CYTOGENETIC EVALUATION OF *SECALE* GERMPLASM FOR FORAGE IMPROVEMENT

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CYTOGENETIC EVALUATION OF *SECALE* GERMPLASM FOR FORAGE IMPROVEMENT

By

Mansour Nicknejad

AN ABSTRACT

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ABSTRACT

CYTOGENETIC EVALUATION OF SECALE GERMPLASM

FOR FORAGE IMPROVEMENT

by Mansour Nicknejad

The genus Secale has a great natural variability which might be utilized in different breeding programs. At present, the only species of this genus which is being utilized is S. cereale or cultivated rye.

Eight species of Secale, each represented by one or many samples, were assembled and grown in the greenhouse or in the field. This collection also contained 92 samples of weedy ryes from Iran. Many inter- and intraspecific crosses were made, and cytologically analyzed. Meiosis was quite regular in weedy ryes from Iran, and seven bivalents were also generally observed in the F_1's of weedy ryes from separate areas. Cytological analysis of F_1's between weedy ryes and cultivated ryes revealed a similar pattern of pairing. Weedy ryes and cultivated ryes behaved similarly when crossed with Secale montanum. In both cases a maximum association of 3 closed bivalents and a chain of 8 chromosomes were observed at metaphase I. It is concluded that the two species differ in three reciprocal translocations.

Cytological analysis of F_2 populations of interspecific crosses between S. cereale x S. montanum revealed only two kinds of plants, those with 7 bivalents at metaphase I and those which were heterozygous for 3 translocations. Morphologically F_2 populations segregated into 1:2:1 ratio, S. cereale, F_1, and S. montanum respectively. Recombinants for major species characters were not found.

The chelating agent EDTA was used in an attempt to break down species barrier at the diploid level. Interspecific F_1 plants were treated during meiotic processes, but no new recombinants were found in F_2 populations grown from seeds harvested from treated F_1 plants. Frequency of interstitial chiasma increased as a result of treatment with EDTA.

Interspecific barriers at the diploid level presented a formidable handicap to the utilization of Secale germplasm in desirable recombinations. To circumvent these barriers, attempts were made to double the chromosome number in interspecific F_1 hybrids, with colchicine or nitrous oxide. Nitrous oxide treatments did not yield any tetraploids. With colchicine treatments tetraploids were induced. Doubled F_1 plants were 50% fertile in the greenhouse when crossed. One doubled F_2 population was cytologically and morphologically analyzed. At metaphase I they showed an average of 10.72 bivalents per

cell. Multivalents higher than chains of four were also observed. Morphologically, the tetraploid F_2 population was quite uniform.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

INTRODUCTION

Cultivated rye, Secale cereale L. is thought to be the least ancient of the cereals. It was unknown to the ancient Egyptians and Greeks. Rye has a wider potential geographic range than wheat, both as to latitude and altitude.

Although other species of Secale are of potential importance, especially for forage breeding purposes, utilization of this genus has been almost completely confined to S. cereale L. or cultivated rye.

After the pioneering work of Vavilov (56) on the origin of cultivated plants, three broad classes of the genus were recognized; wild, weedy, and cultivated rye. He recognized four species in this genus, whereas Roschevitz (47) recognized 14. There is, however, a great store of variability found in Secale germ plasm for both intra and interspecific characteristics. For different purposes it is desirable to utilize the total natural variability present in this genus through recombinations. For example, wild rye, S. montanum Guss. has desirable features such as leafiness, disease resistance, and a perennial habit which may be recombined with the seed characters of good cereale types. Such combinations would be important for forage breeding purposes. Similarly, the variability found in weedy ryes may be utilized in different rye breeding programs designed for grain or forage improvement.

The greatest concentration of Secale germ plasm occurs in southwest Asia. Although different species are somewhat

restricted to special areas, they overlap in their range of distribution. In spite of sympatric distribution in some areas, introgression of species delineating characteristics has not been observed. Therefore, there must be some efficient mechanisms which protect species identity. A study of such mechanisms seems necessary because of their evolutionary importance and to eliminate or circumvent them in successful crosses.

In this study attempts will be made to survey the variability in this genus and to find out the mean or means by which the desirable combinations might be obtained.

PART I

A STUDY OF WEEDY RYE SAMPLES FROM IRAN

1. Review of Literature

Although cereal rye is not cultivated in Iran, weedy representatives are widely distributed in fields of wheat and barley. On hilly sites where the soils are poor these weedy ryes are more competitive than wheat and lead to the popular notion that wheat actually is converted into rye.

There is little or no evidence suggesting that rye has been cultivated in this area in recent or ancient times. There is no special name for rye in the Sanscrit or Semitic languages; it is merely called the "grain in wheat" or the "grain in barley." Thus, it is clear that in southwest Asia, rye was known first as a weed among fields of barley and wheat and not as a distinct crop.

Collections of weedy rye from Iran were first made by Vavilov in 1916. He classified these samples into 23 varieties according to spike color, toughness of rachis, investment of grain, and glume characteristics. In later expeditions he increased the number of varieties to 27. Due to the great variability present in weedy rye from Iran, Vavilov considered Iran as a center of origin for rye. He also suggested that some weedy ryes of barley and wheat fields gave rise to cultivated rye by means of domestication and selection.

Another collection of weedy rye was made by Kuckuck during 1952-54 and this collection was studied by Kranz (27). In his study special reference was made to morphological characteristics of the population in relation to their geographical distribution. When these samples were classified according to Vavilov's method, individual varieties did not have a special distribution, but occurred at random throughout Iran. When frequency of a trait was studied, significant differences were found between populations from different areas and the differences increased with geographic distance. Evolutionary factors, such as isolation, selection, and hybridization were considered to be responsible for this differentiation.

Müntzing (36) studied samples of weedy ryes from Iran and found that two-thirds of the populations contained accessory chromosomes. The frequency of plants with accessory chromosomes, however, was quite low.

Cytogenetic analyses of crosses between and within Secale species on a population level, with reference to geographical distribution, have not been reported in the literature.

2. Materials and Methods

Ninety-two samples of weedy ryes from 50 different geographical locations in Iran were assembled (Fig. 1). These samples were grown in the greenhouse or in the field,

and their morphological characteristics were assessed. Individual spikes were isolated by means of glassine bags a few days before anthesis and crosses were made by changing bags on two spikes. Vernalization of winter types was accomplished by germinating seeds in petri dishes and leaving them in the refrigerator between 34°-40°F for about six weeks.

In studying the cytogenetic relationships of this collection, cytological analyses were carried out on the following materials:

1. Plants grown from original seed samples.
2. F_1 plants of crosses between parents from different geographical areas.
3. F_1 plants of crosses between the weedy ryes and cultivated rye.
4. F_1 plants of crosses between the weedy ryes and perennial rye.

For cytological observations, whole spikes still completely in the boot were fixed in Newcomer's solution made up of the following:

6 parts isopropyl alcohol

3 parts propionic acid

1 part acetone

1 part petroleum ether

1 part dioxane

The fixed materials were kept in the refrigerator and used at various intervals up to seven months.

Most observations were made from temporary propionic-carmine smears. Some permanent slides were made by floating the cover slip in 95 per cent alcohol for a few hours and remounting them with diaphane.

3. Results

Individual plants showed different degrees of self-fertility when bagged under greenhouse conditions, ranging from 0.0 to 27 per cent. This might be an adaptive feature of weedy rye isolated after continuous cultivation in wheat fields for many generations.

Most samples of weedy ryes were susceptible to rust and mildew, and only two showed complete resistance to both diseases under greenhouse conditions. In the field, however, these two samples also showed susceptibility.

When samples were grown in the greenhouse, no spikelets were detected with three florets, whereas the same samples showed a high number of such plants when grown in the field.

Meiosis was quite regular in weedy ryes from various areas and the diploid number $2n = 14$ was observed in all cases.

Seven bivalents were also generally observed in the F_1's of weedy ryes from separate areas. Several cases of asynapsis of chromosomes were detected. Sometimes three pairs of chromosomes did not pair and resulted in six univalents

and four bivalents. Such a case was found in crosses of two neighboring samples.

Cytological analyses of F_1's between weedy ryes and cultivated ryes also revealed a similar pattern of pairing and seven bivalents were formed. Two accessory chromosomes were detected in crosses which had in common a cultivated rye from Denmark. No accessory chromosomes were found in the original samples or in crosses between samples from Iran.

Hybrids between different samples of weedy rye and mountain rye (S. montanum) showed the same cytological properties as hybrids of cultivated rye and mountain rye, the results of which will be discussed later.

4. Discussion

In spite of the great variability exhibited by weedy rye when compared to cultivated rye, the karyotype is identical through the range of distribution in Iran. Not only are weedy ryes similar to each other but they are also similar to cultivated rye as far as their gross karyotypic structure is concerned. Cultivated ryes from different sources were also similar in this respect, and there is no report in the available literature that varieties of cultivated rye differ in their gross chromosomal structure.

All the materials studied were diploid which is especially important when we consider that in other genera of the tribe Hordeae, such as Triticum, evolution has been

achieved through polyploidy, and structural modifications such as translocations differentiate varieties.

Uniformity of karyotype in weedy rye throughout Iran and the gross structural similarity to cultivated rye emphasizes an adaptive value related to cultivation. Whenever rye comes under cultivation there is only one karyotype which can survive, and that exists in all latitudes and altitudes where rye is grown intentionally or unintentionally.

Cultivation as an important factor in the evolution of crop plants was emphasized by Vavilov (56) and its constituent factors outlined as follows by Darlington (10):

(a) Tillage conditions which lead to the selection of larger forms which are better competitors at higher fertility levels.

(b) Sowing conditions which lead to the selection of forms with even and rapid germination and also to selection of forms lacking the special mechanism for self-propagation.

(c) Harvesting conditions which lead to the selection of forms with tough rachises.

Under cultivation special forms of rye of one karyotype were developed. In southwest Asia rye met such conditions in fields of wheat and barley as a weed. Receiving similar management as wheat and barley, rye became more similar to the wheat and barley plants, and the grain became more difficult to separate. When wheat was carried to higher altitudes and wider latitudes, rye, being more winter hardy

and better adapted to sandy soils, predominated in certain areas and gradually became an independent crop.

