CHROMOSOME BEHAVIOR IN A GENUS CROSS^{1*}

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Crosses between different genera of plants are rare and a satisfactory cytological study of such hybrids has never been made. A comparison of chromosome behavior in genus and species crosses is of interest in connection with a comparison of genetic and taxonomic relationships. A cytological analysis should also show the reason why crosses of genera are almost completely or quite sterile and whether there is any possibility of obtaining fertile progeny.

Crosses were made between *Aegilops cylindrica* Host. and *Triticum vulgare* Vill. var. *lutescens* Korn., agronomic variety Marquis. *Aegilops cylindrica* is a wild grass distributed in Italy, the Balkans, South Russia and Asia Minor (PERCIVAL 1921). The culms are hollow and thin-walled and the outer glumes are rounded on the back. The rachis does not disarticulate into separate spikelets, as is the case in certain wild wheats, but is brittle as in *T. Spelta*. The terminal spikelets bear one or two long awns.

Marquis is one of the most important varieties of *T. vulgare*. It is an awnless spring wheat of high quality. The culms are 95 to 115 cm tall, which is about twice the height of the culms of *Aegilops cylindrica*. The heads of the two parents and the F₁ hybrid are shown in plate 1. This photograph was obtained from Doctor E. F. GAINES, who happened to have the same hybrid in his experimental plots at WASHINGTON STATE COLLEGE.

The cross of these two genera resulted in several seeds. These were planted in the greenhouse at BUSSEY INSTITUTION in the fall of 1921. Two F₁ plants were obtained. These plants were unusually vigorous in vegetative development, but were completely sterile. The sterility of the F₁ was also indicated by the appearance of the pollen grains which were obviously imperfect with very few exceptions. The F₁ plants were more or less intermediate in appearance. Doctor GAINES has recently

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made numerous crosses of *Aegilops cylindrica* with several Vulgare wheats and in some crosses about 2 percent of the spikelets set seed.

BELLING'S (1921) method was used to obtain chromosome counts of the parents. *Aegilops cylindrica* has 14 haploid chromosomes and *T. vulgare* has 21 haploid chromosomes. These counts were checked with root-tip preparations of *Aegilops* and sectioned anthers of Marquis wheat (SAX 1922). Camera lucida outlines of the chromosomes in the reduction of the pollen mother cells are shown in plate 1, a.

The F_1 anthers were fixed in a modified Bouin's solution (ALLEN 1916). The material could not be washed and dehydrated at the time, so the anthers were left in the fixing solution almost a year. It was surprising to find that the fixation was unusually good and many excellent preparations were obtained. Sections were cut 10μ to 12μ thick and stained with Haidenhain's iron-haematoxylin. The drawings were done in ink and in all cases were from single sections.

The F_1 plant receives 14 chromosomes from *Aegilops* and 21 chromosomes from the wheat parent. Thus, there should be 35 chromosomes in the somatic cells of the F_1 plant. In species crosses of wheat with the same parental chromosome numbers there are 14 bivalents and 7 univalents in the first reduction division (SAX 1922). One might expect a similar behavior in this genus cross, but such is not the case. At the time of diakinesis in the pollen mother cells there are both paired and unpaired chromosomes, but the number of each cannot be accurately counted at this stage (plate 2, 1). In the metaphase of the first reduction division the bivalents and univalents can be easily distinguished. There are 6 or 7 bivalents surrounded by about 21 univalents. Polar views of the metaphase are shown in plate 2, figures 2, 3 and 4. The grouping of the bivalents surrounded by the univalents is typical and the contrast is made more striking due to the greater size and deeper staining capacity of the bivalents. The number of bivalents is usually 7, but occasionally only 5 or 6 could be identified. The number of single chromosomes that could be counted was often less than 21, but since some of these univalents are frequently at either pole during the metaphase, they may be in adjacent sections or may not be so readily detected. Some of the univalents may not become oriented on the equatorial plate, as shown in plate 2, 5. This behavior is typical, although occasionally the univalents are all on or near the equatorial plate, as shown in plate 2, 6.

