A CYTOGENETIC ANALYSIS OF THE HYBRID SECALE CEREALE L. × SECALE MONTANUM GUSS. AND ITS PROGENY

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WITHIN the genus Secale L. hybrids between various perennial forms have all shown regular meiotic chromosome behavior as have hybrids between different annual strains belonging to *S. cereale* L. In all hybrids between *S. cereale* and perennial forms, however, a translocation figure has been observed at metaphase I in the PMC's. The maximum association is apparently a ring-of-six, but this is seldom observed, which probably accounts for the earlier reports (SCHIEMANN and NURNBERG-KRUGER 1952; PRICE 1955) of a chain-of-six as the maximum association.

Observations on recurring diakinesis configurations in the hybrid *S. cereale* L. × *S. montanum* Guss. have permitted identification of each of the chromosomes within the translocation figure together with the determination of the relative size and position of the translocated segments. New homologies evident in the F₂ segregants from this hybrid have made possible additional interpretations of the structural relationships of the two species.

MATERIALS AND METHODS

Most of the hybrids studied were made during the summers of 1952 and 1953. Since most strains of Secale are self incompatible, it was possible to make the hybrids by bagging together the heads of the two parents or by transferring flowering culms from the male parent to the female parent. Since *S. africanum* is self-compatible, the flowers were emasculated when this species was used as the female parent.

The F₁ population was derived from intercrosses between several different sister F₁ hybrids. For this study all female parents were emasculated before hybridization. Of 550 F₂ seeds sown, 303 seedlings survived until the time for transplantation. As nearly as possible, they were all given the same horticultural treatment before and after transfer to the garden.

Fixations of the entire spike, less the glumes and awns, were made in 1:3 aceto-alcohol. After about 24 hours, they were washed two or three times in 70 percent alcohol and stored under refrigeration.

Most observations were made from temporary aceto-carmine smears. Some permanent slides were prepared by MCCLINTOCK's method (1929) but most of them were prepared by a modification of the alcohol-vapor-chamber method described by BRADLEY (1948).

The analyses of multiple chromosome associations were found to be most satisfactory in cells at diakinesis. At earlier stages the chromosomes are too long and interwoven to allow accurate analysis; at a later stage the contraction is so extreme that associations are often hidden. The interpretations presented in this paper are based only on recurrent associations and in the cases of the F₁ hybrids, only on associations observed in several different plants.

RESULTS

Cytology of the species hybrids: Collections from various sources were utilized in making hybrids between representatives of the major taxonomic groups. All hybrids between perennial forms (members of the series *Kuprijanovia* Roshev., according to ROSHEVITZ 1948), are highly fertile and show good chromosome pairing at meiosis. This is true also for hybrids between the annual representatives of the series *Cerealia* Roshev. (ROSHEVITZ 1948). Hybrids between the perennial and annual species, however, are partly sterile and show a six-chromosome translocation configuration at meiosis.

Since the Mediterranean perennial forms *S. anatolicum* Boiss., *S. dalmaticum* Vis., *S. Kuprijanovia* Grossh., and *S. montanum* Guss., of ROSHEVITZ (1948), are so similar cytologically, genetically and morphologically, they are considered to be ecotypes of one genetic species and are referred to in this paper as *S. montanum*.

Cytology of the F_1 hybrid S. cereale L. × S. montanum Guss.: The maximum association observed at diakinesis and metaphase in the F_1 hybrid consists of four ring-bivalents plus a translocation configuration made up of six chromosomes. Of the our bivalents, three are essentially homomorphic, including the one associated with the nucleolus. The fourth pair, however, shows peculiar stretching in the median region (figs. 3, 6, 12) resulting in a configuration which superficially resembles two isochromosomes. Some of the configurations examined suggest that one of the parental types is deficient for median chromosomal material present in its homologue and that during synapsis it has undergone extreme stretching in this region in response to the forces of homologous pairing in the distal portions.

The translocation configuration: The maximum association during meiosis of the hybrid has previously been reported to consist of a chain of six chromosomes plus four ring-bivalents (SCHIEMANN 1952; PRICE 1955). However, in the current investigation, a few cells have been observed in which a complete ring-of-six is formed (fig. 1; table 1).

From a detailed analysis of the chromosome associations in the F_1 and from the analysis of aberrant F_2 segregants, which will be presented below, it has been possible

TABLE 1

Frequencies of multiple configurations in the hybrid c × m as found in 100 cells of two different plants at MI

Maximum association	Plant no. 516-3	Plant no. 516-10
Ring-of-six	1	0
Chain-of-six	25	26
Chain-of-five	17	18
Chain-of-four	25	16
Two chains-of-three	8	6
Chain-of-three	13	18
Bivalent	11	16
Total	100	100

to establish that the pachytene translocation configuration in the F_1 may be diagrammed as follows:
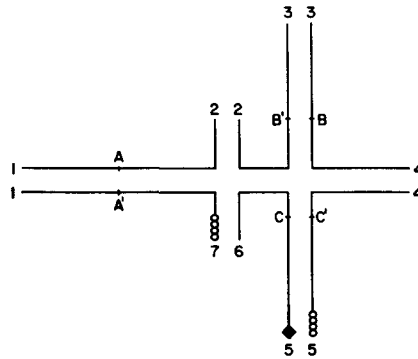
And that the parental chromosomes belonging to the translocation group are therefore:

The arms which are usually free at diakinesis are numbered as 6–7. A satellite unique to the 5-C-6 *cereale* chromosome is shown as ◆——. A duplication segment found within the *montanum* genome is shown at the 5 and 7 arms of the *montanum* chromosome as ——∞∞.