Despite the fact that rye has been an important crop in areas other than southwest Asia, it still has the most diversity in this area. In southwest Asia rye meets the greatest climatic diversity and a multitude of ecological variations. Here, too, natural selection has produced a great diversity of forms. Such a diversity is unknown to cultivated rye elsewhere.

Fig. 1. Map showing locations of weedy rye samples from Iran.

PART II

INTERSPECIFIC HYBRIDIZATION IN *SECALE* AND SPECIES RELATIONSHIPS

1. Review of Literature

Longley and Sando (30) made interspecific crosses between *S. cereale* and *S. montanum* and found that the behavior of chromosomes in such hybrids was generally normal with bivalent formation but that univalents occasionally occurred.

Kostoff (25) found 75 per cent abortive pollen in such hybrids and Duka (13) reported pollen viability ranges between 5.0-38.0 per cent. Kostoff and Duka also reported only bivalent formations with some univalents in hybrids.

Schiemann and Nurnberg-Kruger (48) confirmed the low fertility of interspecific crosses and ascribed it to multivalent formation. They found a maximum association of four bivalents and a translocation chain of six chromosomes.

Jain (20) also observed a maximum association of a chain of six chromosomes plus four bivalents in the same interspecific hybrids.

Riley (46) observed cells with chains of 3, 4, 5, and 6 in similar hybrids. From this he concluded that *S. cereale* differs from *S. montanum* by two large translocations involving three pairs of chromosomes. Thus, he confirmed the observations of Schiemann *et al*. On the evidence of a

single cell with chains of 3 and 5 and another cell with a single chain of 8, he proposed a third translocation difference between the two species.

Price (41) studied the same hybrids and found a maximum association of 6 chromosomes in the first metaphase, as a result of two tranlocations. The translocation configurations were usually open chains and no closed multivalents were found. Inversion bridges were reported in hybrids as well as in their parents.

Price also irradiated pollen from S. montanum with which he pollinated S. cereale. In hybrids produced in this way he was able to detect a new translocation. This translocation involved chromosomes of the original chain of six and another pair of chromosomes.

Stutz (54) found a maximum association of four ring bivalents plus a translocation configuration made up of six chromosomes. He also reported some cells which had a complete ring of six chromosomes.

Ossent (38) found that F_1's of the same crosses were perennial with brittle rachises and the F_2's contained 75 per cent perennials with brittle rachises and one per cent perennials with tough rachises.

2. Materials and Methods

In order to study interspecific relationships in this genus, three different sources of S. montanum, two sources of

S. segetale, one source of S. ancestrale, one source of S. silvestre and many sources of both weedy rye and cultivated rye were used. Crosses were made both in the greenhouse and field. In self-sterile species it was possible to obtain hybrids without emasculation of the female parent and morphological recognition of hybrids made this procedure relatively simple to apply. Spikes were fixed in Newcomer solution and most observations were made from temporary propionic-carmine smears.

3. Results

In crosses between S. montanum and S. cereale, F_1 hybrids were obtained only when S. cereale was used as the female parent. In reciprocal crosses the seeds did not germinate or germinated very poorly and the seedlings died at early stages of development. F_1 plants of interspecific crosses were quite vigorous perennials and similar to their wild parent S. montanum with rachises brittle from the top to the center of the spike. They were later flowering than cultivated rye and slightly earlier than S. montanum. These hybrids had 57.71 per cent stainable pollen and their thousand kernel weight was about 23.6 grams.

Results of cytogenetic analysis of one thousand cells at diakiesis or metaphase I in S. cereale x S. montanum hybrids are shown in Table 1.

TABLE 1. Types of pairing in F_1 interspecific hybrids.

Type of pairing	% P.M.C.
7 II	4.5
6 II + 2 I	1.7
5 II + 1 I + 1 III	12.3
5 II + 1 IV	19.8
4 II + 3 I + 1 III	0.7
4 II + 2 I + 1 IV	0.8
4 II + 1 I + 1 V	13.6
4 II + 2 III	6.4
4 II + 1 VI	37.5
3 II + 2 I + 1 VI	0.7
3 II + 1 I + 1 VII	0.3
3 II + 1 III + 1 V	0.6
3 II + 1 VIII	1.0

More than 93 per cent of the cells analyzed at metaphase or diakinesis showed multivalent formation.

About 40 per cent of bivalents observed at metaphase I were open and 60 per cent closed. In most cases four or less closed bivalents were observed and only in four per cent of the cases as high as five ring bivalents were detected. Six or seven closed bivalents were not observed in any cell. This points out that one of the translocations is quite large and prevents formation of closed bivalents.

The configuration which was observed most frequently was four bivalents, three of which were closed and a chain of six chromosomes (Fig. 5). In such a configuration the chromosomes associated with the nucleolus appear as a bivalent and not associated in the chain of six. Five bivalents, three of which were closed and a chain of four, was next in order of frequency. Other configurations which are the result of modification of original associations were found with frequencies listed in Table 1.

There was no ring formation in multivalents observed as reported previously (Stutz). About two per cent of the cells showed configurations such as chains of VII, III + V and a chain of VIII, all of which indicate existence of a third translocation difference between the two species. The other pair of chromosomes which joins the chain of six in order to make a maximum association of three ring bivalents and a chain of eight is the pair of chromosomes which is associated with the nucleolus (Fig. 4). This explains why with such a high frequency of chains of six chromosomes there was no ring of six.

Since only gametes which contain alternate chromosomes of a translocation multivalent may survive, it is desirable to know how these multivalents are oriented at metaphase I. Results of such study in 273 multivalents are listed in Table 2.

TABLE 2. Multivalent orientation at Metaphase I in F_1 hybrids.

Chain	IV	V	VI
Alternate Segregation	70%	82%	49%
Non-Alternate Segregation	30%	18%	51%

The differences between orientation of multivalents may be due to the morphology of the chromosomes involved in multivalent chains. In a chain of VI, orientation seems to be quite random. In chains of IV it seems that more alternate disjunction takes place and for chains of V it is definitely nonrandom ($\chi^2 = 40.9 > 7.88$). Anaphase was studied in about 600 cells, the results being listed in Table 3.

As the table shows, more than 78 per cent of gametes received equal numbers of chromosomes.

Univalents which were present in more than 30 per cent of the cells (Table 1) had a different behavior at anaphase. In some cases the univalent or univalents passed undivided to one pole in anaphase I (Fig. 6) or they did not go to any pole and lagged behind to form a micronucleus. In other cases univalent(s) divided mitotically (Fig. 7). Then each sister-chromatid moved to opposite poles (Fig. 8). At second anaphase, the behavior of univalents depended upon their behavior at first anaphase. If they did not divide at

TABLE 3. Analysis of anaphase I in F_1 hybrids.

$7_I - 7_I$	$6_I - 8_I$	$6_I - 1_I - 7_I$	$6_I - 2(\frac{1}{2}) - 7_I$	6_I — $2(\frac{1}{2})$ / $2(\frac{1}{2})$ — 6_I	$6\frac{1}{2} - 7\frac{1}{2}$
78.2%	13.4	3.7	2.5	0.9	1.2

TABLE 4. Per cent of tetrads with different numbers of micronuclei in F_1 hybrids.

Number of micronuclei	No M.N.	1 M.N.	2 M.N.	3 M.N.
% tetrads	70	10.5	17.5	2.0

anaphase I then they divided at anaphase II. Otherwise, they were unable to divide again whereupon they usually lagged on the plate and were often lost in the cytoplasm to form micronuclei (Figs. 10, 11, 12).

As the number of micronuclei could be used as an indication of irregularity of meiosis, more than 900 tetrads were analyzed for this purpose and the results are listed in Table 4.

Analysis of F_2 Population

F_2 populations were obtained by growing seeds harvested from F_1 plants whose cytological properties had been analyzed. A population of 38 F_2 plants was chosen for morphological and cytological analysis.

Cytological analysis of the population revealed only two kinds of plants. Of 38 plants analyzed, 17 were structurally homozygous and formed only bivalents with occasional univalents, and 21 plants were structurally heterozygous. In heterozygous plants, cytological analysis revealed exactly the same associations as reported for F_1's (Table 1). There was no single heterozygous plant which showed only a tetravalent association with no chains of six and occasional chains of eight. This shows that these four chromosome pairs segregate in parental combinations.

The homozygotes were of two kinds; ten of which were early flowering annuals with tough rachises, the other seven

were free tillering, late flowering plants with brittle rachises.

Morphologically, the heterozygous plants were essentially similar to F_1's. They were perennial, with brittle rachises, and displayed low fertility. Mean fertility for this group was $\bar{x}$ = 35.7 per cent whereas fertility for homozygotes was $\bar{x}$ = 53.2 per cent.

An asynaptic plant was found in the F_2 population with a high number of univalents in metaphase I. The average number of univalents was 4.81, for pseudobivalents 1.85 and for true bivalents 2.20 per cell. A few multivalents were also observed. There was a great disturbance in the karyotype of this plant as reflected in the chromosome number (Figs. 14, 15), fragmentation (Figs. 17, 18), and distribution at anaphase.

In several cases of asynaptic rye reported in the literature (40) none has been accompanied by chromosome fragmentation. In Scilla sibirica, however, Rees (45) found a similar situation in which asynapsis was in conjunction with spontaneous chromosome breakage. But meiosis was normal in cells which did not have fragments and frequency of cells having fragments was about three per cent.

In the rye plant studied, meiotic disturbance was not always accompanied by fragmentation. When anthers from different florets of the same spike were examined, differences were found. In some florets only asynaptic chromosomes were found, either as univalents or pseudobivalents. In a few

florets true bivalents as well as asynaptic univalents were found (Fig. 16).

A high percentage of tetrads showed micronuclei, and fertility was as low as 5.9 per cent with caryopses being shrunken.

Cytogenetic Analysis of Other Species Intercrosses

Other species of rye used in this study were S. segetale, S. ancestrale, and S. silvestre.

When S. segetale and S. ancestrale were crossed with S. cereale they formed seven bivalents and in crosses with S. montanum they behaved exactly as S. cereale.