Several cases were found where the univalents had lagged behind and divided equationally. In plate 2, 7 there are about 16 lagging univalents which have divided. The lagging univalents can be easily distinguished

DESCRIPTION OF PLATE 1

FIGURE 1.—*Aegilops cylindrica*.

FIGURE 2.—*Aegilops cylindrica* $\times$ *Triticum vulgare* F₁.

FIGURE 3.—*Triticum vulgare* (Marquis).

FIGURE *a*.—Chromosomes of *Aegilops cylindrica* at the time of reduction of pollen mother cells.

FIGURE *b*.—Chromosomes of Marquis wheat at same stage.

from the bivalents because of their size and method of dividing. In most cases, however, the 21 univalents apparently remain at the poles or pass to the poles without dividing. At the telophase there are no chromosomes left on the equatorial plate, but occasionally there appears to be some disintegrating chromatic material in the cytoplasm. A typical case is shown in plate 2, 8. In later stages, however, there are seldom any chromosome fragments or extra nuclei to be found.

The chromosome counts in the metaphase of the second division indicate that the univalents frequently pass to the poles without dividing in the heterotypic division. If the univalents pass to either pole at random there would be from 7 to 28 chromosomes at either pole, but usually (in 81 percent of the cases) 15 to 20. We do find about 17 or 18 chromosomes in the metaphase of the second division in plate 2, 9, and similar counts were obtained in other diads. In the second division there is some lagging of chromosomes in the anaphase, but in most cases the division is comparatively regular (plate 2, figures 10 and 11). If the univalents divide in the first division it is probable that they lag behind and do not divide in the second, while if they do not divide in the first division they will probably divide normally in the second division. Whether the 21 univalent chromosomes divide in the first or second division makes but little difference if they divide but once, as occurs in similar cases in other crosses. In a few cases extra nuclei were formed, but as a rule the second division is regular and normal tetrads are formed (plate 2, figure 12).

DISCUSSION

When species which differ in chromosome number are crossed, the behavior of the chromosomes in the reduction divisions of the F_1 plant varies greatly in different crosses. The behavior of chromosomes in certain species crosses is of interest in comparison with chromosome behavior in the genus cross. The following illustrations indicate the great variability in chromosome behavior in different species hybrids.

1. All chromosomes of the F_1 may fail to pair. *Crepis setosa*, with 4 haploid chromosomes, crossed with *C. capillaris*, with 3 haploid chromosomes, produced an F_1 with 7 chromosomes in the somatic cells. In the reduction division of the F_1 there is no pairing of the chromosomes. The division is irregular and usually the chromosomes pass into the poles without dividing. The F_1 is partially sterile (COLLINS and MANN 1923).

2. A varying number of parental chromosomes may pair in the F_1 reduction division. When *Polypodium aureum* with $34 \pm$ chromosomes is crossed with *P. vulgare*, var. *elegantissimum*, which has $90 \pm$ chromosomes,

DESCRIPTION OF PLATE 2

Preparations from pollen mother cells of the F_1 of *Aegilops cylindrica* $\times$ *Triticum vulgare*. All drawings were made from single sections. Magnification 750 diameters. No reduction.

FIGURE 1.—Paired and unpaired chromosomes in diakinesis.

FIGURE 2.—Polar view of metaphase of heterotypic division. 7 bivalents and 21 univalents.

FIGURE 3.—Heterotypic division with 7 bivalents and 19 univalents.

FIGURE 4.—Heterotypic division with about 6 bivalents and 21 univalents.

FIGURE 5.—Side view of metaphase of heterotypic division showing distribution of bivalents and univalents.

FIGURE 6.—Same as 5 but univalents grouped.

FIGURE 7.—Division of the lagging univalents.

FIGURE 8.—Telophase of heterotypic division.