The origin of the satellite: Most cytological descriptions of *S. cereale* (HASEGAWA 1934; LIMA-DE-FARIA 1952) have reported only one satellited chromosome which is regularly associated with the nucleolus. However, LEVAN (1942) has shown that in addition to the chromosome associated with the nucleolus (S^1), another chromosome (S^2) regularly has a smaller ovoid satellited knob. It is possible that this S^2 chromosome is the same as the satellited chromosome in the translocation figure. However, as can be seen in figures 3, 6–10, the satellite appears to be as wide as the rest of the chromosome, whereas LEVAN reported that the knob of the S^2 chromosome is considerably smaller in diameter than the rest of the chromosome. Since his examinations were made subsequent to cold treatments which accentuated the secondary constriction, his observations are not strictly comparable to these diakinesis illustrations in which no pretreatment was applied. LIMA-DE FARIA (1952) reports terminal knob formations for each of the pachytene chromosomes of *S. cereale*, but there is no indication which one (or ones) of these might appear satellited at diakinesis. If his chromosome VI is the same as the knobbed chromosome belonging to the translocation configuration, the terminal structural rearrangement which he observed at pachytene may in some way be related to the appearance of the knob at diakinesis.
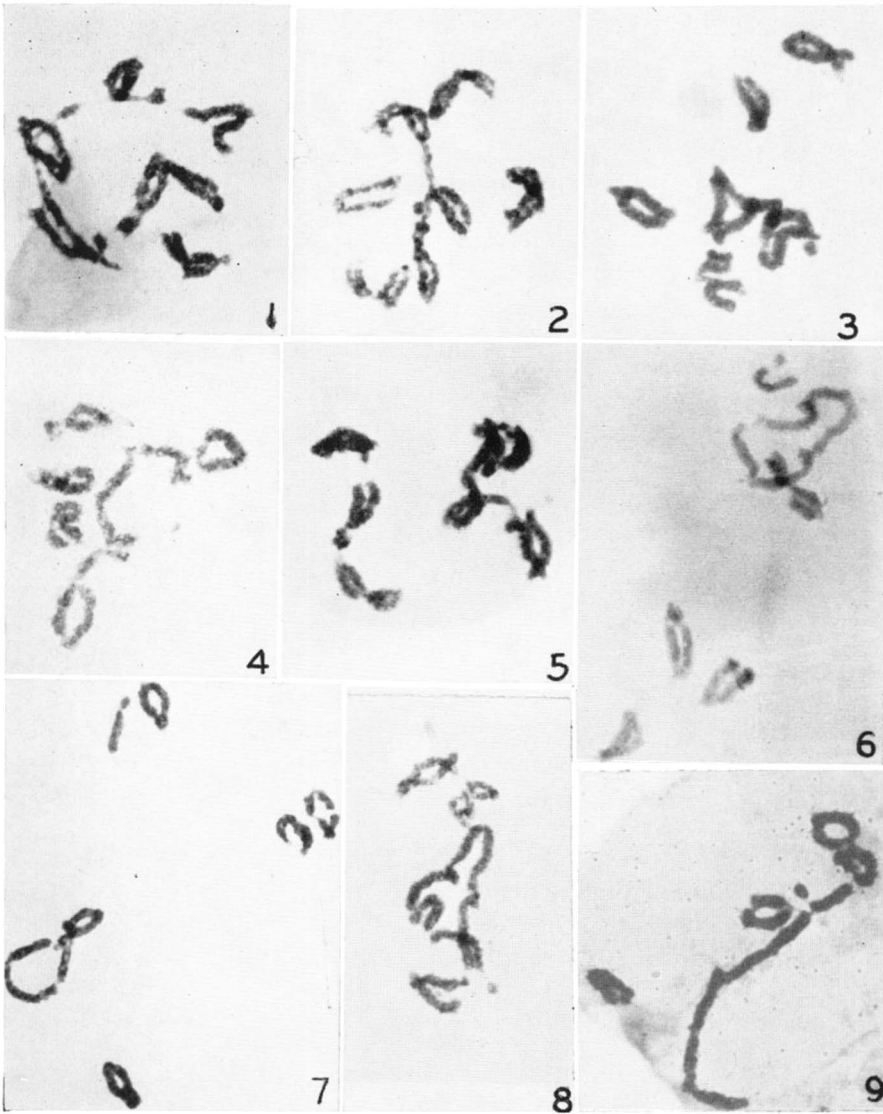
FIGURES 1–9.—Diakinesis in the F₁ hybrid $c \times m$. × 1100. Further descriptions are given within the text. FIGURE 1.—Shows a chiasma in the interstitial region of the two central chromosomes of the chain-of-six (12 o'clock). FIGURE 2.—Chiasmata on both sides of the centromeres of all chromosomes belonging to the translocation complex. FIGURE 3.—Knob of *cereale* C chromosome at 4 o'clock. Peculiar bivalent at 6 o'clock which appears to be medianally heteromorphic. FIGURE 4.—Complete (or nearly complete) terminalization of chiasmata in the four segments of chromosomes B-C′ and in the interstitial region of the C-C′ chromosomes. FIGURE 5.—Chiasmata on both sides of the centromere in chromosomes C-C′. FIGURE 6.—Ring-of-five. Knobbed C chromosome at 12 o'clock. FIGURE 7.—Ring-of-five at 8 o'clock, knobbed C chromosome at 12 o'clock. FIGURE 8.—Knob of *cereale* C-chromosome at 5 o'clock. A tendency for a closed ring-of-six is evident. FIGURE 9.—The knob of the C chromosome is distinct following terminalization of chiasmata in the 5-segments of the C-C′ chromosomes.
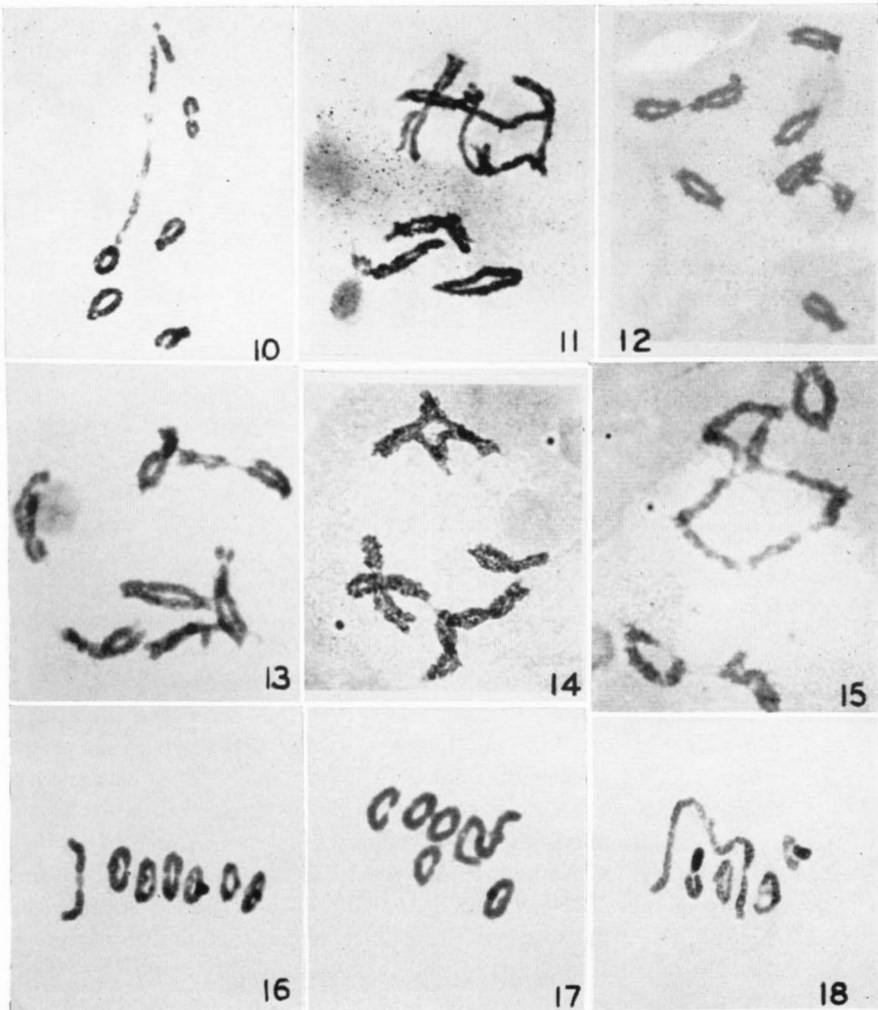
FIGURES 10–18.—The C chromosome of *cereale* with its knob at 12 o'clock. Chiasmata on both sides of the centromeres of the A-A' chromosomes. FIGURE 11.—Ring-of-six. FIGURE 12.—Seven bivalents.

FIGURE 13.—Diakinesis is *S. cereale*. × 1100. The satellited knobs on a pair of chromosomes other than the chromosomes associated with the nucleolus is shown at 3 o'clock.

FIGURE 14.—Diakinesis in the F_2 segregant which forms a ring-of-four in the PMC's: ring-of-four at 12 o'clock. × 1100.

FIGURE 15.—An F_2 segregant which shows a ring-of-eight plus 3 bivalents at meiosis. × 1100.