In the case of S. silvestre the situation was different. This is a self-fertile species which is quite short with narrow leaves and extremely brittle rachises. Attempts to cross this species with S. cereale were unsuccessful in both directions. There is no report in the literature of such a cross being made. When S. silvestre was used as female and S. cereale, variety Gator as male, many embryos were initiated but failed to reach maturity. Many of them aborted prior to the dough stage. When hybrid embryos were dissected and cultured on artificial media some of them developed and produced vigorous plants. In reciprocal crosses with S. cereale as the female, the embryos failed to show signs of development. Incompatibility between the embryo and surrounding tissue appeared to be the cause of failure in

crossing the two species. According to Jain (20) this species crosses easily with S. africanum and gives a 4 II and VI association in the meiotic metaphase of F_1 plants. This indicates that the karyotype of S. silvestre is similar to that of S. cereale.

4. Discussion

In F_1 hybrids between S. montanum and S. africanum seven bivalents with occasional univalents were observed. This shows that the two species are similar in gross chromosomal structure.

Cytological analysis of the hybrids between S. cereale and S. africanum revealed the same pattern of pairing as S. cereale x S. montanum hybrids. In both cases there were three translocation differences between the two species. Two of these translocations were large and caused multivalent formation in many cases, whereas the third one was small and only in about two per cent of cells formed chains of eight.

These translocations do not occur independently; that is, they have some pairs of chromosomes in common. If they did not involve any common pairs of chromosomes they would have produced only three independent tetravalents. This, of course, is not the case and translocations have brought together four pairs of chromosomes in order to form a chain of eight. This must have a selective advantage over three independent translocations. Analysis of F_2 populations

showed that only two kinds of zygotes can form, those which are homozygous and are of S. cereale or S. montanum type and those which are heterozygous and exactly similar to F_1 plants. The ratio was close to 1:2:1 S. cereale, F_1 and S. montanum, respectively. This suggests that in gametes produced by F_1 plants, only parental combinations, as far as these four pairs of chromosomes are concerned, can survive. Other gametes being deficient or duplicated for segments of chromosomes are discarded. Thus in rye, the four pairs of chromosomes involved in translocations are transmitted as a single Mendelian factor and segregate to maintain species identity. In a translocation configuration, the homologous parts of chromosomes which are not between a kinetochore and translocated segment can recombine easily. In interstitial segments crossing-over causes duplications and deficiencies in most cases, especially when multivalents consist of more than four chromosomes, and interstitial chiasma are not frequent. Therefore, we can assume that the genes determining specific characteristics are located in interstitial segments and are transmitted as a linkage group and thus gene exchange, as far as these characters are concerned, is suppressed. Structural heterozygosity is not the only mechanism which keeps the two species separate. In the course of evolution other barriers have also evolved. As was previously mentioned, the cross between the two species is successful only when cereal rye is used as the female. In reciprocal crosses seeds

do not develop or seedlings die in early stages of growth. Therefore, in nature, hybrids of S. cereale and S. montanum can only occur in fields of wheat and barley where cereal rye grows as a weed. The hybrid plants are perennial with brittle rachises. Thus, in major species characteristics, on which ecological adaptation is based, they resemble mountain rye, whereas they occur in habitats suitable for cereal rye. Under these conditions they are vigorously selected against. The F_1 hybrids are selected against, not only because they cannot survive under cultivation, but also because they bloom later than the mass of cereal rye. Being self-sterile and only 28 per cent fertile in cross pollination, back crosses are not very frequent in nature.

How different species of Secale are related is not quite clear. S. africanum seems to be derived from S. montanum. Both are perennial species with similar morphological and cytological characteristics and they intercross easily. The only main difference between the two species is that S. africanum is self-fertile and occupies only one mountain range in South Africa, and is cut off from the main Secale germ plasm which is found in southwest Asia and in the Mediterranean region (Fig. 2).

It is not quite clear how this species occurs a long distance from the main germ plasm of the genus. Anderson (1) suggested that in the geological past the whole continent of Africa was probably exposed to Secale and later unfavorable

conditions eliminated this genus in most parts. Only in South Africa has this species persisted. Such a premise intimates that at least in some other places in Africa with conditions similar to the present habitat of S. africanum and probably identical past geological changes, one should find this or a similar species. There is no such report in the available literature. Therefore, we may assume that long distance dispersal of S. montanum by different means has given rise to S. africanum.

S. cereale is certainly younger than S. montanum. Cereal rye exists only under cultivation as a result of man's activity, a relatively recent event. Cultivation of wheat and barley was practiced by man in the early days in southwest Asia, where S. montanum is endemic. This practice created a special habitat which did not exist previously. Many new variants from S. montanum reached these areas as before, but now some of them found suitable conditions. Probably at first these new forms were only the result of gene mutations. But still S. montanum occupied the surrounding areas, and the new forms which had established themselves in cultivated lands had to be protected from being lost as a result of inter-crosses with mountain rye. Thus selection favored establishment of translocations as a protective device and at the same time as a linkage measure for species characteristics.

With the rather wide distribution that weedy rye has today, it is difficult to see how these genic and structural

changes have been fixed in the whole area. Actually cereale rye started as a weed in fields of wheat and barley. The areas in which these crops were grown at first were not large and consequently the weedy rye populations were small. Natural selection and a small population size could have easily fixed genic and structural changes in the early weedy rye populations.

Wheat, being a valuable crop, was carried by man in different directions to be grown in fertile valleys. Rye grains mixed with the wheat were likewise transferred to different regions. Thus, we may postulate that an originally small weedy rye population, in which some characteristics had been fixed, spread throughout the range of distribution in which we find weedy rye today.

If the above assumption is true, then we might expect to find a great uniformity between weedy ryes from different regions. As far as gross chromosomal structure is concerned we have shown that weedy ryes are similar. There are significant differences in morphological characteristics between populations of weedy rye from different regions. Such a great variability of weedy rye might have been obtained in two ways; first by direct action of natural selection on weedy rye populations in each region; second by introgression of nonspecific characteristics from S. montanum, an endemic in those areas into which weedy rye was introduced. In this way weedy rye could have obtained a great deal of variability from mountain rye which was also adapted to its new habitat.

For S. silvestre the situation is different. This species occupies the uncultivated sandy soils of the Sarmatian basin north of the Black Sea and Caucasia (Fig. 2). This is a completely self-fertile species with flowers which have a great tendency to cleistogamy. It is an annual with the karyotype of S. cereale.

Stutz (54) suggested that S. cereale might have been derived from the products of interspecific hybridization between S. silvestre and S. montanum. Such an assumption seems to be at variance with the known facts about the three species involved. First of all, self-fertility and cleistogamy in S. silvestre prevent hybridization between this species and S. montanum, and if such hybrids are occasionally produced, translocation differences between the two species provide a mechanism which prevents recombination of certain characteristics. Secondly, assuming that successful hybridizations occurred, S. silvestre has little to contribute to the characteristics found in S. cereale. The only major characteristics, which S. silvestre might have contributed to such a hybrid, is an annual habit and it seems more reasonable to assume that such mutants have been selected directly from S. montanum under cultivation.

As an alternative assumption we may propose that S. cereale in its upward movement, whether in wheat or as a distinct crop, was brought in contact with sandy soils which were not cultivated. Under these conditions the tough rachis

of *cereale* types was a great disadvantage and, therefore, mutants with a brittle rachis had a high selective value. Drastic and unfavorable conditions in the sandy desert area promoted self-fertility and a complete reproductive barrier developed protecting this species from crossing with cereal rye from surrounding areas. Morphological changes such as short, thin, and spreading stems with narrow leaves provided the species with better adaptive features in this habitat.

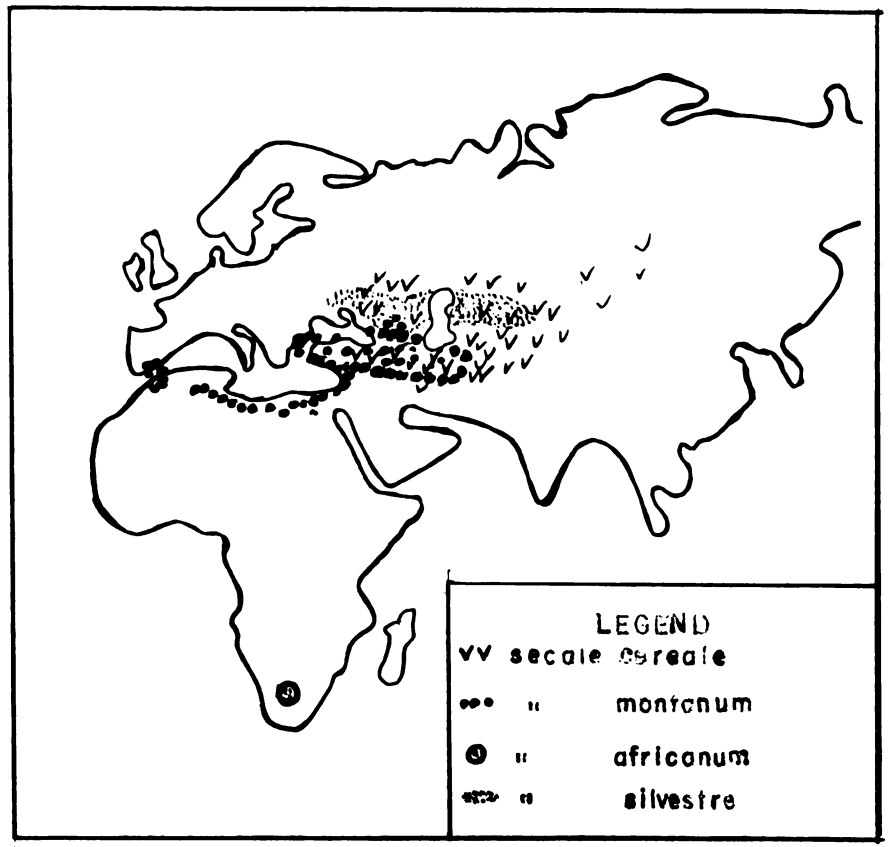

Fig. 2. Map showing distribution of some Secale species (adapted after Vavilov, 1925).

Fig. 3. Metaphase I in F_1 hybrids of S. cereale x S. montanum. 5 II (3 closed) +1 I + 1 III. 951X.

