. 1958; and Hagberg and Tjio, 1950). Microsporogenesis in Hordeum vulgare (2x) was normal (Figure 8) with seven bivalents formed in 74 diakinesis-metaphase I cells (Figure 8 C,D). Bridges (Figure 8 F) were recorded for 38% of the 55 regularly segregating, 7-7, anaphase I cells studied. Of 185 T-I cells (Figure 8 G) and 306 quartets, 8.1% and 8.6%, respectively, contained micronuclei. Pollen stainability and seed set were not determined for diploid Hordeum vulgare.

Hordeum vulgare (4x) is a self-fertile, annual autotetraploid, 2n=28, whose microsporogenesis is represented in Figure 9. Averages of diakinesis-metaphase I chromosome association (Figure 9 C) were 0.03 I, 8.38 II, 0.03 III, and 2.78 IV for 64 cells (Table 8). The frequency of quadrivalents in the material used in this investigation was lower than the mean of 3.9 reported by Morrison and Rajathy (1960a) for their autotetraploids. Approximately 70.0% of the 47 anaphase I cells showed a 14-14 chromosome distribution (Figure 9 D; Table 9), while another 15.0% of the cells displayed lagging chromosomes and precocious centromere division. Micronuclei occurred in 27.2% of the 162 T-I cells and in 60.7% of the 211 quartets (Figure 9 H). Under field conditions, pollen stainability was 67.3%, and seed set was 83.3%. The latter percentage exceeds the 33% and 78% reported for autotetraploids of Hordeum vulgare by Morrison and Rajhathy (1960a) and Tsuchiya (1953), respectively.

The interspecific hybrid, Hordeum jubatum x Hordeum vulgare (2x) was synthesized by Morrison, et al. (1959), Vinogradova (1946,

Figure 8. Stages of Microsporogenesis in Hordeum vulgare (2n=14)

A. Pachytene (2210x)

B. Diplotene (2560x)

C. Diakinesis with seven bivalents (2785x)

D. Metaphase I with seven bivalents (2785x)

E. Anaphase I with a 7-7 distribution (1075x)

F. Anaphase I with a 7-7 distribution and one bridge (2335x)

G. Telophase I (2175x)

H. Late anaphase II (1075x)

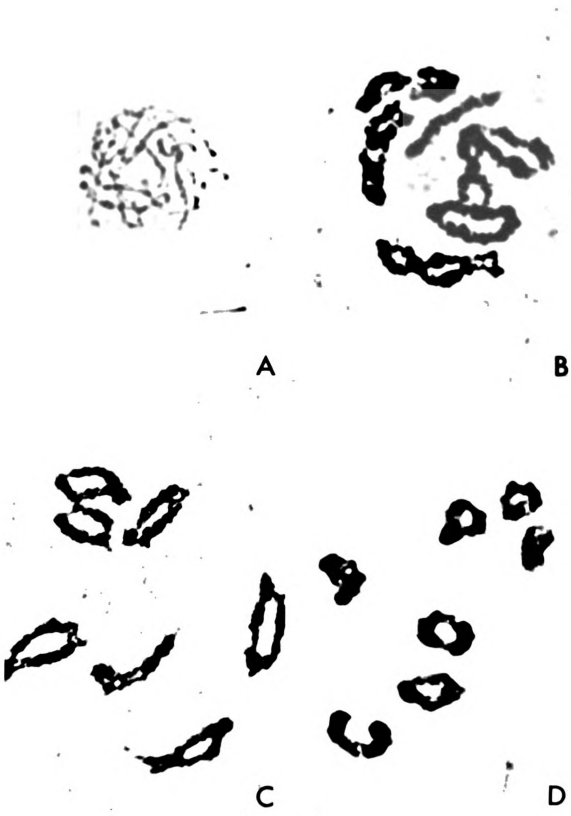


Figure 8

Figure 8 (cont'd.)

Figure 9. Stages of Microsporogenesis in Hordeum vulgare (2n=28)

A. Diplotene (1505x)

B. Diakinesis (2550x)

C. Metaphase I with 10II and (arrows) 2IV (1855x)

D. Anaphase I with a 14-14 distribution (1650x)

E. Prophase II (1075x)

F. Anaphase II (1075x)

G. Telophase II (1075x)

H. Quartet (1750x)

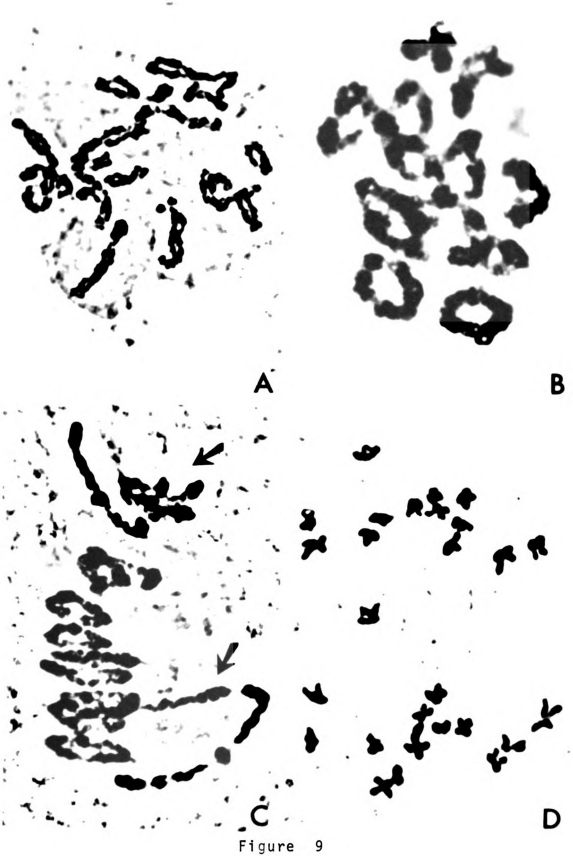


Figure 9

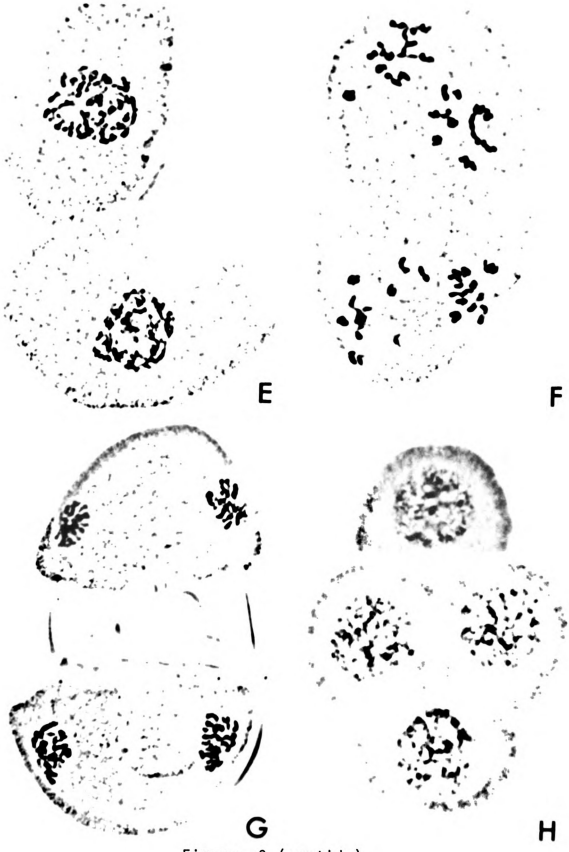


Figure 9 (cont'd.)

TABLE 8

Diakinesis-Metaphase I Chromosome Association in Hordeum vulgare (4x)

	Chromosome Association					
	I	II	III	IV	# of Cells	%
		4		5	4	6.25
		6		4	12	18.75
	1	6	1	3	2	3.12
		8		3	18	28.12
		10		2	22	34.38
		12		1	6	9.38
Total	2	536	2	178	64	
Average	0.03	8.38	0.03	2.78		

cited in Price, 1968, and Smith, 1951), and Quincke (1940) and studied cytologically by Kerber (unpublished data, cited in Wagenaar, 1960) and Rajhathy and Morrison (1959). Kerber's hybrid had a somatic chromosome number of 2n=21 and formed 0-4 bivalents at metaphase I. The hybrid studied by Rajhathy and Morrison (1959) had a variable meiotic chromosome number, 2n=17-22, and averaged 1.1 bivalents for 11 metaphase I cells. In both studies, those chromosomes not involved in bivalent formation remained unassociated.

The reciprocal hybrid, HV x HJ (Figure 2 G), has not been studied previously. The irregularity of HV x HJ microsporogenesis (Figure 10) precluded statistical investigation of meiotic stages subsequent to metaphase I. Approximately 12% of the microspore mother cells examined were aneuploid with chromosome numbers varying from 16 to 22. Perhaps this variation may be attributed to chromosome elimination (Kao and Kasha, 1971; Lange 1971a,b) either by premeiotic chromosomal loss (Figure 10 A) or by meiotic chromosomal disintegration (Figure 10 C). The diakinesis-metaphase I chromosome association (Figure 10 D) presented in Table 10 is based on the analysis of 41 euploid cells which averaged 19.85 I, 0.54 II, and 0.02 III per cell. Foldback and ring univalents and secondary associations (cf. Sadasivaiah and Kasha, 1971) were common in this material. Anaphase I cells with fragmenting univalents, centromere misdivision (Darlington, 1939; Figure 10 F), and multiple poles (Figure 10 G) were occasionally observed, but stages of meiosis II were not distinguishable. The effects of

Figure 10. Stages of Microsporogenesis in HV x HJ (2n=21)

A. Premeiotic telophase with one laggard (2200x)

B. Asynchronous diplotene-diakinesis (925x)

C. Diakinesis with 19 I and (arrow) pseudobivalent; two of the univalents seem to be either uncondensed or disintegrating (1375x)