FIGURES 16–18.—Representative configurations found in one of those plants which showed a high frequency of chains-of-four at meiosis but which occasionally showed chains-of-six. × 1100. FIGURE 16.—Seven bivalents. FIGURE 17.—Chain-of-four plus five bivalents. FIGURE 18.—Chain-of-six plus four bivalents.

Since *S. cereale* var. Merced, one of the parents used in this study, regularly shows at diakinesis a pair of satellited chromosomes in addition to those associated with the nucleolus (fig. 13) and since the other parent, *S. montanum*, shows no comparable satellited chromosome, the knobbed chromosome of the F_1 translocation figure must be derived from the *S. cereale* parent.

Since the knobbed. *cereale* chromosome belonging to the translocation figure is usually detectable as one of the end chromosomes in the chain-of-six, it follows that the ring-of-six usually breaks down to a chain-of-six as the result of the lack of association of two particular chromosomes rather than from a general low chiasma frequency which could have resulted in a breakdown of the ring in any one of the six pairs of homologous arms at random.

Position of the centromeres: The six chromosome translocation figure frequently breaks down into smaller associations even in the absence of increased univalent formation. Since this is not accompanied by a corresponding decrease in the frequency of ring-bivalents, it suggests that there is less homology between the translocated arms than between normal nontranslocated arms. Furthermore, some cells have been observed in which seven ring-bivalents were present (fig. 12). Therefore, the low frequency of pairing in translocated arms may be attributed to the fact that the homologous segments include considerably less than whole chromosome arms.

Evidence for this is also furnished in figures 1, 2, and 5, which illustrate chiasmata on both sides of the centromere in diakinesis. Repulsion of homologues during diakinesis accentuates the position of chiasmata in the end pairs, but it is sometimes difficult to be certain whether the central pair has true chiasmata or if the arms are merely overlapping. Only in cells such as that depicted in figure 1, where the chromosomes are stretched, is it possible to establish definitely the presence of a chiasma.

Since the translocated arms of the chromosomes in the chain-of-six are associated with arms of different chromosomes, any chiasma within the interstitial regions of the central pair is unable to terminalize. The results of such a chiasma should be a loop in the middle of the chain-of-six at metaphase as the centromeres repel each other. Such a loop was often observed but, since many configurations were not definitive, it was not possible to obtain an accurate count of the frequency of central loops in the chain, which would have been useful in estimating the length of the interstitial segment. In general, however, they were rather scarce, suggesting that the distance between the centromere and the point of translocation in these chromosomes is short.

Since closed ring-bivalents are common at the end of the chain which is terminated by the 1-A'-7 *montanum* chromosome, the segments distal to the point of translocation in the A-A' chromosomes must be short. Very few closed ring-bivalents were seen at the other end of the chain which indicates short interstitial segments in the C-C' chromosomes. This, together with the high frequency of chiasmata observed in the "4" arms, indicates that the distal segments in these chromosomes, as well as the distal segments of the B-B' chromosomes, are long. Therefore, since the segments distal to the point of translocation are short in the 1-A-2 chromosome and long in the 3-B'-2 chromosome, a differential region must exist in the latter segment to accomodate that portion which does not associate with the shorter homologous segment

of the 1-A-2 chromosome. A corresponding differential region is present within the long distal segment of the 5-C-6 chromosome.

The presence of chiasmata on both sides of the B-B′ centromere precludes the possibility of a centromere within the differential region as inferred to be present by RILEY (1955) in a comparable hybrid.

Evidence for duplication: Very infrequently, diakinesis cells are found in which a ring-of-five translocation figure is formed (figs. 6 and 7). These particular cells clearly show that the univalent left out of the translocation figure is the knobbed *cereale* chromosome described above.

Since the ring-of-five can form only as a result of crossing over between two chromosomes derived from the same parent, it follows that *S. montanum* must contain a duplication and that the duplicated segments are on those chromosomes which associate with the two arms of the knobbed chromosome of *S. cereale* when the ring-of-six is formed. Since the breakdown of the ring-of-six to the chain-of-six usually occurs at the nonknobbed end of the *S. cereale* chromosome and at the 7 arm of the A′ *montanum* chromosome which is terminated by the duplication segment, it suggests that the duplication segment may be responsible for some of the non-homology which prevents regular association between these arms.

In cells in which the knobbed chromosome of *S. cereale* can be identified, chiasmata are usually formed in the paired segments proximal to the knob. Since the knob often projects beyond the terminalized chiasmata (figs. 8, 9), it must either represent chromatin material unique in *S. cereale* or else a translocated segment from another chromosome pair.

Other evidence for the duplication segments is described below in the analysis of certain F_2 segregants.

Cytology of the F_2 progeny

If, as suggested by McCLINTOCK (1930), THOMPSON and HUTCHESON (1942) and others, only products of alternate disjunction produce viable gametes, F_2 segregants from a translocation heterozygote should fall into two groups: those having translocation configurations, and those in which only bivalents would form at meiosis. However, as shown in table 2, new homologies are evident in some of the F_2 segregants which indicate that under certain circumstances some products of adjacent distribution are viable.

Of the 226 F_2 plants which were examined cytologically, approximately 50 percent showed only bivalents at meiosis. These are presumably parental types with regard to the three pairs of chromosomes which are associated in the translocation configuration of the F_1. Of the 66 progeny which did not flower the first season, only 22 survived the winter, and were examined the following summer. Of these, ten or nearly 50 percent of them, showed seven bivalents at meiosis. There is no reason to suppose, therefore, that of those which did not survive, there was a preponderance of plants which would have formed seven bivalents nor a preponderance of plants which would have formed multiple associations.

Since those plants which showed configurations other than bivalents or chains-of-six must possess chromosomal homologies different from those present in the F_1, they

TABLE 2

Frequencies of F₂ plants (S. cereale × S. montanum) *with different maximum associations at diakinesis*

Maximum configuration	Frequency	Percent
Disomics		
Ring-of-eight	1	0.44
Ring-of-six	6	2.65
Chain-of-six	78	34.41
Ring-of-four	1	0.44
Chain-of-four	3	1.33
Bivalents	121	53.54
Trisomics		
Chain-of-seven	11	4.87
Chain-of-three	5	2.21
Totals	226	99.99

are discussed in more detail below. Eight of the plants which showed maximum associations of chains of six are also discussed separately, since they form the chain-of-six at a very low frequency despite high chiasma frequencies.

Ring-of-four: One plant of the F_2 generation was found to have its meiotic chromosomes associated as five ring bivalents plus a ring-of-four derived from the 6-complex of the F_1.

