IRRADIATION AND INTERSPECIFIC HYBRIDIZATION IN SECALE¹

SAM PRICE

U.S.D.A. Agricultural Research Service, Plant Industry Station, Beltsville, Maryland

Received January 28, 1955

HYBRID sterility does not seriously limit genetic exchange between the species, *Secale cereale* L. and *S. montanum* Guss. F₁ hybrids are vigorous and partially fertile. Though these are taxonomically distinct species, the reproductive barriers which separate them are poorly developed. Such species and their partially sterile hybrids provide excellent material for studying the nature and development of hybrid sterility. This is a study of the cytology and partial sterility of F₁ hybrids produced by crossing *S. cereale* and *S. montanum* using both X-rayed and untreated pollen. The observations are discussed in respect to their bearing on the phenomenon of interspecific hybrid sterility.

Hybrids of *Secale cereale* and *S. montanum* have been reported by TSCHERMAK (1927), LONGLEY and SANDO (1930), KOSTOFF (1932, 1937), DUKA (1935), SMITH (1942), and SCHIEMANN and NÜRNBERG-KRÜGER (1952). TSCHERMAK described the hybrids as moderately fertile, as did SMITH. KOSTOFF found 75 percent aborted pollen. DUKA reported pollen viability ranges between five and 38.3 percent; some plants set a small amount of seed while others did not. SCHIEMANN and NÜRNBERG-KRÜGER found 57.65 percent pollen fertility and 37.23 percent seed set in *S. montanum* × *cereale* hybrids and 56.68 percent pollen fertility and 28.75 percent seed set in reciprocals. In their descriptions of meiosis, LONGLEY and SANDO, KOSTOFF, and DUKA report only bivalents and variable frequencies of univalents. SCHIEMANN and NÜRNBERG-KRÜGER describe a maximum association of four bivalents and a translocation chain of six chromosomes. The translocation configuration frequently occurs in lesser associations, such as a chain of four and a bivalent or a chain of four and two univalents.

MATERIALS AND METHODS

Secale cereale L. var. Merced is a variety of cereal rye commonly cultivated in California. It is an erect annual with a continuous rachis, dense spikes, and broad glaucous leaves. *S. montanum* Guss. is a perennial grass native in southwestern Asia. It has narrow leaves, a rachis which disarticulates quite readily, and it flowers later than Merced rye in Berkeley. Two strains of this species were used. The first was obtained from the Copenhagen Botanical Garden; the location of the original collection is not known. This "Copenhagen" strain has slender culms, a slightly spreading habit, and bright green foliage with little glaucousness. The second strain of *S. montanum* was collected in Iran in 1948 by H. P. OLMO. The "Iran" strain resembles cereal rye in that it is erect and has glaucous leaves and culms. Both species are diploid ($n = 7$) and are self incompatible.

¹ Material extracted from a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Genetics, University of California.

Hybrids obtained from untreated pollinations, hereafter referred to as "control" hybrids, were produced by enclosing emasculated spikes in a paper towel wrapper with one or more spikes of the pollen parent. The method is described in detail by STEBBINS and TOBGY (1944). Hybrids obtained from X-rayed pollinations, hereafter called "r-hybrids", were produced by enclosing irradiated spikes of the male parent in a paper towel wrapper with an emasculated spike of the female parent. A Coolidge X-ray tube operating at three milliamperes and 100 kilovolts was used in irradiating pollen. Culms of the pollen parent were cut when mature pollen was being shed and the spikes were placed fourteen cm from the target. With two ordinary index cards used as filters, the centers of these spikes received approximately 1250r.

The hybrids appear to be intermediate in many of their characters. However, they are mostly erect, have broad leaves, and are taller than either parent. They are extremely variable in their flowering date, some flowering as early as Merced rye. A few did not flower during the first growing season. Plants grown in the field tend to be perennial in Berkeley. Those that did not flower during the first season flowered in the second. The hybrids, Merced rye $\times$ Copenhagen *S. montanum* and their reciprocals, have bright green foliage, while Merced rye $\times$ Iran *S. montanum* and their reciprocals are glaucous.

Pollen was taken for fertility counts soon after the first spike began to exert its anthers. The pollen was stained in cotton blue. Those grains which were filled with starch and stained dark blue were considered functional. At least two hundred pollen grains were scored for each plant, except in the case of plants with aborted anthers which contained only shriveled pollen. In determining the percentage of seed set, not less than fifty florets were examined per plant.

