

CYTOGENETIC STUDIES ON TRITICALE. I. A METHOD
FOR DETERMINING THE EFFECTS OF INDIVIDUAL
SECALE CHROMOSOMES ON TRITICUM¹

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THE production of fertile, true-breeding hybrids which combine the characters of two different species or genera has proven to be of theoretical and practical importance. These hybrids have usually arisen through a doubling of the chromosome complement following the initial cross, this doubling resulting in the presence in the fertile hybrid of two full complements from each parent. Such plants are known as amphidiploids because the chromosome set of each of the two parents is present twice, or in the diploid condition. It is possible, however, to combine characters of two species or genera by a method which involves the addition of one or more chromosomes from one species to the full diploid complement of the other species. When the chromosomes of one species have desirable and undesirable dominant characters distributed among the various chromosomes of the set, these characters are given to the second species without selection in the amphidiploid. If only the chromosomes with the desired dominant characters could be added to the chromosome complement of the second species, the value of the combination for theoretical and practical purposes would be greatly enhanced. Such combinations have been secured between *Secale* and *Triticum*, and the method by which they were obtained should be applicable to other genera where an amphidiploid is available.

The method, in brief, involves the production of an amphidiploid between two species or genera, A and B. The amphidiploid is then crossed to one of the parents—for illustration, species A. The individual obtained from this latter cross would have two full sets of chromosomes from species A and one full set from species B. During meiosis, the two sets from species A would undergo normal pairing and distribution, whereas the set from species B would remain as univalents and would be distributed at random. Thus the gametes from such a plant would contain a full set of chromosomes from species A, and from none to a full set from species B. From such a plant the offspring would contain two full sets of chromosomes from species A and from none to various combinations of the chromosomes of species B.

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CHROMOSOME BEHAVIOR OF THE PARENTAL TYPES

The amphidiploid used in these experiments (produced by E. R. SEARS) consisted of spring rye, *Secale cereale*, and "Chinese" spring wheat, *Triticum vulgare*, and was derived directly from the first generation hybrid by duplication of the chromosomes in somatic tissue. These three types may be seen in figure 1, where a is the rye, c the wheat, and b the amphidiploid. The wheat-rye hybrid has been studied cytologically by KIHARA (1924), THOMPSON (1926), LONGLEY and SANDO (1930), FLORELL (1931), and KATTERMANN (1934). KIHARA reported that the hybrid, at metaphase 1, had from one to three bivalents, and his report was confirmed by THOMPSON, who found the same numbers of bivalents, and who further reported that he believed the bivalents to be the result of autosyndesis among the wheat chromosomes. LONGLEY and SANDO observed no pairing in this cross but found the same number of bivalents, from one to three, in a hybrid involving *Secale montanum*. FLORELL observed that in the wheat-rye hybrid, involving Hybrid 128 wheat and Rosen rye, from one to three bivalents were formed, approximately half the cells having two bivalents. KATTERMANN reported as many as six bivalents in some cells. The amphidiploid between wheat and rye has been made several times, and its cytology has been most thoroughly investigated by MÜNTZING (1939). All the amphidiploids reported thus far have been asynaptic to some degree, univalents occurring at metaphase 1. The amphidiploid used in these experiments had approximately 43 percent of the cells without univalents. No Triticale strain with a perfectly regular meiosis has been reported. As a result of this irregularity, variations occur in the chromosome numbers of the gametes and of the progenies.

For the purposes of the present method of procedure, the production of a plant with two full complements of wheat chromosomes and one full complement of rye chromosomes is absolutely necessary. An amphidiploid sector on a wheat-rye hybrid was backcrossed to wheat. A single plant from this cross was examined cytologically and found to have 21 normally-behaving pairs of wheat chromosomes and seven univalent rye chromosomes. This plant was allowed to self, and from it 70 seeds were obtained.

THE PROGENY OF THE PLANT DIPLOID FOR WHEAT AND
HAPLOID FOR RYE CHROMOSOME COMPLEMENTS

The 70 seeds obtained from the plant which had two wheat complements and one rye complement produced 70 plants. These plants had 21 pairs of wheat chromosomes and various numbers of rye chromosomes, ranging from one to seven. The presence of rye chromosomes in these plants produced very striking effects on the morphology of the plants. All the rye chromosomes occurred as univalents, which were distributed in the random fashion usual for univalents.