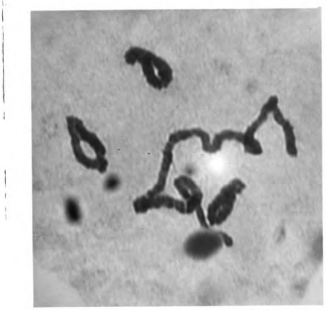

Fig. 4. Metaphase I in F_1 hybrids of S. cereale x S. montanum a chain of 8 plus 3 closed bivalents. 897X.

Fig. 5. Metaphase I of F_1 hybrids in S. cereale x S. montanum. A chain of 6 showing alternate orientation plus 4 bivalents. 938X

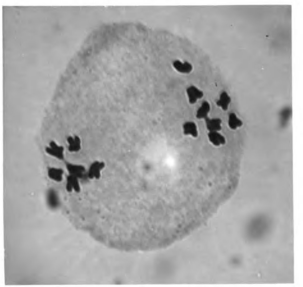

Fig. 6. Anaphase I in F_1 hybrids of S. cereale x S. montanum, showing unequal distribution. 831X.

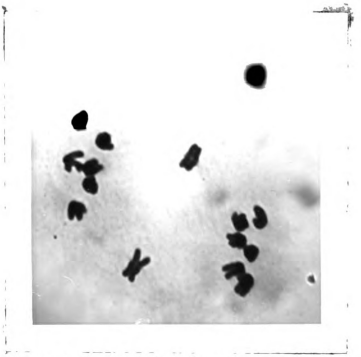

Fig. 7. Anaphase I in F_1 hybrids of S. cereale x S. montanum, lagging univalents start to divide. 970X.

Fig. 8. Anaphase I in F_1 hybrids of S. cereale x S. montanum, lagging univalents have divided. 861X.

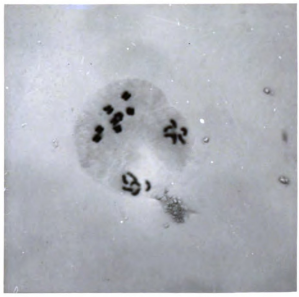

Fig. 9. Anaphase II in F_1 hybrids of S. cereale x S. montanum, unequal distribution at anaphase I and unsynchronized division at anaphase II. 473X.

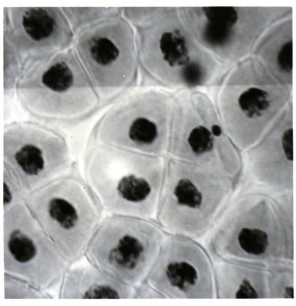

Fig. 10. Micronucleus in tetrads of F_1 interspecific hybrids of S. cereale x S. montanum. 82X.

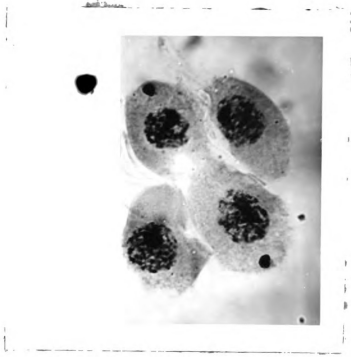

Fig. 11. A tetrad with two micronuclei. 89X.

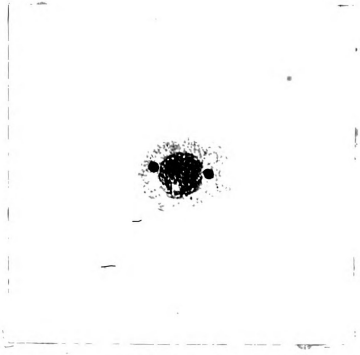

Fig. 12. Microspore with two micronuclei. 78X.

Fig. 13. Microspore with two nuclei. 89X.

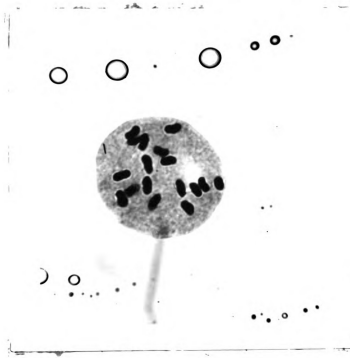

Fig. 14. Seventeen univalents in metaphase I of meiosis in asynaptic rye. 1040X.

Fig. 15. Nine "pseudobivalents" at metaphase I in asynaptic rye. 815X.

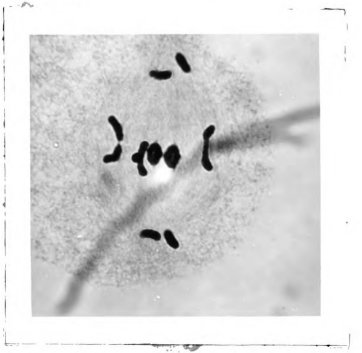

Fig. 16. Five bivalents and four univalents at metaphase I in asynaptic rye. 856X.

Fig. 17. Spontaneous chromosome breakage in asynaptic rye with other disturbances. 846X.

Fig. 18. "Pseudobivalents," univalents, and chromosome fragments in asynaptic rye. 854X.

PART III

USE OF CHELATING AGENTS TO FACILITATE RECOMBINATION

1. Review of Literature

It has been suggested that calcium and magnesium ions play an important role in maintaining chromosome structure.

Mazia (31) reported dispersion of salivary gland chromosomes of D. melanogaster by treatment with chelating agents, such as EDTA, at low ionic concentrations. On the basis of such experiments a particulate structure for chromosomes was suggested, the particles being held together lengthwise by divalent cation bridges. He showed that two conditions should be met if the chromosomes are going to be dispersed: first, treatment with an agent capable of binding Ca^{++} or Mg^{++} ions and, second, provision of a medium of sufficiently low ionic strength. He predicted that chromosome breakage and crossing-over would be sensitive to the ionic environment of the chromosome.

Kaufmann and McDonald (23), on the other hand, attributed the effects of EDTA to alteration in general metabolism of the cell rather than to direct breakage of chromosomes through chelation of divalent cations serving as bridges between macromolecular complexes of protein and nucleic acid. By comparison of the action of EDTA with that of ribonuclease in modifying crossing-over in Drosophila they indicated that the latter agent may be more effective

than the former under specified conditions of treatment.

Steffensen (53) reported induction of chromosomal alterations during meiosis in Tradescantia by raising plants under magnesium deficient conditions. Using Solution I of Hoagland and Arnon; he adjusted the concentration of calcium to three different levels. The first solution contained 2.5 ppm of calcium. After three months clones grown in this solution were transferred to a solution lacking Ca^{++} altogether. The second solution contained 10 ppm of Ca^{++} for the first three months and 2.5 ppm thereafter. The third solution contained 250 ppm of calcium, an optimal supply.

Buds were collected during the sixth and seventh months of incubation.

Steffensen found that plants grown in the first solution contained only abortive cells, none of which were undergoing microspore division. Chromosomal alterations could not be analyzed in these anthers. Plants grown in the second solution, which was suboptimal, exhibited at least 17 times more chromosomal aberration and micronuclei than did plants grown with optimum calcium. From these results, he concluded that bivalent cations may bind together the macromolecular constituents of the chromosome and thereby provide structural stability.

Kirchner (24) removed calcium and magnesium from bacterial cells either by treatment with Versene or by culture in a minimal medium lacking these ions. He obtained an

increase in the frequency of recombination.

Hyde (19) treated non-excised root tips of onion and Vicia faba with .01 M solutions of EDTA. The first noticeable effect within three hours of the treatment was suppression of anaphase movement of chromosomes. Chromosomes swelled in metaphase and late prophase. This swelling was not accompanied by any marked loss of stainability. Therefore, Hyde concluded that EDTA treatment breaks chromatin into smaller units. When he treated root tips with a 1:1 molar ratio of $CaCl_2$ and EDTA the cells continued to divide normally.

Wolff and Luippold (58) soaked seeds of V. faba for varying times in a .001 M solution of EDTA. They found chromosome breakage as a result of the treatment and the amount of chromosome breakage produced by combined calcium deficiency and radiation was more than the additive amount of both when applied alone.

Davidson (11) treated root tips of onion and flowering shoots of Tradescantia with solutions of chelating agents (EDTA and quinaldic acid) and $CaCl_2$, $MgCl_2$, and NaCl. He found various abnormalities at meiosis and mitosis. Chromosome breakage occurred but only in shoots and after a long delay. The chelates did not appear to be as specific in vivo as they were in vitro. Therefore, he concluded that the effects found after treatment could not be attributed to the inactivation of particular cations.

Eversole and Tatum (15) found variation in the amount of crossing-over after treating the cells of biochemical mutant strains of Chlamydomonas reinhardi with a chelating agent or with $MnCl_2$. The frequency of crossing-over was increased by these treatments in two of three chromosome intervals tested. The effect of both agents were reversed by subsequent incubation of treated cells in high concentrations of calcium and magnesium ions. It is suggested that the action of $MnCl_2$ may involve the replacement of Mg by Mn. They concluded that the sensitivity of crossing-over in C. reinhardi to ionic environment is consistent with the theory that chromosomes are composed of macromolecular unit particles linked together by divalent ions.

Caldwell and Burdich (5) reared Drosophila melanogaster on media containing various amounts of EDTA. They studied uptake, viability, and crossing-over resulting from these treatments. They found that crossing-over was increased at all levels of EDTA, the maximum effect being produced at 50 per cent of maximum uptake level where viability was still equal to the control.

2. Materials and Methods

Four solutions of EDTA with concentrations of 0.1 M, 0.01 M, 0.001 M and 0.0001 M were prepared and the pH adjusted to about 7.0.

F_1 plants of crosses between S. cereale and S. montanum

were used which have a maximum association of a chain of eight and three closed bivalents at metaphase I.

Spikes still in the boot stage were chosen for treatment in which meiosis would continue to occur for a few days.

For each treatment, including the control, four plants were allocated. The plan was to apply two different rates of EDTA for each concentration. This was not quite possible, because the culms did not have the same capacity even in the same plant and equal amounts of EDTA did not produce similar observable effects.