The ring-of-four appears in more than 60 percent of the M_I cells in this plant, which indicates a large amount of homology in the translocated arms and therefore the presence of long segments distal to the points of translocation breakage. This is also evident at diakinesis in cells which show chiasmata in these segments (fig. 14). Aside from the unlikely possibility that a new reciprocal translocation has occurred within the F_1, the only way in which such new homology could have been produced appears to be by means of a crossover within the differential region of the F_1 hybrid. Subsequent fertilization of a gamete containing such a crossover chromosome with a parental gamete containing a chromosome having the corresponding terminal segment would yield a plant which could form a closed ring-of-four at meiosis. This may be diagrammed as follows:
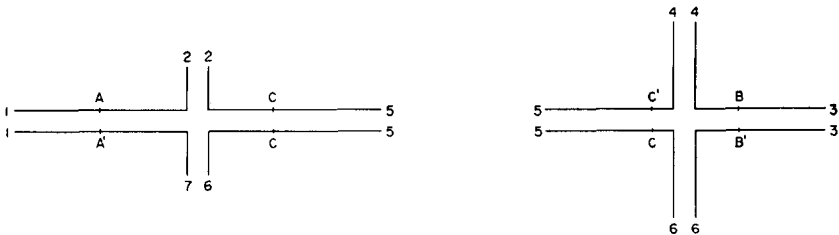
Crossing over in F_1

Viable gametes

A   B
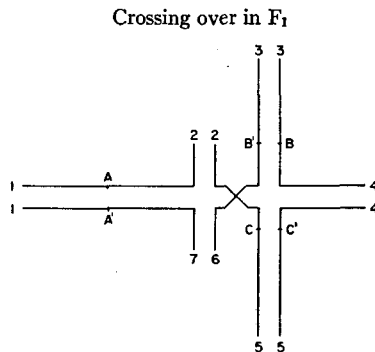
Meiosis in heterozygote with *S. montanum* gamete
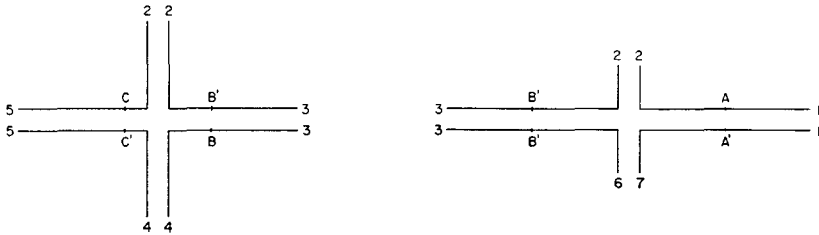
Meiosis in heterozygote with *S. cereale* gamete

Since in the heterozygote of *S. cereale* with the type A gamete and in the heterozygote of *S. montanum* with the type B gamete, the 6–7 arms are the same as those which rarely associate at meiosis in the $F_1 c \times m$, they will not associate here and the maximum association will therefore be a chain-of-four. A ring-of-four will result either with *S. cereale* × type B gamete or *S. montanum* × type A gamete.

The backcross progeny from this ring-of-four segregant × *S. cereale* consists of plants which show a ring-of-four plus five bivalents and of plants which show seven bivalents at meiosis. The gamete which functioned in producing the F_2 segregant with a ring-of-four must therefore have been type B above.

Infrequent chain-of-six: Twelve plants of the F_2 generation showed a translocation figure consisting of a chain of four chromosomes in many cells at metaphase I together with a large number of cells which showed seven bivalents and a very few cells in which there was a translocation figure consisting of a chain of six chromosomes. In some cells the seven bivalents all appeared as closed ring bivalents. Representative configurations are shown in figures 16 to 18.

Since there is no accompanying increase in frequency of univalents or open bivalents, the very low incidence of this chain-of-six indicates that it does not result from the same homologies that formed the chain-of-six configuration in the F_1 hybrid. This is also indicated by the high incidence of cells in which seven ring bivalents form.

As shown above, crossing over within the differential region of the translocation

figure of the F_1 could give rise to gametes which, when fertilized by a particular parental gamete, could produce a zygote with a chain-of-four configuration at meiosis. However, this would not concurrently furnish the new homologies necessary to provide for the few associations of six which occur in these plants. Such new chromosome patterns apparently could have arisen only as the result of new translocations or of aberrant disjunction.

The occurrence of 12 different plants, each of which shows this same meiotic behavior, rules out the possibility that new translocations were responsible for their altered chromosome homologies. Even if new reciprocal translocations appeared at a very high rate, they would be expected to occur more or less at random.

The only obvious alternative explanation for their origin appears to be, therefore, that some aberrant disjunction has incorporated new chromosome types within viable gametes. However, aberrant disjunctions resulting in gametes with seven chromosomes must necessarily entail deficiencies and duplications. That large duplications can be tolerated is indicated by the high frequency of trisomics found in the F_2 population (Table 2), but transmission of large deficiencies is expected to be rare or impossible. However, because of the presence of a duplicated segment on two different and otherwise non-homologous chromosomes of the *montanum* genome, gametes resulting from adjacent disjunction in which these two chromosomes segregated to opposite poles would not suffer a deficiency for this segment. As shown above, one of these "duplication" chromosomes is situated at one end of the chain-of-six in the F_1 translocation figure, the other one is in the penultimate position at the other end of the chain.

A gamete could therefore result from adjacent disjunction which would be deficient only for the segment which lies between the terminal duplication segment and the point of translocation of the A' *montanum* chromosome. Since the entire distal segment, including the duplication segment, is short, the deficient region would be relatively small. Such a deficient gamete would contain the following chromosomes:
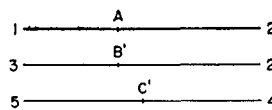
Upon fertilization with a parental *cereale* gamete, a plant would be produced in which a chain-of-four would be present, at MI. This might be diagrammed as follows:
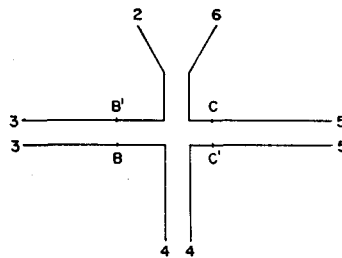
The "2" and "6" segments are homologous only in the region which was interstitial in the F_1 configuration. Since this region is bounded on both sides by non-homologous segments, very little association would be expected within it even though this region were fairly long. Consequently, this configuration would usually appear as a chain-of-four at MI.

Since three "2" arms would be present, association of any two of these at a time might be expected. If the "2" arm of one of the chromosomes in the bivalent associated with the "2" arm of the translocation figure, a chain-of-six would be formed. Since such a configuration could result only when zygotene pairing was initiated between the "2" segments of chromosomes "A" and "B'", it should not form very often.

The expected MI configurations from the above model would consist therefore of a preponderance of chains-of-four, occasional chains-of-six and quite a few cells having maximum associations of trivalents or bivalents. The discovery of 12 different plants whose meiotic configurations fit those expected from this model suggests that the model is correct.

If the viable gamete resulting from the adjacent disjunction described above were fertilized by a parental *montanum* gamete, the meiotic chromosomes would usually be associated as bivalents, one of which would regularly be a rod bivalent. Occasional trivalents and chains-of-four would form due to the presence of three terminal "2" segments.

A number of plants have been observed in which rod bivalents are common, but in most instances it is not possible to determine whether or not they result from a low chiasma frequency or as the result of reduced homology as described above. The rare association of chains of four in plants which normally form seven ring bivalents has been established for only three plants. However, others may present the same configurations but too infrequently to have been recognized as potential chain-of-four types.

Ring-of-six: Six plants of the F_2 population showed a high frequency of ring-of-six translocation-configurations at diakinesis (fig. 19). In four of these plants a few cells showed a closed ring of five with a sixth chromosome associated with the ring-of-five at approximately the position at which two adjacent chromosomes were joined by a terminalized chiasma (figs. 20, 21, 22).

The regular presence of a ring-of-six in diakinesis of plants which are segregants from an F_1 hybrid in which rings-of-six are seldom found, implies that more homology must be present in the segregants than was present in the F_1 hybrid. It further suggests that this increased homology is located in the terminal segments which are usually unassociated in the F_1. Since six different plants all show this same behavior, the mechanism by which such new homologies were obtained must have been a simple one and must have occurred fairly commonly in the F_1. This would rule out the possibility of new translocations or other irregular chromosomal behavior and would suggest crossing over as the most likely explanation for the production of the new homologies.

