# A GENOME ANALYSIS OF AEGILOPS JUVENALIS (THELL.) EIG AND AEGILOPS CRASSA BOISS.

by

JOHN HENRY MELNYK

A Thesis

Submitted to the Faculty of Graduate Studies and Research

in Partial Fulfilment of the Requirements

for the Degree of

MASTER OF SCIENCE

THE UNIVERSITY OF MANITOBA

April 1957

ACKNOWLEDGMENTS

Grateful acknowledgments are made to Dr. R. C. McGinnis, Cytogeneticist, Cereal Breeding Laboratory, Winnipeg, for his patient guidance throughout this study; to Dr. R. F. Peterson, Officer-in-Charge, Cereal Breeding Laboratory, Winnipeg, for the facilities and support provided by the laboratory which made this study possible; to Mr. W. E. Clark, Laboratory of Plant Pathology, Winnipeg, for the photographic plates; and to Miss E. Magner for typing of the manuscript.

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## ABSTRACT

A cytological determination of the genome complements of two hexaploid species *Aegilops juvenalis* and *Aegilops crassa* was conducted. A total of twelve interspecific $F_1$ hybrids involving *Ae. juvenalis* and a total of thirteen $F_1$ hybrids of *Ae. crassa* with ten other species of *Aegilops*, two of *Triticum* and one of *Haynaldia*, were examined. An embryo culture technique was employed in germination of the hybrid seed. Cytological data on twenty-one of the twenty-five hybrids studied had not been reported previously. The amount and kind of pairing observed at metaphase I of meiosis was used as a basis for determining the genome relationships between the parent species.

This study confirmed earlier reports that *Ae. juvenalis* contains the $C^u$ genome of *Ae. umbellulata* Zhuk. and the D genome of *Ae. squarrosa* L. It also provided evidence that the third genome is the S, derived from *Ae. speltoides* Tausch var. *ligustica* Fiori. The new symbol $S^j$ was proposed.

This study also confirmed earlier reports that *Ae. crassa* contains the D genome of *Ae. squarrosa* L. and the $M^{cr}$ genome. Evidence was found that *Ae. crassa* also contains the S genome of *Ae. speltoides*.

On the basis of evidence from several sources, the suggestion was made that *Ae. mutica* Boiss. may belong to the S group rather than the M group.

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# INTRODUCTION

The genus Aegilops together with Triticum, Agropyron, Secale and Haynaldia occurs in the sub-tribe Triticinae, tribe Hordeae of the grass family Gramineae. Aegilops is composed of diploids, tetraploids and hexaploids, with a basic chromosome number of seven as in wheat. For some time it was believed that this genus had participated in the phylogeny of wheat but it was only recently that evidence was found in support of this belief. This led to an increased interest in the classification of Aegilops species according to their genomic constitution. Main studies are being conducted in Japan by Kihara, in the United States by Sears and in Canada by McGinnis.

Although interspecific and intergeneric hybrids involving Aegilops species have been studied cytologically for about thirty years, it was only lately that their genomic relationships have been understood. There are twenty-five named species in this genus whose diploids have been arranged into three different groups according to their genomic relationships. These groups are sub-divided into genomes, each of which is believed to have arisen through evolution from a common genome of its group. Each diploid species has been assigned a symbol representing its genome. Each polyploid is composed of combinations of these genomes and all but three species have been studied extensively with regard to their genomic constitution. The present study was designed to contribute further knowledge on the genomic constitution of two hexaploid species, Ae. juvenalis (Thell.) Eig and Ae. crassa Boiss.

## REVIEW OF LITERATURE

Over 250 interspecific and intergeneric crosses involving Aegilops species have been reported. A review of these crosses has been summarized in tables by Kihara (13) and Kostoff (19) so that only those involving Ae. juvenalis and Ae. crassa will be considered in this paper.

Kihara (12, 14) proposed a list of nine diplcid species which were used to determine the genomes and to classify the species in the genus Aegilops. The nine species were classified into three groups, C, M, and S as shown in Table I.

TABLE I

Genome Classification of Diploid Aegilops Species

| Group | Species | Genome |
|---|---|---|
| C | Ae. caudata | C |
| | Ae. umbellulata | $C^u$ |
| M | Ae. comosa incl. Ae. Heldrechii | M |
| | Ae. uniaristata | $M^u$ |
| | Ae. mutica | $M^t$ |
| | Ae. squarrosa | D |
| S | Ae. speltoides incl. Ae. Aucheri | S |
| | Ae. bicornis | $S^b$ |
| | Ae. longissima incl. Ae. sharonensis | $S^l$ |

These groups are sub-divided into genomes, each of which arose through evolution from a common genome of its group. For example the C group is exemplified by Ae. caudata but it also contains the $C^u$ genome of Ae. umbellulata. The M group is composed of four divergent genomes, the M, D, $M^u$ and $M^t$. The S group consists of three different genomes, the S, $S^b$ and $S^l$. These evolutionary genomes are now only partly homologous with the genome which is representative of their

group. However, their chromosomes pair with sufficient frequency to establish their relationship.

As would be expected the genomes within each group are homoeologous. Furthermore, there is a varying degree of homology between any two genomes of different groups as shown by Kihara (13) though this homology is relatively weak in most cases (Fig. 1).

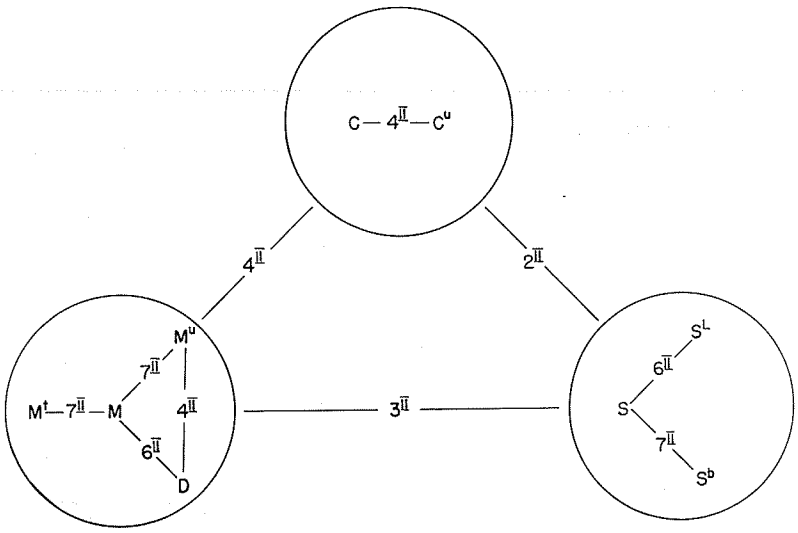



Fig. 1. Inter- and intra-group relationships for three *Aegilops* groups.

A review of the literature pertinent to genome relationships in *Ae. juvenalis* and *Ae. crassa* is presented separately as follows.

1. Ae. juvenalis (Thell.) Eig (= Ae. turcomanica Rosh.)

This species was first described in 1907 by Thellung who named it Triticum juvenale. Later, Eig (7) reclassified it as Ae. juvenalis. At about the same time Roshevitz (27) described Ae. turcomanica which was subsequently recognized to be Ae. juvenalis.

Until recently very little information on genome relationships in Ae. juvenalis was available. Eig (7) placed this species in the same section as Ae. squarrosa (D) on a taxonomical and morphological basis. Kihara (14) gave Ae. juvenalis the tentative genome designation D($M^{j}C^{u}$?).

Senjaninova-Korczagina (32) in a karyosystematical study of the genus Aegilops found that the $C^{u}$ genome was present in a number of the tetraploid and hexaploid species. There was strong evidence to show that Ae. juvenalis carries this genome also. Her studies revealed a sharp difference between the sets of chromosomes of Ae. juvenalis and Ae. crassa. This report did not refer to the constitution of the remaining two genomes of Ae. juvenalis.

McGinnis (22) presented the first cytological analyses of $F_1$ hybrids involving Ae. juvenalis with three Aegilops species and two Triticum species. In this study he showed evidence which confirmed earlier theories regarding the presence of the D genome. This evidence was derived from crosses of Ae. juvenalis with T. durum (AB) and T. vulgare (ABD). The juvenalis-durum hybrid showed an average bivalent association of only 3.72 per cell whereas the juvenalis-vulgare hybrid showed an average of 7.67 bivalents, which is sufficient to indicate the presence of a genome in common to both Ae. juvenalis and T. vulgare. Since T. vulgare differs from T. durum by its possession of the D genome, the high degree of pairing in the juvenalis-

vulgare cross was attributed to the presence of this genome.

McGinnis (22) found an average of 5.89 bivalent associations in a cross of Ae. juvenalis x Ae. ventricosa ($DM^{V}$). This low frequency of pairing was considered to be due to the evolutionary changes which had taken place in the D genomes of the two species.

McGinnis (21) suggested Ae. juvenalis contains the $C^{u}$ genome of Ae. umbellulata. This hypothesis was based on evidence derived from crosses of Ae. juvenalis with Ae. variabilis ($C^{u}S^{v}$) and Ae. ovata ($C^{u}M^{o}$). The juvenalis-variabilis cross showed an average of 10.76 bivalents and the juvenalis-ovata cross showed an average of 8.62 bivalents. Both of these crosses revealed sufficient pairing between chromosomes to indicate the presence of one common genome, which was believed to be the $C^{u}$ genome. The only other report of a cross with Ae. juvenalis which gives pairing frequencies was made by Favorsky (8) in which he described an $F_1$ hybrid having Agropyron intermedium as the other parent. This cross showed an average of 14 bivalents.

2. Aegilops crassa Boiss.

Ae. crassa was described by Boissier (4) in 1846. Kihara (14) assigned the symbols $DM^{cr}{}_{+}(?)$ to this species indicating that the third genome had not been determined. He showed evidence that the D genome of Ae. squarrosa contributed to the evolution of this species. The tetraploid species of Ae. crassa was given the genome designation $DM^{cr}$ by Kihara (14). Taxonomically, Eig (7) classified Ae. crassa in the same section as Ae. squarrosa. Some of the crosses reported by Kihara and others are shown in Table II. The pairing observed in several of these hybrids supports the presence of the D genome, but little evidence was found to substantiate the presence of the $M^{cr}$ genome in Ae. crassa.