Spikelets were fixed in 1:3 acetic alcohol and were stored in 70 percent alcohol. Pollen mother cells were smeared in iron-aceto-carmin. Observations were made on smears permanized by a modification of the alcohol vapor chamber method described by BRADLEY (1948). In each plant 50 metaphase I cells, 100 anaphase I cells, 100 anaphase II half-quartets, and 100 sporads were studied if possible. Where these numbers were not obtainable, fewer cells were accepted.

In testing the significance of differences and the significance of correlations, where variances and covariances are calculated by the product moment method, accurate statements of probability can be made only if the sample is drawn from a normally distributed population, or if the data can be transformed to fit a normal distribution. The data presented here are not normal, nor can they be transformed to fit a normal curve. To avoid the assumption of normality, ranking methods are employed for statistical analyses. Although ranking is an inefficient use of data, this method provides tests about which definite probabilities can be stated. The test of significance of differences used here was devised by WILCOXON (1945) and modified by MANN and WHITNEY (1947). A correction for tied values was given by HEMELRIJK (1952). For determining correlations, the rank correlation coefficient, *Tau*, was computed as described by KENDALL (1948). *Tau*, like the better known correlation coefficient, *r*, ranges from -1 to $+1$. KENDALL also described the method of testing if *Tau* differs significantly from zero.

RESULTS

Meiosis and fertility in parental strains

Meiosis was studied in five plants of each of the parental strains (table 1). Ten *Secale cereale*, five Copenhagen, and seven Iran *S. montanum* plants were available for fertility studies.

Irregularities in meiosis occur at low frequencies in *S. cereale*. A high frequency of laggards was found in one plant. Inversion bridges and fragments occurred in this and one other plant. Plants which exhibited no other irregularities produced a small percentage of microspores which contained micronuclei. In ten plants of Merced rye the mean pollen fertility was 71.0 percent and ranged from zero to 92 percent. Mean seed set was 71.7 percent and ranged between 56 and 86 percent.

Meiotic irregularities in the strain of *S. montanum* from Copenhagen occurred in low frequencies, but were found in every plant. A few univalents were observed in four of the five. An inversion bridge was found at anaphase I in one plant, and an anaphase II bridge was found in another. Laggards were seen in the second division in two plants, and micronuclei were found in the microspores of all five plants. In this strain the mean pollen fertility was 80.5 percent and ranged from 72.3 to 88.1 percent. The mean seed set was 22.0 percent and ranged from eight to 34 percent.

Two of the five Iran *S. montanum* plants had serious meiotic disturbances. In one the first division proceeded quite regularly, but the second anaphase was characterized by extreme stickiness. The other irregular plant exhibited a high frequency of lagging chromosomes at anaphase I and bridges at anaphase II. Meiosis in the remaining three plants was generally normal but with low frequencies of irregularities. This Iran strain had 64.7 percent fertile pollen. The range in pollen fertility was zero to 96.4. The mean seed set was 33.7 percent with a range of two to 60 percent.

Meiosis in control hybrids

Meiosis was studied in a random sample of ten Merced *Secale cereale* × Copenhagen *S. montanum* and reciprocal hybrids. Ten randomly chosen Merced *S. cereale* × Iran *S. montanum* were also studied. Metaphase I, anaphase I, anaphase II, and sporad stages were observed in detail. Summaries of meiosis in these control hybrids are given in table 1.

Copenhagen vs. Iran hybrids

Ranking analyses were used to compare meiosis in the Merced *S. cereale* × Iran *S. montanum* hybrids with meiosis in hybrids produced in crosses between Merced *S. cereale* and Copenhagen *S. montanum*. The following were used as indices of meiotic chromosome behavior: (1) univalent frequency; (2) chiasmata per cell; (3) percentage of alternately arranged translocation configurations; (4) frequency of anaphase I cells with bridges; (5) frequency of cells with laggards at anaphase I; (6) frequency of second anaphase half-quartets with bridges; (7) frequency of anaphase II half-quartets with laggards; and (8) microspore frequency. Although they involved different strains of the *S. montanum* parent, these two samples of the interspecific hybrids