D. Metaphase I with 21 univalents (1300x)

E. Anaphase I (1350x)

F. Anaphase I with univalent fragmentation (1500x)

G. Anaphase I with tetrapolar segregation (1400x)

H. Quartet with an (arrow) extra microcell (1600x)

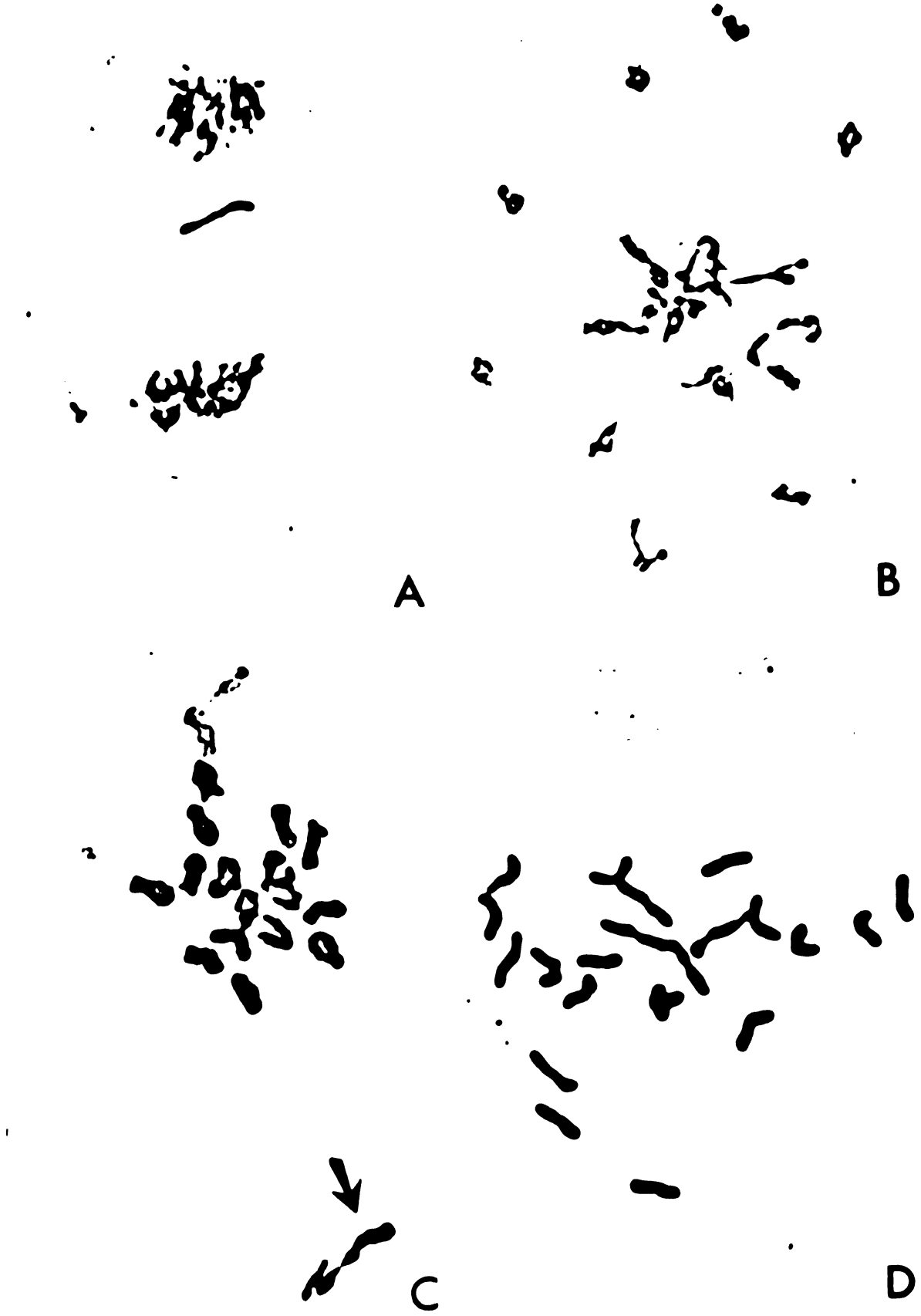


Figure 10

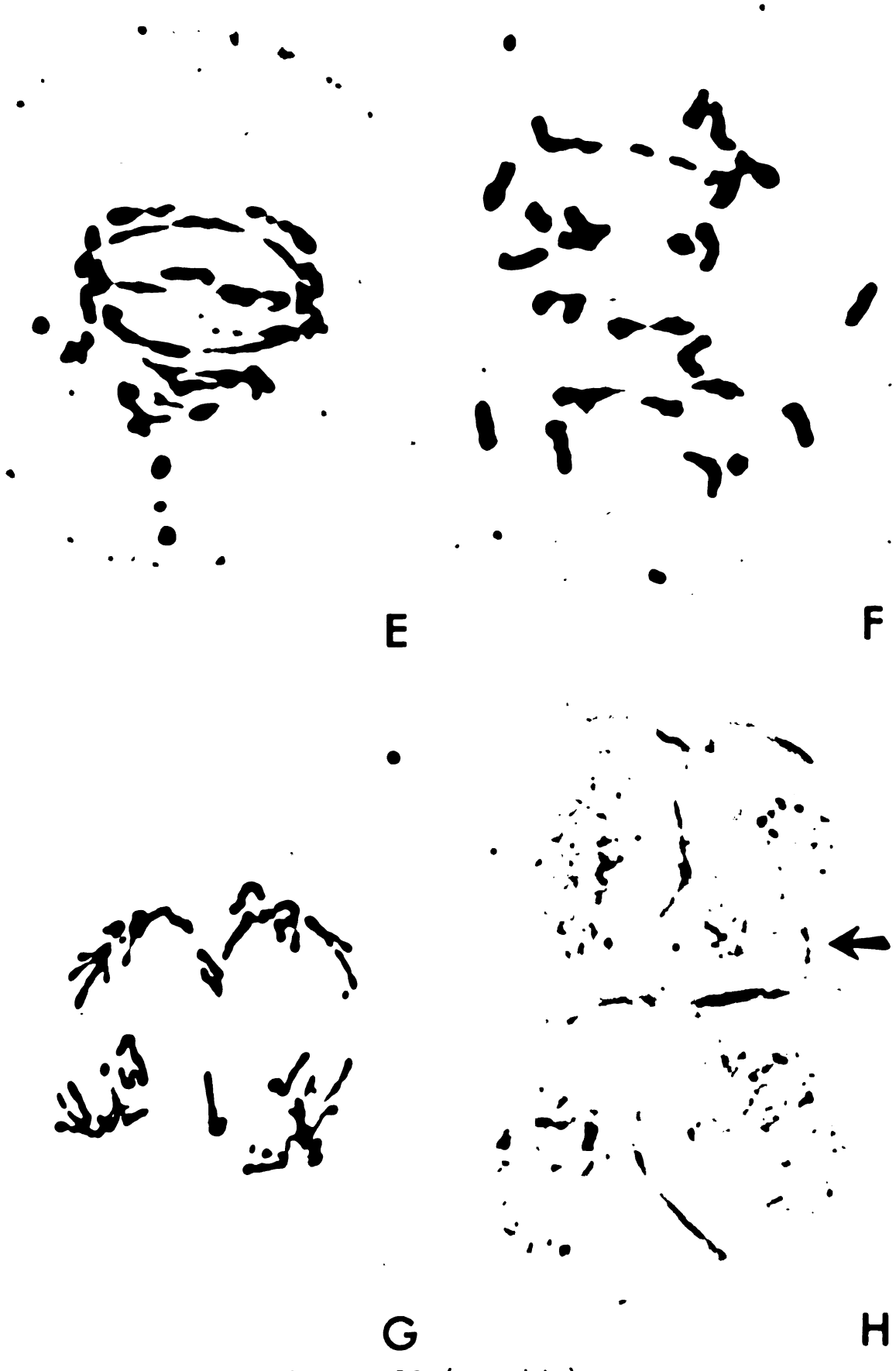


Figure 10 (cont'd.)

TABLE 9

Anaphase I Chromosome Distribution in Hordeum vulgare (4x)

	Groupings				
	Pole	Laggards on Plate	Pole	# of Cells	%
	10	5	13	1	2.13
	12	4	12	2	4.26
	12	3	13	1	2.13
	12		16	1	2.13
	13	1	14	3	6.38
	13		15	6	12.76
	14		14	33	70.21
Total	637	19	660	47	
Average	13.55	0.40	14.04		

TABLE 10

Diakinesis-Metaphase I Chromosome Association in HV x HJ

	Chromosome Association					
	I	II	III	IV	# of Cells	%
	16	1	1		1	2.44
	17	2			3	7.32
	19	1			15	36.58
	21				22	53.66
Total	814	22	1		41	
Average	19.85	0.54	0.02			

multipolar cell division (Tai, 1970) are evident in the quartet and microcell pictured in Figure 10 H. Other irregularities of HV x HJ meiotic behavior will be developed following the presentation of AHPV microsporogenesis. Pollen stainability and seed set in HV x HJ were zero regardless of growth conditions.

The hybrid of the amphiploid crossed to Hordeum vulgare (4X), AHPV (Figure 2 H), exhibited irregular microsporogenesis which was accentuated by adverse environmental conditions (Sax, 1937). Initial collections of AHPV spikes grown under summer field and greenhouse conditions had no analyzable microspore mother cells in stages beyond metaphase I. Spikes collected six weeks after AHPV was transferred to the 21 C growth chamber contained a few cells of each meiotic stage (Figure 11). Diakinesis-metaphase I chromosome association (Figure 11 C; Table 11) averaged 16.47 I, 10.10 II, 0.73 III, and 0.73 IV for 30 cells. The anaphase I cells frequently displayed univalent fragmentation. Micronuclei were present in all T-I cells (Figure 11 D), stages of meiosis II (Figure 11 E,F), and quartets (Figure 11 G); and the majority of the quartets had microcells (Figure 11 H). Empty pollen grains whose contents are assumed to have disintegrated were more prevalent than full ones. Pollen stainability was zero, and an 8-fold range in volume was noted from the smallest and largest grains examined. Seed set for AHPV was also zero.

The irregular meiotic behavior previously mentioned for HV x HJ and AHPV is depicted in Figure 12. Similar behavior has been

Figure 11. Stages of Microsporogenesis in AHPV (2n=42)