TABLE II

Interspecific and Intergeneric Hybrids with Ae. crassa

| $F_1$ Hybrids | Bivalents (Mode) | Author |
|---|---|---|
| Ae. crassa ($DM^{cr}$+?) x Ae. squarrosa (D) | 6-10(7) | Kihara (12) |
| " " " x Ae. crassa ($DM^{cr}$) | 14 | " " |
| " " " x Ae. ventricosa ($DM^{v}$) | 6-9 | " & Lilienfeld (17) |
| " " " x " " " | 8-10 | Kihara (12) |
| " " " x Ae. uniaristata ($M^{u}$) | 6-9 | " " |
| " " " x Ae. ovata ($C^{u}M^{o}$) | 4-9(7) | " & Lilienfeld (17) |
| " " " x Ae. bicornis ($S^{b}$) | 3-6 | Kihara (12) |
| " " " x T. dicoccoides (AB) | 0-5 | Longley & Sando (20) |
| " " " x T. dicoccum (AB) | 0-3(0) | " " " |
| " " " x T. durum (AB) | 0-4(0) | " " " |
| " " " x T. turgidum (AB) | 0-4 | " " " |
| " " " x T. polonicum (AB) | 0-4 | " " " |
| " " " x T. vulgare (ABD) | 0-7 | " " " |
| " " " x T. spelta (ABD) | 6 | " " " |
| " " " x " " " | 7 | Kihara (11) |
| " " " x Agr. intermedium | 14 | Favorsky (8) |
| " crassa ($DM^{cr}$) x Ae. squarrosa (D) | 4-8 | Kihara (12) |
| " " " x Ae. bicornis ($S^{b}$) | 2-7(5) | " " |
| " biuncialis ($C^{u}M^{b}$) x Ae. crassa ($DM^{cr}$+?) | 3-8(7) | " " |
| " triaristata ($C^{u}M^{t}$+?) x Ae. " " | 7-8(7) | " " |
| " columnaris ($C^{u}M^{c}$) x Ae. crassa ($DM^{cr}$+?) | 4-8(7) | " " |
| " ventricosa ($DM^{v}$) x " " ($DM^{cr}$) | 7 | " " |
| " triuncialis ($CC^{u}$) x " " ($DM^{cr}$+?) | 3-10(6) | Miczynski (23) |

# MATERIALS AND METHODS

## Parent species

Fifteen species of *Aegilops*, two of *Triticum*, and one of *Haynaldia* were used in the crossing program of this study. They are listed below with their diploid chromosome numbers and genomes as designated by Kihara (12, 14).

| | 2n chrom. no. | Genomes |
|---|---|---|
| *Aegilops caudata* L. var. *polyathera* Boiss. | 14 | C |
| *Aegilops umbellulata* Zhuk. | 14 | $C^u$ |
| *Aegilops triuncialis* L. | 28 | $CC^u$ |
| *Aegilops columnaris* Zhuk. | 28 | $C^uM^c$ |
| *Aegilops variabilis* Eig | 28 | $C^uS^v$ |
| *Aegilops cylindrica* Host | 28 | CD |
| *Aegilops ventricosa* Tausch | 28 | $DM^v$ |
| *Aegilops crassa* Boiss. | 42 | $DM^{cr_4}$(?) |
| *Aegilops juvenalis* (Thell.) Eig | 42 | $C^uD(M^j?)$ |
| *Aegilops mutica* Boiss. | 14 | $M^t$ |
| *Aegilops uniaristata* Vis. | 14 | $M^u$ |
| *Aegilops speltoides* Tausch var. *ligustica* Fiori (incl. *Ae. Aucheri* Boiss.) | 14 | S |
| *Aegilops longissima* Schw. et Musch. (incl. *Ae. sharonensis* Eig) | 14 | $S^l$ |
| *Triticum aestivum* L. var. Redman | 42 | ABD |
| *Triticum durum* Desf. var. *hordeiforme* (Carleton) | 28 | AB |
| *Haynaldia villosa* Schur | 14 | V |
| *Aegilops squarrosa* L | 14 | D |

F1 Hybrids

A total of twelve interspecific hybrids were established from crosses involving Ae. juvenalis as the female parent and certain of the above species as the pollen parents. These crosses were as follows:

Ae. juvenalis x Ae. caudata
" " x Ae. umbellulata
" " x Ae. uniaristata
" " x Ae. mutica
" " x Ae. Aucheri
" " x Ae. speltoides
" " x Ae. longissima
" " x Ae. sharonensis
" " x Ae. cylindrica
" " x Ae. columnaris
" " x Ae. triuncialis
" " x Ae. crassa (2n=42)

A total of twelve interspecific and intergeneric hybrids were established from crosses involving Ae. crassa as the female parent. The successful crosses were as follows:

Ae. crassa x Ae. caudata
" " x Ae. umbellulata
" " x Ae. variabilis
" " x Ae. cylindrica
" " x Ae. ventricosa
" " x Ae. speltoides
" " x Ae. sharonensis
" " x Ae. squarrosa

| | | |
|---|---|---|
| Ae. crassa | x | Ae. Aucheri |
| " " | x | Ae. mutica |
| " " | x | T. durum |
| " " | x | T. aestivum |
| " " | x | H. villosa |

Crossing techniques

The parent species were grown in a greenhouse in six-inch clay pots. Crosses were made over a period from the fall of 1954 to the winter of 1956. Ae. juvenalis and Ae. crassa were used as the female parents in all of the crosses with other species. In the cross involving these two species, Ae. juvenalis was used as the female parent.

The crosses were made at various periods of the day. The emasculated heads were bagged and were subsequently pollinated two to three days later in most cases. In some cases pollination followed emasculation immediately. The bags were left on the pollinated heads for ten to twelve days after pollination.

Early in the program when crossed seed was obtained, the kernels were stored for two to three months to break dormancy. When certain of these seeds were planted in pots in the greenhouse, a low incidence of emergence was encountered. Therefore, attempts were made to germinate other crossed seeds on moist filter paper in a petri plate but this method also revealed a low incidence of germination. To overcome this low germination, embryos of the hybrid seed were excised and grown on a nutrient medium. In the course of this work a rapid method of culturing embryos was developed. With the aid of embryo cultures, $F_1$ hybrids were established from sixteen crosses which had not been reported earlier.

Embryo culture techniques

In preliminary experiments in which the recognized culture technique was followed as described by Brink and his associates (5), it was found that sterilization of the green seed or embryo in as low a concentration as 1 p.p.m. of mercuric chloride solution suppressed lateral root development. In further experiments, where sterilization was eliminated, root growth appeared normal and contamination of cultures occurred but rarely. This suggested that an embryo under greenhouse conditions is free of contaminants and that sterilization was unnecessary. Thereafter sterilization of the seed or embryo was omitted. Contamination of the embryo or culture medium by air-borne organisms was avoided by working in a small dust-free room. A damp cloth was placed over the working area as an aid in controlling floating spores. In three cases, embryo cultures were made in the greenhouse. The benches and floors were watered previously to control spore movement and no contamination of the cultures occurred. The instruments, a scalpel and a pair of fine-pointed forceps, were kept sterile in a jar of 95% ethyl alcohol.

The seed was held between the thumb and index finger of the left hand and an incision was made around the embryo within 1 mm. of the scutellum. If the seed was at the correct stage of development (15 to 20 days after pollination), the pericarp could be peeled back in an entire section with the forceps. In this operation meticulous care was taken to avoid contact with the embryo in case the forceps or scalpel were contaminated. The embryo, which sits freely in a hollow on the endosperm, was lifted off with the point of the scalpel, which was previously flamed long enough to burn off the alcohol. Another method of lifting the embryo

involved the use of a small platinum loop. A hollow was cut out on the surface of an agar slant with the loop and the tacky loop was then touched to the embryo. The embryo adhered firmly to the loop if the pericarp was peeled back far enough. The embryo was then placed in the hollow on the agar with the epicotyl pointed toward the open end of the slant. Square bottles, three inches in height, with metal screw-caps were used for the slants. Difco Orchid Agar was found to be a satisfactory medium in this work. It was convenient to use because it required only the addition of water, thus eliminating the time-consuming work of preparing media from formulae. The cultures were kept at room temperature under diffused light until the seedlings grew the full length of the bottle. The seedlings were then transferred to six-inch pots with a mixture of three parts of soil to one part of sand and were grown in the greenhouse.

Scoring of pairing frequencies

Slides were prepared of pollen mother cells at metaphase I of meiosis using the aceto-carmine method of Smith (34). The preparations were kept semi-permanent by sealing the cover slips with a mixture of gum-mastic and paraffin. Later the preparations were made permanent by the tertiary-butyl alcohol method (34).

In the analyses of $F_1$ hybrids, records were kept of the number of univalents, open and closed bivalents, trivalents, and higher multi-valents that occurred per pollen mother cell at Metaphase I of meiosis. In order to facilitate comparisons of results between the different hybrids and to make possible a clearer evaluation of the pairing frequencies of individual hybrids, the complexities of pairing were reduced to a common denominator of a unit termed a bivalent association as suggested by Kihara (10). Thus

a closed or open bivalent was interpreted as one bivalent association, a trivalent equalled one bivalent association, a quadrivalent equalled two bivalent associations, and a pentavalent equalled two bivalent associations. In most hybrids 200 pollen mother cells were examined and scored but in some fewer cells were analyzed.

Cytological examinations were made on a Leitz Ortholux microscope at a magnification of 600 diameters. Photomicrographs were taken with a Leitz Aristophot camera on Eastman's Contrast Process Panchromatic film at 550 diameters.

## RESULTS

The ease with which *Ae. juvenalis* and *Ae. crassa* hybridized with each of the species used in this study varied considerably. On the basis of general observations there seemed to be no correlation between the ease of hybridization and the degree of relationship of either *Ae. juvenalis* or *Ae. crassa* to these species. For instance, although *Ae. juvenalis* contains the $C^u$ genome of *Ae. umbellulata*, the cross between these two species was made with great difficulty. On the other hand, *Ae. crassa* hybridized readily with *H. villosa* whose relationship is believed to be remote.

The capacity of the hybrid seed to germinate also varied. A few such as the *juvenalis-speltoides*, *juvenalis-cylindrica*, *juvenalis-crassa*, *crassa-umbellulata*, *crassa-cylindrica*, *crassa-ventricosa* and the *crassa-Triticum* hybrids germinated readily from the whole kernel. Others required embryo cultures to induce germination.

The cytological data of the $F_1$ hybrids involving *Ae. juvenalis* and *Ae. crassa* respectively are presented in Tables III and IV. Summaries of their genome homologies are listed in Tables V and VI. In Plates I to IV inclusive, typical metaphase figures of each of the hybrids are presented.