TABLE 1
Mean observations on meiosis in parental strains and control hybrids

	No. of plants	Metaphase I						Anaphase I			Anaphase II		Micro- spores			
		Associations (per cell)						Percent cells with:			Percent half- quartets with:			Percent n = 7 nuclei	Percent with micro- nuclei	
		I	II	III	IV	V	VI	Xmata per cell	Percent alt. trans.	bridges	lags.	Unequal distrib.	bridges			lags.
<i>S. cereale</i> var. Merced	5	0.21	6.89					14.4		1.9	1.2	0.4	0	2.4	96.8	1.1
<i>S. montanum</i> , Copenhagen	5	0.24	6.88					11.9		0.4	2.8	0.8	2.8	3.2	95.6	1.7
<i>S. montanum</i> , Iran	5	0.06	6.97					13.3		1.6	3.6	0.4	2.9*	2.4*	76.2	1.1
Copenhagen control hybrids	10	0.44	4.45	0.14	0.21	0.16	0.43	12.6	61.6	0.9	15.6	15.1	0.2	19.6	66.0	8.2
Iran control hybrids	10	0.24	4.48	0.14	0.24	0.10	0.49	11.9	66.0	0.6	10.4	20.0	0.3	14.1	69.0	7.0
Pollen-sterile control hybrids	14	0.38	4.38	0.10	0.19	0.19	0.46	12.2	62.7	1.2	14.7	13.5	0.6†	16.1†	65.3†	10.3

*Mean of 4 plants.

†Mean of 13 plants.

did not differ significantly ($P > .05$) from each other in any of the eight meiotic characteristics.

The representative sample: control hybrids

Since the two samples of control hybrids did not differ in the comparisons above, they are considered together as a single representative sample in all further comparisons and descriptions. The most outstanding feature of the first metaphase in these control hybrids was the presence of a translocation configuration. The maximum number of chromosomes associated in the configuration was six (figs. 1 and 2), and this maximum was found in several cells in every control hybrid. Chains of three (fig. 3), four, and five (fig. 4) were observed, and occasional cells were seen with associations no greater than bivalents. The translocation configurations were usually open chains. "Frying pan" and Y-shaped associations were found infrequently, but closed rings were not seen.

Good pairing in these hybrids was indicated by low frequencies of univalents. In

FIGURE 1.—Diplotene in control hybrid 178-23, four bivalents and a translocation chain of six chromosomes. $\times 1100$.

FIGURE 2.—Metaphase I in control hybrid 176-6, four bivalents and an alternate translocation chain of six chromosomes. $\times 1100$.

FIGURE 3.—Metaphase I in control hybrid 176-6, a univalent, five bivalents, and a translocation chain of three chromosomes. $\times 1100$.

FIGURE 4.—Metaphase I in pollen sterile control hybrid 180-3, a fragment, one univalent, four bivalents, and a translocation chain of five chromosomes. $\times 1100$.

FIGURE 5.—Metaphase I in r-hybrid 174B-1, three bivalents, a translocation chain of three, and a translocation chain of five chromosomes. $\times 1100$.

FIGURE 6.—Metaphase I in r-hybrid 171-1, three bivalents and an alternate translocation chain of eight chromosomes. $\times 1100$.

FIGURE 7.—Metaphase I in r-hybrid 172-14, three bivalents and an adjacent translocation chain of eight chromosomes. $\times 1100$.

FIGS. 1-7

metaphase I cells there was a close correspondence between high univalent frequency and incomplete six chromosome chains suggesting that most univalents were chromosomes that should have formed a part of the translocation configuration.

Inversion bridges occurred in control hybrids, but in no greater frequencies than in the parental strains. Univalents of metaphase I appeared as laggards at anaphase I. They divided in either the first or the second anaphase. As a result of adjacent disjunction of translocation chains and irregular univalent behavior, unequal numbers of chromosomes were distributed to the poles in about seventeen percent of the first anaphase cells. Distribution in these irregular cells was usually 6:8, but rarely five chromosomes moved to one pole and nine to the other.

Occasional bridges were encountered at anaphase II. Approximately seventeen percent of the half-quartets contained laggards, and only about 67 percent of the nuclei received exactly seven chromosomes in the second division. Micronuclei occurred in about eight percent of the microspores.

Pollen sterile control hybrids

Twenty-five of the 91 control hybrids that flowered in the first season were pollen sterile. Meiosis was studied in fourteen of these (table 1). Taking as meiotic indices the eight characteristics of meiosis used in the test described above, these pollen sterile control hybrids were compared with the representative sample. No significant differences were found ($P > .05$). However, one pollen sterile control hybrid exhibited a fragment in eight of the 50 metaphase I cells examined (fig. 4). With this single exception it may be concluded that no more meiotic irregularities occurred in pollen sterile control hybrids than in the representative sample.

Meiosis in r-hybrids

Two r-hybrids from the cross, Merced rye $\times$ Copenhagen *Secale montanum* (r) and four from the reciprocal cross, Copenhagen *S. montanum* $\times$ Merced rye (r) were available for studies of meiosis. Random samples of ten Merced rye $\times$ Iran *S. montanum* (r) and of ten Iran *S. montanum* $\times$ Merced rye (r) hybrids were also studied. Since no differences were demonstrated between control hybrids which involved different strains of *S. montanum*, and since no reciprocal differences were evident, the 26 r-hybrids are considered as a single representative sample. Summaries of meiosis in these r-hybrids are given in table 2.