A. Pachytene (1215x)

B. Diplotene (1400x)

C. Metaphase I with 19I, 10II, and (arrow) III (1200x)

D. Telophase I with eight micronuclei (815x)

E. Prophase II with one micronucleus (1800x)

F. Anaphase II with a 15.5-16.5 distribution and one micronucleus (1275x)

G. Quartet with three micronuclei (815x)

H. Quartet with one microcell and with micronuclei (925x)

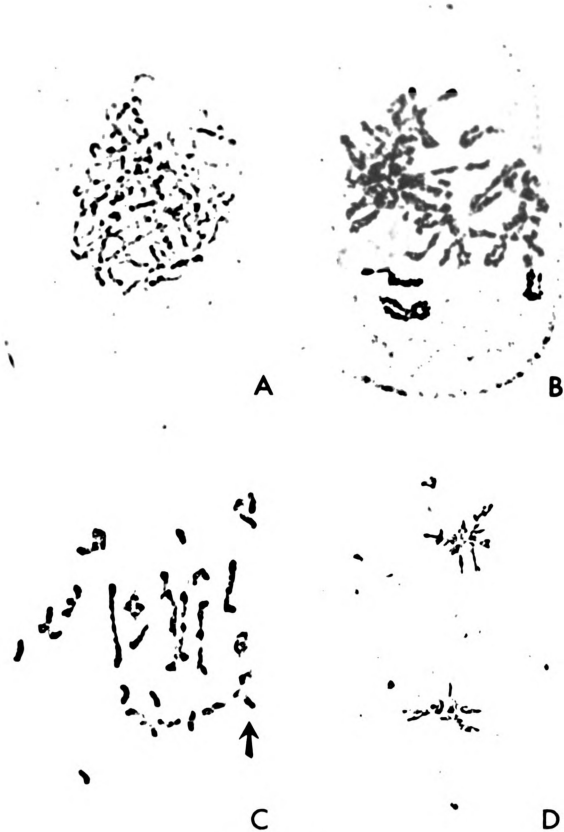


Figure 11

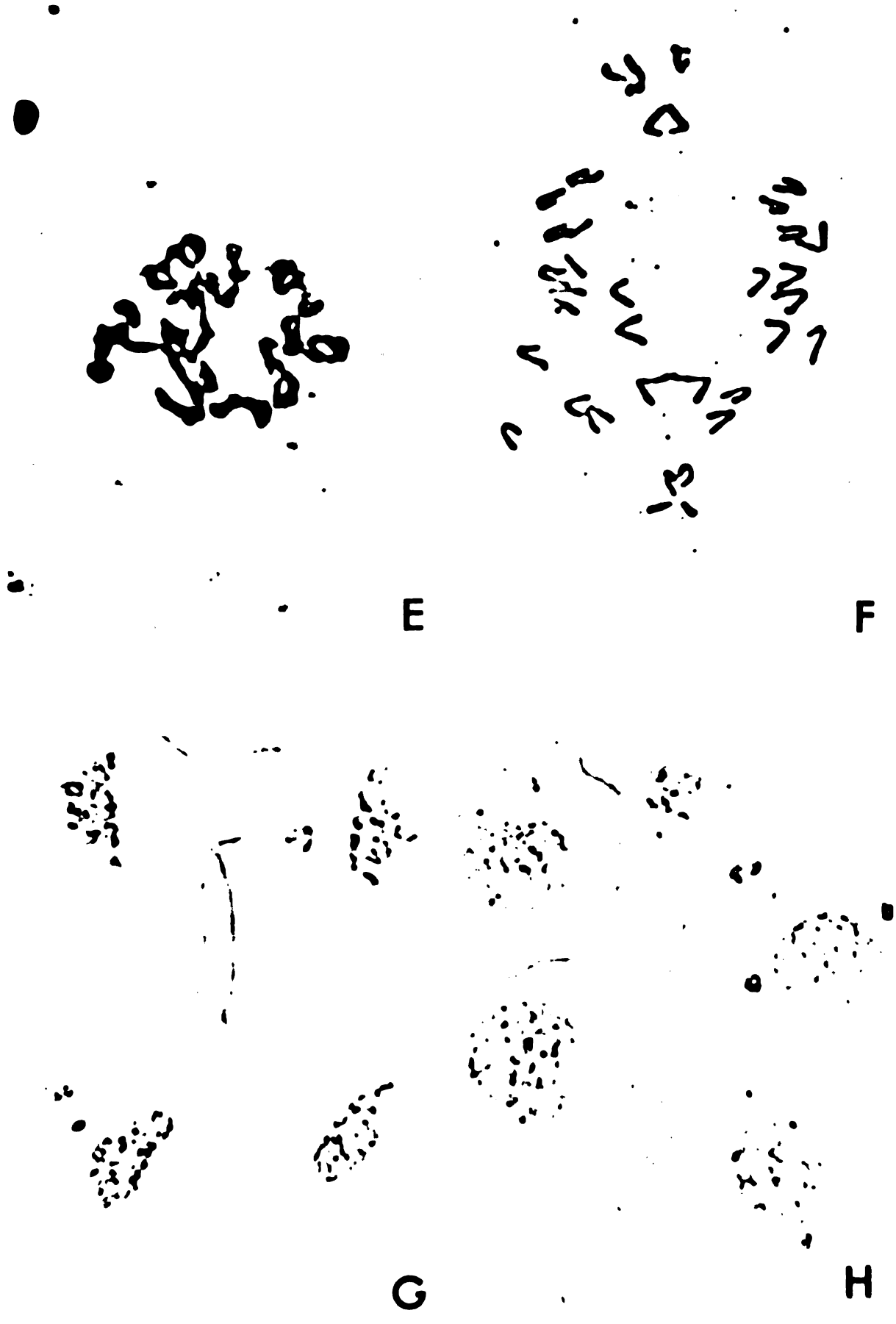


Figure 11 (cont'd.)

TABLE 11

Diakinesis-Metaphase I Chromosome Association in AHPV

Chromosome Association					
I	II	III	IV	# of cells	%
8	14	2	1	1	3.33
10	7	2	3	1	3.33
12	6	2	3	1	3.33
12	11		2	1	3.33
13	9	1	2	1	3.33
13	11	1	1	1	3.33
14	10		2	1	3.33
14	12		1	3	10.00
14	14			3	10.00
15	9	1		1	3.33
15	12	1		1	3.33
16	10	2		1	3.33
16	13			1	3.33
17	9	1	1	1	3.33
17	11	1		1	3.33
19	8	1	1	1	3.33
19	10	1		1	3.33
20	6	2	1	1	3.33
20	9		1	1	3.33
21	6	3		1	3.33
21	9		1	1	3.33
22	8		1	2	6.67
22	10			2	6.67
24	9			1	3.33
Total 494	303	22	22	30	
Average 16.47	10.1	0.73	0.73		