Concentrations of 0.1 M were too strong and in most cases killed the spike in the boot, and 0.0001 M did not produce any observable effects. Therefore, they were both discontinued.

Hypodermic needles were used to inject the solution into the plants. An attempt was made first to inject solutions into the base of spikes, but this was found impossible to achieve. Unless the amount was very small the solution was pressed out by the boot. Internodes of the culm were found to be a suitable place for injection, since they are hollow and the nodes are solid.

In order to inject the solution in the culm, the needle was first inserted at the top of the internode to allow air to escape while solution was injected into the lower part of that internode. Glue was applied to both holes and it prevented leakage of the injected solution. The capacity of internodes varied from less than 0.1 ml to more than 1.0 ml,

depending on the vigor of the plant. Therefore, the capacity of the internode was taken as the approximate vigor of the spike and in each case it was filled with solution. Cytological observations were carried on, on the spikes, which showed visible damage when they were fixed for observation.

All the plants used for treatment were grown in the greenhouse. Spikes of treated culms were bagged together for interpollination. Seeds from these F_1 plants were grown in the field in early spring. Many of these F_2 plants stayed vegetative and did not produce spikes, those which produced spikes were cytologically analyzed.

3. Results

Treated culms showed symptoms after 24-48 hours. First an injured region appeared along the midrib of the leaves above the treated internode; then the edges of the leaves began to curve inward. Gradually injured spots appeared over the whole surface of the leaf and chlorosis developed. In most cases the first three leaves from the top died.

Chemical analyses were carried out by spectographic methods in order to determine whether changes in chemical composition had occurred in treated plants. Three parts were taken and analyzed separately; the first including the spike plus two top leaves, the second the third leaf, and the third the first internode. Results of the analysis are listed in Table 5. Samples were taken when visible damage from treatment was obvious.

TABLE 5. A comparison between amounts of Ca, Mg, and P in control and treated plants as measured by spectographic method.

		% Ca	% Mg	% P
Spike plus 1st and 2nd leaf:				
	treated	0.70	0.35	0.73
	control	0.48	0.26	0.85
3rd leaf:				
	treated	0.75	0.42	0.73
	control	0.57	0.36	0.91
1st internode:				
	treated	0.26	0.22	0.80
	control	0.26	0.22	0.95

As shown in the table, the amount of calcium and magnesium was greater in treated parts than in the control, except for the first internode in which the amount remained unchanged. In fact, the difference between control and treated plants decreases as less active parts of the plant are compared. The largest differences were found between young leaves. No differences were observed between treated and control internodes.

Cytological analysis. EDTA treatments produced changes in the morphology of the chromosomes. These changes ranged from complete agglutination to only very slight changes. In many cases, metaphase chromosomes had swollen and fragmentation was not observed in any case. Analysis of metaphase I in pollen mother cells revealed the same configurations as in controls and the treatment did not seem

to have produced any gross structural changes such as breakage, new translocations, and inversions. During the analysis, however, it was found that the number of interstitial chiasmata had increased. These are the chiasmata which form in a translocation configuration in that part of the chromosomes between the point of interchange and the centromere. According to Darlington (8), these interstitial chiasmata cannot terminalize; therefore, they are arrested between the point of interchange and the centromere. Several workers have given views in support or rejection of this hypothesis, but none seem to be conclusive. Among those who have rejected Darlington's hypothesis is Price (42) who worked with an interspecific hybrid of S. cereale L. x S. montanum, Guss., the same material used in this experiment. He found a special multivalent in the first metaphase which he calls "frying pan." This association was found in an irradiated hybrid plant obtained by pollinating S. cereale with x-rayed pollen of S. montanum. This "frying pan" configuration is exactly the same as is shown in Fig. 27. He suggests that this configuration is the result of terminalization of an interstitial chiasma. Thus, he refutes Darlington's hypothesis that terminalization of chiasmata is arrested at points where homologies change. In a diagram as reproduced on page 49, he shows how this terminalization takes place. As far as terminalization of interstitial chiasma is concerned, configurations observed in this study confirm Price's views.

But as to how this terminalization takes place, a somewhat different point of view may be suggested. Fig. 28 and its diagrammatic interpretation on page 50 suggest that there is no three-chromosome terminal chiasma as diagrammed by Price. Fig. 28 shows that a multivalent association opens when an interstitial chiasma is terminalized. This breaking of multivalents is the result of terminalization of regular chiasmata formed between two homologous parts of non-homologous chromosomes in a translocation configuration. Thus chromatids involved in interstitial chiasma have to pass through two chiasmata in order to terminalize and this is accompanied by opening of the multivalent at least one point. Therefore, it seems reasonable to assume that interstitial chiasmata terminalize later than normal chiasmata. Such a delay makes them more suitable for counting than regular chiasmata.

When the frequency of interstitial chiasmata in controls and treated materials were compared, marked differences were found. In the controls, they occurred in 4.8 per cent of multivalents with a coefficient of variability of 32 per cent. In treated materials corresponding values were 23.4 and 67.0 per cent, respectively. These chiasmata occurred in chains of III, IV, V, and VI.

Anaphase I analyses revealed more unequal chromosome distribution in the treated material than in the controls. The results, however, were not very consistent and considerable variation was found from plant to plant. Table 6 shows the result of anaphase analyses.

TABLE 6. A comparison of anaphase I distribution in control and treated plants of F_1 interspecific hybrids.

	7 I - 7 I	6 I - 8 I	Others
Control	72.8%	13.4%	13.8%
Treated	58.9	30.4	10.7

It seems reasonable to assume that higher chiasma frequency in treated materials maintains chromosome associations for a longer time and this may enhance chances of unequal distribution especially in multivalents.

The amount of seed set in treated F_1 plants was 15 per cent which is about half of the controls with 27.7 per cent. This can be interpreted as the result of increased meiotic disturbances, in structurally heterozygous plants, due to EDTA treatments. But when structurally homozygous plants of cultivated rye, variety Gator, were treated with 0.010 M and 0.001 M EDTA the amounts of seed set was 17.8 and 38.7 per cent, respectively. Controls of the same variety had a seed set of 51.8 per cent under similar conditions. Cytological analyses of treated Gator plants did not reveal any visible changes. Therefore, reduction of seed set in treated F_1 plants is due not only to increased meiotic irregularities, but may be attributed to a general metabolic disturbance.

Cytological analysis of F_2 plants obtained from seeds

harvested from treated F_1 plants showed no significant difference from those of the control F_2 populations. Of 41 plants analyzed, 18 were structurally heterozygous and morphologically similar to F_1's and 23 were structurally homozygous. Weight of 1000 caryopses was calculated, the mean being 19.2 grams and coefficient of variability 25.1 per cent. Therefore, there was no significant difference between treated and control F_2 populations with regard to the seed weight or its variance.

4. Discussion

With the knowledge that three translocations are the major factors which keep the two species separate, attempts were made to induce new translocations or to reverse natural ones.

It is suggested that Ca^{++} and Mg^{++} play an important role in maintaining chromosome structure. Plants deficient in these ions can be produced by growing them in an artificial medium lacking these ions. But this is a long process and causes an over-all disturbance in plant metabolism. Rapid inactivation of metallic ions in solutions can be achieved by chelating agents. These form chelate-metal complexes and though soluble, are not ionized. EDTA shows a marked affinity for calcium ions.

In this experiment EDTA did not produce any chromosome breakage and two cells each with one additional translocation

cannot be indicative of such effects. As the treated cells are the last or close to the last ones in their lineage to form microspores, any effective treatment will produce very limited cells with modified chromosomes. It has been shown by several workers that chelating agents produce more recombination of genetic material in bacteria and Drosophila. Information postulating chiasma formation as the cytological mechanism for genetic recombination are not conclusive. Formation of interstitial chiasma in structurally heterozygous hybrids of S. cereale x S. montanum and their increase by means of EDTA seemed at first to be a suitable case for such investigation. If more recombinant types had been recovered in F_2 populations from treated F_1's than in F_2 populations from control F_1 plants, then we could have established a positive correlation between chiasma frequency and genetic recombination. This was not the case. Recombinants did not appear either from cytological analysis of treated F_2's or from their morphological characteristics. Systems of three translocation differences makes it very improbable for products of interstitial crossing-over to be recovered. There should be other cross-overs in the same chain to compensate for chromosomal unbalance produced by the first interstitial cross-over. As the treated F_1 plants had only 15 per cent seed set or about half of that of the controls, those gametes which survived may have been those with parental combinations.

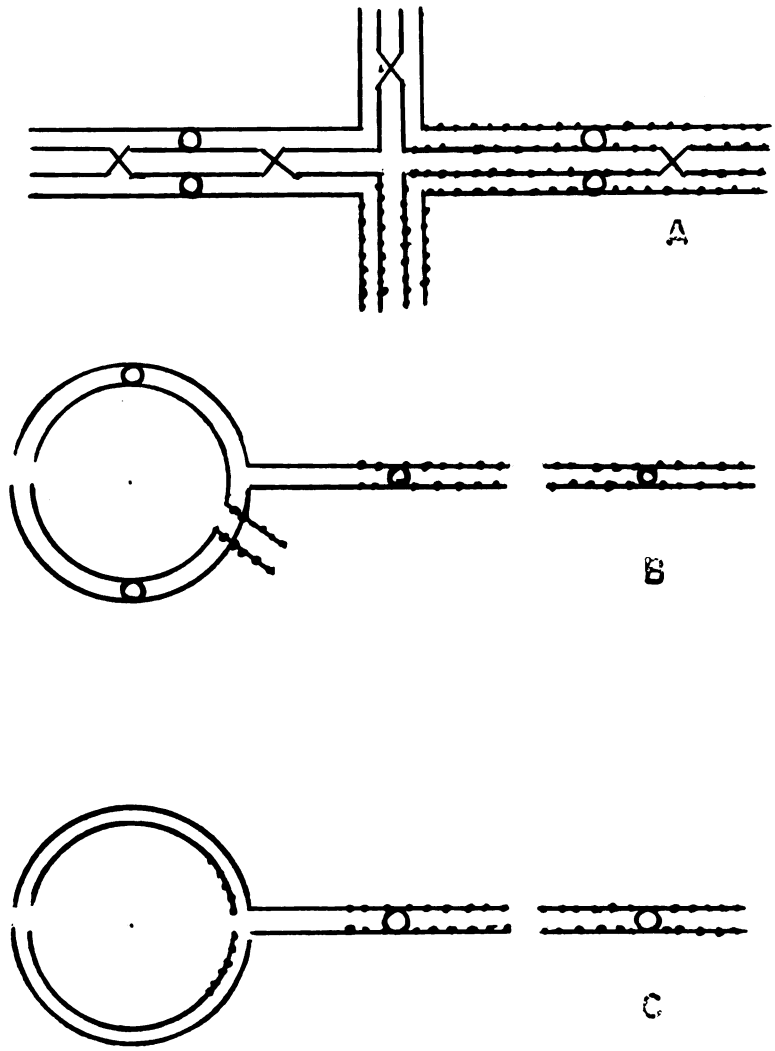

Fig. 19. Diagrams of 4 chromosome associations in a translocation heterozygote. A, pachytene association with chiasmata located so that a "frying pan" configuration would result at first metaphase; B, first metaphase with terminalization of proximal chiasma arrested at the point of interchange; C, first metaphase with terminalization completed despite changes of chromosome homology. (After Price, 1959.)