The representative sample: r-hybrids

The most frequently observed chromosome alterations observed in the r-hybrids were additional reciprocal translocations. First metaphase cells with more than six chromosomes involved in translocation configurations (figs. 5, 6, 7, 8, and 10) provide evidence that new translocations have been induced. Newly induced translocations were found in seventeen of the 26 r-hybrids. In only one of these, No. 168-1, was the induced translocation independent of the original translocation configuration found in control hybrids. Evidence for this induced alteration appeared in only one cell in which the chromosome association was $1_I + 2_{II} + 1_{IV} + 1_V$ (fig. 8).

One plant, No. 171-2, contained two induced translocations which involved at

TABLE 2
Meiosis in r-hybrids

Plant no.	Fertilities		Metaphase I										Anaphase I			Anaphase II			Micro-spores		
	Percent pollen fert.	Percent seed set	Associations (per cell)										Percent cells with:			Percent half-quartets with:				Percent n = 7 micro-nuclei	
			I	II	III	IV	V	VI	VII	VIII	IX	X	bridges	lags	unequal distrib.	bridges	lags.				
165-4	0	68.0	0.18	4.96	0.08	0.66	0.06	0.02					11.3	80.4	1.1	6.7	4.4	0	23.6	55.9	7.0
166-1	0	16.0	0.50	5.14	0.18	0.38	0.04	0.16					12.8	50.0	0.8	5.3	7.1	3.0	10.0	77.0	12.6
166-2	0	34.0	0.28	5.00	0.06	0.26	0.10	0.20					11.4	85.3	0.9	2.8	2.8	0	8.0	90.0	3.8
166-3*	0	4.0	4.12	3.64	0.41	0.24	0.02	0.02		0.02			6.0	74.3	2.6	26.3	34.2	1.0	51.0	25.0	11.9
166-4	0	0	1.08	5.42		0.02							9.3	100.0	0	31.7	15.3	—	—	—	6.8
166-5*	0	0	0.28	5.02	0.25	0.32	0.02	0.18		0.04			10.8	86.8	9.9	6.9	7.9	0	51.7	46.5	13.6
166-6	0	4.0	0.38	4.86	0.16	0.42	0.10	0.20					11.2	75.0	4.4	7.9	7.0	1.8	22.2	75.9	6.2
167-2*	0	54.0	0.56	4.52	0.20	0.36	0.16	0.26					11.0	83.6	0	20.1	9.6	0	2.2	87.7	3.1
167-4*	0	0	0.92	3.54	0.34	0.48	0.20	0.12	0.06	0.12			11.4	32.6	0	4.2	21.3	0	6.8	64.8	5.4
168-1*	5.5	0	0.16	4.78	0.02	0.38	0.06	0.40					11.1	76.7	6.3	5.4	7.2	1.0	7.0	94.0	6.8
170B-1*	0	0	2.20	4.14	0.50	0.30	0.14	0.02					8.4	80.8	20.3	—	—	Fragmentation	—	—	32.5
170B-3*	48.8	28.0	0.08	4.10	0.14	0.16	0.06	0.62		0.08			11.9	60.3	4.7	9.5	23.8	2.0	4.0	82.0	6.6
171-2*	9.8	10.0	0.28	2.60	0.14	0.22	0.04	0.14	0.10	0.24	0.04	0.32	11.6	43.3	1.9	7.7	35.9	1.7	10.7	64.2	5.8
171-3	29.5	32.0	0.20	4.28	0.08	0.19	0.16	0.58					12.2	72.0	0.9	5.5	18.3	0	11.0	85.0	4.2
172-3*	0	0	0.04	3.60		0.13	0.34	0.04	0.47	0.47			11.6	56.5	0	1.6	32.7	0	6.5	64.2	1.2
172-6*	0	0	0.96	2.64	0.10	0.56	0.02	0.26	0.08	†			10.0	72.0	1.7	—	—	0	—	39.7	29.4
172-7*	11.1	10.0	0.02	3.14	0.04	0.06		0.08	0.02	0.84			13.9	25.0	0	11.9	37.3	0	0	88.0	4.4
172-11*	26.8	10.0	0.06	3.76	0.06	1.56		0.08	0.02				12.7	88.7	0	0.9	3.9	0.9	5.7	75.0	3.4
172-14*	2.4	13.5	0.01	3.33	0.12	0.12	0.08	0.22	0.06	0.54			12.4	59.6	2.8	6.6	30.1	0	0	74.3	2.2
172-15*	13.5	6.0	0.10	3.34	0.16	0.16	0.08	0.18	0.02	0.56			12.3	58.6	0	5.0	27.0	0	0	84.3	5.0
174A-1*	5.0	18.0	0.90	4.08	0.44	0.24	0.20	0.16	0.02	†			10.2	79.3	26.9	29.2	13.8	0	45.1	37.0	24.7
174B-1*	1.0	2.0	1.04	3.54	0.16	0.12	0.10	0.12	0.46	0.06			11.2	58.0	1.6	42.6	18.8	—	—	—	7.0
182-1*	12.5	0	0.46	3.82	0.30	0.22	0.10	0.20	0.14	0.18			10.4	57.8	3.7	19.3	19.6	2.7	34.3	40.2	4.7
183-1	43.0	20.0	0.30	4.24	0.26	0.12	0.12	0.56					11.5	69.8	2.7	7.3	26.6	0	26.1	61.5	4.9
184-1	39.5	16.0	0.84	4.44	0.16	0.12	0.28	0.38					12.1	59.5	2.8	1.9	3.8	0	1.9	87.2	7.2
184-2	43.0	20.0	0.88	4.18	0.10	0.32	0.30	0.28					11.7	62.0	6.7	18.2	2.8	0	38.0	29.5	19.6