As mentioned above, rings-of-six are rarely observed in the F_1 hybrid. However, since they do occur, it implies that crossing over may take place between the segments

which are usually unassociated. If these segments are heteromorphic, crossing over between them could produce segregants in which these segments were identical. This would furnish additional homology which would permit greater association than was present in the F_1.

Since one of the ends, which is usually free in the F_1 hybrid, is terminated by the "duplication" segment of a *montanum* chromosome, two different homozygous F_2 segregants could result from crossing over between the homologous portions of these ends. One of these would be homozygous for the duplication segment of the *montanum* chromosome, the other for the nonduplication segment of the *cereale* chromosome. Fertilization by the appropriate parental type gamete could produce plants which would show rings of six at meiosis as follows:
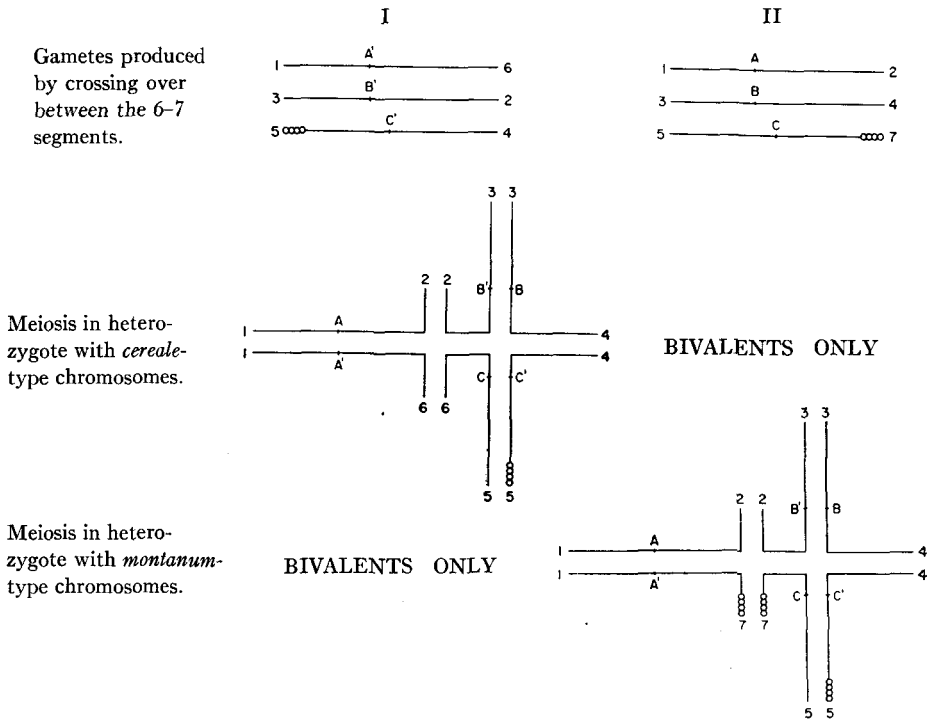
Since the "7" arms are probably long and completely homologous, as are the "6" arms, these configurations should usually appear as closed rings-of-six at diakinesis.

Since in plants in which the ring-of-six is formed following fertilization with a *montanum* gamete, the *montanum* duplication segment is present in triplicate, a few cells could show association between the end of the "5" arm carrying the duplication and the end of one of the "7" arms. If a chiasma should form between the "5" end of the C′ chromosome and the "7" arm of the A′ chromosome, a ring-of-five could be formed similar to that observed in the F_1. However, if concurrent with the formation of the chiasmata which form the closed ring-of-five, a chiasma should form be-

tween a portion of the two "7" arms, the closed ring-of-five would have an attached sixth chromosome. Diagrammatically, this would appear as follows at pachytene:
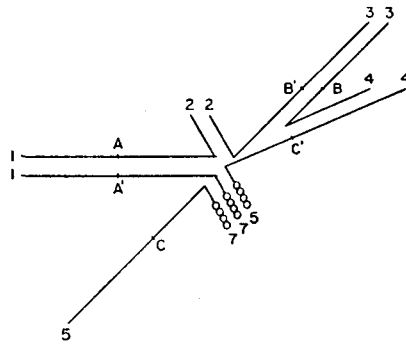
As noted above, diakinesis configurations showing such associations have been found in four of the six plants which form the ring-of-six (figs. 20, 21, 22).

Up to this point considerable evidence has been presented which supports the suggestion that a duplication is present in the *montanum* genome. The presence of the ring-of-five at diakinesis in the F_1 hybrid; the evidence that products of adjacent distribution could explain the high frequency of plants in which metaphase cells show predominantly chains-of-four and very infrequently chains-of-six; and the presence of segregants showing a ring-of-five having an attached sixth chromosome, are all explicable only on the basis of the presence of a duplication. Further evidence is furnished by one of the F_2 segregants which forms a ring-of-six at diakinesis. This particular plant was observed to have some cells in which a ring chromosome and a rod chromosome were laggards at telophase II (figs. 23, 24). The most likely origin of these chromosomes is as equational-division products of a partial isochromosome in which chiasmata formed between the two homologous ends of the same chromosome during prophase I. This can be diagrammed as follows:
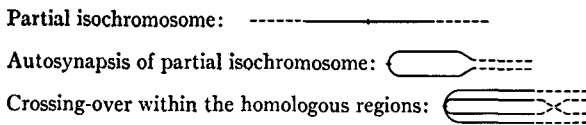
If this passed as a unit to one of the telophase I cells the equational separation at anaphase II could yield a rod chromosome and a ring chromosome similar to those observed.

If, as suggested by SEARS (personal communication) from his observations on isochromosomes in wheat, chiasmata in the paired arms of a univalent are resolved prior to anaphase I, the telophase II ring would indicate reverse homology in the two arms of the partial isochromosome.