Figure 12. Irregular Meiotic Behavior in HV x HJ and AHPV

A. Post-metaphase I cell with 12 nuclei (1450x)

B. Budding cell with four nuclei (2325x)

C. Cell and bud containing chromatin (1400x)

D. Cell and bud containing chromatin (1950x)

E. Cell and separated bud (2000x)

F. Cell with two buds and pollen 'pore' formation (1625x)

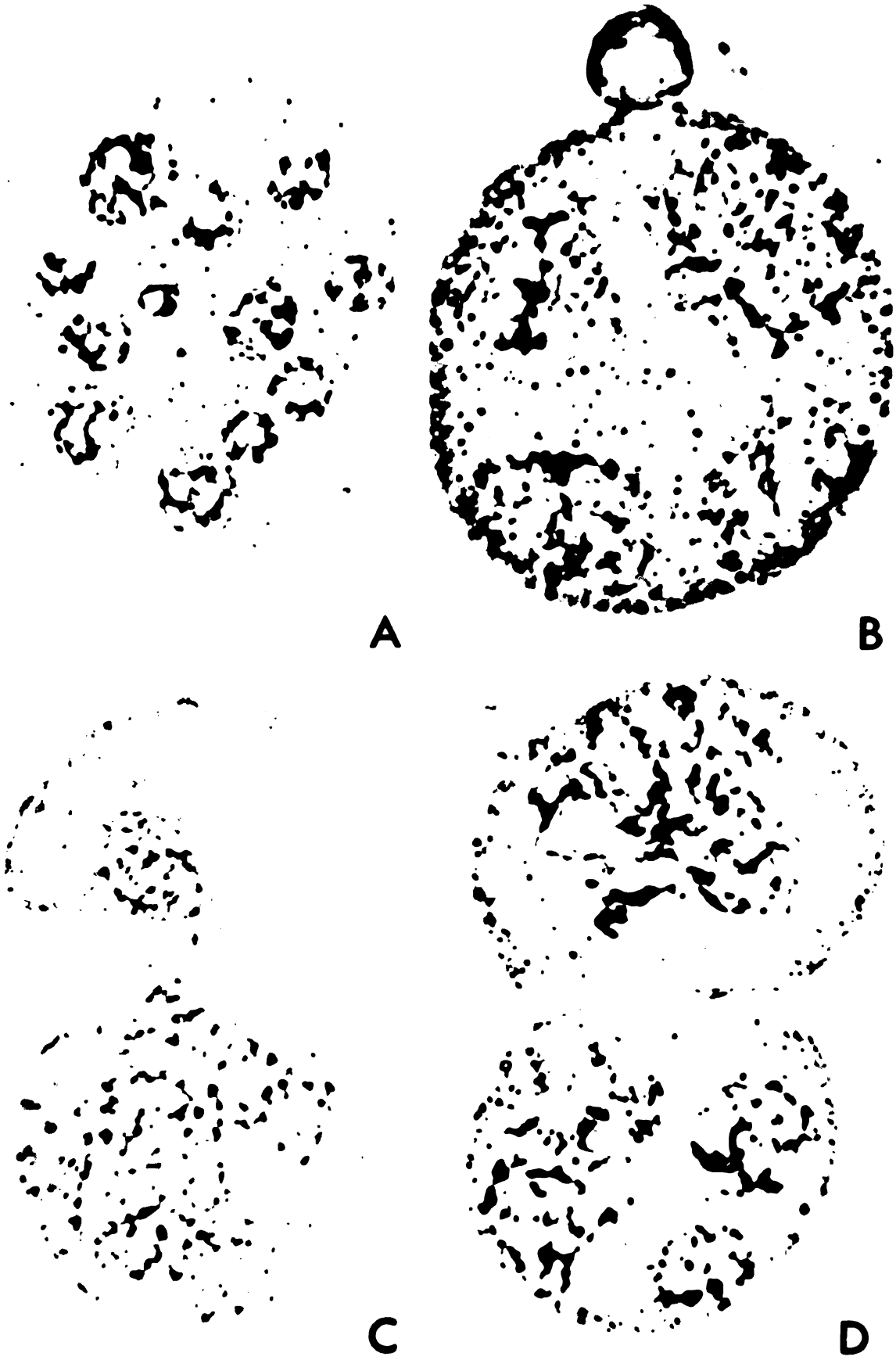


Figure 12

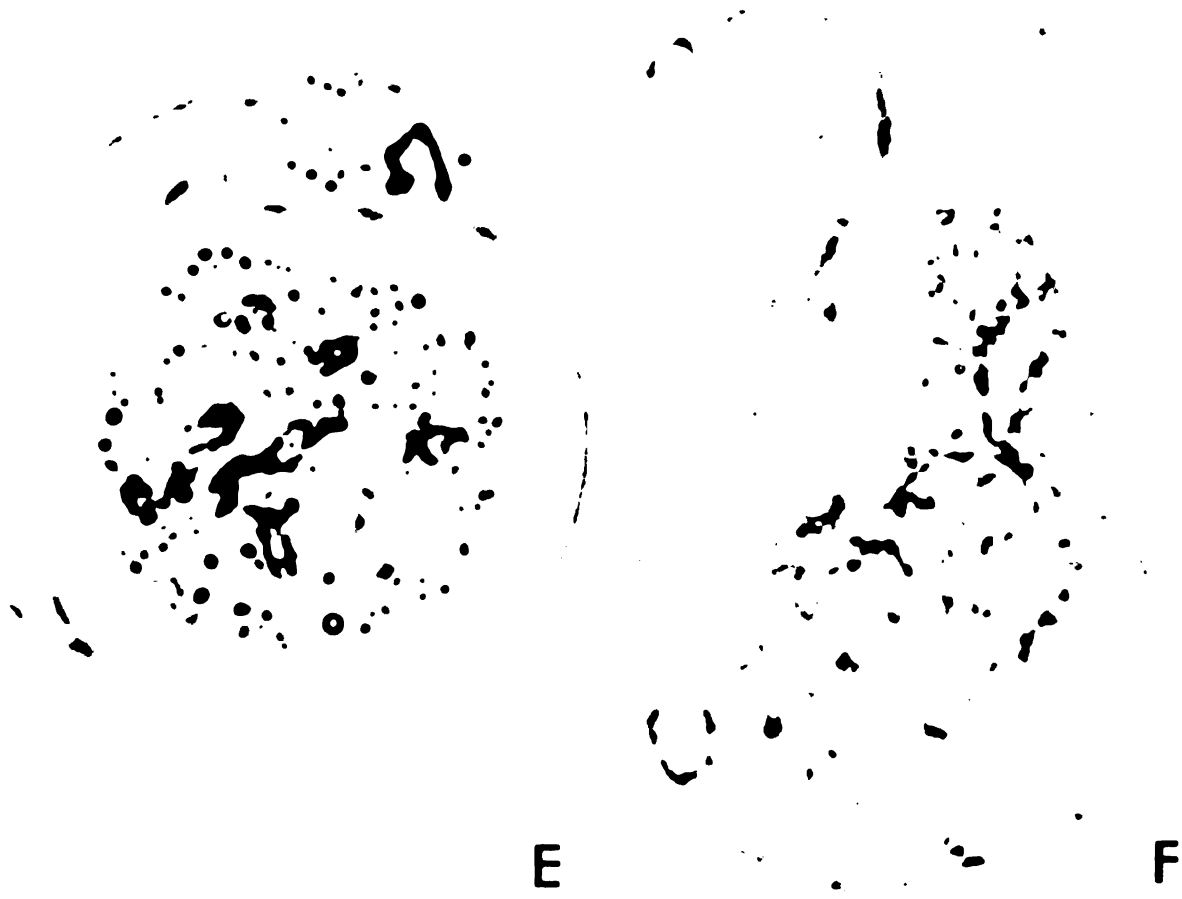


Figure 12 (cont'd.)

reported in hybrids or haploids by Sayed, et al. (1973), Bennett, et al., (1972), Wagenaar (1959), Crowder (1953), Levan (1941, 1942), Nordenskiold (1941), Sax (1937), and Gaines and Aase (1926). Meiosis was interrupted in both plants as soon as the microspore mother cells reached metaphase I. It appeared that the spindle failed to form in these cells for neither alignment of the chromosomes on the metaphase plate nor chromosomal movement towards the poles was observed. Subsequently, groups of adjacent chromosomes formed up to 12 nuclei per cell (Figure 12 A). In a few cells, erratic cell wall formation compartmentalized the nuclei; however, most cells remained multinucleate, became rounded, and proceeded to produce buds (Figure 12 B-F). Nuclei or dispersed chromatin were often observed in both the original cell and its bud (Figure 12 C-E). Figure 12 E shows distinct chromosomes in an original cell and its severed bud. In the later stages of budding (Figure 12 F), pollen 'pore' formation was evident.

Cytomixis, the transfer of chromatin from one cell to another (Gates, 1911), was observed in some of the microspore mother cells of all the plants used in this investigation. The frequency of this phenomenon and the stage (leptotene to metaphase I) at which it was seen (Figure 7 A) varied from spike to spike as well as from plant to plant. The literature contains many light and electron microscopic studies on cytomixis (Schnaider and Pardi, 1972; Tai and Vickery, 1972; Bhandari, et al. 1969; Heslop-Harrison, 1966; Sadasivaiah and Magoon, 1965; Weiling, 1965; Kamra, 1960; Tarkowska,

1960; Bopp-Hassenkamp, 1959; Takats, 1959; and Sarvella, 1958). In an article on cytomixis in Hordeum vulgare, Kamra (1960) reported that up to 40% of the microspore mother cells displayed cytomixis. The amount of chromatin transferred ranged from a fragment to the entire complement of a cell.

When cytomixis occurred in the early prophase stages of microspore mother cells of their Mimulus glabratus hybrids, Tai and Vickery (1972) assumed that cytomixis was initiated during the mitotic division preceding meiosis. To check this hypothesis, the premeiotic mitoses of Hordeum vulgare (4x) and AHPA, both of which showed cytomixis in approximately 80% of the microspore mother cells, were analyzed. Premeiotic mitosis in both plants seemed to be normal; no aberrant behavior which might preface cytomixis was identified. Heslop-Harrison (1966) and Tarkowska (1960) stated that cytomixis was a hydrostatic phenomenon caused by either chemical or mechanical stress. Accordingly, a fixation experiment was attempted on two of the plants in this study. It was found that the frequency of cytomixis in HV x HJ and AHPV was reduced from approximately 70.0% to 5.0% by simply aspirating freshly harvested spikes immediately after they were placed in Newcomer's. This tends to corroborate the recent interpretation of cytomixis (Heslop-Harrison, 1966; Tarkowska, 1960; and Takats, 1959), i.e. that cytomixis represents an artifact of handling and fixation rather than a naturally occurring, evolutionary phenomenon.

Tables summarizing the data for all plants used in this investigation are presented for diakinesis-metaphase I chromosome association (Table 12), frequency of micronuclei in dyads (Table 13), frequency of micronuclei in quartets (Table 14), and pollen stainability and seed set (Table 15). These tables will be referenced in the discussion.

TABLE 12

Summary of Diakinesis-Metaphase I Chromosome Association

Plant	Mean Chromosome Association I	II	III	IV	# of Cells
Agropyron sericeum		14.00			92
x Agrohordeum pilosilemma	13.32	5.89	0.89	0.05	38
Hordeum jubatum		14.00			40
HV x HJ	19.85	0.54	0.02		41
Hordeum vulgare (2x)	7.00				74
Amphiploid	2.43	19.80	0.70	2.97	30
AHPA	6.58	9.68	0.97	3.29	31
AHPV	16.47	10.10	0.73	0.73	30
Hordeum vulgare (4x)	0.03	8.38	0.03	2.78	64

TABLE 13

Frequency of Micronuclei in Telophase I Cells

Plant	# Scored	% T-I Cell-MN	Micronuclei/T-I Cell	
			Range	Mean
Agropyron sericeum	187	11.8	1-2	1.18
x Agrohordeum pilosilemma	61	90.2	1-6	2.33
Hordeum jubatum	96	17.7	1-2	1.18
HV x HJ	*			
Hordeum vulgare (2x)	185	8.1	1-2	1.07
Amphiploid	403	91.1	1-11	3.35
AHPA	254	92.5	1-9	3.13
AHPV	*			
Hordeum vulgare (4x)	162	27.2	1-3	1.43

*Irregular meiosis, see text.

TABLE 14

Frequency of Micronuclei in Quartets

Plant	# Scored	% Quartet-MN	Micronuclei/Quartet Range	Mean
Agropyron sericeum	807	7.2	1-10	2.81
x *Agrohordeum pilosilemma*	572	100.	1-16	8.47
Hordeum jubatum	207	11.1	1-2	1.39
HV x HJ	*			
Hordeum vulgare (2x)	306	8.6	1-2	1.23
Amphiploid	937	95.7	1-20	7.40
AHPA	1018	99.4	1-17	8.24
AHPV	*			
Hordeum vulgare (4x)	211	60.7	1-11	2.69

* Irregular meiosis, see text.

TABLE 15

Pollen Stainability and Seed Set

Plant	Pollen Stainability		# Seed Set
	Grains Scored	% Stainable	
Agropyron sericeum	1000	84.0	88.6
x Agrohordeum pilosilemma	1000	0	0
Hordeum jubatum	2289 *	84.8	93.7
HV x HJ	1000	0	0
Amphiploid	2000	51.8	54.9
AHPA	1200	50.6	25.4
AHPV	1000	0	0
Hordeum vulgare (4x)	1000	67.3	83.3

* Data from G. Starks, Department of Botany and Plant Pathology, Michigan State University

DISCUSSION

Cytological analysis of microsporogenesis in Agropyron sericeum revealed that 14 bivalents were formed at metaphase I (Table 12). The complete absence of multivalents evidenced in both this investigation and that of Hodgson (1964) suggests that only homologous pairing occurs and that Agropyron sericeum is an allotetraploid. Hordeum jubatum displays identical metaphase I behavior (Table 12) but is believed to be a segmental allotetraploid. This designation was proposed by Wagenaar (1959, 1960) after a thorough study of the chromosome behavior of hybrids between Hordeum jubatum and Secale cereale L. In Wagenaar's hybrids, the smaller chromosomes of Hordeum jubatum usually paired autosyndetically rather than with the chromosomes of the Secale cereale complement. Whether Hordeum jubatum displayed autosyndetic pairing with chiasma in that cross with Secale cereale or some phenomenon analogous to distributive pairing (Grell, 1967) based on chromosomal size differences reamins open to question. Starks and Tai (1974), in an article on Hordeum jubatum x Hordeum compressum Griseb. hybrids, agreed with Wagenaar's interpretation of Hordeum jubatum as a segmental allotetraploid. In addition they proposed that homologous versus homeologous chromosome association

in Hordeum jubatum is genetically controlled and suggested that the genome formula, AAA'A', be assigned to Hordeum jubatum. In order to determine the genome formula for Agropyron sericeum, the chromosome associations of X Agrohordeum pilosilemma were analyzed.

The average chromosome association for X Agrohordeum pilosilemma (Table 2) was 13.32 I, 5.89 II, 0.89 III, and 0.05 IV, which approaches a maximum pairing configuration of 14 I + 7 II. If autosyndetic pairing occurred in Hordeum jubatum, it may be assumed that the genomes of Agropyron sericeum did not pair with one another, the behavior expected of genomes from a strict allotetraploid. The single quadrivalent recorded for X Agrohordeum pilosilemma is believed to represent two loosely associated bivalents, a pseudoquadrivalent (Walters, 1954). The presence of 1-3 III in 55% of the hybrid cells suggests that one Agropyron sericeum genome is partially homologous with one of the genomes of Hordeum jubatum. From the results of this study, the genome formula, A"A"BB, is assigned to Agropyron sericeum, and, AA'A"B, to X Agrohordeum pilosilemma. Possible pairing relationships of the parents and hybrid are presented in Figure 13. Subscripts S and J delineate the genomes of Agropyron sericeum and Hordeum jubatum, respectively.