FIGURE 9.—Metaphase of second division with about 18 chromosomes on each equatorial plate.

FIGURES 10,11.—Late anaphase and telophase of homeotypic division.

FIGURE 12.—Normal tetrad.

an F_1 plant is produced which has $124 \pm$ somatic chromosomes. In the first reduction division there are 95 to 125 chromosomes, indicating that a varying number of the parental chromosomes pair. The division is irregular and the F_1 is quite sterile (FARMER and DIGBY 1910).

3. All chromosomes of one parent may pair with some of the chromosomes of the other parent. In crosses of 2 species of *Drosera* with 10 and 20 chromosomes, respectively, the 10 chromosomes of one parent pair with 10 of the other parent, leaving 10 single chromosomes (ROSENBERG 1909). In rose hybrids bivalents and univalents are found in multiples of 7. Certain hybrids have 7 bivalents and 7, 14, 21 or 28 univalents (BLACKBURN and HARRISON 1921, TÄCKHOLM 1922). In crosses of *Triticum durum* or *T. polonicum* with *T. vulgare* the 14 chromosomes of *T. durum* pair with 14 chromosomes of *T. vulgare*, leaving 7 single *vulgare* chromosomes (KIYARA 1919, SAX 1922). The bivalents divide normally but the univalents are irregularly distributed. Various degrees of sterility occur in the F_1 of such hybrids.

4. The chromosomes contributed by one parent may not pair with those of the other parent, but pairing may occur among the chromosomes contributed by only one of the parents. This method of chromosome behavior is found in crosses of *Crepis setosa* ($n=4$) with *C. biennis* ($n=20$). In the F_1 meiosis the 20 *biennis* chromosomes pair with each other while the 4 *setosa* chromosomes remain unpaired. The divisions of the pollen mother cells are comparatively regular.

5. All of the chromosomes may pair, even when one or two extra sets of chromosomes are present. In triploid *Daturas* there are 36 somatic chromosomes. In the first reduction division the chromosomes are grouped in 12 sets of 3 chromosomes each. In the first division of the pollen mother cells 2 chromosomes of each set go to one pole and the remaining chromosome goes to the other pole. In tetraploid plants the chromosomes unite in groups of four (BELLING and BLAKESLEE 1923). The triploid plants are partially sterile but tetraploids are almost completely fertile.

The behavior of the chromosomes in the genus cross of *Aegilops* $\times$ *Triticum* does not differ greatly from the behavior in certain species crosses. In this cross 7 *Aegilops* chromosomes presumably pair with 7 wheat chromosomes, leaving 21 single chromosomes, 7 of which are from *Aegilops* and 14 from the wheat parent. The bivalents divide normally, but the univalents are apparently distributed at random without dividing in one of the reduction divisions. The discussion of chromosome behavior in the cross of *Aegilops* $\times$ *T. vulgare* will be based on the

typical chromosome behavior for calculating chromosome recombinations.

Sterility in these crosses can be attributed to the incompatibility of certain chromosomes and to differences in parental chromosome number which results in univalent chromosomes at the time of the reduction divisions in the F_1 hybrids. In certain cases at least the gametic and somatic perfection increases as the chromosome number approaches some multiple of a basic chromosome number. In *Triticum* and *Rosa* this number is 7. The random distribution of univalents results in a frequency distribution of gametic chromosome numbers in which the parental numbers, or numbers which are a multiple of the basic number, are seldom recovered.