* Plants which exhibit induced translocations.

† 0.06 Cent. frag.

‡ One frag.

FIGS. 8-13

least one chromosome of the original chain of six and extended the configuration to a maximum of ten chromosomes (fig. 10). Fifteen r-hybrids contained one newly induced chromosome exchange which also involved chromosomes of the original translocation configuration. In one of these, No. 172-11, no configuration of more than four chromosomes was seen. However, many cells had associations of $3_{II} + 2_{IV}$. The newly induced exchange involved the chromosomes of the original chain of six but in such a way that the maximum association of $3_{II} + 1_{VIII}$ was not obtained.

Plants 166-5 and 174A-1 had high frequencies of inversion bridges as well as an induced translocation. Plant 166-3 may have also contained an induced inversion in addition to an induced chromosome exchange. Despite fewer chiasmata, this r-hybrid maintained an inversion bridge frequency comparable to hybrids which had better pairing.

Plant 170B-1 contained an induced translocation and exhibited fragmentation in first and second anaphase (fig. 9). Many bridges were encountered in both anaphase stages, but these were not inversion bridges. Accurate observation of fragmentation was possible in 44 anaphase II cells. Fragmentation was lacking in only five of these; a range of one to fourteen fragments was found in the remaining 39 cells.

In addition to an induced translocation, r-hybrid 172-6 contained a small centric ring fragment which was all that remained of one of the chromosomes. Thirteen chromosomes plus this centric fragment (dividing) can be seen in figure 12. At pachytene the fragment was seen to be a ring (fig. 11). That it contained a centromere was shown by its behavior which was very much like the behavior of univalent chromosomes. Although it sometimes lagged behind, it usually divided at first anaphase (fig. 12). In anaphase II the daughter fragments moved to the poles with the chromosomes or lagged behind. In some cells they appeared to take the place of the missing chromosome, being distributed to the poles that received only six whole chromosomes (fig. 13). A high frequency of micronuclei suggested that the fragment was often left out of telophase II nuclei.

Nine of the 26 r-hybrids lacked evidence of induced translocations. In one of these, No. 166-4, bivalent pairing suggested more chromosome homology than was found in the control hybrids. A translocation configuration was seen in only one cell; this was a chain of four chromosomes. Although the univalent frequency was high (1.08 per cell) and the chiasma frequency was correspondingly low (9.32 per cell), 32 of 50 metaphase I cells contained seven bivalents. One or more structural alterations among the six chromosomes of the original translocation configuration could be postulated to explain the meiotic behavior in this plant. Induced retranslocations

FIGURE 8.—Metaphase I in r-hybrid 168-1, a univalent, two bivalents, a "frying pan" translocation configuration of four, and a translocation chain of five chromosomes. $\times 1100$.

FIGURE 9.—Chromosome fragmentation at second anaphase in r-hybrid 170B-1. $\times 1100$.

FIGURE 10.—Metaphase I in r-hybrid 171-2, two bivalents and a translocation configuration of ten chromosomes. $\times 1100$.

FIGURE 11.—Centric ring fragment at pachytene in r-hybrid 172-6. $\times 1100$.

FIGURE 12.—Centric ring fragment dividing at first anaphase in r-hybrid 172-6. $\times 1100$.