Indirect support for the supposition that Agropyron sericeum is an allotetraploid with a genome formula partially homologous to a genome of Hordeum jubatum was found in the cytotaxonomic literature. Taxonomically Agropyron sericeum is closely related to two other northern, slender wheatgrasses, Agropyron latiglume

FIGURE 13

Possible Pairing Relationships among the Genomes of Agropyron sericeum, Hordeum jubatum, X Agrohordeum pilosilemma, the Amphiploid, and AHPA, Assuming Genetic Control of Pairing

AGROPYRON SERICEUM

$$\frac{A'_S\ A'_S}{7II}\ \frac{B_S\ B_S}{7II}$$

HORDEUM JUBATUM

$$\frac{A_J\ A_J}{7II}\ \frac{A''_J\ A''_J}{7II}$$

X AGROHORDEUM PILOSILEMMA

$$\frac{\dfrac{A_J\ A'_J\ A''_S}{III}\ \dfrac{B_S}{7I}}{6II+6I}$$

AMPHIPLOID

$$\frac{\dfrac{A_J\ A_J\ A'_J\ A'_J\ A''_S\ A''_S}{3IV+3II}\ \dfrac{B_S\ B_S}{7II}}{\dfrac{}{3II}\ \dfrac{III+I}{3II}\ \dfrac{}{3II}}$$

AHPA

$$\frac{\dfrac{A^*_J\ A^*_J\ A^*_S\ A''_S}{3IV}\ \dfrac{B_S\ B_S}{7II}}{\dfrac{III+I}{3II+3I}\ \dfrac{}{3I}}$$

and Agropyron trachycaulum (Bowden, 1965; Mitchell and Hodgson, 1965a). Dewey (1966) noted close similarities between the genomes of Agropyron latiglume and Agropyron trachycaulum, both allotetraploids which carry the basic Agropyron spicatum (Pursh) Scribn. & Smith genome (Stebbins and Snyder, 1956). In a later paper, Dewey (1971) wrote, "Although Hordeum species do not contain a genome drived from Agropyron, a modified Hordeum genome apparently occurs in Agropyron...". In a recent study of X Agrohordeum macounii, Huang (1975) stated that one of the genomes of allotetraploid Agropyron trachycaulum was homoelogous to a Hordeum jubatum genome. Future cytogenetic investigations will answer the obvious question: what are the chromosome associations among Agropyron latiglume, Agropyron sericeum, and Agropyron trachycaulum and between Agropyron latiglume and Hordeum jubatum.

Since the optimum pairing configuration for X Agrohordeum pilosilemma would be 7 I + 7 III and the data (Table 2) show limited trivalent formation, it is suggested that the extent of multivalent formation in the amphiploid and the backcross of the amphiploid to Agropyron sericeum, AHPA, may offer more accurate indices of chromosome homology. Chromosome association in the amphiploid averaged 3.67 multivalents per cell (Table 4) with 1-3 III in 57% of the cells and 1-5 IV in 100%. Multivalent formation in AHPA averaged 4.26 (Table 6) with 1-4 III in 61% of the cells and 1-6 IV in 100%. Considering that multivalent formation is governed by the size and number of chromosomes per cell, chiasma frequency and distribution, environment, and genetic control of pairing (Thomas and

Kaltsikes, 1972; Morrison and Rajhathy, 1960 b; Hovin, 1958; Grun, 1952; Sears, 1941; and Myers and Hill, 1940), the amphiploid with a maximum association of 3I, 15II, III, and 5 IV, and AHPA with 4I, 7II, and 6IV approach their predicted maximum pairing configurations, 14 II + 7 IV and 7 II + 7 IV, respectively.

Figure 13 shows the possible pairing relationships and genome formula, AAA'A'A"A"BB, of the amphiploid. As the amphiploid was created by colchicine treatment, its genomes appear in duplicate and were interpreted to pair as follows. The BB genome from Agropryon sericeum paired homologously. The segmentally homologous AA, A'A' and A"A" genomes from Agropyron sericeum and Hordeum jubatum paired either autosyndetically as bivalents or autoallosyndetically as multivalents. Since at least one chiasma per chromosome pair is required for multivalent formation (Darlington, 1929), segmentally homologous parts must have been exchanged between A and A' of Hordeum jubatum and A or A' and A" of Hordeum jubatum and Agropyron sericeum approximately 57% of the time. Consequently, chromosomes with an Agropyron centromere may carry a Hordeum telomere; the converse situation would also exist. For the purpose of demonstrating pairing relationships in Figure 13, those genomes bearing exchanged chromosomes are designated A*. The exchange chromosomes may be assumed to assort independently and to pass into the gametes produced by the amphiploid.

The constitution of these gametes is reflected in the genome formula of the subsequent hybrid, AHPA (Figure 13) and in its pairing

configuration. Chromosome pairing in some plants is believed to depend on telomere recognition. The literature on this subject began with the work of Cleland and Blakeslee (1931) who proposed that chromosome pairing was initiated at or near the ends of chromosomes. The fusion of homologous, telocentric heterochromatin during pachytene was reported by Kostoff (1938) in Triticum, by Thomas and Revell (1942) in Cicer, and by Kasha and Burnham (1965) in barley. Recent articles include theoretical papers by Wagenaar (1969), Comings (1968), Jones (1968), Sved (1966), and Walters (1954) as well as papers by Godin and Stack (1975), Ashley and Wagenaar (1972), Wagenaar and Sadasivaiah (1969), Brown and Stack (1968), Kumar and Natarajan (1966), Wagenaar (1960), Riley and Chapman (1957), and Ostergren and Vigfusson (1953), which contain supporting data.

In AHPA, the BB genomes from Agropyron sericeum paired homologously and the four A genomes from Agropyron sericeum and Hordeum jubatum paired as fully as telomere recognition in a hexaploid cell allows. The average 6.58 univalents observed in the AHPA cells may have resulted from mechanical obstruction to complete pairing (Sears, 1941), the failure of chiasma to form between A* chromosomes with one or two matched telomeres but different centromeric regions (Swanson, 1940) or the occurence of multivalents in all of the cells (Sears, 1941).

Genetic influence on chromosome pairing has been reported by Starks and Tai (1974), Gottschalk (1973), Ellis, et al. (1973), Driscoll (1972), Douglas and Brown (1971), Harlan, et al. (1970),

Feldman (1966), Rajhathy, et al. (1964), and Riley and Chapman (1958). Starks and Tai (1974) proposed that control of pairing in Hordeum jubatum x Hordeum compressum hybrids is governed by a gene or genes on the Hordeum jubatum A genome and that the type of pairing results from a dosage effect, i.e., a single dose allows homeologous pairing whereas a double dose promotes homologous pairing. This hypothesis was employed to construct the maximum pairing configurations for X Agrohordeum pilosilemma, the amphiploid, and AHPA, and can be used to explain the possible pairing relationships seen in Figure 13. Pairing in X Agrohordeum pilosilemma and in AHPA with one dose of the A genes is generally homeologous, and in the amphiploid with two doses is primarily homologous.

The frequency of Agropyron sericeum (27.9%), Hordeum jubatum (10.0%), and diploid Hordeum vulgare (38.0%) anaphase I cells displaying bridges was rather high. Hodgson (1964) reported a 10% frequency in Agropyron sericeum, and Redmann and Borgaonkar (1966), 2-5%, in Hordeum jubatum. It seems probable that late separating bivalents, especially in the case of Hordeum vulgare (2x), were interpreted as bridges, thus inflating the frequencies reported in this investigation.

X Agrohordeum pilosilemma showed asynchrony, similar to that reported in Triticale (Thomas and Kaltsikes, 1972), during anaphase I with 4-14 chromosomes, univalents and bivalents, lagging on the metaphase plate. This behavior may be attributable to a differential duration of meiotic stages (Bennett, 1971; Bennett, et al. 1971;

and Riley, 1968) or lack of homology between the kinetochores and spindle organizers (Tai, 1970) of Agropyron sericeum and Hordeum jubatum; the meiotic times of the parental species have not been determined. The late-aligning univalents (cf. Wagenaar and Bray, 1973) of X Agrohordeum pilosilemma, the amphiploid, and AHPA commonly underwent precocious centromere division, a meiotic phenomenon generally correlated with the presence of unpaired chromosomes in hybrids, haploids, polyploids, or asynaptics (Clayberg, 1959). Precocious centromere division involves the separation of chromosomes into "chromatids" during anaphase I and the fragmentation of some of these "chromatids" during anaphase II. If either the "chromatids" or fragments are not included in their respective daughter nuclei, they become micronuclei scorable in either T-I cells or quartets. Precocious centromere division was described in haploid wheat by Gaines and Aase (1926), in triploid maize by McClintock (1929), in Aegilotritricum by Kihara (1931), and in an asynaptic wheat hybrid by Smith (1936). The occurence of this phenomenon has been widely reported (Dewey, 1972; Sadasivaiah and Kasha, 1971; Lange, 1971 a; Tai and Dewey, 1966; Rajhathy and Morrison, 1959; Wagenaar, 1959; Lima-de-Faria, 1956; Dowrick, 1953; Walters, 1950; Elliott and Love, 1948; and Stebbins, et al. 1946). The precocious centromere division observed in univalents and bivalents of X Agrohordeum pilosilemma, and in univalents of the amphiploid and AHPA is believed to have added significantly to the number of micronuclei found in their T-I cells and quartets.

Whether precocious centromere division results from lack of complement balance, asynchrony or late alignment of the chromosomes on the metaphase plate, or is an inheritable character in these plants remains unknown.

The frequency of micronuclei in the T-I cells and quartets of Agropyron sericeum, Hordeum jubatum, X Agrohordeum pilosilemma, the amphiploid, and AHPA (Tables 13, 14) may be related to cytological irregularities such as univalent frequency, bridge-fragment formation, precocious centromere division, laggards, and chromosomes excluded in the various meiotic stages. Likewise, pollen stainability and seed set (Table 15) may be related, although in polyploids, not necessarily correlated with the forementioned cytological irregularities. Overall fertility, as predicted from frequencies of pollen stainability and seed set, is determined by a combination of genetic, environmental, physiological, and cytological factors (Hsam and Larter, 1973; Weimarck, 1973; Merker, 1971; Rommel, 1961; Stebbins, 1950; and Muntzing, 1939).

Irregular meiotic behavior was observed in both Hordeum vulgare (2X) x Hordeum jubatum, HV x HJ, and the amphiploid x Hordeum vulgare (4X), AHPV, despite the relatively stable constitutions and regular meiosis of their respective parents (Table 12). The euploid chromosome association of HV x HJ which averaged 19.85 I, 0.54 II, and 0.02 III illustrates essentially asynaptic behavior. The rod bivalents and trivalent recorded in this study and the bivalents and trivalents reported for the reciprocal hybrid (Kerber, cited in Wagenaar, 1960;

Rajhathy and Morrison, 1959) may represent persistent secondary associations, pseudochiasma resulting from heterochromatic fusion of paired telomeres (see references, discussion AHPA). Obvious secondary associations were noted in approximately 90.0% of the HV x HJ metaphase cells, and 98% of these associations were end-to-end. Since genome suppression of chromosome pairing has been reported in other interspecific crosses involving Hordeum vulgare; Hordeum bulbosum x H. vulgare (Lange, 1971 a), (Hordeum compressum x H. stenostachys)^2 x H. vulgare (Rajhathy, et al. 1964), (Hordeum pusillum x H. californicum) x H. vulgare (Rajhathy, et al. 1964), (Hordeum jubatum x H. brachyantherum) x H. vulgare (Rajhathy and Morrison, 1959), and Hordeum depressum x H. vulgare (Morrison and Rajhathy, 1959); it is suggested that genome interaction is responsible for the asynapsis displayed in HV x HJ.