In either case, the origin of a partial isochromosome from the F_1 translocation figure can be explained on the basis of crossing over between the homologous portion of the "6" and "7" segments concurrently with a crossover between the "5" arms.
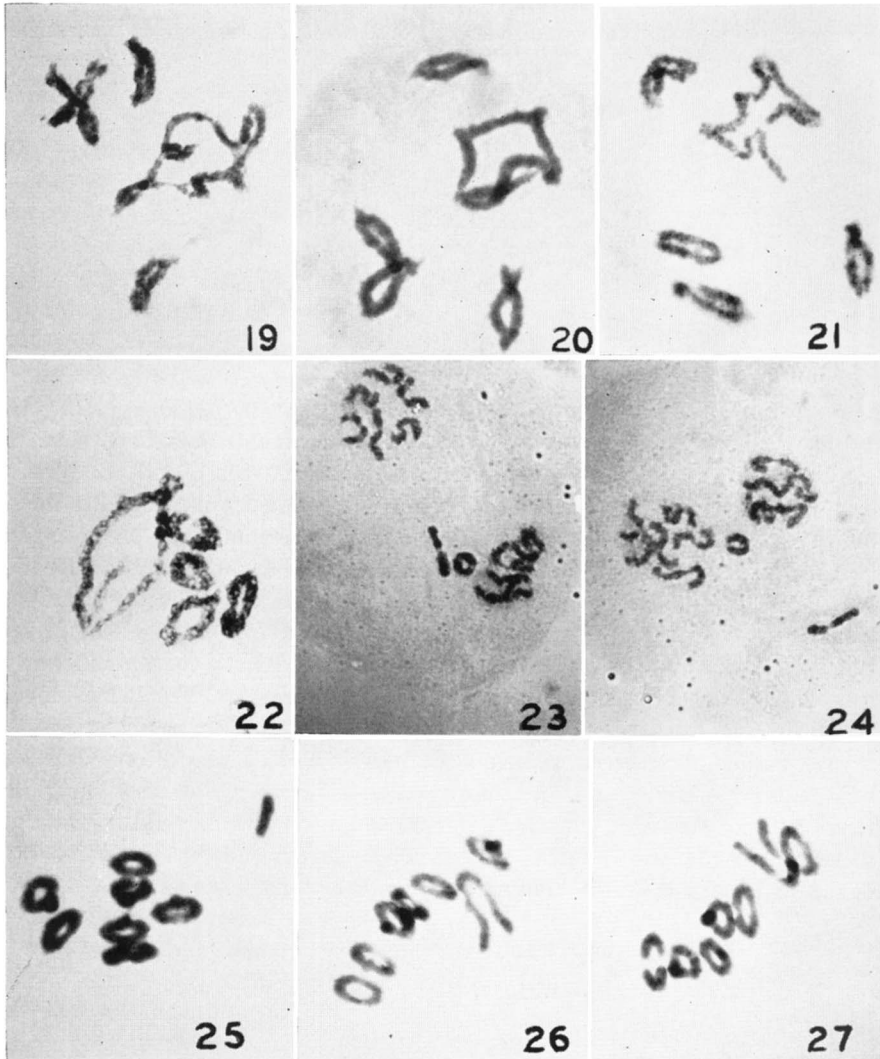
FIGURES 19–24.—Representative configurations found in those plants which formed rings-of-six at a high frequency in their PMC's. × 1100. FIGURE 19.—Ring-of-six. FIGURES 20, 21, 22.—Show a ring-of-five with a "tail". FIGURES 23 and 24.—Show a rod and a "ring" chromosome at telophase II.

FIGURES 25–27.—Representative configurations found within a single trisomic plant. × 1100.

These two crossovers would yield a "C" chromosome having the "duplication" *montanum* segment on both ends which could, upon autosynapsis, yield rod and ring chromosomes at telophase II such as observed in the F_2 segregant described above.

Ring-of-eight: In the PMC's of one F_2 plant, ring-of-eight configurations were observed at diakinesis (fig. 15). This may have resulted from new structural rearrangements produced by a new translocation in the F_1 hybrid involving one of the

chromosomes of the translocation group and a chromosome of one of the bivalents. However, it can also be explained on the basis of crossing over between the distal segment of one of the chromosomes of the translocation figure and a homologous interstitial segment of one of the chromosomes of a bivalent. The rare chain-of-eight observed by RILEY (1955) in meiosis of a comparable hybrid supports such a suggestion.

As noted above, the knobbed *cereale* chromosome in the translocation figure of the F_1 often shows affinity for one of the other bivalents, although no definite chiasmata have been observed. If actual homology exists between the knob and this other bivalent, it would suggest that in relation to the *montanum* genome, the knob represents a translocation from another chromosome of the *cereale* complement. The bivalent would thus be heteromorphic for the region involved in the translocation.

If this assumption were correct, and it should be emphasized that it is an assumption only, no good cytological proof having been found, then occasional pairing and crossing over between the knob of the *cereale* chromosome and the homologous interstitial region of the *montanum* chromosome could result in altered chromosome structure which could give rise to a plant which would form a ring-of-eight in meiosis.

Trisomics: Sixteen trisomics were found in the F_2 population, 11 of which showed a translocation configuration at meiosis (figs. 25, 26, 27). In each of these, the extra chromosome was associated with the translocation group, indicating that the extra chromosome is usually derived by nondisjunction of chromosomes within the translocation configuration.

Since two of the terminal segments in the translocation configuration of the F_1 usually fail to form chiasmata, the configuration in a segregant will not be the same for each chromosome in the group. If, for instance, a trisomic segregant is heterozygous for the translocation configuration, and in addition has an extra chromosome "C", the two "C" chromosomes will usually pair forming a bivalent, leaving a configuration consisting of five chromosomes. If the extra chromosome, however, is the *montanum* "C'" chromosome, a bivalent with the "C'" chromosome of the translocation figure and a chain-of-four plus a univalent will result. Similarly, the presence of an extra "B" or "B'" chromosome will result in a chain-of-three plus six bivalents. An extra "A'" chromosome will result in a chain-of-five plus four bivalents.

Since the failure of association of the "A'" and "C" chromosomes in the translocation figure of the F_1 is not absolute and since the extra chromosomes may associate other than as bivalents, higher associations than those described will occasionally result in each case. However, the relative frequencies of the various configurations should furnish some indication as to which chromosome of the translocation figure corresponds to the extra chromosome.

As shown in table 3, eight of the 11 plants which have an extra chromosome in addition to the translocation configuration show a high frequency of chains-of-five. On the basis of the above predictions this would correspond to the expected behavior of those plants which had an extra "A'" or "C" chromosome. The two plants which usually show chains-of-four at diakinesis would correspond to those expected in which either an extra "C'" or an extra "A" chromosome is present. The single plant in which the usual metaphase configuration exhibits a chain-of-three would correspond

TABLE 3

Configurations observed in trisomic segregants at MI

Multiple associations	Bivalents	Univalents	No. of plants
Chain-of-five*	5		8
Chain-of-four†	5	1	2
Chain-of-three‡	6		1
§	7	1	5
Total plants			16

* Occasional chain-of-seven plus four bivalents.
† Occasional chain-of-five plus five bivalents.
‡ Occasional chain-of-four plus five bivalents plus one univalent.
§ Occasional chain-of-three plus six bivalents.

to translocation heterozygotes having an extra "B" or "B'" chromosome. These instances are too few to permit the use of their frequencies as an index to the frequencies with which each of the various chromosomes of the translocation figure might be included as an extra chromosome in a trisomic segregant. However, the preponderance of those types of trisomics expected from the presence of an extra "C" or "A'" chromosome suggests that these two chromosomes, which are situated at the end of the metaphase-I chain in the F_1 hybrid, are more likely to be included as an extra chromosome, in a nondisjunctional gamete, than are any of the other chromosomes belonging to the translocation figure.

Morphological characters scored for the F_2 segregants

Several morphological characters were scored for the various F_2 segregants to determine if any of these might be solely or predominantly associated with the translocation figure. The characters measured were: (1) annuality *vs.* perenniality, (2) earliness as measured by the number of days before anthesis, (3) growth habit with reference to the erectness of the culms, (4) culm maturity in terms of simultaneous *vs.* continuous maturation, (5) leaf rigidity in terms of the laxness of the blade, and (6) position of the leaves on the culms with regard to whether they are cauline or basal.

Of these, the annuality *vs.* perenniality appears to be the only one which segregated as a monofactorial character. Sixty-six of the 303 F_2 segregants were considered perennial since they did not produce flowering culms during the first season. This many perennials does not differ significantly from that expected on the basis of a 3:1 segregation ($\chi^2 = 1.67$). Unfortunately, 44 of these 66 perennials did not survive the winter so were unavailable for cytological examination. From a study of meiosis in the remaining 22 it was found that nine showed a translocation configuration similar to that of the F_1, ten showed seven bivalents, one was a trisomic and one showed a chain-of-four translocation configuration. Since there is no reason to expect that those perennials which did not survive would have been very different cytologically from those examined, it appears that the factor or factors responsible for perenniality segregate independent of the translocation group.