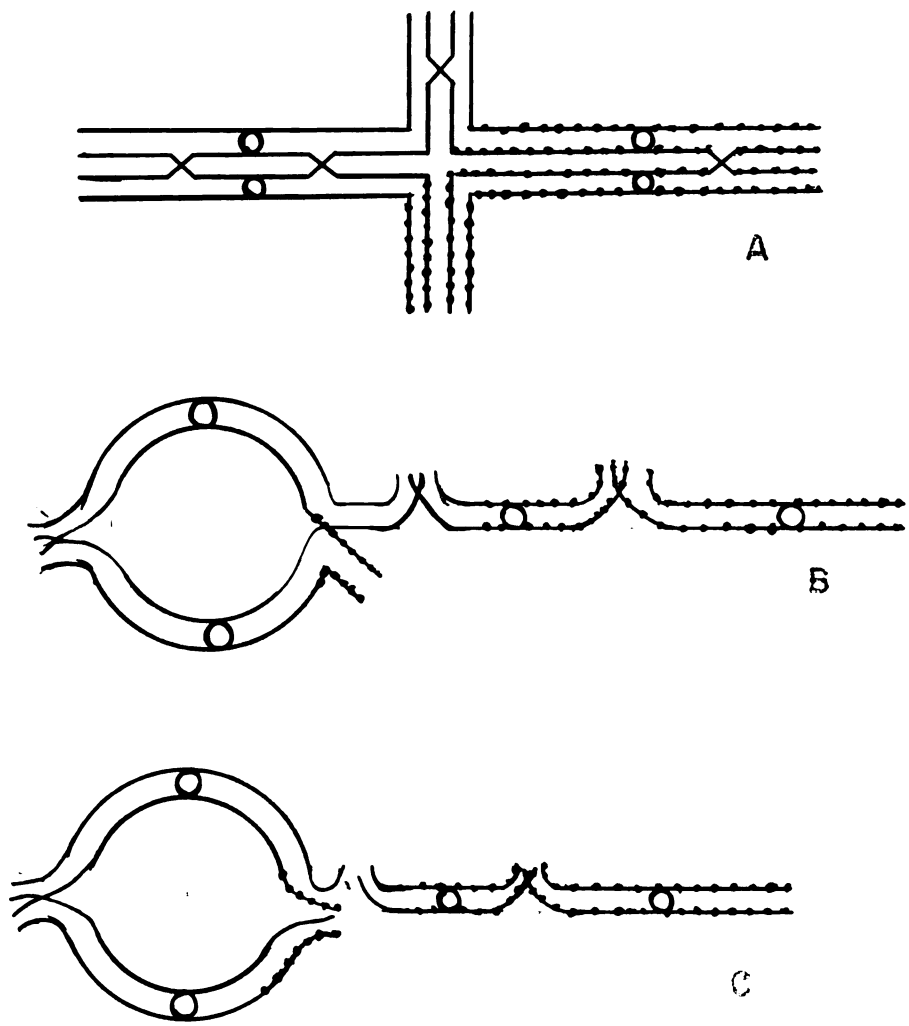

Fig. 20. Diagrams of 4 chromosome associations in a translocation heterozygote. A, pachytene association, B, metaphase I, and C, the same association at a somewhat later stage. Complete terminalization of interstitial chiasma is accompanied by opening of the "frying pan" configuration.

Fig. 21. Diakinesis in F_1 hybrids of S. cereale x S. montanum. 849X.

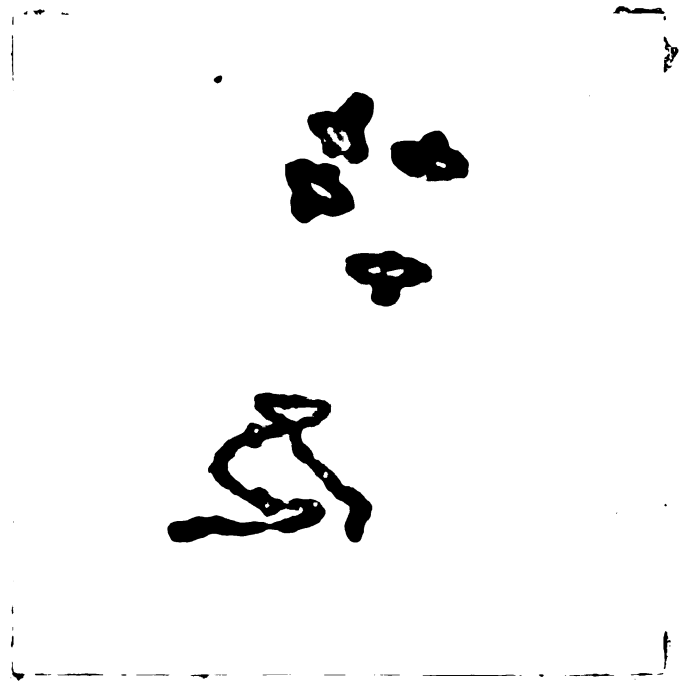

Fig. 22. Metaphase I in F_1 hybrids of S. cereale x S. montanum. Four closed bivalents and an interstitial chiasma in chain of six. 1263X.

Fig. 23. Metaphase I in F_1 hybrids. Four bivalents plus a chain of six with an interstitial chiasma. A later stage than Fig. 22. 1202X.

Fig. 24. Interstitial chiasma in a chain of four.

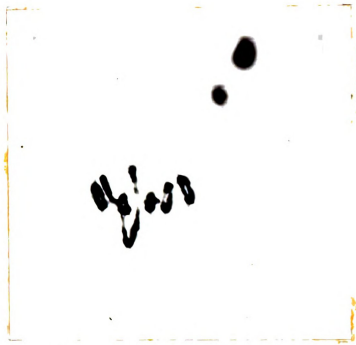

Fig. 25. Interstitial chiasma in a chain of six. 754X.

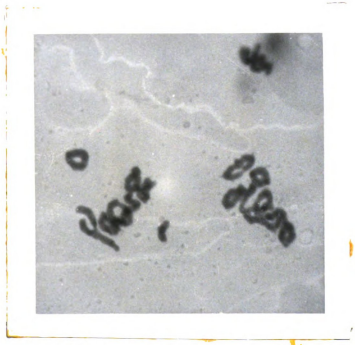

Fig. 26. Two interstitial chiasmata in chains of III and IV. 844X.

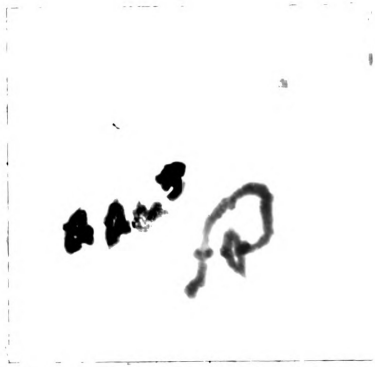

Fig. 27. Four closed bivalents plus a "frying pan" configuration made up of six chromosomes. 1254X.

Fig. 28. Open "frying pan" configuration. 824X.

PART IV

ALLOPOLYPLOIDY INDUCTION

1. Review of Literature

In the process of organic evolution, nature has long utilized polyploidy in creating new species. Recently plant breeders have used this method to produce more suitable plants. In flowering plants, nearly half the species, and in the Gramineae about 75 per cent of the species owe their origin to polyploidy. In general, the origin of polyploids in the Gramineae has been through hybridization and subsequent chromosome doubling of the hybrids. With such a high occurrence of polyploidy in the grass family, differentiation in Secale has occurred entirely at the diploid level (2n = 14). Lack of polyploid series and almost complete barriers to recombination of specific characteristics at the diploid level encourages allopolyploidy induction in this genus.

In order to produce polyploids artificially different methods have been utilized such as tissue wounding, grafting, heat treatment, colchicine techniques and nitrous oxide.

Blakeslee and Avery (3) opened a new era in polyploidy investigation when they announced in 1937 the discovery of colchicine as a powerful chemical agent in inducing polyploidy in plants. Since that time many polyploid plants have been produced by this method. Colchicine techniques usually produce plants which have polyploid chimeras. Attempts have

been made to double the embryonic cells as close to the first mitotic division of the zygote as possible. This may result in plants whose chromosomes are doubled in all or most of the tissue. For this purpose gases such as nitrous oxide which have a good penetrating power have been utilized.

Östergren (39) in 1944 treated Crepis capillaris with N_2O under pressure of ten atmospheres for four-six hours at the first or second zygotic division. By this method he obtained a fair number of polyploids.

Nygren (37), also using nitrous oxide, was able to induce polyploidy in Melandrium.

Cooper (7) used nitrous oxide at 50, 75 and 100 pounds pressure in order to induce polyploidy in Vicia. Plants receiving 75 pounds pressure or more did not set any seeds. No tetraploids were found in the samples of treated materials.

The first autotetraploid plants in rye were induced by Dorsey (12) in 1936, using the heat shock method developed by Randolph (43). After the discovery of the colchicine technique many more autotetraploid ryes were produced, especially in Sweden.