One plant among the 70 is interesting in that it had 28 univalent chromosomes at the first meiotic division. Presumably, this plant resulted from the parthenogenetic development of an egg which had received the full set of wheat chromosomes plus all seven of the rye chromosomes. This plant, then, should have the same constitution as a first-generation wheat-rye hybrid, since it had a haploid set of wheat and a haploid set of rye chromosomes.

Twenty-nine of the 70 plants were selected for carrying on the rye chromosomes to the next generation. These plants were quite fertile, possessed certain rye characters, and showed 21 pairs of normally-behaving wheat chromosomes plus one or two rye univalents at metaphase I. The plants were selfed by bagging the heads before anthesis. From the 29 progenies of these plants, a single plant which carried the character under consideration, was selected from each of 18 progenies, and these 18 plants were selfed. The selfing was carried on, in this generation, to reduce the number of rye chromosomes to one. Since the rye chromosomes were present as univalents, they tended to be lost at meiosis for two reasons: (1) univalents are often lost in the cytoplasm at meiosis, where they form micronuclei; (2) each univalent is distributed to but half the gametes, and so the number decreases in successive generations. These 18 plants consisted of nine plants which had hairy-neck, two which had tapered heads, and seven which had awns. These characters were selected for study because their distribution among the 70 plants indicated that they were in different chromosomes. The progenies of the 18 plants were examined cytologically.

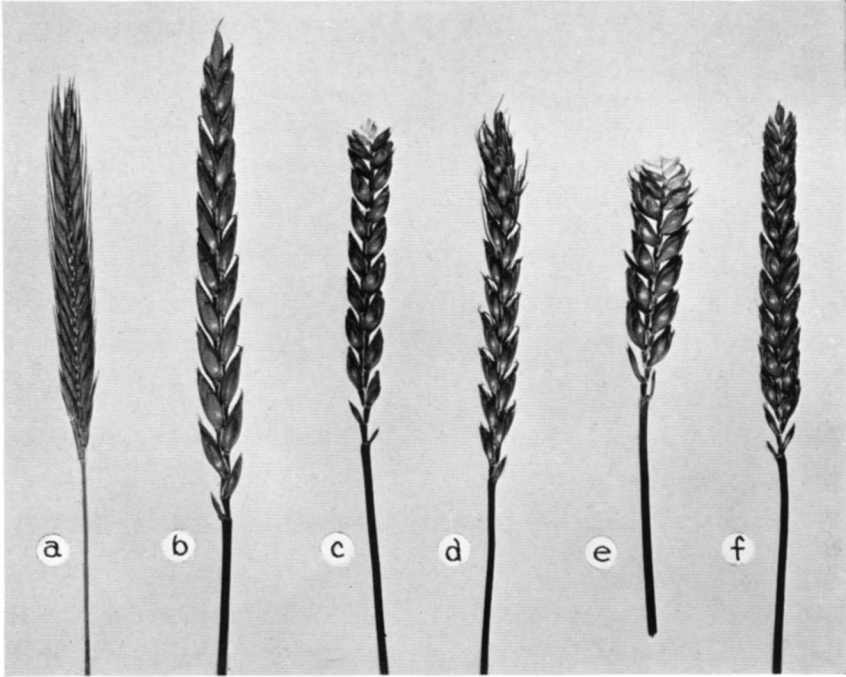
In the progenies of eight of the nine hairy-neck plants, approximately one third of the individuals had hairy-neck. In the progeny of the remaining hairy-neck plant, consisting of 19 individuals, all the plants had hairy-neck. Upon examination, all these 19 individuals proved to have at least 22 pairs of chromosomes, that is, 21 wheat bivalents plus one rye bivalent. Three of the 19 plants had 23 pairs of chromosomes. In the progenies which were segregating for hairy-neck, all but two of the affected plants had the rye chromosome present as a univalent. These two plants had 22 pairs of chromosomes. They were disomic for the rye chromosome and similar in appearance to the plants of the progeny which had all 22-chromosome plants. The rye chromosome, when disomic, did not always form a bivalent but was present in a small percentage of cells as two univalents, and when present as a bivalent, often separated when the wheat chromosomes were at anaphase.