FIGURE 13.—Regular distribution of centric fragments at second anaphase in r-hybrid 172-6. $\times 1100$.

would provide a convenient explanation, but inversions could be responsible if they seriously disturbed pairing in the chromosome arms in which they occurred.

Although none of the remaining eight r-hybrids exhibited newly induced translocations, and none of them exceeded the range of control hybrids in the frequency of inversion bridges, slight effects upon meiosis were suggested. Plants 165-4 and 166-1 had low frequencies of chains of six and high frequencies of bivalents. The chiasma frequency (11.22 per cell) in r-hybrid 166-2 was below the range in control hybrids. In this same plant unequal chromosome distribution in anaphase I was less frequent than in any control. In plant 183-1 the frequency of unequal distribution in this stage exceeded that of any control hybrid. Although these are slight differences, they suggest that mutations, deletions, or minute structural rearrangements may have been induced. These plants were compared as a group with the control hybrids in respect to the same eight meiotic characteristics used in earlier tests. The comparison indicated that significantly more r-hybrids contained inversion bridges than did the controls ($P < .01$). No other significant differences were found.

If a large number of differences are tested, as has been done here, some may be significant due to chance alone. However, it seems more than a fortuitous circumstance that the only significant difference here is in the comparison of inversion bridges, the one difference most likely to have been predicted. A difference as great as this would occur by chance in fewer than one out of a hundred tests. Among 20 randomly chosen control hybrids, only nine showed inversion bridges; of the nine r-hybrids lacking induced translocations, eight showed bridges. Induced inversions surely existed in some of these r-hybrids. The inverted segments must have been very small.

Pollen sterile r-hybrids

Preliminary studies of pollen fertility suggested that the progeny of r-hybridizations were less fertile than those of the controls. This seemed to imply that more induced chromosomal alterations might be found among pollen sterile r-hybrids than among those with functional pollen. If the occurrence of new translocations is taken as a measure of induced chromosomal aberration, this expectation is not realized. Twelve of the 26 r-hybrids studied were pollen sterile. Seven (58.3 percent) of these had induced translocations as compared with seventeen (65.3 percent) in the whole sample of 26.

Fertilities studies

The percentage of stainable pollen and the percentage of florets containing seed were determined for 90 control hybrids and for 41 r-hybrids. The mean fertilities of these and of the parental strains are recorded in table 3.

In control hybrids, mean pollen fertility and mean seed set were 18.6 and 20.4 percent respectively. The r-hybrids were 12.2 percent pollen fertile and set seed in 10.7 percent of their florets. The frequency distributions (table 3) show a wide range of variability in pollen fertility and seed set in both control and r-hybrids. A marked shift toward low fertility is seen in the distributions for r-hybrids. In view of the many chromosome alterations induced in the r-hybrids, this apparent reduction in fertility was not unexpected. However, when the differences between control and

TABLE 3

Pollen fertility and seed set distributions in parental strains, control-, and r-hybrids

	Percent										n	Mean	Range
	0-9.9	10-19.9	20-29.9	30-39.9	40-49.9	50-59.9	60-69.9	70-79.9	80-89.9	90-100			
<i>Secale cereale</i>													
P. F.	1			1				3	3	2	10	71.0	0-92.0
S. S.						1	3	3	3		10	71.7	56.0-86.0
<i>Copen. S. montanum</i>													
P. F.								2	3		5	86.5	72.3-88.1
S. S.	1	1	1	2							5	22.0	8.0-34.0
<i>Iran S. montanum</i>													
P. F.	1				1		1	2		2	7	64.7	0-96.4
S. S.	1	1	1	1	1		2				7	33.7	2.0-60.0
Control hybrids													
P. F.	33	14	17	16	6	4					90	18.6	0-58.7
S. S.	15	30	27	13	3	1		1			90	20.4	0-74.0
r-hybrids													
P. F.	23	8	4	3	3						41	12.2	0-48.9
S. S.	24	10	3	2		1	1				41	10.7	0-68.0

r-hybrids were subjected to tests of significance, only the difference in seed set was significant ($P < .01$). These results are especially surprising since chromosome abnormalities that can be transmitted through eggs are frequently pollen lethals. The original variation was so great that the effect of irradiation on pollen fertility could not be demonstrated.

To test the possibility of independent seed set and pollen fertility, the rank correlation coefficient, *Tau*, was computed. The correlation between seed set and pollen fertility was not significantly different from zero in the control hybrids ($Tau = 0.110$; $P > .05$). The correlation in r-hybrids was small but significant ($Tau = 0.275$; $P < .05$).