TABLE III

Frequencies of Bivalents and Higher Associations of Chromosomes in Hybrids Involving *Aegilops juvenalis*

| Ae. *juvenalis* x | Univalents Av. | Univalents Range | Closed bivalents Av. | Closed bivalents Range | Open bivalents Av. | Open bivalents Range | Total pairs Av. | Total pairs Range | Trivalents Av. | Trivalents Range | Quadrivalents Av. | Quadrivalents Range | Higher Multiv. Av. | Higher Multiv. Max[1] | Av. biv. assoc.[2] | Cells exam. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aec. *caudata* (C) | 14.70 | 6-24 | .08 | 0-3 | 4.88 | 2-9 | 4.96 | 2-9 | .99 | 0-3 | .11 | 0-1 | .00 | $C^{IV}$ | 6.16 | 200 |
| " *umbellulata* ($C^u$) | 6.39 | 1-14 | 4.46 | 1-8 | 3.59 | 0-7 | 8.05 | 4-11 | 1.42 | 0-4 | .28 | 0-2 | .03 | $C^{VI}$ | 10.09 | 200 |
| " *uniaristata* ($M^u$) | 18.50 | 13-26 | .02 | 0-1 | 2.80 | 0-7 | 2.82 | 0-7 | 1.15 | 0-3 | .08 | 0-1 | .02 | $C^{V}$ | 4.17 | 200 |
| " *mutica* ($M^t$) | 14.21 | 6-22 | .08 | 0-1 | 4.66 | 1-10 | 4.74 | 1-10 | 1.08 | 0-4 | .25 | 0-3 | .02 | $C^{V}$ | 6.34 | 200 |
| " *Aucheri* (S) | 8.32 | 2-16 | .20 | 0-3 | 3.38 | 0-9 | 3.58 | 0-9 | 1.89 | 0-5 | .84 | 0-4 | .62 | $C^{XI}$ | 8.71 | 200 |
| " *speltoides* (S) | 10.24 | 4-17 | .92 | 0-4 | 4.60 | 0-9 | 5.52 | 1-10 | 1.57 | 0-4 | .40 | 0-2 | .08 | $C^{V}$ | 8.04 | 200 |
| " *longissima* ($S^l$) | 15.86 | 12-23 | .08 | 0-2 | 3.23 | 0-7 | 3.31 | 0-8 | 1.28 | 0-3 | .36 | 0-2 | .01 | $C^{V}$ | 5.30 | 200 |
| " *sharonensis* ($S^l$) | 13.14 | 9-22 | .42 | 0-2 | 4.17 | 0-8 | 4.59 | 0-8 | 1.40 | 0-4 | .35 | 0-2 | .03 | $C^{VI}$ | 6.75 | 200 |
| " *cylindrica* (CD) | 14.35 | 3-24 | 1.09 | 0-3 | 5.97 | 2-11 | 7.06 | 3-11 | 1.53 | 0-4 | .39 | 0-3 | .11 | $C^{VII}$ | 9.53 | 200 |
| " *columnaris* ($C^uM^c$) | 12.95 | 7-21 | 3.57 | 1-7 | 4.24 | 1-9 | 7.81 | 3-13 | 1.79 | 0-4 | .22 | 0-2 | .04 | $C^{VI}$ | 10.12 | 200 |
| " *triuncialis* ($CC^u$) | 12.94 | 7-21 | 2.27 | 0-6 | 5.37 | 1-11 | 7.64 | 3-13 | 1.70 | 0-6 | .34 | 0-2 | .02 | $C^{VII}$ | 10.18 | 130 |
| " *crassa* ($DM^{cr}$+?) | 14.05 | 9-24 | 5.38 | 0-10 | 3.52 | 1-12 | 8.90 | 4-15 | 2.01 | 0-5 | .59 | 0-2 | .28 | $C^{IX}$ | 12.82 | 200 |

[1] C = Chain

[2] Bivalent = 1 bivalent association
Trivalent = 1 bivalent association
Quadrivalent = 2 bivalent associations

TABLE IV

Frequencies of Bivalents and Higher Associations of Chromosomes in Hybrids Involving *Aegilops crassa*

| *Ae. crassa* x | Univalents | | Closed bivalents | | Open bivalents | | Total pairs | | Trivalents | | Quadrivalents | | Higher Multiv. | | Av. biv. assoc.[2] | Cells exam. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Range | Av. | Max[1] | | |
| *Ae. caudata* (C) | 13.28 | 8-20 | 2.02 | 0-5 | 4.05 | 0-9 | 6.07 | 3-9 | .76 | 0-3 | .05 | 0-1 | .01 | $C^{V}$ | 6.96 | 166 |
| " *cylindrica* (CD) | 13.12 | 8-18 | 3.73 | 1-7 | 4.39 | 0-9 | 8.12 | 2-13 | 1.42 | 0-4 | .32 | 0-2 | .02 | $C^{V}$ | 10.22 | 100 |
| " *umbellulata* ($C^{u}$) | 13.94 | 7-21 | 1.46 | 0-5 | 4.06 | 1-9 | 5.52 | 2-9 | .81 | 0-3 | .09 | 0-1 | .03 | $C^{VI}$ | 6.84 | 200 |
| " *variabilis* ($C^{u}S^{v}$) | 19.67 | 12-25 | 1.50 | 0-5 | 4.29 | 1-9 | 5.79 | 2-9 | .98 | 0-3 | .17 | 0-2 | .03 | $C^{VI}$ | 7.12 | 200 |
| " *ventricosa* ($DM^{v}$) | 16.56 | 10-25 | 2.74 | 0-6 | 4.26 | 1-9 | 7.00 | 2-11 | 1.60 | 0-4 | .14 | 0-2 | .02 | $C^{V}$ | 8.92 | 200 |
| " *sharonensis* ($S^{1}$) | 10.88 | 5-18 | 2.34 | 0-5 | 4.44 | 0-9 | 6.78 | 2-10 | .94 | 0-4 | .15 | 0-2 | .04 | $C^{V}$ | 8.08 | 200 |
| " *Aucheri* (S) | 10.57 | 3-18 | 3.32 | 2-6 | 2.54 | 0-8 | 5.86 | 2-11 | 1.11 | 0-4 | .36 | 0-3 | .18 | $C^{VII}$ | 8.10 | 200 |
| " *speltoides* (S) | 8.00 | 3-12 | 3.42 | 0-6 | 3.05 | 2-7 | 6.47 | 4-9 | 1.42 | 0-3 | .38 | 0-2 | .08 | $C^{V}$ | 8.81 | 24 |
| " *mutica* ($M^{t}$) | 11.14 | 5-18 | 2.28 | 0-4 | 3.58 | 0-6 | 5.86 | 3-10 | 1.30 | 0-3 | .60 | 0-1 | .04 | $C^{V}$ | 7.74 | 50 |
| *T. aestivum* (ABD) | 24.53 | 16-32 | 2.24 | 0-5 | 4.22 | 1-8 | 6.45 | 3-11 | 1.31 | 0-4 | .12 | 0-2 | .02 | $C^{VI}$ | 8.03 | 200 |
| *T. durum* (AB) | 32.91 | 27-35 | 0.00 | --- | 1.05 | 0-4 | 1.05 | 0-4 | 0.00 | --- | .00 | --- | .00 | --- | 1.05 | 200 |
| *H. villosa* (V) | 14.20 | 10-20 | 1.86 | 0-5 | 3.86 | 1-7 | 5.72 | 2-9 | .73 | 0-3 | .01 | 0-1 | .00 | --- | 7.46 | 200 |
| *Ae. squarrosa* (D) | 10.21 | 5-15 | 4.42 | 2-7 | 2.61 | 0-7 | 7.03 | 5-10 | 1.03 | 0-3 | .24 | 0-1 | .03 | $C^{VI}$ | 8.37 | 100 |

[1] C = Chain

[2] Bivalent = 1 bivalent association
Trivalent = 1 " "
Quadrivalent = 2 " "

TABLE V

Genome homologies in $F_1$ hybrids with *Ae. juvenalis* ♀

| Hybrid | Genomes | Av. biv.* assoc. | Homologous genomes | Genomes in common |
|---|---|---|---|---|
| *Ae. juvenalis* x | $C^uDS$ | | | |
| *Ae. caudata* | C | 6 | 0 | |
| " *umbellulata* | $C^u$ | 10 | 1 | $C^u$ |
| " *uniaristata* | $M^u$ | 4 | 0 | |
| " *mutica* | $M^t$ | 6 | | |
| " *Aucheri* | S | 9 | 1 | S |
| " *speltoides* | S | 8 | 1 | S |
| " *longissima* | $S^l$ | 5 | 0 | |
| " *sharonensis* | $S^l$ | 7 | 0 | |
| " *cylindrica* | CD | 10 | 1 | D |
| " *columnaris* | $C^uM^c$ | 10 | 1 | $C^u$ |
| " *triuncialis* | $CC^u$ | 10 | 1 | $C^u$ |
| " *crassa* | $DM^{cr}S$ | 13 | 2 | DS |

* To nearest whole integer.