In the F_1 hybrid of *Triticum durum* $\times$ *T. vulgare* it was assumed that the 14 chromosomes of the *durum* parent paired with 14 of the 21 *vulgare* chromosomes. This assumption is based on the fact that in general the maternal and paternal chromosomes pair, and on the genetic behavior of F_2 segregates. *Crepis biennis* with 20 chromosomes does not differ greatly from certain species with 4 or 5 chromosomes. Back-crosses of *C. setosa* $\times$ *C. biennis* with *biennis* give segregates with 10 *biennis* chromosomes and the *biennis* type of plants (COLLINS and MANN 1922). In view of these facts it is not improbable that *biennis* contains 4 similar sets of chromosomes. In wheat, on the other hand, the species with 7, 14 and 21 chromosomes differ greatly in taxonomic, ecological and economic characters (SAX 1923). A study of crosses between the 14- and 21-chromosome groups shows that the 7 *vulgare* chromosomes which do not pair in the reduction division of the F_1 plant, determine the typical *vulgare* characters and are therefore probably different in genetic constitution from the 14 *vulgare* chromosomes which pair. It is possible, however, that the *vulgare* characters are due simply to duplication of hereditary factors. The fact that all 14-chromosome segregates in F_2 are of the Emmer type would indicate that the 14 *vulgare* chromosomes which pair are similar to the 14 *durum* chromosomes and would therefore be expected to pair with them.

The assumption that sets of chromosomes in wheat do not pair with each other is substantiated by the chromosome behavior in the cross of *Aegilops* $\times$ *T. vulgare*. In this cross there are 21 single chromosomes, 14 of which must be from one of the two parents, whether the 7 bivalents consist of 7 *Aegilops* and 7 wheat chromosomes or consist of 2 sets of wheat chromosomes.

The sterility of the F_1 hybrid between *Aegilops cylindrica* $\times$ *Triticum vulgare* can probably be attributed to chromosome incompatibility

and to the irregular behavior of the chromosomes in the reduction divisions. If the 21 univalents pass at random to one pole or the other, according to the laws of chance, then the F_1 gametes will contain from 7 to 28 chromosomes. In hybrids of wheat species with different chromosome numbers the gametes with an intermediate chromosome number are more or less sterile and the functional gametes have a chromosome number which approaches a multiple of 7. If all gametes with chromosome numbers in multiples of 7 survive in the *Aegilops* $\times$ *T. vulgare* cross, then there would be 1 functional gamete in 2,097,152 with 7 chromosomes, 1 in 18 with 14 chromosomes, 1 in 18 with 21 chromosomes, and 1 in 2,097,152 with 28 chromosomes. However, there is reason to believe that each one of the sets of 7 chromosomes may consist of specific chromosomes which cannot usually be interchanged with those of another set. The chance of getting only the 7 chromosomes of any one set at one pole is only 1 in 38,760 times. Evidently there would be little chance of obtaining functional gametes if only the above combinations survive. The fact that a few seeds are obtained in certain *Aegilops* $\times$ wheat crosses would indicate that functional chromosome combinations are not entirely confined to the above limitations. It will be of interest to follow the chromosome behavior in these F_2 segregates.

If single chromosomes are assorted at random, it is theoretically possible in this genus cross to get a segregate with a chromosome number greater than that of either parent. The union of two 28 chromosome gametes would result in an octoploid species. In the wheat species there is in general an increase in adaptability and economic value as the chromosome number increases from 7 to 21. A new species with 28 chromosomes might be of even greater value than existing varieties.

Although it is improbable that a species with 28 chromosomes would ever be obtained from the cross of *Aegilops* $\times$ *Triticum vulgare*, the possibilities of a similar behavior in other crosses is of interest in connection with the origin of certain wheat species. PERCIVAL (1921) believes that *Triticum vulgare* originated from a cross of *Aegilops* with a wheat species of the Emmer group. According to PERCIVAL,

“crossing between mutants of the same specific prototype is, I think, sufficient to account for many, if not all, of the moderate number of forms found among the races of the Emmer series, but the extraordinary complexity and almost endless number of varieties and intermediate forms of the *vulgare* race can only be satisfactorily explained by the assumption of its hybrid origin from two or more distinct species.”

The characters which distinguish the Vulgare group from the Emmer group are all found in *Aegilops ovata* and *Aegilops cylindrica*.