This indicates an induced addition to that part of the genetic variation that was common to both pollen fertility and seed set. Most of the induced mutations that affect fertility, whether genic or chromosomal, had similar influences in both male and female gametophytes.

It is regrettable that the data were not amenable to an analysis of variance to determine if there were fertility differences between hybrid families, and to estimate the environmental portion of the variation in fertility. Nevertheless, means were computed for control hybrid families (table 4). Since many families were small, some of the variation in family means undoubtedly is due to random fluctuations, but if only the families of five or more individuals are considered, the range of fertility remains variable. If environmental differences were the only causes of variation, family means would tend to be equal. The wide range of family mean fertilities sug-

TABLE 4

Control hybrid family mean fertilities ranked in order of pollen fertility

Family	No. of plants	Mean percent pollen fert.	Mean percent seed set
180	5	0.00	20.80
178	23	6.58	14.17
179	11	10.46	21.82
176	11	13.56	26.36
177	8	28.67	19.00
169	26	29.61	23.69
185	2	35.78	18.00
186	2	37.86	11.00
187	1	51.14	28.00
173	1	58.71	18.00

TABLE 5

Rank correlation coefficient (Tau) values: Correlations of fertility with meiosis in control and r-hybrids

Variables correlated	<i>Tau</i> control hybrids	<i>Tau</i> r-hybrids
Pollen fertility with:		
% Irregular met. I	0.005	-0.073
% Irregular ana. I	0.156	0.170
% n = 7 nuclei ana. II	0.191	0.074
% Spores with micronuclei	0.143	0.045
Seed set with:		
% Irregular met. I	-0.288	-0.203
% Irregular ana. I	0.118	-0.128
% n = 7 nuclei ana. II	-0.070	-0.004
% Spores with micronuclei	-0.053	-0.124

gests that much of the variation in control hybrids is genetic rather than environmental.

Since much of the variability in fertility appears to be genetic, it is desirable to know if that part of the variability which independently influences pollen fertility and seed set is genetic also. An indication that part of the independent variation is genetic is found again in a consideration of family fertility means of the control hybrids. It will be noticed that the families in table 4 are listed in order of the mean pollen fertility. If the correlation between mean pollen fertility and mean seed set were absolute, this arrangement would automatically succeed in arranging the values for seed set in the same order. In table 4 mean seed set appears to be independent of pollen fertility.

In earlier sections it was pointed out that no differences in meiotic irregularities were demonstrated when pollen sterile control hybrids were compared with the representative sample of control hybrids, nor were detectable induced translocations more frequent in pollen sterile r-hybrids than in the representative sample of r-hybrids. This suggests a lack of correlation between observable meiotic irregularities and fertility. Rank correlation coefficients were computed for correlations between both

pollen fertility and seed set and each of the four following indices of meiotic irregularity for both control and r-hybrids: (1) percentage of metaphase I cells with univalents and/or adjacent translocation configurations; (2) percentage of anaphase I cells with bridges, laggards, and/or unequal chromosome distribution; (3) percentage of anaphase II nuclei receiving seven chromosomes; and (4) the percentage of microspores which contained micronuclei. None of the correlations differed significantly from zero ($P > .05$). The values of *Tau* are listed in table 5. Since variation in the fertility of control hybrids appears to be largely genetic rather than environmental, this lack of correlation between fertility and chromosome behavior points to genic sterility. Genic sterility is the likely source of independent seed set and pollen fertility.

DISCUSSION

Since X-rays are capable of producing the same kind of structural changes that have differentiated the chromosomes of diverging species, the use of irradiated pollen in interspecific hybridization is comparable to crossing species that are even more distantly related. The significant reduction in seed set and the probable, though not demonstrable, effect on pollen viability, suggests a marked increase in hybrid sterility in the r-hybrids of *Secale cereale* and *S. montanum*. Undoubtedly sterility in some of these r-hybrids is the result of induced deficiencies or even lethal gene mutations, and is not comparable with the sterility of other interspecific hybrids. However, the induced translocations and inversions in these *Secale* hybrids are comparable with those that have been responsible for the repatterning of genes in many species. Their contributions to sterility are derived from the same phenomena that produce chromosomal sterility in many other hybrids. Structural alterations and their role in the reproductive isolation of species are thoroughly discussed by STEBBINS (1950, Ch. III and VI) and by DOBZHANSKY (1951, Ch. VIII). The loss of genetic material and the associated centric ring fragment in one r-hybrid is reminiscent of the losses that reduced the basic chromosome number in *Crepis* (TOBGY 1943; SHERMAN 1946). In another r-hybrid, evidence for structural heterozygosity was found in only one first metaphase cell, and pairing suggests more chromosome homology than is observed in control hybrids. If an induced chromosome rearrangement is responsible, this plant represents a step in the direction of "cryptic" structural hybridity (STEBBINS 1945, 1950).