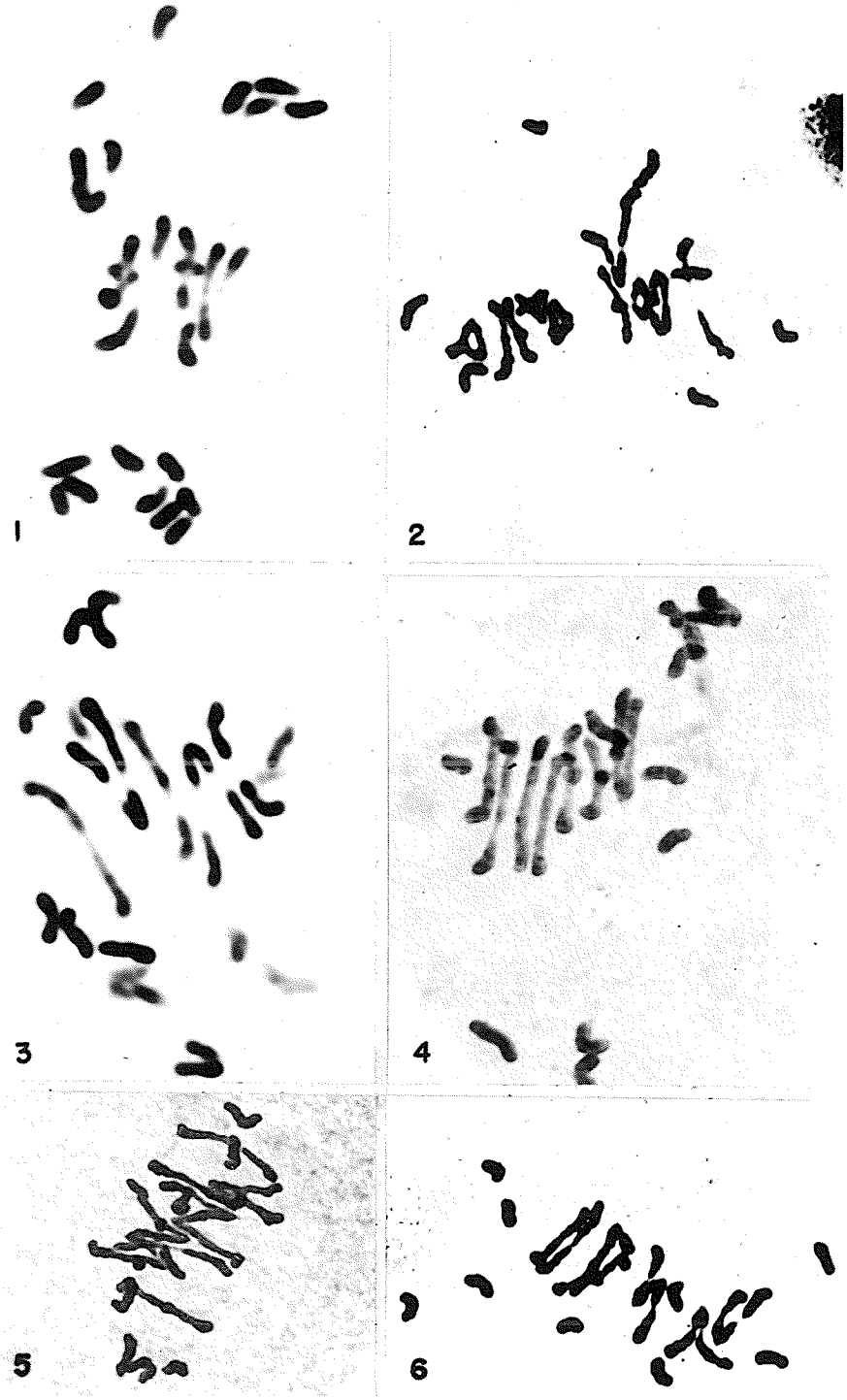
TABLE VI

Genome homologies in $F_1$ hybrids with *Ae. crassa* ♀

| Hybrid | Genomes | Av. biv.* assoc. | Homologous genomes | Genomes in common |
|---|---|---|---|---|
| *Ae. crassa* x | $DM^{cr}S$ | | | |
| *Ae. caudata* | C | 7 | 0 | |
| " *cylindrica* | CD | 10 | 1 | D |
| " *umbellulata* | $C^u$ | 7 | 0 | |
| " *variabilis* | $C^uS^v$ | 7 | 0 | |
| " *ventricosa* | $DM^v$ | 9 | 1 | D |
| " *sharonensis* | $S^l$ | 8 | 0 | |
| " *Aucheri* | S | 8 | 1 | S |
| " *speltoides* | S | 9 | 1 | S |
| " *mutica* | $M^t$ | 8 | 1 ? | $M^t$ ? |
| *T. aestivum* | ABD | 8 | 1 | D |
| *T. durum* | AB | 1 | 0 | |
| *H. villosa* | V | 7 | 0 | |
| *Ae. squarrosa* | D | 8 | 1 | D |

* To nearest whole integer.

Plate I



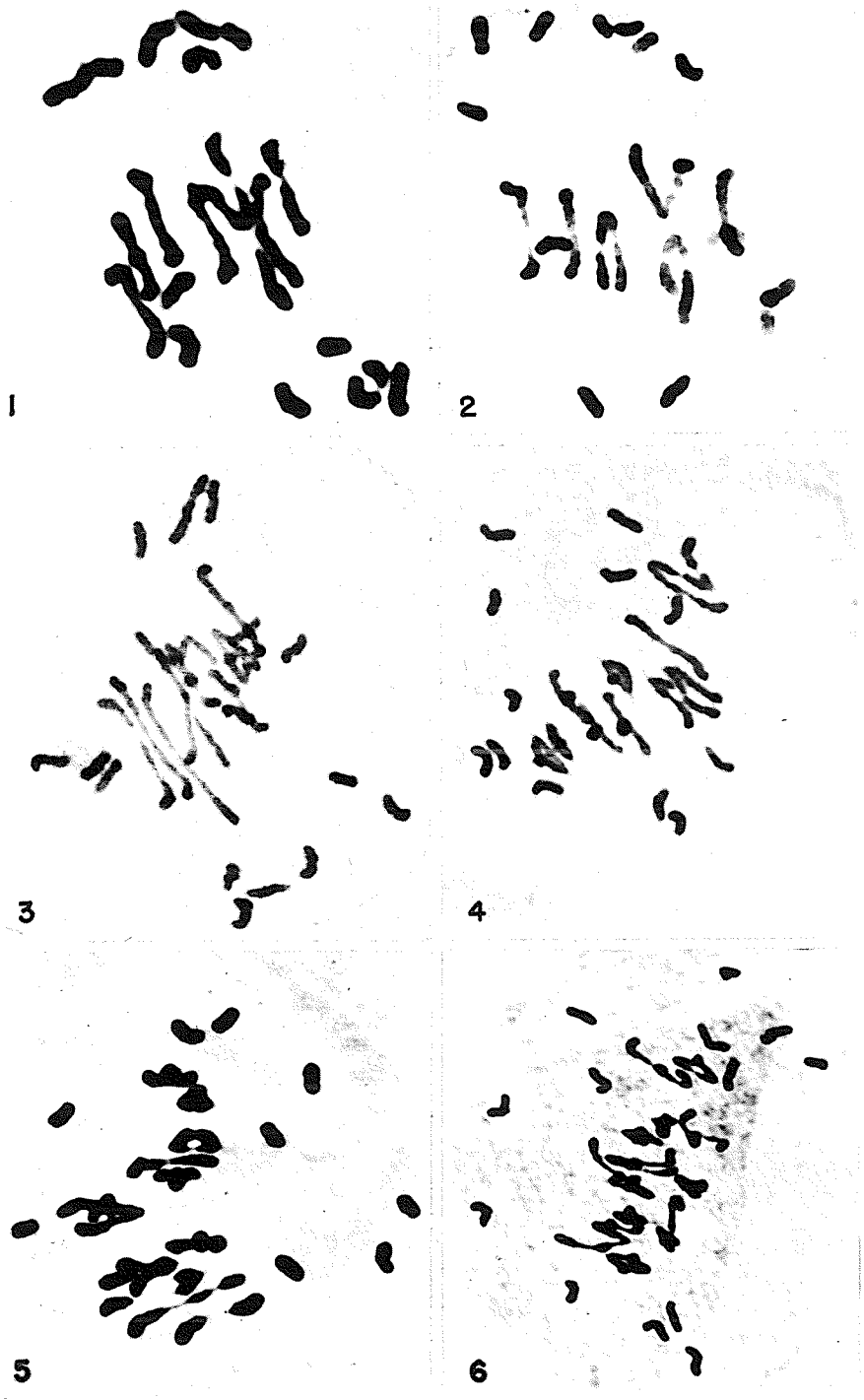

Pairing at metaphase I in $F_1$ hybrids. ✱

| | | | |
|---|---|---|---|
| Fig. 1. | Ae. juvenalis | x Ae. caudata | $C^{III}$ $(5^o)^{II}$ $15^I$ |
| Fig. 2. | Ae. juvenalis | x Ae. umbellulata | $C^{III}$ $(4^o 5^c)^{II}$ $7^I$ |
| Fig. 3. | Ae. juvenalis | x Ae. uniaristata | $2\,C^{III}$ $(3^o)^{II}$ $16^I$ |
| Fig. 4. | Ae. juvenalis | x Ae. mutica | $C^{III}$ $(6^o)^{II}$ $13^I$ |
| Fig. 5. | Ae. juvenalis | x Ae. Aucheri | $C^V$, $C^{IV}$, $C^{III}$, $(4^o)^{II}$ $8^I$ |
| Fig. 6. | Ae. juvenalis | x Ae. speltoides | $C^{IV}$ $2C^{III}$ $(4^o)^{II}$ $10^I$ |

Magnification approximately 650X

✱ C - Chain, o - open, c - closed.