The aneuploidy (2N = 16-22) revealed in microspore mother cells of HV x HJ is attributed to premeiotic loss of chromosomes by chromosome elimination. Chromosome elimination subsequent to fertilization in interspecific hybrids was first suggested by Schooler (1963, cited in Subrahmanyam and Kasha, 1973) and has been reported for Hordeum lechleri x H. vulgare (Rajhathy, et al. 1964) and Hordeum bulbosum x H. vulgare (Lange, 1971 a,b; Kao and Kasha, 1971; Kasha and Sadasivaiah, 1971; and Subrahmanyam and Kasha, 1973). In these investigations of Hordeum bulbosum x H. vulgare, it was proposed that the balance between genetic factors of the two parents regulated the stability or elimination of chromosomes. Whereas a

1:2 genome ratio (Hordeum vulgare : H. bulbosum) is relatively stable, a 1:1 hybrid may lose chromosomes until it becomes a pure haploid or dihaploid Hordeum vulgare. Chromosome elimination in the 1:1 hybrid may begin in the embryo and continue during plant maturation. The exact mechanism of chromosome elimination or timing of chromosome loss in HV x HJ has not been determined; however, lagging chromosomes at premeiotic telophase (Figure 10 A) and uncondensed or disintegrating univalents at diakinesis (Figure 10 C) were noted in the material analyzed here. A series of Hordeum jubatum x H. vulgare hybrids, similar to those studied in Hordeum bulbosum x H. vulgare (Lange, 1971 a, b: Kasha and Sadasivaiah, 1971) would be necessary to determine which chromosomes are being eliminated, the mechanism through which this occurs, and which genome genetically controls this process.

The average chromosome association for AHPV, 16.47 I, 10.1 II, 0.73 III, and 0.73 IV, was comparable to the pairing observed in X Agrohordeum pilosilemma (Table 12) which seems to indicate that the genomes of Hordeum vulgare are isolated from the other genomes by homology or through genes controlling pairing. As secondary associations were quite prevalent among obvious univalents and the modal class was 14 I + 14 II, most of the AHPV multivalents are believed to be loosely-associated I + II, II + II, and I + III combinations. Interchromosomal synapsis was particularly difficult to distinguish from secondary association in HV x HJ and AHPV. The metaphase I chromosomes of HV x HJ always assumed an appearance more typical of mitosis and never assembled on the metaphase plate (Figure 10 D):

metaphase I in AHPV was defined by the presence of a few bivalents on the metaphase plate and numerous univalents scattered throughout the cytoplasm (Figure 11 C). Since the putative influence of the Hordeum vulgare genome on pairing was not evidenced in AHPV, in the manner previously described for other hybrids (see HV x HJ discussion), the genome formula, AA'A"BVV, is tentatively assigned to AHPV. Then, by extrapolation, the genome formula for HV x HJ becomes AA'V.

Centromere misdivision, a term coined by Darlington (1939), occurs in the anaphase I cells of both HV x HJ and AHPV. Apparently the telomeres of a univalent acquire centromeric activity (neocentromere, Rhoades, 1952) and bring about the transverse division of the chromosome; the products of this division are telocentrics, isochromosomes, acentrics and accessory chromosomes (Rieger, et al. 1968; Sayed, et al. 1973). Centromere misdivision has been studied in Bromus (Walters, 1952), maize (Rhoades and Vilkomerson, 1942), rye (Ostergren and Prakken, 1946) and wheat (Sears, 1952; Sanchez-Monge, 1950). The irregularity of meiosis II in HV x HJ and AHPV precluded both identification of the products of centromere misdivision and analysis of the disposition of these products in the T-I cells and quartets.

Multipolar cell division is a spontaneous or induced spindle apparatus abnormality resulting in genome separation during either mitosis or meiosis (Chen, 1975). This inheritable phenomenon, which occurs in both plant and animal tissues, has been described in several grasses (Chen, 1975; Huang, 1975; Dewey, 1974; Maguire, 1974; Sosniklina, 1973; Tai, 1970; Kabarity, 1966; Nielson and Nath, 1961;

and Walters, 1958, 1960). In an article on Agropyron cristatum (L.) Gaertn., Tai (1970) proposed that multipolar cell division occurs via genome specific spindle organizers, cell organelles which govern chromosome migration and cytokinesis, and provides an evolutionary mechanism for haploidization in higher plant polyploids. In this investigation, multipolar spindles were first detected in late anaphase I cells of the hybrids, X Agrohordeum pilosilemma, HV x HJ, and AHPV. Figures 5-G and 10-G show tripolar and quadripolar spindles, respectively. Genome separation is manifested in the quartets of X Agrohordeum pilosilemma (Figure 5 K) by binucleate microspores and of HV x HJ and AHPV by supernumerary cytokinesis producing extra microcells (Figures 10-H, 11-H).

The irregular meiotic behavior (Figure 12) originally observed in HV x HJ and AHPV (spindle suppression, reformed nuclei, erratic cytokinesis, and budding) may be explained as the effect of temperature stress on an unbalanced genome incurred under summer field and greenhouse conditions. Gene-independent asynapsis provoked by high temperatures has been reported in cotton (Douglas and Brown, 1971), wheat (Riley, 1968), Tradescantia (Dowrick, 1957; Sax, 1937) and Uvularia (Dowrick, 1957). Riley (1968) views such asynapsis as a result of altered meiotic timing and states that high-temperature shortens prophase I preventing stable, homologous, zygotene pairing which usually leads to synapsis. Sax (1937) reported that temperature shock in Tradescantia induced asynapsis and budding, conditions that lasted up to several months after the experiment. In wheat and rye

grown at 25 C, Bennett, et al. (1972) observed the termination of meiosis after the first division, dyads with greatly thickened wall and germ pores, and abnormal, persistent tapetal cells. They attributed the meiotic irregularity to temperature-mediated physiological failure. Wagenaar (1959) reported spindle suppression during second meiotic division in Hordeum jubatum x Secale cereale hybrids. Irregular nuclear formation, budding, and cell degeneration were ascribed to abnormal physiologic conditions created by combining the genomes of these two genera. It is proposed that genome interaction between Hordeum jubatum and Hordeum vulgare in HV x HJ and AHPV presents a similar physio-genetic incompatibility. Temperature stress accentuated the meiotic failure in these plants, but even under ideal growth conditions, asynapsis in HV x HJ and multipolar cell division, centromere misdivision, and the low level of synapsis in AHPV assures sterility, the absence of pollen stainability and seed set (Table 15).

SUMMARY

Cytogenetic investigation of microsporogenesis in Agropyron sericeum, Hordeum jubatum, their spontaneous hybrid, X Agrohordeum pilosilemma, its amphiploid, and the backcross of the amphiploid to Agropyron sericeum elucidated the genome relationships of Agropyron sericeum and Hordeum jubatum. The tetraploid parental species, Agropyron sericeum and Hordeum jubatum, share a partially homologous genome which affects the pairing relationships evidenced in their hybrids. The genome formulae assigned to these plants are: Agropyron sericeum, A"A"BB; Hordeum jubatum, AAA'A'; X Agrohordeum pilosilemma, AA'A"B; the amphiploid, AAA'A'A"A"BB; and the amphiploid x Agropyron sericeum, AA'A"A"BB. Observed pairing configurations were compatible with the expected maximum pairing configurations predicted under the assumption of genetic control of pairing with dosage effects. This is interpreted as further support for the hypothesis that pairing in the hybrids of Hordeum jubatum is controlled by its A genome; one dose of A allows homeologous pairing and two doses of A promotes homologous association.

Microsporogenesis in the hybrids, Hordeum vulgare (2X) x Hordeum jubatum and the amphiploid x Hordeum vulgare (4X) was also investigated. Hordeum vulgare x Hordeum jubatum was found to display asynaptic

behavior that is believed to represent a physiogenetic incompatibility. The pairing configuration of the amphiploid x Hordeum vulgare was comparable to the pairing seen in X Agrohordeum pilosilemma indicating that the genomes of Hordeum vulgare are effectively isolated from the genomes of both Agropyron sericeum and Hordeum jubatum either by homology or through genes controlling pairing. The genome formulae, AA'V and AA'A"BVV, were tentatively assigned to Hordeum vulgare (2X) x Hordeum jubatum and the amphiploid x Hordeum vulgare (4X), respectively.

LITERATURE CITED

LITERATURE CITED

Aase, H.C. and L. Powers. 1926. Chromosome numbers in crop plants. Amer. J. Bot. 13:367-372.

Ashley, T. and E.B. Wagenaar. 1972. End-to-end attachment of haploid chromosomes of Ornithogalum virens. Can. J. Genet. Cytol. 14:716-717.

Ashman, R.B. and W.S. Boyle. 1955. A cytological study of the induced octaploid of an Agropyron-Hordeum hybrid. J. Hered. 46:297-301.

Bennett, M.D. 1971. The duration of meiosis. Proc. Royal Soc. London B 178:277-299.

Bennett, M.D., V. Chapman, and R. Riley. 1971. The duration of meiosis in pollen mother cells of wheat, rye, and Triticale. Proc. Royal Soc. London B 178:259-275.

Bennett, M.D., J.B. Smith, and R. Kemble. 1972. The effect of temperature on meiosis and pollen development in wheat and rye. Can. J. Genet. Cytol. 14:615-624.

Bhandari, N.N., S.L. Tandon, and S. Jain. 1969. Some observations on the cytology and cytomixis in Canavalia DC. Cytologia 34: 22-28.

Booher, L.E. and R.M. Tryon. 1948. A study of Elymus in Minnesota. Rhodora 50:80-91.

Bopp-Hassenkamp, G. 1959. 'Cytomixis' im elektronenmikroskopischen Bild. Expt. Cell Res. 18:182-184.

Bowden, W.M. 1967. Taxonomy of intergeneric hybrids of the tribe Triticeae from North America. Can. J. Bot. 45:711-724.

Bowden, W.M. 1965. Cytotaxonomy of the species and interspecific hybrids of the genus Agropyron in Canada and neighboring areas. Can. J. Bot. 43:1421-1448.

Bowden, W.M. 1962. Cytotaxonomy of the native and adventive species of Hordeum, Eremopyrum, Secale, Sitanion, and Triticum in Canada. Can. J. Bot. 40:1675-1711.

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

Bowden, W.M. 1959. Chromosome numbers and taxonomic notes on northern grasses. Can. J. Bot. 37:1143-1151.

Boyle, W.S. and A.H. Holmgren. 1955. A cytogenetic study of natural and controlled hybrids between Agropyron trachycaulum and Hordeum jubatum. Genetics 40:539-545

Brink, R.A., D.C. Cooper, and L.E. Ausherman. 1944. A hybrid between Hordeum jubatum and Secale cereale. J. Hered. 35:67-76.