TABLE 4

Means and variances of F_2 plants which show only bivalents at meiosis and of plants which show an F_1-type translocation figure

	Plants with 7II (n = 120)		Plants with F_1-type configuration (n = 70)		Significance of difference of means	
	$\bar{x}$	s^2	$\bar{x}$	s^2	t	P
Culm maturity	1.48	2.18	2.51	2.20	4.62	<0.01
Position of leaves	0.86	0.95	0.91	0.98	0.34	>0.70
Growth habit	3.73	2.57	4.33	3.01	2.36	<0.02
Rigidity of leaves	0.56	0.53	0.80	0.66	2.03	<0.05
Earliness	3.11	2.27	3.33	2.37	0.96	>0.40
Hybrid index	9.73	22.50	11.79	28.11	2.68	<0.01

The "P" values were obtained from FISHER's "t-table" (1950).

Using ANDERSON's (1949) methods, the other characters were scored from zero (*cereale* type) to various higher numbers representing the *montanum* type. A hybrid index for the combined scores was prepared for each plant. The distributions of the various characters with respect to the cytological behavior are shown in table 4.

As shown in table 4 there is no significant difference between the variance of F_2 segregants with seven bivalents and F_2 segregants having the multiple association. This precludes the detection of any association of these characters with the chromosomes of the translocation figure. However, since the means are significantly different for these two cytologically different groups, for three of the characters which were measured as well as for the hybrid index, it suggests that they are distinct populations.

In each case where the means are different, the plants which have the translocation figure, and are therefore heterozygous for the three component chromosomes, have a larger mean than the plants which are homozygous for these three chromosomes. However, since the mean of the F_1 hybrid is in all cases intermediate between the two parents, the larger mean of heterozygous F_2 segregants cannot be interpreted as an heterotic effect nor as the result of dominance.

Since both "culm maturity" and "growth habit" are not entirely independent of perenniality, it may be assumed that the 44 plants which did not flower the first season and which did not survive the dormant season would have all been scored primarily as *montanum*-like for these characters if they had reached maturity. If these perennials consisted of equal numbers of plants having the translocation figure and of plants having only bivalents, the omission of these perennials might have a greater influence on the mean of the heterozygote population than on the mean of the homozygote population, because the aggregate population of the former is smaller than that of the latter (due to the extraction of all multiple associations other than that shown by the F_1 hybrid). If this were true, the larger mean of the plants with the translocation figure would be due to the effect of those perennials which were included in the analysis. When the means were calculated for sub-populations in which those plants which did not flower the first season were excluded, this is suggested for "growth habit" but not for "culm maturity".

DISCUSSION

Variation in chromosome number and chromosome patterns in populations of rye have been reported by GOTOH (1924, 1932), EMME (1927), LEWITSKY (1929, 1931), MÜNTZING and PRAKKEN (1941) and others. Although some of these populations were reportedly free from recent contact with perennial species, the possibility that products of previous hybridizations have been retained cannot be excluded. Since rye is very prolific and since trisomics, duplications and some deficiencies are readily transmitted, as shown above, the cytological effects of a single hybridization with a perennial species could conceivably be perpetuated for many generations in a rye field. POPOFF (1939) suggested this to be the source of the aberrations which he found in 14 varieties of cultivated rye and further suggested that such hybridization may have given some of the variation which was responsible for the production of new types.

HASEGAWA (1934), MÜNTZING (1946, 1950), LIMA-DE-FARIA (1952), and others have shown that many populations have a specific type of extra chromosome different from any of the other chromosomes. Certain features of the behavior of these standard-fragments suggest that they may also have resulted from interspecific hybridization. As shown above, in the hybrid between *S. cereale* and *S. montanum*, crossing over may sometimes occur between a terminal segment of a *cereale* chromosome and an interstitial homologous region of a *montanum* chromosome. Such an exchange could produce a chromosome with a subterminal centromere which would correspond to the dimensions of the standard fragment. Since MÜNTZING (1950) reported a high incidence of plants having the standard fragment chromosome from populations obtained from Turkey, the same geographic area in which cereal rye and *S. montanum* are sympatric, and since the populations with the highest frequencies were those which were weedy types in wheat fields, and which may therefore represent introgressant populations, it suggests that the standard fragment may result from crossing over in interspecific hybrids.

The ease with which the hybrids between *S. cereale* and *S. montanum* can be obtained suggests that such interspecific hybridization may be of common occurrence wherever these two species are sympatric. Successive backcrosses onto the *cereale* types could and probably do furnish much of the extensive variation found in rye populations. The comparatively uniform populations of perennial Secale, however, suggest that products of introgression from *cereale* into *montanum* are less successful than the parental forms. However, a few ecotypes which have been described as cereal-like perennials may represent instances where the progenies of such backcrosses have been perpetuated by man. *S. daralgesi* Thum. which was found by THUMANIAN in the mountains of Armenia may have originated in this way. It is a perennial form with large grains and a stiff rachis. THUMANIAN (1938) considers it to be a relic of an old cultivated form, whereas ROSHEVITZ (1948) regards it as the product of prolonged selection from *S. kuprijanovia*. DERZAVIN (1935) reports that some forms of *S. kuprijanovia* have been selected for local cultivation in other areas. The increased earliness, larger grain and erect growth of these cultivated perennials suggest the presence of *S. cereale* in their ancestry. However, selection for introgres-

sants into *montanum* is probably rare, and because of the sterility which would accompany such introgression, natural selection would tend to strengthen any incipient barriers.

Therefore, since the variability resulting from introgression of *montanum* into *cereale* has a selective value and since the variability resulting from introgression of *cereale* into *montanum* has a selective disadvantage, natural selection should favor those mechanisms which will encourage the former and hinder the latter. An indication of such barriers is furnished by the reciprocal fertility differential in $c \times m$ and $m \times c$ hybrids. PRICE (1955) reported 78 percent seed germination in $c \times m$ hybrids and only 18 percent germination in the reciprocal $m \times c$ hybrids using the Copenhagen strain of *montanum*. In hybrids with a perennial form from Iran, the $c \times m$ hybrids showed 84 percent germination, the $m \times c$ hybrids only four percent germination. In crosses between *S. cereale* variety King II and *S. montanum*, RILEY (1955) obtained germination of hybrid seed only when *S. cereale* was the female parent.

From a study of a small F_2 population (21 plants) from a comparable $c \times m$ hybrid, RILEY (1955) concluded that only parental-type gametes are functional in the F_1 and that the translocation arrangement therefore functions in rigidly preserving the identity of the species. He further contends that the important differentiating characters of the species are located in the proximal regions of the chromosomes belonging to the translocation group and are therefore preserved from recombination. However, the results of the present study do not support these observations.

With a six-chromosome translocation figure only one pair of centromeres, at most, could exist in the differential region. However, as shown above, there is no centromere within the differential region of the $c \times m$ hybrid used in this study. There are, therefore, no median chromosome sections which would be protected from crossing over and, as shown above, even the differential region of the translocation configuration shows recombination. At best, therefore, the three chromosomes belonging to each species can be considered as one linkage group and even this is often altered when viable gametes are produced following adjacent disjunction (a situation permitted by the duplication segment present in *S. montanum*).

RILEY's (1955) observation that perenniality is due to a dominant factor located within the translocation group of chromosomes is also at variance with the results of this study, which might indicate a real difference between the linkage groups of the plants which he studied and of those reported herein. As noted above, perenniality was found to segregate as a simple recessive factor and showed no correlation with the translocated chromosomes. Other morphological characters studied which distinguished the two forms, including earliness, growth habit, rhythm of maturation and position of leaves, likewise showed no correlation with the translocation chromosomes. There is, therefore, no apparent reason for assuming either that certain morphological characters associated with one or the other species has been responsible for the establishment of the translocation pattern nor that the translocations have been important in preserving species identity. The establishment of the two translocation events may therefore have been fortuitous.