Plate II



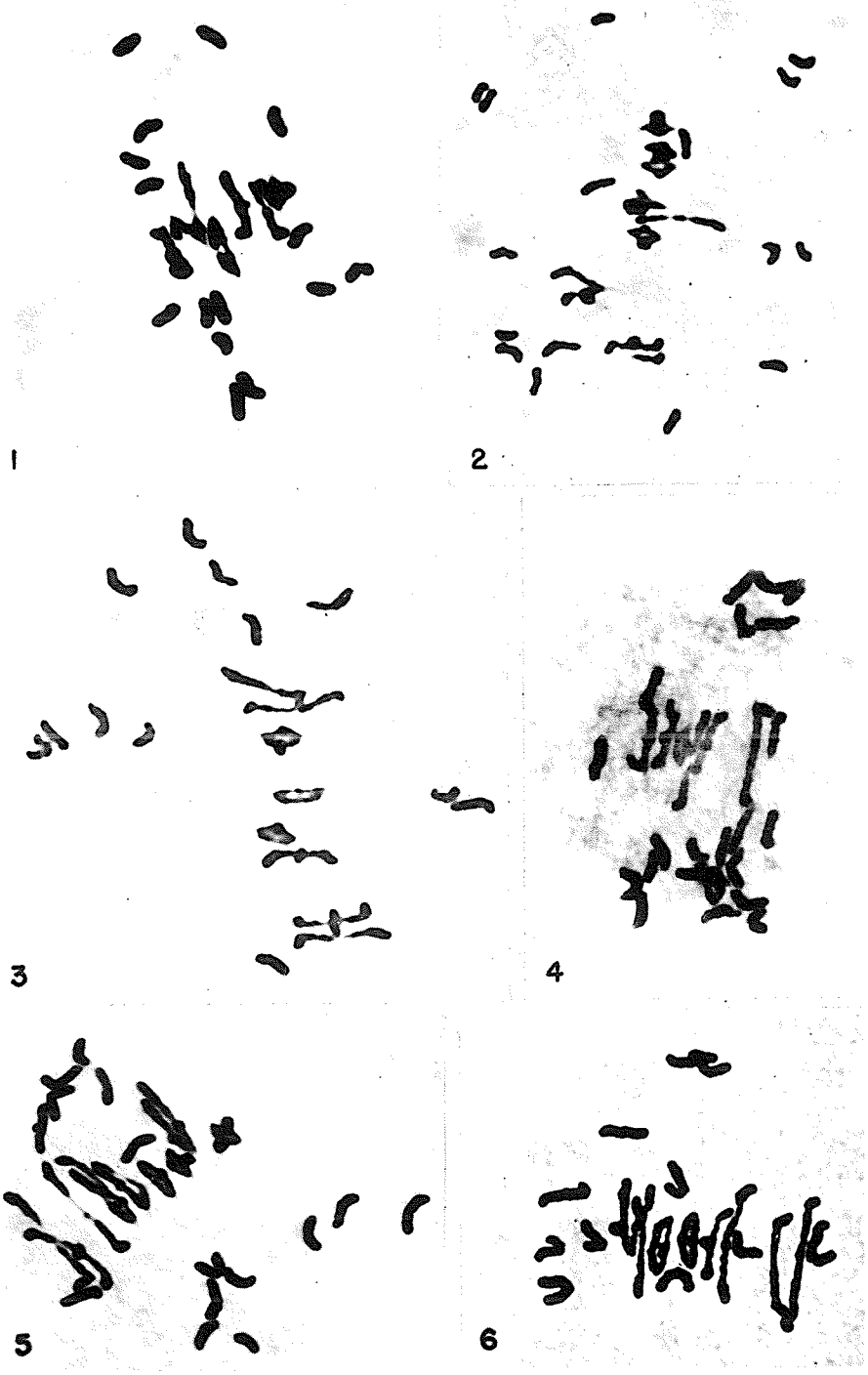

Pairing at metaphase I in $F_1$ hybrids. ✯

| | | | |
|---|---|---|---|
| Fig. 1. | Ae. juvenalis | x Ae. longissima | $C^{IV}$ $(5^{o})^{II}$ $14^{I}$ |
| Fig. 2. | Ae. juvenalis | x Ae. sharonensis | $3C^{III}$ $(3^{o})^{II}$ $13^{I}$ |
| Fig. 3. | Ae. juvenalis | x Ae. cylindrica | $(8^{o}1^{c})^{II}$ $17^{I}$ |
| Fig. 4. | Ae. juvenalis | x Ae. columnaris | $C^{IV}$ $2C^{III}$ $(4^{o}3^{c})^{II}$ $11^{I}$ |
| Fig. 5. | Ae. juvenalis | x Ae. triuncialis | $3C^{III}$ $(5^{o}3^{c})^{II}$ $10^{I}$ |
| Fig. 6. | Ae. juvenalis | x Ae. crassa | $C^{IV}$ $2C^{III}$ $(2^{o}7^{c})^{II}$ $14^{I}$ |

Magnification approximately 650X

✯ C - Chain, o - open, c - closed.

Plate III



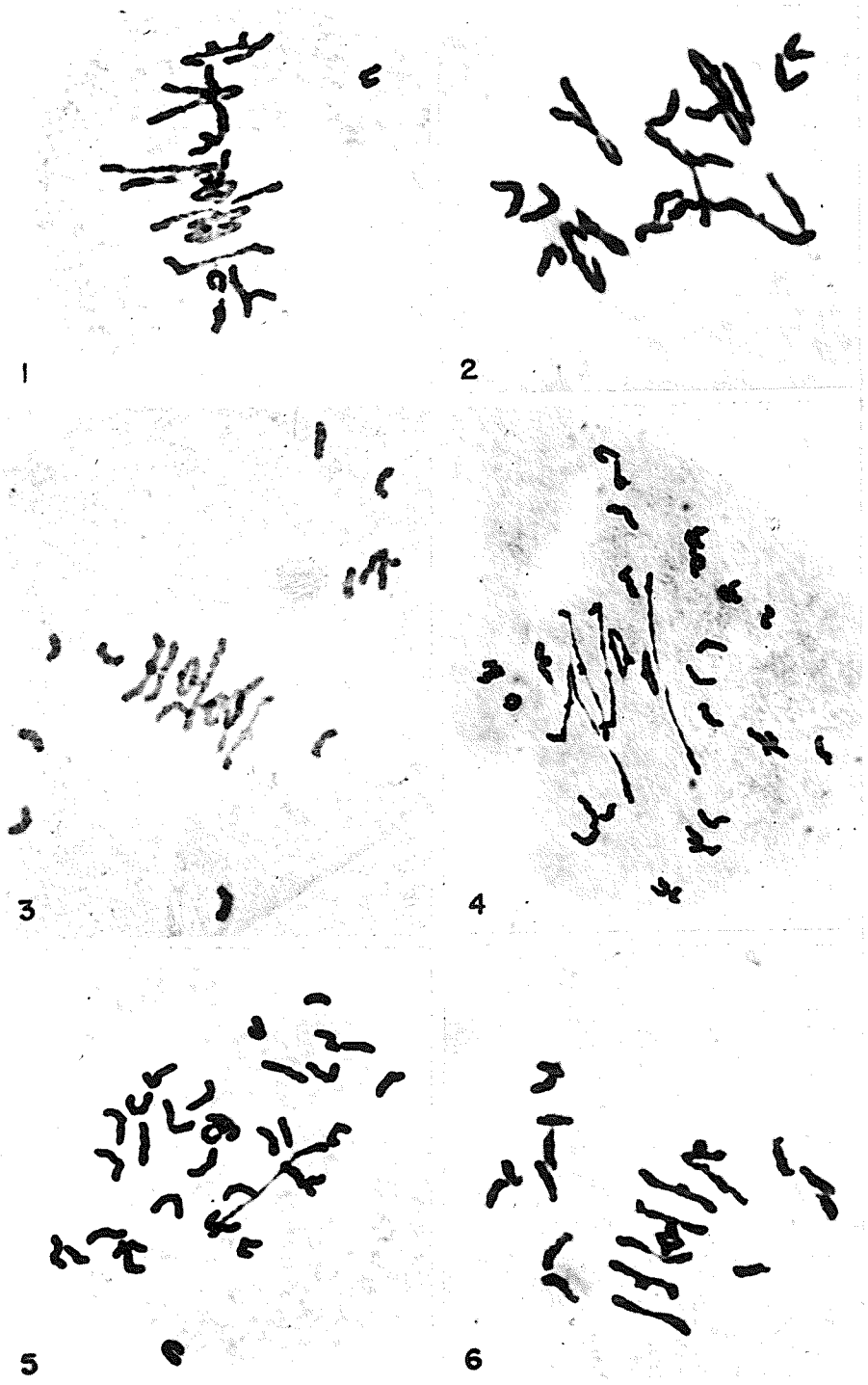

Pairing at metaphase I in $F_1$ hybrids. ✱

| | | | |
|---|---|---|---|
| Fig. 1. | Ae. crassa x Ae. caudata | $C^{III}$ $(3^{o}2^{c})^{II}$ $15^{I}$ |
| Fig. 2. | Ae. crassa x Ae. cylindrica | $C^{III}$ $(3^{o}5^{c})^{II}$ $16^{I}$ |
| Fig. 3. | Ae. crassa x Ae. umbellulata | $(5^{o}3^{c})^{II}$ $12^{I}$ |
| Fig. 4. | Ae. crassa x Ae. variabilis | $(6^{o}1^{c})^{II}$ $21^{I}$ |
| Fig. 5. | Ae. crassa x Ae. ventricosa | $2C^{III}$ $(3^{o}4^{c})^{II}$ $15^{I}$ |
| Fig. 6. | Ae. crassa x Ae. sharonensis | $C^{III}$ $(5^{o}2^{c})^{II}$ $11^{I}$ |

Magnification approximately 650X

✱ C - Chain, o - open, c - closed.

Plate IV




Pairing at metaphase I in $F_1$ hybrids. ✱

| | | |
|---|---|---|
| Fig. 1. | Ae. crassa x Ae. Aucheri | $3c^{III}$ $(2^{o}3^{c})^{II}$ $9^{I}$ |
| Fig. 2. | Ae. crassa x Ae. speltoides | $c^{III}$ $(3^{o}4^{c})^{II}$ $11^{I}$ |
| Fig. 3. | Ae. crassa x Ae. mutica | $(5^{o}3^{c})^{II}$ $12^{I}$ |
| Fig. 4. | Ae. crassa x T. aestivum | $c^{III}$ $(3^{o}3^{c})^{II}$ $27^{I}$ |
| Fig. 5. | Ae. crassa x T. durum | $(1^{o})^{II}$ $33^{I}$ |
| Fig. 6. | Ae. crassa x H. villosa | $c^{III}$ $(5^{o}1^{c})^{II}$ $13^{I}$ |

Magnification approximately 650X

✱ C - Chain, o - open, c - closed.

# DISCUSSION AND CONCLUSIONS

## Significance of pairing.

It will be readily appreciated that a genome analysis of a hexaploid species cannot demonstrate precisely the genomic relationship between the hexaploid and a given diploid, tetraploid, or another hexaploid species. The complexity of chromosomal pairing tends to obscure the true relationship of any two genomes in hybrids from different species or genera. However, such an analysis, if conducted on a large enough scale, gives a fairly accurate indication of genome relationships.

There are several possibilities some or all of which may contribute to the chromosome pairing as seen in metaphase I of meiosis. The first of these is the pairing derived from true homology between the chromosomes of a genome of one parent and those of a corresponding genome of the other parent. This type of pairing is characterized by a high frequency of closed bivalents and visible chiasmata in the open bivalents. Evidence of this nature, therefore, is a strong indication of a true relationship between the genomes in question.

The second possibility is the pairing incurred by the homoeology between chromosomes of dissimilar genomes of the respective parents, that is, non-homologous pairing of chromosomes from different parents. For instance, in the $F_1$ hybrid of *Ae. sharonensis* ($S^l$) x *Ae. umbellulata* ($C^u$), up to four open bivalents were found by Melnyk and McGinnis (unpub.). These bivalents were paired end to end and chiasmata were rarely seen. Such pairing may result from chromosomal and genic similarities retained by these species during their divergent evolution from a common ancestor, resulting in strong segmental attractions. This is of little significance in terms of genome

homology and where a high frequency of such bivalents is encountered discretion must be used in interpretation in order to avoid faulty conclusions.

The third possibility which contributes to the complexity of pairing is the homoeology of the chromosomes of the genomes derived from a particular parent. The nature of the pairing in this instance may be much the same as that in the foregoing example. No direct evidence on the extent of such pairing is available for the species used in this study except in the case of Ae. ovata ($C^uM^o$), mentioned by Sears (31) wherein a haploid plant was found by Matsumura to have a maximum of three bivalents per cell.

Another source of evidence of autosyndetic pairing has been derived from crosses with rye species as indicated by Kihara (11). Rye chromosomes do not pair with Aegilops chromosomes, therefore any pairing that occurred was among the Aegilops chromosomes. Kihara (11) reported that the C and S genomes showed the weakest affinities, whereas the C and $C^u$ genomes of Ae. triuncialis showed the strongest affinities. He pointed out that all non-homologous genomes generally showed segmental similarities in at least one part of their chromosomes in the absence of their true homologues. However, in the presence of the homologous genomes, homoeologous pairing was suppressed.

An assumption concerning the extent of such autosyndetic pairing may be made on the basis of the knowledge of non-homologous pairing in the interspecific diploid crosses reported by Kihara (12) and Sears (29). For instance, if a hexaploid is thought to have the genome formula $C^uDS$, the amount of pairing exhibited by the $C^u$ and D genomes in the cross $C^uDS \times S$ may be inferred from the pairing occurring in the diploid cross $C^u \times D$.

This pairing may be somewhat lower, however, due to evolutionary modifications in the critical genomes.