Both of the above species of *Aegilops* have 14 haploid chromosomes. In a cross of *Aegilops* with a wheat of the Emmer series there would be 28 chromosomes in the somatic cells of the F_1 . If 7 *Aegilops* chromosomes paired with 7 wheat chromosomes, as seems probable from the behavior of chromosomes in the cross of *A. cylindrica* $\times$ *T. vulgare*, there would be 7 bivalents and 14 univalents at the time of the reduction division. The random distribution of univalents would result in a 21-chromosome gamete once in 16,384 times. The chances of combining two 21-chromosome gametes are of course rather small. However, in the semi-arid climate of the Mediterranean region cross-pollination probably occurs rather frequently, and in the course of long periods of time such crosses and recombinations might occur which would result in a species of the *vulgare* type. This hypothesis is in accord with the taxonomic, genetic, and cytological relationships of the wheat species and related genera.

The chromosome behavior in the cross of *A. cylindrica* $\times$ *T. vulgare* offers an interesting comparison of genetic and taxonomic relationships. These two genera differ greatly in morphological characters and in adaptability to climatic conditions. In every respect they seem to merit different generic classification. Certainly the differences are as great as the differences between *T. vulgare* and *Secale cereale*. Although these two genera differ greatly in taxonomic characters they are apparently more closely related genetically than certain species, as indicated by the greater compatibility of the parental chromosomes in F_1 hybrids. In this genus cross 7 chromosomes of *Aegilops* are apparently compatible with 7 chromosomes of *T. vulgare*. On the other hand a cross of two species of *Crepis* with 3 and 4 haploid chromosomes, respectively, the chromosomes are so incompatible that the F_1 plant dies at an early stage (BABCOCK and COLLINS 1920). In another cross of *Crepis* species with 3 and 4 chromosomes, respectively, the F_1 is vigorous but the chromosomes are so incompatible that they do not pair in the reduction division of the pollen mother cells (COLLINS and MANN 1923). These species of *Crepis* are apparently more similar in taxonomic characters than are *A. cylindrica* and *T. vulgare*, but the chromosomes are more incompatible than in the genus cross. Evidently genus and species classification is not always in accord with chromosome relationships.

SUMMARY

A cross of *Aegilops cylindrica*, a wild grass found in the Mediterranean region, with Marquis wheat, one of the leading varieties of *Triticum vulgare*, resulted in vigorous, but completely sterile, F_1 plants.

Aegilops cylindrica has 14 haploid chromosomes while the wheat variety used has 21 gametic chromosomes. Thus the F_1 plant has 35 chromosomes in the somatic cells. In the first reduction division of the pollen mother cells of the F_1 plant there are about 7 bivalent and 21 univalent chromosomes, due presumably to the pairing of 7 *Aegilops* chromosomes with 7 wheat chromosomes. If the single chromosomes are distributed at random in the reduction divisions as appears probable, then the chromosome number of the gametes will vary from 7 to 28.

Sterility in this genus cross is due to the incompatibility of certain chromosomes and to chromosome differences in the parents, both of which factors result in univalent chromosomes in the reduction divisions of the F_1 hybrid. With random distribution of the 21 single chromosomes there is little chance of obtaining gametes in multiples of 7. If only gametes with a chromosome number in multiples of 7 survive, as is the case in crosses of wheat species, then most of the sterility can be accounted for. If each set of 7 chromosomes must consist of specific individuals then the chances of recovering fertile gametes are extremely small.

Theoretically, it is possible for a new species with 28 chromosomes to be derived from this genus cross, but the chances for such an occurrence are extremely small.

The behavior of the chromosomes in this cross suggests the possible origin of the *vulgare* wheats from a cross of *Aegilops* with a wheat species of the Emmer series.

The chromosomes of the two genera, *Aegilops* and *Triticum*, are more compatible with each other, as indicated by the number which pair, than the chromosomes of certain species within a given genus.

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