Chin (6) analyzed metaphase I of autotetraploid rye and found that the maximum number of quadrivalents was four in one cell. He also observed bridges at the first anaphase. The material under study was directly obtained from the diploid parent by doubling the chromosome number and no selection had been practiced at the tetraploid level.

Tetraploids had a seed set of about 23.8 per cent.

Müntzing (35) studied rye autotetraploids for several different characteristics and compared them to their diploid parents. These tetraploids had been selected for seven generations for desirable agronomic characteristics. At metaphase I of this material, an average of four quadrivalents were found per cell with many univalents. Tetraploid plants were about 15 per cent taller than diploids and their degree of tillering was reduced about 12 per cent. Tetraploids had fewer flowers per spike than diploids but their caryopsis weight exceeded those of the diploids by more than 50 per cent. In the best tetraploid varieties the average seed set was about 62 per cent, whereas the corresponding value for diploids was 80 per cent.

Morrison (33) studied autotetraploid rye selected for fertility over a 15 year period. He did not find any significant difference between this rye and "Steel-rye" produced by Müntzing and selected for fertility for seven generations with respect to meiotic behavior. Therefore, he concluded that selection for increased fertility was not accompanied by changes in pairing behavior. Hilpert (18), on the other hand, believed that good fertility was correlated with regular meiosis.

Although several workers have tried to combine desirable characteristics of different rye species at the diploid level, there is no report of utilizing allopolyploidy for this purpose.

2. Methods and Materials

In order to induce polyploidy two methods were employed:

1. Nitrous oxide under pressure,
2. Colchicine.

Nitrous oxide was applied under five different pressures; namely, 75, 100, 125, 150, and 200 pounds per square inch. Duration of treatments was 6, 12, 18, and 24 hours. Heads were bagged individually and pollinated when completely receptive. Treatments started 12, 24, 48, and 72 hours after pollination. Plants in pots were treated by putting them in an air-tight chamber. None of the plants died as a result of treatment. No seeds were obtained from plants receiving pressures of 125 pounds or more. In seeds obtained from 75 and 100 pound treatments, no polyploids were found.

The second method employed for polyploidy induction was colchicine treatment.

Interspecific hybrid seeds were germinated in petri dishes. When coleoptiles and roots were about 2-5 mm long, they were immersed in a thin layer of 0.1 or 0.2 per cent of aqueous colchicine for 3 or 1.5 hours, respectively. Petri dishes were placed in an oven with a 27^{o}C temperature during treatment. After treatment, seeds were washed thoroughly with water for about ten minutes and then spread

over moist blotting paper in petri dishes at room temperature for about four days and then transplanted to flats in the greenhouse. Fifty-six plants out of 180 seeds treated reached maturity in which seven tetraploid heads were detected. Tetraploid heads were found as sectors and in no case was the whole plant doubled. Morphologically, tetraploid sectors were quite distinct from diploid parts by having larger leaves, thicker and longer stems.

Of the seven tetraploid heads produced, five were crossed to Mota rye, an autotetraploid, because they bloomed at different times and it was not possible to cross doubled F_1's together. The other two bloomed simultaneously and were intercrossed. Seeds obtained from these two heads were planted in an isolated area in the field.

It seems worthwhile to mention that the following year exactly the same colchicine technique was employed and despite a larger number of seeds treated, no tetraploid heads were detected. The difference seems to be due to the cold period which followed colchicine treatment in the second year, but which was not applied during the first colchicine treatment. The cold period employed for the purpose of vernalization of winter types seems to retard development of tetraploid sectors and results in their abortion.

3. Results

Detailed cytological analysis of doubled F_1 plants was not possible due to the limited amount of pollen mother

cells available. Eleven cells which were analyzed showed an average of 1.1 univalents, 9.4 bivalents, 0.63 trivalents, and 1.0 tetravalent. Analysis of 322 microspores showed that 23 per cent had one, 21 per cent had 2, 0.9 per cent had 3, and 84 per cent had no micronuclei. When flowers of the two doubled heads were crossed, the average seed set was about 51 per cent. Thus, compared to diploid F_1's with an average seed set of 27.7 per cent, tetraploids showed an improvement of 80 per cent in the first generation.

In the F_2 generation rather detailed cytogenetic analysis of the doubled hybrids was carried out.

At the prophase stage the tetraploids showed only one nucleolus instead of two (Fig. 32) and no cells were found which contained more than one nucleolus. Thus, doubling did not seem to have affected number of nucleoli.

Analysis of all cells at metaphase I was not possible, due to the large number of chromosomes and their overlapping. The results of metaphase I analyses of 100 cells in which the chromosomes were well spread and readily countable are shown in Table 7. This table shows the frequency of different multivalents found in 100 cells and not the combination of different multivalents in each cell. The first column in the table indicates the chromosome configurations, the second frequency of their occurrence, the third their relative frequency, and the fourth the average number of each multivalent per cell.

TABLE 7. Metaphase I analysis of doubled F_2 hybrids.

Configurations	Frequency	Per Cent	Average Configuration Per Cell
I	74	5.6	0.74
II	1072	81.9	10.72
III	70	5.3	0.70
IV	64	4.9	0.64
V	14	1.1	0.14
VI	10	0.76	0.10
VII	5	0.38	0.05
	1309	99.99	28 I or 14 II

On the average more than ten bivalents were found in each cell at metaphase I. In many other cells in which complete analysis was not possible, at least seven bivalents could be detected. The average number of quadrivalents per cell was 0.64 and in no case were seven quadrivalents present. Quadrivalents were mostly oriented in zig-zag chain configurations and rings of IV were not observed. Multivalents higher than quadrivalents were also observed. These were due, perhaps, to homologies caused by translocations.

One hundred sixty-three cells were analyzed at anaphase I and the results are shown in Table 8. The first

column shows the kind of distribution, and the second column their relative frequency. Behavior of univalents was the same as described for diploid rye. Other irregularities were detected which are listed in Table 8.

TABLE 8. Chromosome distribution at anaphase I of doubled F_2 hybrid rye.

Distribution	Frequency
14 - 14	59.5%
13 - 15	17.8
12 - 16	3.1
13 - 1 - 14	3.1
13 - 2 - 13	2.4
13 2/2 - 13 2/2	1.8
13 1/2 - 14 1/2	8.0
13 - 14 2/2	1.8
12 2/2 - 14 2/2	1.2
12 - 2 - 14	1.2

Microspore analysis showed that 76 per cent had no micronuclei, 21 per cent had one, and 3 per cent had two. The total number of microspores counted was 412.

Pollen grains were much larger in tetraploids than in their diploid parents. Tetraploids had 87.21 per cent stainable pollen grains whereas the corresponding value for

diploid F_1's was 45.5 per cent. Thus, tetraploidy increased the amount of stainable pollen 92 per cent over their diploid parents.

Seed fertility in the tetraploid F_2's averaged 40.4 per cent compared to 27.7 per cent for diploid hybrids and represents a 45 per cent improvement in the amount of seed set. Average thousand kernel weight in the tetraploids was 31.1 grams, whereas that of diploid hybrids was 23.6 grams, an increase of about 32 per cent in seed weight. Average height of tetraploid plants was 63.6 inches, the corresponding value for diploid F_1's was 48.2, an increase of 32 per cent in plant height due to polyploidy. Average diameter of culms measured in the middle of the second internode was 5.72 mm, that of the parent diploid 3.20 mm or about 79 per cent increase in culm diameter.

The above tetraploids were compared with their related diploid F_2's from the same original crosses with respect to five agronomically important characteristics. The results of such comparisons are listed in Table 9. Plant height, culm diameter, and kernel weight were larger for the tetraploids. Tillering and fertility were relatively constant between the diploid and tetraploids. As far as fertility was concerned, tetraploid plants were very uniform in flowering and during this period unfavorable weather prevailed. In a favorable condition, higher fertility would be expected. It seems more reasonable to expect higher

higher fertility, especially when we consider that doubled F_1's had about 51 per cent seed set when pollinated under bags, in the greenhouse. In the fields some tetraploid plants had fertility as high as 68 per cent and variability between the heads of the same plants was very low in all cases studied.

TABLE 9. Comparison of means and coefficients of variability for several characteristics in diploid and tetraploid F_2's of interspecific crosses.

	Diploid		Tetraploid	
	Mean	Coefficient of Variability	Mean	Coefficient of Variability
Plant height (in.)	45.86	19.07	63.63	7.4
Tillering	40.35	53.5	41.63	43.5
Stem diameter (mm)	3.36	24.7	5.72	13.11
Thousand kernel weight (grams)	21.9	27.2	31.1	12.67
Fertility (per cent)	43.5	56.1	40.4	37.88

A comparison of coefficients of variability for characteristics in the diploids and tetraploids reveals less variability in all cases for each characteristic in the tetraploids than in the diploids.

A comparison of three characteristics whose means were increased in the tetraploids; namely, plant height, culm diameter, and 1000 kernel weight reveals how means and

variances have changed in tetraploids when compared to diploids (Fig. 29). In this figure means are plotted on the vertical axis and variance on the horizontal axis for each of the above characteristics. For all of these three cases, tetraploid means have increased and variances have decreased. Variance decreases also for tillering and fertility whose means remain unchanged in the diploid and tetraploid F_2's.

It should be pointed out that chromosome doubling in cultivated rye causes a considerable reduction in degree of tillering. In this case allotetraploidy seems to compensate for that disadvantageous effect.

Tetraploid F_2's were partially perennial and continued to send up new shoots and form heads which again produced seeds. When we consider that diploid F_1's are strongly perennial, then doubling the chromosome number has actually changed this characteristic from dominant to an intermediate condition.

In contrast to diploid hybrids which have brittle or semibrittle heads, which usually break before they are harvested, the doubled F_2 hybrids had persistent heads which remained intact during harvest and showed only slight brittleness at the top after drying. For all practical purposes, they may be considered non-brittle. A similar situation was observed in doubled F_1's. Thus, for this character doubling the chromosome number in hybrids has reversed dominance almost completely.