The fourth possible source of pairing is intragenomic, that is, of chromosomal associations within a single genome. Katayama (9) and Smith (33) reported a haploid of T. monococcum in which they found an accasional bivalent. This type of pairing is probably of minor concern in the present study. There is a strong tendency of the chromosomes to exist in pairs during meiotic prophase and, lacking homologous partners, the non-homologous chromosomes may attract each other.

Secondary pairing as described by Ribbands (26) and evaluated by Person (25) was not recorded in this study. The side by side associations of univalents, termed quasi-bivalents by Oestegren and Vigfusson (24), were considered by Person to be a result of true homology. He suggested that the formation of typical bivalents and quasi-bivalents were determined by the presence or absence of chiasmata. If crossing-over occurs, a bivalent is formed. If crossing-over fails to occur, a quasi-bivalent results. This type of pairing has a bearing on the present study in that the number of chromosomal associations reported herein are lower than they actually might have been if quasi-bivalent associations had been considered.

In view of the complications described above, it was necessary to establish a set of basic assumptions which would be used as a guide in the analysis and interpretation of homologies in this study. The three hypotheses proposed by McGinnis (22) were adopted and are outlined below:

1. Where closed bivalents occur regularly, true homology must exist.
2. Up to three or four bivalents may be accounted for through homoeologous pairing.

3. Where seven or more pairs occur in a high frequency of cells, one genome is likely to be common to both parents.

$F_1$ hybrids with Ae. juvenalis

1. Ae. juvenalis x Ae. umbellulata ($C^u$)

A relatively high frequency of pairing was found in this hybrid. There was an average bivalent association of 10.09 of which 4.46 were closed bivalents (Table III). This evidence indicates that the $C^u$ genome is present in Ae. juvenalis. This concludion is in agreement with the evidence of Senjaninova-Korczagina (32) and McGinnis (21). The former author based her conclusion on karyomorphological studies of the species in Aegilops which showed that Ae. juvenalis contains five groups of chromosomes which are almost identical to those of Ae. umbellulata. McGinnis (21) based his conclusions on the chromosome pairing observed in crosses of this species with two Aegilops tetraploid species which contain the $C^u$ genome (cf. rev. lit.).

2. Ae. juvenalis x Ae. caudata (C)

This hybrid showed an average bivalent association of 6.16 per cell (Table III). The pairing was composed largely of open bivalents with few chiasmata indicating that there was only weak homology between the caudata and the juvenalis chromosomes. The low number of closed bivalents, an average of .08, is also indicative of remote homology. The amount of pairing observed may be accounted for by the pairing between the C genome of Ae. caudata and the $C^u$ genome of Ae. juvenalis. Studies by Kihara (12) and Sears (29) of caudata-umbellulata hybrids showed an average of five and four pairs respectively. In addition, the homoeologous pairing of the remaining two genomes of Ae. juvenalis could have contributed to the total pairing.

3. Ae. juvenalis x Ae. triuncialis ($CC^u$)

An average bivalent association of 10.18 including 2.27 closed bivalents was observed in this hybrid (Table III). These data support the theory that the $C^u$ genome is common to these species. The number of closed bivalents was lower in this hybrid than in the juvenalis-umbellulata hybrid but this could be attributed to evolutionary changes which resulted in weakened homologies. An example of such evolutionary effects was shown by Kihara and Kondo (15) in the $F_1$ hybrid of the cross Ae. triuncialis ($CC^u$) x Ae. caudata-umbellulata ($CC^u$), the latter being an amphidiploid produced synthetically. This hybrid showed only twelve bivalents in the majority of pollen mother cells whereas fourteen bivalents were expected in view of the fact that Ae. triuncialis is an allotetraploid composed of the Ae. caudata and Ae. umbellulata genomes.

4. Ae. juvenalis x Ae. columnaris ($C^uM^c$)

The $F_1$ hybrid of this cross revealed an average bivalent association of 10.12 with an average of 3.58 closed bivalents (Table III). This frequency of pairing was approximately the same as that shown by the juvenalis-umbellulata hybrid. Since the excess of pairing beyond the seven bivalent associations, which are required to establish a genome in common was largely composed of homoeologous chromosomes in the remaining two genomes of Ae. juvenalis, the D and S, it is not likely that the $M^c$ genome of Ae. columnaris participated to any appreciable extent in the pairing. Consequently, it may be concluded that the results of this cross support the hypothesis of the $C^u$ genome homology, but does not show any evidence of the presence of an M genome in Ae. juvenalis.

5. Ae. juvenalis x Ae. uniaristata ($M^u$)

The average number of bivalent associations in this cross was 4.16 of which .02 were closed (Table III). Although Kihara (14) suggested that Ae. juvenalis contains the $M^j$ genome, the present study does not support this hypothesis. The low pairing frequency observed herein must be taken as contradictory rather than supporting evidence for its presence. The pairing that did occur was probably the result of homoeology between the D and $M^u$ genomes as would be expected on the basis of the report by Kihara (11) of the cross Ae. uniaristata ($M^u$) x Ae. squarrosa (D) in which he found a range of 0-6 bivalents with a mode of four and 0-1 closed bivalents.

Cytological evidence by McGinnis (22) based on a cross of Ae. juvenalis x Ae. ventricosa ($DM^v$) showed only an average of 5.89 bivalent associations. This amount of pairing barely supports the hypothesis regarding the presence of the D genome and certainly offers no support for the presence of the M genome. In his juvenalis-ovata ($C^uM^o$) hybrid an average of 8.62 bivalent associations were observed and considered as evidence for the $C^u$ genome in the juvenalis parent. This again rules out the possibility of the presence of an M genome in this species.

6. Ae. juvenalis x Ae. mutica ($M^t$)

A surprisingly high frequency of pairing was observed in the $F_1$ hybrid of this cross. There was an average of 6.33 bivalent associations including .08 closed bivalents (Table III). On the basis of the crosses involving Ae. juvenalis with species containing the M genome a much lower incidence of pairing would be anticipated.

It is difficult to establish the relationship of Ae. mutica to the other species in the genus Aegilops since only one cross has been reported

with it. This cross, Ae. comosa (M) x Ae. mutica ($M^t$) analyzed by Kihara & Lilienfeld (17) showed a range of 3 to 7 bivalents with an average of 7 which included 3 to 4 closed bivalents. Their analysis of Ae. comosa (M) x Ae. squarrosa (D) showed almost identical results indicating that these 3 species are closely related. However, the juvenalis-mutica hybrid showed pairing frequencies which closely approximated those of the S group hybrids. An examination of Senjaninova-Korczagina's ideograms (32) revealed that the karyotype of Ae. mutica is almost as closely related to Ae. Aucheri as it is to Ae. comosa. The morphology of the heads of the species in the M group typified by Ae. comosa is very different from that of Ae. mutica.

Zhukovsky (36) noted that Ae. mutica closely approaches Ae. longissima ($S^l$) and Ae. bicornis ($S^b$) in structure and that it grows along with Ae. speltoides in central Asia Minor.

Additional evidence which suggests that Ae. mutica may be in the S group is inferred from a cross of T. durum (AB) x Ae. mutica ($M^t$) made by Kihara and Lilienfeld (17). They reported a range of 2-8 bivalents (mode 6-7) with 0-3 closed bivalents. These results were almost identical to those obtained from their cross of Ae. Aucheri (S) x T. durum (AB). This hybrid showed a range of 3-8 bivalents (mode 6-7) with 0-4 closed bivalents. It would appear that the homology reported in the durum-mutica hybrid would be between the B and $M^t$ genomes on the basis of the evidence of Sarkar & Stebbins (28) that the B genome of wheat originated from Ae. speltoides (S). These authors also pointed out that karyotypes of somatic chromosomes with a characteristic morphology, which are found in the B genome of tetraploid emmer wheat, exist only in Ae. speltoides and Ae. mutica among the diploid species of Aegilops.

The foregoing evidence suggests that Ae. mutica is not as closely related to the M group as its symbol indicates and that it may belong to the S group. An analysis of hybrids involving Ae. mutica with members of the S group will be necessary to establish its position with regard to these two groups.

7. Ae. juvenalis x Ae. speltoides (S)

The average number of bivalent associations in this cross was 8.04 including .92 closed bivalents (Table III). This is sufficient to indicate that the genome of Ae. speltoides is represented in Ae. juvenalis. The low number of closed bivalents and the frequent occurrence of trivalents suggest that considerable genic and structural changes have occurred in this genome resulting in a reduced degree of chromosome homology in the hybrid. Of the four hybrids of Ae. juvenalis with the species in the S group, this cross appears to come the closest to the requirements of the assumptions established earlier. It may be inferred that Ae. juvenalis contains a modified S genome. The new symbol $S^J$ is suggested by the author.

8. Ae. juvenalis x Ae. Aucheri (S)

The $F_1$ hybrid from this cross revealed 8.71 bivalent associations on the average including .19 closed bivalents (Table III). The number of bivalent associations in this cross approximated those of the juvenalis-speltoides hybrid as was anticipated in view of the close relationship of Ae. Aucheri to Ae. speltoides. The number of closed bivalents in the juvenalis-Aucheri hybrid was somewhat lower than in the juvenalis-speltoides hybrid but the frequency of trivalents and other multivalents was greater. The multivalents ranged in complexity from a chain of four to a chain of eleven, with only one cell of the 200 analyzed lacking in multivalents.

Such complex associations were not observed in the juvenalis-speltoides hybrid and it is difficult to account for them.

On the basis of the number of bivalent associations, the results of this cross supports the hypothesis that the S genome is present in Ae. juvenalis.

9. Ae. juvenalis x Ae. longissima ($S^l$)

An average of 5.30 bivalent associations was observed in this hybrid including .08 closed bivalents (Table III). This evidence suggests that Ae. longissima is more distantly related to Ae. juvenalis than either Ae. speltoides or Ae. Aucheri. The low average of bivalent associations and particularly the low number of closed bivalents indicate that Ae. longissima is not directly involved in the parentage of Ae. juvenalis. However, the pairing frequencies observed give indirect evidence in support of the theory that the S genome is present in Ae. juvenalis.

10. Ae. juvenalis x Ae. sharonensis ($S^l$)

Ae. sharonensis is considered to be a variety of Ae. longissima differing from it by only one translocation according to Tanaka (35). The juvenalis-sharonensis hybrid had an average of 6.75 bivalent associations which is 1.45 higher than the juvenalis-longissima hybrid (Table III). Otherwise the pairing frequencies were not greatly different. The same conclusion may be formed for this genome as for the genome of Ae. longissima, that is, that Ae. sharonensis was not involved directly in the parentage of Ae. juvenalis.

11. Ae. juvenalis x Ae. cylindrica (CD)

An average of 9.53 bivalent associations were revealed in this hybrid including 1.09 closed bivalents (Table III). This evidence supports the theory of previous workers that Ae. juvenalis contains the D genome.