Brown, W.V. and S.M. Stack. 1968. Somatic pairing as a regular preliminary to meiosis. Bull. Torrey Bot. Club 95:369-378.

Chen, Y. 1975. Multipolar cell division in Agropyron cristatum (L.) Gaertn. (Gramineae). Ph.D. Dissertation, Michigan State University. 90pp.

Clayberg, C.D. 1959. Cytogenetic studies of precocious meiotic centromere division in Lycopersicon esculentum Mill. Genetics 44:1335-1346.

Cleland, R.E. and A.F. Blakeslee. 1931. Segmental interchange, the basis of chromosomal attachments in Oenothera. Cytologia 2:175-232.

Comings, D.E. 1968. The rationale for an ordered arrangement of chromatin in the interphase nucleus. Amer. J. Human Genet. 20: 440-460.

Cooper, D.C. and R.A. Brink. 1944. Collapse of the seed following the mating of Hordeum jubatum x Secale cereale. Genetics 29: 370-390.

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

Crowder, L.V. 1953. Interspecific and intergeneric hybrids of Festuca and Lolium. J. Hered. 44:195-203.

Darlington, C.D. 1939. Misdivision and the genetics of the centromere. J. Genet. 37: 341-364.

Darlington, C.D. 1929. Chromosome behavior and structural hybridity in the Tradescantiae. J. Genet. 21:207-

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

Dewey, D.R. 1974. Hybrids and induced amphiploids of Elymus canadensis x Agropyron libanoticum. Amer. J. Bot. 61:181-187.

Dewey, D.R. 1972. Cytogenetics of Elymus angustus and its hybrids with Elymus giganteus, Elymus cinereus, and Agropyron repens. Bot. Gax. 133:57-64.

Dewey, D.R. 1971. Genome relations among Agropyron spicatum, A. scribneri, Hordeum brachyantherum, and H. arizonicum. Bull. Torrey Bot. Club 98:200-206.

Dewey, D.R. 1966. Synthetic Agropyron-Elymus hybrids. I. Elymus canadensis x Agropyron subsecundum. Amer. J. Bot. 53:87-94.

Doebel, P., R. Rieger, and A. Michaelis. 1973. The giemsa banding patterns of the standard and reconstructed karyotypes of Vicia faba. Chromosoma 43:409-422.

Douglas, C.R. and M.S. Brown. 1971. A study of triploid and 3X-1 aneuploid plants in the genus Gossypium. Amer. J. Bot. 58: 65-71.

Dowrick, G.J. 1957. The influence of temperature on meiosis. Heredity 11:37-49.

Dowrick, G.J. 1953. The chromosomes of Chrysanthemum. III. Meiosis in C. atratum. Heredity 7:219-226.

Driscoll, C.J. 1972. Genetic suppression of homeologous chromosome pairing in hexaploid wheat. Can. J. Genet. Cytol. 14:39-42.

Elliott, F.C. and R.M. Love. 1948. The significance of meiotic chromosome behavior in breeding smooth bromegrass, Bromus inermis Leyss. J. Amer. Soc. Agron. 40:335-351.

Ellis, W.M., B.O. Lee, and D.M. Calder. 1973. Chromosome pairing in Poa annua L. Can. J. Genet. Cytol. 15:549-551.

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

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

Gaines, E.F. and H.E. Aase. 1926. A haploid wheat plant. Amer. J. Bot. 13:373-385.

Gates, R.R. 1911. Pollenformation in Oenothera gigas. Ann. Bot. 25:909-940.

Gill, B.S. and G. Kimber. 1974. A giemsa C-banding technique for cereal chromosomes. Cereal Res. Comm. 2:87-94.

Godin, D.E. and S.M. Stack. 1975. Heterochromatic connectives between the chromosomes of Secale cereale. Can. J. Genet. Cytol. 17:269-273.

Gottschalk, W. 1973. The genetic control of meiosis. Genetics 74:s99.

Grell, R.F. 1967. Pairing at the chromosomal level. J. Cell Physiol. 701:s89-112.

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

Grun, P. 1952. Effect of environment upon chromosomal pairing of some species and hybrids of Poa. Amer. J. Bot. 39:318-323.

Hagberg, A. and J.H. Tjio. 1950. Cytological localization of the translocation point for the barley mutant erectoides. Hereditas 36:487-491.

Harlan, J.R. 1971. On the origin of barley: a second look. Barley Genetics II. Proc. Sec. Int. Barley Genet. Symp. Washington State University Press, Pullman. pp. 45-50.

Harland, J.R., J.M.J. DeWet, S.M. Nark, and R.J. Lambert. 1970. Chromosome pairing in maize-Tripsacum hybrids. Science 167: 1247-1248.

Heslop-Harrison, J. 1966. Cytoplasmic connections between angiosperm meiocytes. Ann. Bot. N.S. 30:221-230.

Hitchcock, A.S. and A. Chase. 1950. Manual of Grasses of the United States. U.S. Dept. Agr. Misc. Publ. No. 200. 1051 pp.

Hodgson, H.J. 1964. Cytology, morphology, and amino-acid characterization of the putative intergeneric hybrid, Agroelymus palmerensis, and its presumed parents. Crop. Sci. 4:199-203.

Hodgson, H.J. 1956. Cytology, morphology, and amino-acid characterization of a putative Agroelymus and its presumed parents. Iowa State College J. Sci. 30:383.

Hovin, A. 1958. Meiotic chromosome pairing in amphihaploid Poa annua L. Amer. J. Bot. 45:131-138.

Hsam, S.L.K. and E.N. Larter. 1973. Identification of cytological and agronomic characters affecting the reproductive behavior of hexaploid Triticale. Can. J. Genet. Cytol. 15:197-204.

Huang, R. 1975. The genomes of Agropyron trachycaulum (Link) Malte and Hordeum jubatum L. and their hybridization with Hordeum vulgare L. MS Thesis, Michigan State University. 62 pp.

Hulten, E. 1942. Flora of Alaska and Yukon. II. Monocotyledoneae (Glumiflorae, Spathiflorae). C.W.K. Gleerup, Lund, Sweden. pp. 129-412.

Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill Book Co., Inc., New York. 523 pp.

Jones, G.H. 1968. Meiotic errors in rye related to chiasma formation. Mutation Res. 5:385-395.

Kabarity, A. 1966. Induction of multipolar spindles in the meiosis of Triticum aestivum as effected by acetone. Cytologia 31:457-460.

Kamra, O.P. 1960. Chromatin extrusion and cytomixis in pollen mother cells of Hordeum. Hereditas 46:592-600.

Kao, K.N. and K.J. Kasha. 1971. Haploidy from interspecific crosses with tetraploid barley. Barley Genetics II. Proc. Sec. Int. Barley Genet. Symp. Washington State University Press, Pullman. pp. 82-87.

Kasha, K. and C.R. Burnham. 1965. The location of interchange breakpoints in barley. II. Chromosome pairing and the intercross method. Can. J. Genet. Cytol. 7:620-632.

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

Kihara, H. 1931. Genomanalyse bei Triticum und Aegilops II. Aegilotriticum und Aegilops cylindrica. Cytologia 2:106-157

Keller, W. 1948. Wanted: a paragon for the range. Grass Yearbook of Agriculture, U.S. Dept. Agr. Washington DC pp. 347-351.

Kerber, E.R. Unpublished data.

Konzak, C.F., L.F. Randolph, and N.F. Jenssen. 1951. Embryo culture of barley species hybrids. J. Hered. 42:125-134.

Kostoff, D. 1938. Heterochromatin at the distal ends of the chromosomes of Triticum monococcum Nature 141:690-691.

Kumar, S. and A.T. Natarajan. 1966. Kinetics of two-break chromosome exchanges and the spatial arrangements of chromosome strands in the interphase nucleus. Nature 209:796-797.

Lange, W. 1971 a. Crosses between Hordeum vulgare L. and H. bulbosum L. I. Production, morphology, and meiosis of hybrids, haploids, and dihaploids. Euphytica 20:14-29.

Lange, W. 1971 b. Crosses between Hordeum vulgare L. and H. bulbosum L. II. Elimination of chromosomes in hybrid tissues. Euphytica 20:181-194.

LePage, E. 1953. Nouvelles notes sur des hybrides de Graminees. Nat. Can. 80:189-199.

LePage, E. 1952. Etudes sur quelques plantes americaines. II. Hybrides intergeneriques: Agrohordeum et Agroelymus. Nat. Can. 79:241-266.

Levan, A. 1942. Studies on the meiotic mechanism of haploid rye. Hereditas 28:177-211.

Levan, A. 1941. Syncyte formation in the pollen-mother-cells of haploid Phleum pratense. Hereditas 27:243-252.

Lima-de-Faria, A. 1956. The role of the kinetochore in chromosome organization. Hereditas 42:85-160.

Love, A. and D.L. Love. 1961. Chromosome numbers of Central and Northwest European plant species. Op. Botanica Soc. Bot. Lund. 5:1-580.

McClintock, B. 1929. A cytological and genetical study of triploid maize. Genetics 14:180-222.

Maguire, M.P. 1974. Chemically induced abnormal chromosome behavior at meiosis in maize. Chromosoma 48:213-233.

Merker, A. 1971. Cytogenetic investigations in hexaploid Triticale. I. Meiosis, aneuploidy, and fertility. Hereditas 68:281-290.

Merritt, J.F. and J.A. Burns. 1974. Chromosome banding in Nicotiana otophora without denaturation and renaturation. J. Hered. 65: 101-103.

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

Mitchell, W.W. and H.J. Hodgson. 1965b. The status of hybridization between Agropyron sericeum and Elymus sibericus in Alaska. Can. J. Bot. 43:885-895.

Mitchell, W.W. and A.C. Wilton. 1964. The Hordeum jubatum-caespitosum-brachy-antherum complex in Alaska. Madrono 17:269-280.

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

Morrison, J.W. and T. Rajhathy. 1960a. Chromosome behavior in autotetraploid cereals and grasses. Chromosoma 11:297-309.

Morrison, J.W. and T. Rajhathy. 1960b. Frequency of quadrivalents in autotetraploid plants. Nature 187:528-530.

Morrison, J.W. and T. Rajhathy. 1959. Cytogenetic studies in the genus Hordeum. III. Pairing in some interspecific and intergeneric hybrids. Can. J. Genet. Cytol. 1:65-77.

Morrison, J.W., A.E. Hannah, R. Loiselle, and S. Symko. 1959. Cytogenetic studies in the genus Hordeum. II. Interspecific and intergeneric crosses. Can. J. Plant Sci. 39:375-383.

Muntzing, A. 1939. Studies on the properties and the ways of production of rye-wheat amphidiploids. Hereditas 25:387-430.

Myers, W.M. and H.D. Hill. 1940. Studies of chromosomal association and behavior and the occurence of aneuploidy in autotetraploid grass species, orchard grass, tall oat grass, and crested wheatgrass. Bot. Gaz. 102:236-255.