Since the contrasting patterns differ by two separate translocations and since both of these chromosomal patterns are represented in populations which are now very

widespread geographically, the origin of one from the other or of both from an ancestral type, probably occurred only once and prior to the geographic dispersal of at least one of the types.

According to ROSHEVITZ (1948) some perennial types have occupied their present habitats since the Miocene epoch of the Tertiary period. Therefore, since the same chromosome pattern is found in all of these perennial forms, including the species *S. africanum* which is separated from the rest of the genus by the continent of Africa, its origin must have been at least 40 million years ago. Most investigators (HITCHCOCK and CHASE 1910; VAVILOV 1917; SCHIEMANN 1940) consider cereal rye to be of recent origin, dating back only a few centuries. The wild weedy types which apparently have the same chromosome arrangements as cereal rye, may be somewhat older, but since they occupy only habitats created by man, they are probably relatively very young as compared to the perennial forms. It appears, therefore, that it would have been impossible for the perennial forms to have been derived from the cereal or weedy rye types.

In order for the *cereale* pattern to have been derived from *S. montanum* a very strong selective advantage for each of the two new rearrangements would have been necessary to counteract the sterility barrier attending the translocation heterozygote. Due to the swamping effect of the parental type gametes, the new rearrangements would have been kept in the heterozygous, and therefore highly sterile, condition for a long period of time. Furthermore, when the rearranged chromosomes became homozygous, inbreeding depression, common in the cross-fertilized Secale species, would again have furnished a strong selective disadvantage.

It seems unlikely, therefore, that either of these two chromosome patterns is ancestral to the other. This is further attested by the fact that no pattern intermediate between the two has been found in natural populations. Since such an intermediate pattern must have had a very strong selective advantage for its establishment, it should be represented in some modern populations, especially in view of the apparently recent origin of the *S. cereale* pattern since which there have been no apparent catastrophic climatic or edaphic changes in the regions in which the intermediate rearrangement would necessarily have been well adapted.

It appears to the writer, therefore, that a third taxon must have been involved in the origin of the two chromosomal patterns. According to ROSHEVITZ (1948) *S. silvestre* Host., an annual species now occupying sandy soils of the Sarmatian basin, is as old as the earliest perennial forms. He suggests that it has been separated from the perennial forms since the Miocene epoch of the Tertiary period when the ancient Tethys basin became separated into the Sarmatian and the Mediterranean Sea. Such a major geographic alteration with its accompanying edaphic and climatic changes would have provided the ideal conditions for splitting up a sympatric population into separate isolates. If the ancient representative Secale population contained various chromosome patterns, or if segregant isolates from the original population should fortuitously fix different chromosomal arrangements, two divergent patterns could have become established with or without any accompanying selective advantage of such arrangements.

The chromosomal arrangements have not yet been determined for *S. silvestre*,

but if it has the same patterns as do the other annual forms, it may represent one of the ancestors of cereal rye. Such an assumption is not at variance with the known distributions and characteristics of the current members of the genus. *S. silvestre* occupies the sandy soils of the Sarmatian basin. Series *Kuprijanovia* is limited to the mountains of the Mediterranean (except *S. africanum* which is endemic to South Africa). The range of most of the cultivated and weedy ryes (Series *Cerealia* Roshev.) is that area which lies between the ranges of the *Silvestria* and *Kuprijanovia* series, namely the Caucasus, Asia Minor, Kurdistan, Northern Iran, Afghanistan and the eastern part of Kasakstan (according to ROSHEVITZ, 1948).

Since both *S. silvestre* and the perennial forms have occupied, for several millions of years, the same habitats which they now occupy, it may be concluded that they are well adapted to these habitats and that excessive new variability within them would be at a selective disadvantage. The annual cereal types, however, occupy new and variable habitats created by man and would therefore be favored by considerable variability.

This suggests that the series *Cerealia* may have been derived from the products of interspecific hybridization between *S. silvestre* and some member (or members) of the *Kuprijanovia* series. Since the parental species are well adapted to their particular environments, such new combinations could be established only in new habitats such as those which have been furnished in these areas by man. The hardiness and vigor of the perennial types, accompanied by the annual characters of *S. silvestre* could have resulted in an aggressive weedy pioneer in wheat and barley fields. Subsequent selection in the grain fields would have resulted in a stiff rachis, larger grain, stiffer straw, etc., characteristic of cultivated rye. On this assumption and as postulated by VAVILOV (1917) and SCHIEMANN (1932), cereal rye may have been derived by selection from the weedy-rye types.

SUMMARY

1. In the PMC's of the hybrids *S. cereale* L. $\times$ *S. montanum* Guss. and *S. cereale* L. $\times$ *S. africanum* Stapf., a translocation configuration involving six chromosomes is formed. In the hybrid *S. montanum* $\times$ *S. africanum*, only bivalents are formed.

2. At diakinesis the translocation figure is usually observed as a chain-of-six, but occasionally a ring-of-six is found.

3. The failure of the ring-of-six to form in the hybrid $c \times m$ is due to the failure of chiasmata to form in one particular arm of the multiple association.

4. Each of the translocated segments is considerably shorter than an entire chromosome arm so that homology with the same chromosome continues on both sides of the centromere. None of the centromeres lie within the differential region.

5. The recovery of an F_2 segregant which formed a ring-of-four at meiosis indicates that there is crossing over within the differential segment of the F_1 translocation figure.

6. A segment of one of the *montanum* chromosomes belonging to the translocation figure is duplicated on another *montanum* chromosome which is also a member of the translocation group.

7. Second generation segregants which usually form chains of four but which

occasionally form chains of six at meiosis are interpreted as the product of adjacent disjunction in the translocated group of the F_1 hybrid. Such nondisjunctional gametes would be deficient for a portion of the distal segment which rarely associates in the six-chromosome complex and duplicate for one of the other short arms of the translocation figure.

8. Second generation segregants which form rings of six at a high frequency at diakinesis indicate the presence of considerable homology in the distal segments which usually fail to associate in the F_1. Such new homology is considered to have been derived from crossing over within these segments at meiosis of the F_1 hybrid.

9. One of the F_2 plants which showed a high frequency of rings-of-six at meiosis occasionally had a ring chromosome and a rod chromosome at T-2. This is interpreted as having resulted from crossing over within a partial isochromosome. Such a partial isochromosome could be formed following crossing over at each end of the *cereale* chromosome which associates distally with the two *montanum* chromosomes possessing the duplication.

10. A high frequency of trisomics is produced in the F_2 progeny of the $c \times m$ hybrid. Most of these could be shown to have an extra chromosome belonging to the translocation group. The chromosomes located at the end of the F_1 chain-of-six configuration are included as the extra chromosome in trisomics more often than any of the other chromosomes.

11. No evidence for association of morphological characters with the distribution of the translocated chromosomes could be established for the characters which were scored.

12. *S. cereale* is considered to have been derived from weedy rye types which in turn are considered to have resulted from interspecific hybridization between *S. silvestre* and *S. montanum*.

13. The extreme variability currently found in rye and weedy rye populations is considered to have resulted from introgression of *S. montanum* into rye populations.

14. Extra chromosomes in rye populations are also considered to have resulted from interspecific hybridization.

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