The low number of closed bivalents suggests that these two species differ considerably in the composition of their D genomes or that their specific combination leads to some degree of asynapsis. The additional pairing over and above the seven pairs accounted for by the $^{D}$ genomes may be attributed to pairing of chromosomes of the $^{C}$ genome of Ae. cylindrica and the $C^{u}$ of Ae. juvenalis.

12. Ae. juvenalis x Ae. crassa ($DM^{cr}$+?)

In the $F_1$ hybrid of this cross an average of 12.82 bivalent associations was recorded including 5.38 closed bivalents (Table III). Since both species contain the D genome, approximately seven of the bivalent associations may be attributed to this genome. The remaining 5.82 associations may be due to the presence of a second genome common to both species but in modified forms, or it may be the result of homoeology of the other genomes of the two species. The discussion of this aspect will be found in the section on $F_1$ hybrids involving Ae. crassa.

$F_1$ hybrids with *Ae. crassa*

The genome analysis of *Ae. crassa* presented an interesting situation in that the majority of the species used in the crosses appeared to have contributed a genome in the evolution of *Ae. crassa*. In at least nine crosses involving widely separated species with *Ae. crassa*, average bivalent associations of from seven to eight were recorded (Table IV). Such a situation might be explained by assuming that *Ae. crassa* is in part autopolyploid, having one of the genomes represented twice in the gametophase. If this were so, the $F_1$ hybrid of a cross between *Ae. crassa* and any diploid species having a non-homologous genome would display an average of seven bivalent associations formed by autosyndetic pairing. An hypothetical example of such a cross could be as follows: *Ae. crassa* ($DDM^{cr}$) x *Ae. speltoides* (S). The $F_1$ hybrid would reveal an average of seven bivalent associations composed largely of pairing between chromosomes of the two D genomes.

There are several reasons that may be advanced to reject the partial autopolyploidy hypothesis. The first of these is based on the frequency of closed bivalents. If autopolyploidy was involved, a much greater number of closed bivalents would be expected than that observed. The second reason is based on the frequency of the total number of bivalents, both closed and open. Should *Ae. crassa* be partly autopolyploid, a cross with a diploid containing the homologous third genome, that is the genome not involved in autopolyploidy, would reveal close to fourteen bivalent associations at metaphase I. An example of such a cross would be as follows: *Ae. crassa* ($DDM^{cr}$) x *Ae. uniaristata* ($M^u$). None of the crosses with diploids in this

study (Table IV) or in studies of previous workers (Table II) showed such a high number of associations. The third reason is based on the frequency of trivalents. Should Ae. crassa be partly autotetraploid, it would be expected that crosses with species containing the genome represented twice in Ae. crassa would reveal close to seven trivalents. An example of such a cross could be as follows: Ae. crassa ($DDM^{cr}$) x Ae. squarrosa (D). None of the results of this study showed such high trivalent frequencies.

The conclusion was made, therefore, that Ae. crassa was not formed through partial autopolyploidy but rather by a combination of genomes from different species, that is through allopolyploidy. A discussion of the individual crosses is presented below.

1. Ae. crassa x Ae. caudata (C)

The $F_1$ hybrid of this cross showed an average of 6.96 bivalent associations which included 2.02 closed bivalents (Table IV). On the basis of this evidence alone it might be concluded that Ae. crassa contains the C genome. However, as the analyses of crosses with other species progressed, similar pairing frequencies were observed. It was concluded from the results of this study and from Kihara's reports (Table II) that Ae. caudata is not involved in the parentage of Ae. crassa. This conclusion was influenced to some extent by Kihara's report (12, 14) that Ae. crassa contains the D and $M^{cr}$ genomes. If the $M^{cr}$ designation is correct, one would expect that a considerable amount of pairing would occur between the D and $M^{cr}$ genomes of Ae. crassa (c.f. rev. lit. fig. 1). Therefore crosses with Ae. crassa and any species containing a non-homologous genome would show in most cases about six pairs formed by the D and $M^{cr}$ genomes. This may be a partial explanation for the high number of bivalent associations observed in many

of the wide crosses made with this species. It might be assumed that it was largely this type of pairing that was encountered in the crassa-caudata hybrid.

2. Ae. crassa x Ae. cylindrica (CD)

There was an average of 10.22 bivalent associations observed in this hybrid including 3.73 closed bivalents (Table IV). Approximately seven of these pairs were likely derived from the D genomes of the respective parents. The remaining three pairs probably occurred through homoeology of the C genome of Ae. cylindrica with one or both of the other two crassa genomes. Homoeology between the two crassa genomes may have been a source of pairing also. If the C genome of Ae. cylindrica was homologous with a genome of Ae. crassa one would expect to find about fourteen pairs at metaphase I. Therefore the low average of bivalent associations in this hybrid indicates that the C genome was not involved in the parentage of Ae. crassa.

3. Ae. crassa x Ae. umbellulata ($C^u$)

The $F_1$ hybrid of this cross showed pairing frequencies similar to those of the crassa-caudata hybrid. There was an average of 6.84 bivalent associations including 1.46 closed bivalents (Table IV). Theoretically such similar results might be expected if the caudata and umbellulata genomes were very similar. However, Sears (29) found an average of only 3.60 bivalents in his caudata-umbellulata hybrid. This dissimilarity of their genomes was not reflected in the cytological analyses of the hybrids of Ae. crassa with these two species. This may be explained by the suggestion made earlier that autosyndetic pairing of chromosomes from the D and $M^{cr}$ genomes of Ae. crassa will form approximately six pairs in most

of the hybrids from crosses with species containing non-homologous genomes. It was concluded that the $C^u$ genome of Ae. umbellulata had no homologous counterpart in the genome complement of Ae. crassa.

4. Ae. crassa x Ae. variabilis ($C^uS^v$)

The average number of bivalent associations observed in this hybrid was 7.12 which included an average of 1.50 closed bivalents (Table IV). This evidence suggests that one of the genomes of Ae. variabilis, in a modified form, may be present in Ae. crassa. Since the possibility of the presence of the $C^u$ genome in this species has been excluded, it is likely that the $S^v$ genome is homoelogous with one of the crassa genomes. However it is also possible that the pairing observed was due to inter-genomic homoeology and that neither of these genomes are directly involved in the genomic constitution of Ae. crassa. Evidence which is to follow tends to support the former possibility.

5. Ae. crassa x Ae. Aucheri (S)

An average of 8.10 bivalent associations including 3.32 closed bivalents was observed in this cross (Table IV). This extent of pairing is sufficient to conclude that Ae. crassa contains the S genome. The incomplete pairing shown in the $F_1$ hybrid is a reflection of the evolutionary changes which have taken place in either or both of the S genomes of the respective parents. However, the frequency of close bivalents is high in comparison with that of other crassa x diploid crosses and is significant in the determination of genome homology.

6. Ae. crassa x Ae. speltoides (S)

Only 24 cells were available for analysis in the $F_1$ hybrid of this cross. However, the results generally agreed with the data obtained from the other crosses in the S group, especially with that of Ae. Aucheri (S).

There was an average of 8.81 bivalent associations which included 3.42 closed bivalents (Table IV). It would appear from the consistent pairing results of the crosses in the S group with Ae. crassa that the S genome is present in this species.

7. Ae. crassa x Ae. sharonensis ($S^l$)

An average of 8.08 bivalent associations with 2.34 closed bivalents was recorded for the $F_1$ hybrid of this cross (Table IV). Generally these results support the hypothesis that the S genome is present in Ae. crassa. The frequency of closed bivalents is a unit lower but the frequency of open bivalents is almost two units higher than that of the crassa-Aucheri cross.

Further evidence to support this hypothesis was found in the $F_1$ hybrid of hexaploid Ae. crassa x Ae. bicornis ($S^b$) of Kihara (12). He reported a range of three to six bivalents with approximately three closed pairs in this hybrid.

Senjaninova-Korczagina (32) in her karyomorphological study of the genus Aegilops found chromosomal characteristics in Ae. crassa which were found only in Ae. bicornis and suggested the formation of Ae. crassa involved this species.

8. Ae. crassa x Ae. mutica ($M^t$)

The $F_1$ hybrid of this cross had an average of 7.74 bivalents including 2.28 closed bivalents (Table IV). These pairing frequencies were considered to be indicative of true homology between the mutica and crassa genomes. These results may be interpreted in either of two ways depending on the validity of the classification of the mutica genome. If it is assumed that the genome designation $M^t$ for Ae. mutica is incorrect

and that this species actually belongs to the S group as suggested earlier then the conclusion would be that this evidence supports the theory that Ae. crassa contains an S genome. If the $M^t$ designation is correct the conclusion could be that the evidence supports the theory proposed by Kihara (14) that Ae. crassa has the $M^{cr}$ genome.

Kihara based his proposal on crosses which involved Ae. crassa and several Aegilops species, some of which contained both the $C^u$ genome and variations of the M genome (Table II). These species were Ae. ovata ($C^uM^o$), Ae. triaristata ($C^uM^t$+?), Ae. columnaris ($C^uM^c$) and Ae. biuncialis ($C^uM^b$). The crosses with these species showed a mode of 7 bivalents at metaphase I. Another cross which appears to support Kihara's proposal was Ae. crassa x Ae. uniaristata ($M^u$) which showed a range of six to nine bivalents (Table II). However the cross Ae. crassa x Ae. ventricosa ($DM^v$) (Table II) gave results which are inconsistent with this proposal. Since both parent species contain a D and an M genome, approximately fourteen bivalents should appear on the average at metaphase I. This cross revealed only six to nine bivalents indicating that only one genome is common to these species.

9. Ae. crassa x Ae. ventricosa ($DM^v$)

An average of 8.92 bivalent associations was recorded for this cross, including 2.74 closed bivalents (Table IV). This agreed with the results reported by Kihara and Lilienfeld (17) and Kihara (12). As indicated earlier, the presence of a D and an M genome in the parents of this hybrid should produce close to fourteen bivalents at metaphase I. Since the presence of the D genome in Ae. crassa has been adequately demonstrated (12), approximately seven pairs might be attributed to this genome. This low frequency of pairing does not appear to be sufficient to admit the presence

of an M genome in Ae. crassa, therefore the $M^{cr}$ designation given by Kihara should be regarded as tentative until hybrids have been analyzed of crosses between Ae. crassa and Ae. comosa (M) or Ae. Heldreichii (M).

10. Ae. crassa x H. villosa (V)

The $F_1$ hybrid of this cross had an average of 7.46 bivalent associations including 1.86 closed bivalents (Table IV). Since this amount of pairing is indicative of genome homology, the possibility exists that the V genome may be closely related to one of the genomes of Ae. crassa. The reports of pairing frequencies between H. villosa and certain Aegilops species indicate that little or no homology exists between their chromosomes (1, 2, 3, 11). However, crosses of H. villosa with all of the diploid species representative of the basic genomes in the Aegilops have not been made. Until this is done, the possibility that the genome of H. villosa is closely related to one of these genomes cannot be excluded.