Nakao, M. 1911. Cytological studies on the nuclear division of the pollenmother-cells of some cereals and their hybrids. Jour. Coll. Agr. Tohoku Imp. Univ. 4:173-190.

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

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

Nielson, E.L. and J. Nath. 1961. Somatic instability in derivatives from Agroelymus turneri resembling Agropyron repens. Amer. J. Bot. 48:345-349.

Nordenskiold, H. 1941. Cytological studies in triploid Phleum. Bot. Not. 12-32.

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

Ostergren, G. and R. Prakken. 1946. Behavior on the spindle of the actively mobile chromosome ends of rye. Hereditas 32:473-494.

Ostergren, G. and E. Vigfusson. 1953. On position correlations of univalents and quasi-bivalents formed by sticky chromosomes. Hereditas 34:33-50.

Percival, J. 1930. Cytological studies of some hybrids of Aegilops sp. x wheats, and some hybrids between different species of Aegilops. J. Genet. 22:201-278.

Person, C. 1955. An analytical study of chromosome behavior in a wheat haploid. Can. J. Bot. 33:11-30.

Price, P.B. 1968. Interspecific and intergeneric crosses of barley. In: Barley: Origin, Botany, Culture, Winterhardiness, Genetics, Utilization, Pests. USDA Agr. Handbook No. 338, Washington pp. 85-95.

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

Rajhathy, T. and J.W. Morrison. 1961. Cytogenetic studies in the genus Hordeum. V. Hordeum jubatum and the New World species. Can. J. Genet. Cytol. 3:378-390.

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

Rajhathy, T., J.W. Morrison, and S. Symko. 1964. Interspecific and intergeneric hybrids in Hordeum. Barley Genetics I. Proc. First Int. Barley Genet. Symp. Wageningen. pp. 195-212.

Redmann, R.E. and D.S. Borgaonkar, 1966. A cytological study of the Hordeae in the Dakotas. Cytologia 31:231-219

Rhoades, M.M. 1952. Preferential segregation in maize. In: J.W. Gowen, Heterosis. Iowa State College Press, Ames. 66 pp.

Rhoades, M.M. and H. Vilkomerson. 1942. On the anaphase movement of chromosomes. Proc. Nat. Acad. Sci. 28:443-436.

Richardson, M.M. 1935. Meiosis in Crepis. II. Failure of pairing in Crepis capillaris (L.). J. Genet. 31:119-143.

Rieger, R., A. Michaelis, and M.M. Green. 1968. A Glossary of Genetics and Cytogenetics. Springer-Verlag New York Inc., New York. 507 pp.

Riley, R. 1968. The basic and applied genetics of chromosome pairing. Proc. Third Int. Wheat Genet. Symp. pp. 185-195.

Riley, R. and V. Chapman. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713-715.

Riley, R. and V. Chapman. 1957. Haploids and polyhaploids in Aegilops and Triticum. Heredity 11:195-207.

Rommel, M. 1961. Aneuploidy, seed set, and sterility in artificially induced autotetraploid Hordeum vulgare. Can. J. Genet. Cytol. 3:272-282.

Sadasivaiah, R.S. and K.J. Kasha. 1971. Meiosis in haploid barley - an interpretation of nonhomologous chromosome associations. Chromosoma 35:247-263.

Sadasivaiah, R.S. and M.L. Magoon. 1965. Studies on cytomixis and the loss of chromosomes in meiotic cells of Sorghum. Genet. Iberica 17:103-115.

Sanchez-Monge, E. 1950. Two types of misdivision of the centromere. Nature 165:80-81.

Sarvella, P. 1958. Cytomixis and the loss of chromosomes in meiotic and somatic cells of Gossypium. Cytologia 23:14-24.

Sarvella, P., J.B. Holmgren, and R.A. Nilan. 1958. Analysis of barley pachytene chromosomes. Nucleus 1:183-204.

Sax, K. 1937. Effect of variation in temperature on nuclear and cell division in Tradescantia. Amer. J. Bot. 24:218-225.

Sayed, H.I., S.B. Helgason, and E.N. Larter. 1973. Fragments with accessory chromosome characteristics in cultivated barley (Hordeum vulgare). Can. J. Genet. Cytol. 15:625-633.

Schooler, A.B. 1962. Interspecific hybrids of Hordeum. 1962 Barley Newsl. 6:47-48.

Schooler, A.B., J. Nelson, and A. Arfsten. 1966. Hordeum jubatum L. crosses with Hordeum compressum Griseb. Crop Sci. 6:187-190.

Schweizer, D. 1973. Differential staining of plant chromosomes with giemsa. Chromosoma 40:307-320.

Sears, E.R. 1952. Misdivision of univalents in common wheat. Chromosoma 4:535-550.

Sears, E.R. 1941. Chromosome pairing and fertility in hybrids and amphidiploids in the Triticinae. Miss. Agr. Exp. Sta. Res. Bull. 337. 20 pp.

Shnaider, T. and J. Pardi. 1972. (Some abnormalities of microsporogenesis in the hybrid progeny produced by crossing a spring wheat mutant and the original variety). Elsti. Nsv. Tead. Akad. Toim. Biol. 21:335-339.

Smith, D.C. 1944. Pollination and seed formation in grasses. J. Agr. Res. 68:79-95.

Smith, L. 1951. Cytology and genetics of barley. Bot. Rev. 17:1-355.

Smith, L. 1936. Cytogenetic studies in Triticum monococcum L. and T. aegilopoides Bal. Univ. Miss. Agr. Exp. Sta. Res. Bull. 248: 1-48.

Sosniklina, S.P. 1973. Genetic control of the behavior of chromosomes in meiosis in inbred lines of diploid rye (Secale cereale L.). II. Abnormalities on the stages of anaphase I, II, and tetrads. Genetika 9:21-30.

Stack, S.M. and C.R. Clark. 1973. Pericentromeric chromosome banding in higher plants. Can. J. Genet. Cytol. 16:663-668.

Starks, G.D. 1975. Personal communication.

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

Stebbins, G.L. 1950. Variation and Evolution in Plants. Columbia University Press, New York. 643 pp.

Stebbins, G.L. and L.A. Snyder. 1956. Artificial and natural hybrids in the Gramineae, tribe Hordeae. IX. Hybrids between western and eastern North American species. Amer. J. Bot. 43:305-312.

Stebbins, G.L., J.I. Valencia, and R.M. Valencia. 1946. Artificial and natural hybrids in the Gramineae, tribe Hordeae. II. Agropyron, Elymus, and Hordeum. Amer. J. Bot. 33:579-586.

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

Sved, J.A. Telomere attachment of chromosomes, some genetical and cytological consequences. Genetics. 53:747-756.

Swanson, G.P. 1940. The distribution of inversions in Tradescantia. Genetics 25:438-465.

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

Tai, W. 1967. A cytogenetic investigation of diploid members of the Mimulus glabratus complex. Ph.D. Dissertations, University of Utah. 169 pp.

Tai, W. and D.R. Dewey. 1966. Morphology, cytology, and fertility of diploid and colchicine induced tetraploid crested wheatgrass. Crop Sci. 6:223-226.

Tai, W. and R.K. Vickery. 1972. Unusual cytological patterns in microsporogenesis and pollen development of evolutionary significance in the Mimulus glabratus complex (Scrophulariaceae). Amer. J. Bot. 59:488-493.

Takats, S.T. 1959. Chromatin extrusion and DNA transfer during microsporogenesis. Chromosoma 10:430-453.

Tarkowska, J. 1960. (Cytomixis in the epidermis of scales and leaves and in the meristems of the root apex of Allium cepa L.). Acta Soc. Bot. Poland 29:149-168.

Thomas, J.B. and P.J. Kaltsikes. 1972. Genotypic and cytological influences on the meiosis of hexaploid Triticale. Can. J. Genet. Cytol. 14:889-898.

Thomas, P.T. and S.H. Revell. 1946. Secondary association and heterochromatic attraction. Ann. Bot. 9:159-164.

Tsuchiya, T. 1960. Cytogenetic studies of trisomics in barley. Japan. J. Bot. 17:177-213.

Tsuchiya, T. 1953. Fertility of autotetraploids and their hybrids in barley. I. Meiosis and fertility in some autotetraploids. Seiken Ziho 6:46-52.

Verma, S.C. and H. Rees. 1974. Giemsa staining and distribution of heterochromatin in rye chromosomes. Heredity 132:118-122.

Vinogradova, N.M. 1946. (Hybridization between cultivated barley and wild species of Hordeum). Trudy Zonal. Inst. Zernavovo Khoziaistva Nechernozemnoi Polosy SSR 13:134-137.

Vosa and Marchi, C.G. and P. Marchi. 1972. Quinacrine fluorescence and giemsa banding in plants. Nat. New Biol. 237:191-192.

Wagenaar, E.B. 1969. End-to-end chromosome attachments in mitotic interphase and their possible significance to meiotic chromosome pairing. Chromosoma 26:410-426.

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

Wagenaar, E.B. 1959. Intergeneric hybrids between Hordeum jubatum L. and Secale cereale L. J. Hered. 50:195-202.

Wagenaar, E.B. and D.F. Bray. 1973. The ultrastructure of kinetochores of unpaired chromosomes in a wheat hybrid. Can. J. Genet. Cytol. 15:801-806.

Wagenaar, E.B. and R.S. Sadasivaiah. 1969. End-to-end, chainlike associations of paired pachytene chromosomes of Crepis capillaris. Can. J. Genet. Cytol. 11:403-408.

Walters, M.S. 1960. Rates of meiosis, spindle irregularities, and microsporocyte division in Bromus trinii x B. carinatus. Chromosoma 11:167-204.

Walters, M.S. 1958. Aberrant chromosome movement and spindle formation in meiosis of Bromus hybrids: an interpretation of spindle organization. Amer. J. Bot. 45:271-289.

Walters, M.S. 1954. A study of pseudobivalents in meiosis of two interspecific hybrids of Bromus. Amer. J. Bot. 41:160-171.

Walters, M.S. 1952. Spontaneous chromosome breakage and reunion of meiotic chromosomes in the hybrid Bromus trinii x B. pseudolaevipes. Genetics 37:8-25.

Walters, M.S. 1950. Spontaneous breakage and reunion of meiotic chromosomes in the hybrid Bromus trinii x B. maritimus. Genetics 35:11-37.

Weiling, F. 1965. Licht und elektronenmikroskopische beobachtungen zum problem der cytomixis sowie irher molichen bezechung zur potocytose, untersuchungen bei Cucurbita arten und Lycopersicum esculentum. Planta Arch. Wiss. Bot. 67:182-212.

Weimarck, A. 1973. Cytogenetical behavior in octaploid Triticale. I. Meiosis, aneuploidy, and fertility. Hereditas 74:103-118.

Wiebe, G.A. 1968. Introduction. In: Barley; Origin, Botany, Culture, Winter-hardiness, Genetics, Utilization, Pests. USDA, Agr. Handbook No. 338, Washington. p. 1.