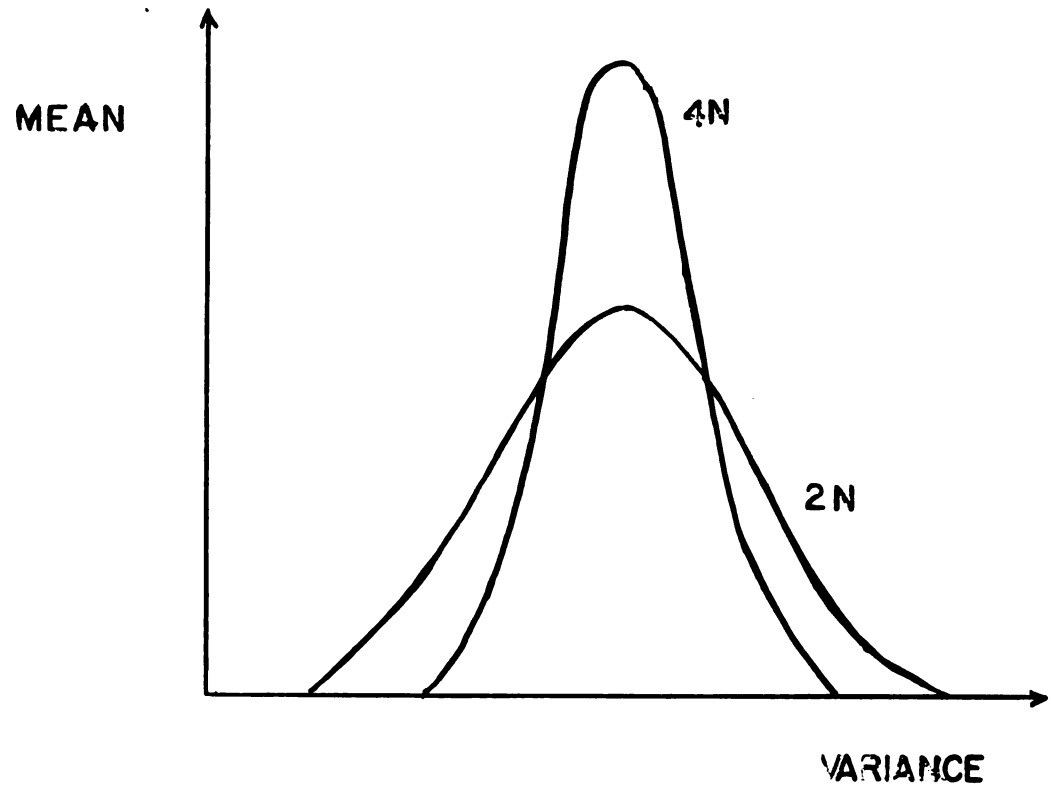

Fig. 29. Diagram comparing means and variances for three quantitative characters, namely plant height, culm diameter, and 1000 kernel weight, in diploid and tetraploid F_2 populations from interspecific crosses. (Adapted after Stebbins, 1956.)

4. Discussion

If reproductive barriers at the diploid level present a formidable handicap to recombination of desirable characteristics in interspecific crosses of Secale, it is possible by doubling the chromosome number in hybrids to overcome these barriers. Nature has utilized this device extensively to circumvent the reproductive barriers existing between species. In fact, most naturally known amphidiploids have appeared in hybrid cultures. In such cases, hybridization leads to the sharing of gene combinations, and polyploidy fixes these combinations with their heterosis, and at the same time insures the reproductive potential by restoring fertility. In Secale, natural polyploids have not been reported. This, of course, does not mean that in nature these polyploids have never been produced or are produced in a very low frequency. Rather, after occurrence they may not establish themselves and are easily eliminated. For example, polyploids have been very successful in wheat. The reason is certainly in the different genetic systems which these genera possess and evolution in each of them has taken different pathways. Rye is naturally a highly cross-pollinated genus and a high potential for recombination can be maintained in this way, whereas in wheat, because of self-pollination, larger numbers of chromosomes are necessary to produce a high recombination index. In rye occasional natural tetraploids will outcross to diploids, and the triploid embryos abort, whereas in wheat

self-pollination preserves these tetraploids.

The fact that three pairs of chromosomes in S. cereale and S. montanum are identical as far as their gross morphological structure is concerned, make their doubled hybrids a segmental allopolyploid and not an amphidiploid. The other four pairs of chromosomes which are not similar in the two species, have translocation differences. This is why we find at metaphase I of doubled hybrids neither 14 bivalents, as would be the case for amphidiploids, nor many tetravalents as might be expected in an autotetraploid. They show an average of 10.72 bivalents, which includes the eight pairs of non-similar chromosomes with some additional ones. By means of selection for more regular meiosis and higher fertility, it might be possible to increase the number of bivalents close to 14 in later generations. Occasional chains of V, VI, and VIII were observed in metaphase I which are the result of association of similar segments in different pairs of translocated chromosomes. Multivalents higher than chains or rings of four usually do not occur in autotetraploids of rye.

In spite of the strong self-incompatibility which exists in diploid S. montanum, S. cereale, and their hybrids, allotetraploids exhibited different degrees of self-compatibility which in some cases reached 22 per cent when individual spikes were isolated under glassine bags. Selection for this characteristic would certainly result in more self-compatible races. Such self-compatible races would be more useful in

areas where weather conditions are not usually favorable for cross-pollination. This is especially important for tetraploid rye which is much more uniform in pollination time and a short period of unfavorable weather during this critical period may cause serious losses. Such self-compatible races will not degenerate very fast as a result of inbreeding, as diploids usually do; because they carry a greater load of heterozygosity and have a higher recombination index. Changes in genetic systems brought about by selection for self-fertility would certainly be of practical and theoretical importance.

Higher fertility of allotetraploids in comparison to diploid hybrids confirms the fact that partial sterility of the F_1 plants is not genic, rather it is chromosomal. By doubling the chromosome number of the F_1 hybrids, each chromosome finds an exact homologue, pairing and segregation becomes more regular, and, as a consequence, higher fertility is achieved.

A comparison of variances in diploid F_2's and tetraploid F_2's showed that tetraploids were buffered more in their reaction to environmental factors than diploids. While diploids readily release their variability in the form of new genotypes, tetraploids have their heterozygosity distributed among all individuals. As a partial compensation for lack of genotypic variations, the whole population is similarly buffered against the environment. Such a developmental

flexibility permits the population to become adapted to its environment without losing its genetic variability. Thus, tetraploids and especially amphidiploids, obtain a great selective advantage in areas where drastic changes of environmental factors prevail. In such areas the intensity and direction of natural selection changes periodically and diploid species whose genetic variation is released in the form of new individuals soon exhaust their store of variability. Due to their buffering capacity, polyploids may be favored in the survival of the species.

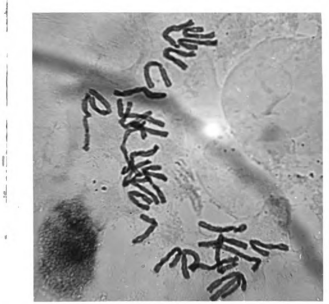

Fig. 30. Twenty-eight mitotic chromosomes of allotetraploid rye at metaphase. 936X.

Fig. 31. Mitotic anaphase of allotetraploid rye. 776X.

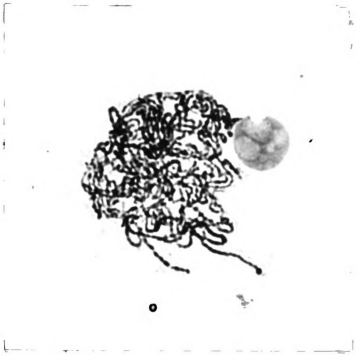

Fig. 32. Prophase in pollen mother cells of allotetraploid rye. One nucleolus present. 977X.

Fig. 33. Prophase in PMC's of allotetraploid rye, "bouquet" stage. 735X.

Fig. 34. Metaphase I in PMC's of allotetraploid rye. An aneuploid plant with 11 II and a chain of 7 chromosomes. 817X.

Fig. 35. Metaphase I in PMC's of allotetraploid rye. Eleven II plus a chain of 6. 873X.

Fig. 36. Metaphase I in PMC's of allotetraploid rye, 8 II, 3 I, plus a chain of V and a chain of IV. 849X.

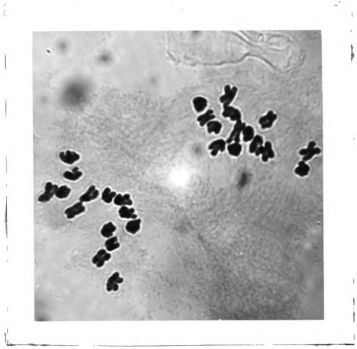

Fig. 37. Anaphase I in PMC's of allotetraploid rye. 14-14 distribution. 977X.

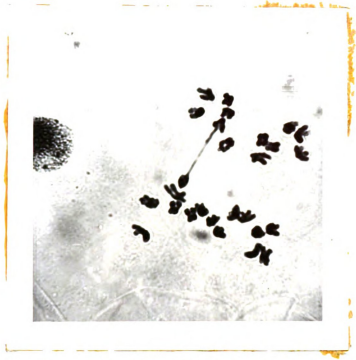

Fig. 38. Anaphase I in PMC's of allotetraploid rye. Aneuploid plant with 12-15 distribution. 933X.

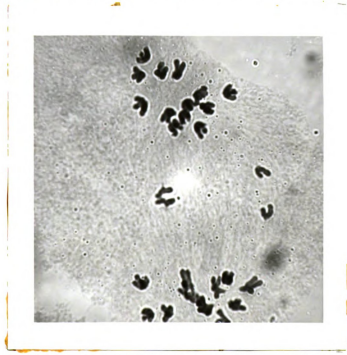

Fig. 39. Lagging univalents dividing at anaphase I. 917X.

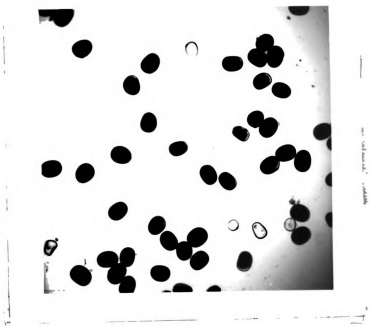

Fig. 40. Stainable and non-stainable pollen grains from allotetraploid rye. 85X.

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