Considering the alternate possibility that H. villosa is not closely related to any of the Aegilops species, it must be assumed, that the pairing observed in the crassa-villosa hybrid was largely the result of homoeology between the D and $M^{cr}$ genomes of Ae. crassa as suggested earlier. Such an assumption may lead to the objection that in some of the interspecific crosses involving Ae. crassa, which had an average of seven bivalent associations, the pairing was all autosyndetic. However, the occurrance of differential affinity as postulated by Darlington (6) explains such homoeologous pairing in the absence of true homologues and the preference for true pairing in the presence of homologues.

In the final analysis of this problem, therefore, it was necessary to distinguish between homoeologous or autosyndetic pairing and pairing of

true homologues. This was accomplished in the present study by a careful consideration of the frequency of closed bivalents which, it is generally agreed, is a strong indication of close relationship. Where conclusions have been made in this study regarding the presence of homologous genomes, they have been supported by a relatively high frequency of closed bivalents in most cases.

11. Ae. crassa x T. aestivum (ABD)

The results of this cross generally agreed with those of Longley and Sando (20) who found a range of 0 to 7 bivalents with 4 to 7 bivalents being most frequent. The present studies showed an average of 8.03 bivalent associations including 2.24 closed bivalents (Table IV). The low frequency of closed bivalents indicates the extent to which the D genomes of these two species differ.

12. Ae. crassa x T. durum (AB)

A high incidence of asynapsis was observed in the $F_1$ hybrid of this cross. There was an average of 1.05 bivalent associations all of which were open (Table IV). No closed bivalents or multivalents were seen in the 200 cells which were analyzed. These results are similar to those of crosses between Ae. crassa and members of the emmer group which were reported by earlier workers (20).

On the basis of inter- and intra-genome homologies in the Aegilops as demonstrated by non-homologous diploid crosses and illustrated by the chart in Fig. 1 (cf. rev. lit.) it would be expected that approximately four to six pairs would occur on the average in this hybrid. Since the low incidence of pairing observed could be accounted for by the homoeology the A and B genomes of T. durum (18), it could be concluded that the Ae. crassa

chromosomes did not pair autosyndetically. This is contradictory to the evidence from other crosses in thus study. No satisfactory explanation for this discrepancy can be advanced at this time, although the possibility of the presence of a gene which suppresses pairing might be considered.

13. Ae. juvenalis x Ae. crassa.

The results of this cross were considered briefly in the preceding section. It was suggested that the occurrence of an average of 12.82 bivalent associations in this hybrid indicates that two genomes may be common to these species. Since the results of the crosses involving Ae. crassa indicate that the D, $M^{cr}$ and S genomes may be present in this species, it may now be proposed that Ae. juvenalis and Ae. crassa have the D and S genomes in common and differ mainly by their $C^{u}$ and $M^{cr}$ genomes. It is apparent from the low frequency of closed bivalents that their respective D and S genomes are not completely homologous. The reduced amount of pairing and the frequent occurrence of trivalents and higher associations are evidence of genic and structural changes which occurred during their evolution.

14. Ae. crassa x Ae. squarrosa (D)

The $F_1$ hybrid of this cross had an average of 8.37 bivalent associations including 4.42 closed and 2.61 open bivalents (Table IV). This study strongly supports the earlier reports (cf. rev. lit.) that Ae. crassa contains the D genome particularly since the average of closed bivalents was so high. Since up to seven closed bivalents were observed in some cells, a capacity for complete homology of the two genomes of the respective parents is indicated.

Concluding remarks

Determination of the genomic constitution of hexaploid Aegilops species

by the diploid analyser method used in this study is complicated by the inter-relationships of the constituent genomes of these polyploids. Consequently, the method probably should not be considered completely reliable in all cases but rather should be used as a guide to indicate which genomes might be present. Considerable variation can be found in the response of species to this method as has been demonstrated in the present study. The pairing frequencies observed in hybrids involving Ae. juvenalis in this study left little doubt that the genomes of this species are $C^u$, D and $S^j$. In all crosses involving Ae. juvenalis with a species having one of these genomes the pairing was significantly higher than in crosses with other species. On the other hand, the consistently high pairing frequencies in hybrids involving Ae. crassa gave rise to difficulties in the identification of the genomes contributing to this species. There was rather clear evidence for the presence of the D genome and almost as good evidence for the presence of the S genome in Ae. crassa. However the data do not clearly indicate that the $M^{cr}$ genome is present as suggested by Kihara. In fact the data support the presence of the C genome equally as well as the $M^{cr}$. Thus there is evidence that the true genomic constitution of Ae. crassa might actually be CDS. The cross Ae. crassa x Ae. comosa (M) would help to resolve this question and should be studied before any definite conclusions can be made.

When the genomic constitution of these two species has been finally established, they should be synthesized through hybridization and chromosome doubling using the existing diploid species which show the closest genome homologies. The synthetic species should then be crossed with the natural species to determine the degree of evolutionary modification that has occured in the genomes of these species.

REFERENCES


1. BERG von, H. K. Beitrag zur Genomanalyse in der Getreidegruppe Der Züchter 9: 157-163. 1937.

2. BLEIER, H. Zytologische untersuchungen an seltenen Getreide- und Rübenbastarden. Zeit. ind. Abst. u. Vererbgsl., Suppl. 1: 447-452. 1928.

3. _____. Cytologie von Art- und Gattungbastarden des Getreides. Der Züchter 2: 12-22. 1930.

4. BOISSIER, K. Diagnoses plantarum orientalium novarum; Ser. I, 7: p. 129. 1846.

5. BRINK, R. A., COOPER, D. C., AUSHERMAN, L. E., A hybrid between Hordeum jubatum and Secale cereale. J. Heredity 35: 67-75. 1944.

6. DARLINGTON, C. D., Recent Advances in Cytology. London: J. & A. Churchill Ltd., 671 pp. 1937.

7. EIG, A. Monographische - kritische Übersicht der Gattung Aegilops. Repert. Spec. nov. Regn. veg. Beih. 55: 1-228. 1929.

8. FAVORSKY, N. V. Reduction division of $F_1$ hybrids of some Aegilops with Agropyrum intermedium. Soc. Grain Farming, Saratov 6: 115-125. 1936.

9. KATAYAMA, Y. Haploid formation by X-rays in Triticum monococcum. Cytologia 5: 235-237. 1934.

10. KIHARA, H. Conjugation of homologous chromosomes in the genus hybrids Triticum x Ageilops and species hybrids of Aegilops. Cytologia 1: 1-15. 1929.

11. _____. Genomanalyse bei Triticum und Aegilops VII. Übersicht über die Ergebnisse der Jahre 1934-36. Mem. Coll. Agric., Kyoto Imp. Univ. 41: 1-61. 1937.

12. _____. Genomanalyse bei Triticum und Aegilops. II. Systematischer Aufbau der Gattung Aegilops auf genomanalytischer Grundlage. Cytologia 14: 135-144. 1949.

13. _____. Table showing the possible and successful combinations of species hybrids in the Aegilops. Wheat Information Service 1: 13. 1954.

14. _____. Considerations on the evolution and distribution of Aegilops species based on the analyser-method. Cytologia 19: 336-357. 1954.

15. KIHARA, H. and KONDO, N. Studies on amphidiploids of Aegilops caudata x Aegilops umbellulata induced by colchicine. Rep. Kihara Inst. Biol. Res. (Seiken Ziho) No. 2: 24-42. 1943.

16. _____ and LILIENFELD, F. Genomanalyse bei Triticum und Aegilops. IV. Untersuchungen an Aegilops x Triticum und Aegilops x Aegilops - Bastarden. Cytologia 3: 384-456. 1932.

17. _____. Genomanalyse bei Triticum und Aegilops. VI. Weitere untersuchungen an Aegilops x Triticum und Aegilops x Aegilops - Bastarden. Cytologia 6: 195-216. 1935.

18. _____ and NISHIYAMA, I. Genomanalyse bei Triticum und Aegilops. I Genomaffinitaten in tri-, tetra-, und pentaploiden weizen-bastarden. Cytologia I: 263-284. 1930.

19. KOSTOFF, D. Wheat phylesis and wheat breeding from a cytogenetic point of view. Bib. Genetica 13: 149-224. 1941.

20. LONGLEY, A. E. and SANDO, W. J. Nuclear divisions in the pollen mother cells of Triticum, Aegilops and Secale and their hybrids. J. Agr. Res. 40: 683-719. 1930.

21. McGINNIS, R. C. Genome analysis of Aegilops juvenalis. Agr. Inst. Rev. May-June: 36-37. 1954 (Abst.)

22. _____. Genome analysis of Aegilops juvenalis. Can. J. Agr. Sci. 36: 284-291. 1956.

23. MICZYNSKI, K. Genetic studies in the genus Aegilops. II. The morphology and cytology of the interspecific hybrids. Bul. Acad. Polon. Sci. Lettres. Ser. B., 1: 51-83.

24. OSTERGREN, G. and VIGFUSSON, E. On position correlations of univalents and quasibivalents formed by sticky univalents. Hereditas 39: 33-50. 1953.

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

26. RIBBANDS, C. R. The consequences of structural hybridity at meiosis in Lilium x testaceum. J. Genet. 35: 1-24. 1937.

27. ROSHEVITZ, R. A new species of the genus Aegilops L. Bul. Appl. Bot. Gen. and Pl. Br. 18: 413-415. 1927-28.

28. SARKAR, P. and STEBBINS, G. L. Morphological evidence concerning the origin of the B genome in common wheat. Am. J. Bot. 43: 297-304. 1956.

29. SEARS, E. R. Chromosome pairing and fertility in hybrids and amphidiploids in the Triticinae. Mo. Agric. Exp. Sta. Res. Bul. 337, 20 pp. 1941.

30. SEARS, E. R. The amphidiploids Aegilops cylindrica x Triticum durum and Ae. ventricosa x T. durum and their hybrids with T. aestivum. J. Agr. Res. 68: 135-144. 1944.

31. _____. The cytology and genetics of the wheats and their relatives. Adv. Gen. 2: 239-270. 1948.

32. SENJANINOVA - KORCZAGINA, M. Karyo-systematical investigation of the genus Aegilops L. Bul. Appl. Bot. Gen. and Pl. Br. Ser. 2: 1-90. 1932.

33. SMITH, Luther. Haploidy in einkorn. J. Agr. Res. 73: 291-301. 1946.

34. _____. The acetocarmine smear technic. Stain Tech. 22: 17-31. 1947.

35. TANAKA, M. Chromosome pairing in hybrids between Ae. sharonensis and some species of Aegilops and Triticum. Wheat Information Service 2: 7-8. 1955.