The hairy-neck chromosome had a marked effect upon the plant, other than the characteristic pubescence just below the head. Since all the plants which had the character were nearly identical in appearance, it is very probable that but one of the rye chromosomes carries the factor or factors involved in producing the character. Those plants which were monosomic

for this chromosome were slightly shorter than normal wheat plants and had heads with broader, coarser spikelets. When the chromosome was disomic, these alterations were exaggerated, and the plants were approximately two thirds the height of normal wheat, and had coarser, broader spikelets than the plants monosomic for the chromosome. These differences may be seen by comparing c, a normal head, with e, a head disomic for the hairy-neck chromosome, in figure 1. A comparison of the amounts of pubescence on the necks of plants monosomic and disomic for the rye chromosome and on the neck of the amphidiploid is interesting, in that such a comparison shows that the amphidiploid has the least pubescence, although it has the rye chromosome present in two doses. This comparison would seem to indicate that no accurate predictions of the degree of manifestation of any character can be made from the amphidiploid. The derived type may be more like one parent than like the amphidiploid, although both have identical doses of the chromosome concerned.

The rye chromosome which carries the factors or factor producing tapered head, imparts to the individual possessing it a head without any compacting at the tip, such as is characteristic of the wheat parent. The two progenies having this character were found to be different, one retaining several extra rye chromosomes. This one was not studied. The other progeny consisted of 30 plants, five of which had the character. Four of these five were monosomic for the chromosome, and the other plant was disomic for it. This rye bivalent was less stable than the hairy-neck bivalent, occurring in many cells as two univalents. The univalents can always be identified as rye chromosomes—and not wheat—by their larger size and consistent form. The effect of this chromosome on the plant, other than that of producing the tapered head, was to increase the height of the plant so that individuals carrying the chromosome as a monosome were slightly taller than normal wheat plants, and the plant which carried the chromosome as a disome was over a third taller. The effect of this chromosome, therefore, seemed to be additive, since the disomic was taller than the monosomic plant. The difference, however, was not so marked as with the hairy-neck chromosome.

The term "tapered" was applied to this character because the heads of the affected plants did not have the compacted tip characteristic of the parental wheat, but tapered continuously from basal to apical spikelets. A comparison of head c—which is normal—and f, which carries the chromosome as a disome, will show the difference. At least one other rye chromosome, however, has a similar effect, and the term was used for this chromosome because no clearly separable effect, other than "tapered," was detectable, whereas another chromosome which produces the same effect carries, in addition, a factor for producing awns.



DESCRIPTION OF FIGURE 1

Heads of plants of various chromosome constitutions. ($\times \frac{3}{2}$)

- a. *Secale cereale* involved in amphidiploid illustrated at b.
- b. Amphidiploid involving a, *Secale cereale*, and c, *Triticum vulgare* var. "Chinese spring."
- c. *Triticum vulgare* var. "Chinese spring" involved in amphidiploid illustrated at b.
- d. Triticum to which has been added the *Secale* chromosome producing awns. The chromosome is present as a disome.
- e. Triticum to which has been added the *Secale* chromosome producing hairy-neck. The chromosome is present as a disome.
- f. Triticum to which has been added the *Secale* chromosome producing tapered-head. The chromosome is present as a disome.

The seven progenies from awned plants did not fall into distinct classes; five were segregating, with approximately one third of the individuals awned, one had seven awned plants and two normal wheat plants; and one progeny consisting of 13 individuals had all awned plants. The segregating progenies had an extra univalent chromosome, and the group with all awned plants had an extra bivalent. This bivalent seemed to be fairly regular in behavior, and although it sometimes separated late, most cells at anaphase 1 had 22 chromosomes at each anaphase pole.

The chromosome bearing the factor or factors for awns had the most striking effect on the plant, especially when disomic. The plant is usually shorter than normal wheat, has no compacting of spikelets at the tip, has many fine, dark green leaves when young, is inclined to be spreading in habit, and possesses awns approximately one fifth the length of those of the rye parent. The awns are not uniform throughout the head, being nearly absent from some spikelets, and generally longest at the tip of the head, as may be seen in *d* of figure 1.

In one segregating progeny a plant was discovered which had 23 bivalents and one univalent. This plant had both awns and hairy-neck, so that two of the three chromosomes represented may be accounted for by phenotypic effects. No effect of the other chromosome or chromosome pair was observable.

In addition to the progeny of the single plant secured by backcrossing the amphidiploid to the wheat parent, the progeny of one other plant of similar parentage was grown. Of this progeny seven plants were examined cytologically. One of these had 22 pairs of chromosomes plus two univalents,—an instance which indicates that the bivalents may be added directly in the progeny of the backcrossed plant, although none of the original 70 plants had such an extra bivalent.

THE INTRODUCTION OF RYE BIVALENTS

Thus far all attempts to secure rye characters in wheat-like plants have been made by using the F_1 hybrids in backcrosses to wheat. This method has, in some respects, proved quite practicable, as the results of LEIGHTY and TAYLOR (1924), of FLORELL (1931), of TAYLOR (1939), of KATTERMANN (1934), (1935), (1937), (1938), and of LEDINGHAM and THOMPSON (1938) indicate. Any additions to the basic wheat complement obtained by this method may, however, consist of wheat chromosomes or chromosome pairs and not of rye, as LEDINGHAM and THOMPSON concluded from their studies on progenies of wheat-rye hybrids backcrossed to wheat. KATTERMANN's extensive studies on plants secured by this method of using the F_1 hybrid indicate that the female gametes of the hybrid may have from 17 to 31 chromosomes. While variations occur in the gametes of the am-

phidiploid, none of this order are indicated by the data of MÜNTZING (1939). The hybrid, from a cytological viewpoint, at least, is comparable to a haploid; and SEARS' (1939) studies on the progeny of a haploid wheat indicate that duplications of whole chromosomes occur in the female gametes. These duplications in the hybrid should involve wheat chromosomes three times as often as rye. Further, the haploid was found by SEARS to produce very aberrant progeny—monosomes, trisomes, and segmental interchanges—all of which would usually be undesirable where the process of addition is to be carefully controlled.

The origin of the present wheat plants disomic for a rye chromosome is capable of two interpretations. Since none of the 70 original plants—secured from the plant with two wheat complements and one rye complement—had pairs of rye chromosomes, these must have occurred in a later generation. That the chromosome is not usually transmitted through the pollen may readily be concluded, since in segregating progenies disomic plants are rare; and no pairs were found among the 70 plants where a maximum probability exists for finding pairs. These disomic plants, however, may occasionally occur as the result of a monosomic pollen grain competing successfully with normal pollen. The other possibility is that in a plant with a single rye chromosome non-disjunction—or some other meiotic anomaly—may occur to produce a disomic egg which, when fertilized by a normal pollen grain, would produce a disomic plant. Either of these methods is possible, and each may be tested by appropriate backcrosses to normal gametes of eggs and pollen from monosomic plants.

Should these disomic plants prove not to be true-breeding—and so resemble those of LEDINGHAM and THOMPSON—they should nevertheless, by chance distribution, make available monosomic and disomic plants in appreciable numbers in succeeding generations.

Another method could be used to secure these results. If the plants with rye univalents were pollinated by the amphidiploid, each univalent would be supplied with its homologue. Those chromosomes without homologues would be eliminated by successive selfings.

The failure of the rye chromosomes to pair as regularly as the wheat chromosomes is difficult to explain. It may represent some inability of rye to operate normally in a cell which is almost wholly wheat. The tendency to asynapsis may also be due to the absolute homozygosity of the rye chromosome and the consequent uncovering of recessive factors which have deleterious effects on the regularity of meiosis. However, all such factors now known affect meiosis as a whole and not merely the single chromosome which bears them.

USES OF THE DERIVED TYPES

These plants with single chromosomes from one species added to the full unaltered chromosome complement of a wholly different species, have several obvious practical and theoretical uses. They could be used to determine efficiently what factors dominant to the genome upon which they were superposed were linked together in the single chromosome. By crossing two plants homozygous for different chromosomes, the interaction of these two known chromosomes could be studied. By using the plants in which single chromosomes had been isolated, various tests for disease resistance, hardiness, and various other factors important economically, could be made to determine if the trait desired were located in a single chromosome or were a property of the whole complement. Such information should indicate whether any single property desirable in one genome could be transferred to the other through rare non-homologous pairing or through induced translocations. Such useful transfer is obviously impracticable where the desirable character is a property of the whole genome or of many factors located in different chromosomes.

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SUMMARY

By backcrossing an amphidiploid to one of its constituent species and selfing for several generations, individuals were produced with a single chromosome pair from one species in the amphidiploid added to the unaltered chromosome complement of the other species. These additions represent three different rye chromosomes, phenotypically detectable, which have been added to an unaltered wheat genome. The method should be applicable to other genera where an amphidiploid is available and may have some value in practical and theoretical genetics.

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