Since chromosome fragmentation has been observed in inbred strains of *Secale cereale* (LAMM 1936; MÜNTZING and AKDIK 1948), the fragmentation in an r-hybrid reported here may not be a product of irradiation. However, fragmentation in this plant may be fully comparable with that in *Triticum* which is controlled by an X-ray induced recessive mutation (SMITH 1939). Interaction of genotypes was proposed as the cause of fragmentation in the meiotic divisions of the hybrids, *Tradescantia canaliculata* $\times$ *T. humilis* (GILES 1940), *Bromus Trinii* $\times$ *B. maritimus* (WALTERS 1950), and *B. marginatus* $\times$ *B. pseudolaevipes* (WALTERS 1952).

Rearrangement of chromosome segments by irradiation is an effective way to produce the same sort of sterility that is found in so many interspecific hybrids. GERASSIMOVA (1939) has produced a strain of *Crepis tectorum* that differs from the

original strain in that each of its chromosomes has been altered by an induced translocation. The new strain is intra-fertile, but when crossed with the original strain produces hybrids that are only about 30 percent fertile. This demonstration of an artificially produced chromosomal sterility barrier suggests a possible use of irradiation in plant breeding. If an improved variety were to be grown in the proximity of unimproved parental material, as a range grass might be, its desirable qualities would be diluted and lost through interbreeding with the parental strain. If a sterility barrier were induced, the new variety could maintain its integrity.

The *Secale cereale* $\times$ *S. montanum* control hybrids studied here exhibit both genic and chromosomal sterility. The lack of correlations between chromosome behavior and fertility suggests that much of the partial sterility is genic and is highly variable. Nevertheless, chromosomal sterility must be present. Genetically unbalanced gametes are produced as a result of about 36 percent adjacent disjunction in translocation configurations. Only about 67 percent of the gametes produced by these hybrids receive the proper number of chromosomes.

The indication that at least part of the genic pollen sterility is independent of the genic sterility that influences seed set is of special interest. This independent variation is unusual in interspecific F_1 hybrids. The writer is unaware of other such instances. However, independent segregation of pollen and seed fertility occurs in the C_2 generation of synthetic allopolyploids of *Elymus glaucus* $\times$ *Sitanion jubatum* and *Elymus glaucus* $\times$ *Sitanion hystrix* hybrids (STEBBINS and VAARAMA 1954) and in F_4 derivatives of the hybrid, *Paraixeris denticulata* $\times$ *Crepidiastrum platyphyllum* (ONO 1950).

Since genetic sterility has been demonstrated in *Secale* (MÜNTZING 1946, PUTT 1954), it may be suggested that the partial sterility barrier between *Secale cereale* and *S. montanum* is built of the sterility factors that exist within the species. STEBBINS (1950) points out that even in their most significant characteristic, sterility, interspecific hybrids differ quantitatively rather than qualitatively from hybrids within species. KLINGSTEDT (1939) suggests the possibility that sterility genes which are recessive in intraspecific matings might behave as dominants in interspecific hybrids. With this assumption, individuals of two species, if heterozygous for several recessive genes, would produce sterile hybrids when crossed. DOBZHANSKY (1951) objects to this proposal as a general explanation on the grounds that hybrid sterility of this sort would be extremely variable depending upon the strains of the parental species used. The idea of a constant degree of interspecific hybrid sterility is also expressed by STEBBINS (1950, p. 257), "The distinguishing feature of interspecific hybrid sterility is that it is always present and differs relatively little depending on which individuals of parental species are used for crossing." This generalization may well apply to hybrids between species in which a sterility barrier is well established. However, the barrier between *Secale cereale* and *S. montanum* is only weakly developed, and the F_1 hybrid sterility is extremely variable. The wide range of family means suggests that the degree of sterility does depend on the individuals used as parents. The variable sterility in these hybrids might represent the initial stage of development of a sterility barrier between the species. If so, DOBZHANSKY'S objection to schemes such as KLINGSTEDT'S is not valid here. The hypothesis that hybrid sterility originates from sterility factors already existing within species should not be disregarded.

DOBZHANSKY's own model of the simplest genetic basis for hybrid sterility can be used as a possible model for this origin of a species barrier. His model assumes complementary genes as the basis for hybrid sterility. Beginning with a fertile population of the genotype, *aabb*, a species with the genotype, *AAbb*, might arise by mutation and gene fixation. A second species might diverge with the genotype, *aaBB*. These species are fertile but the hybrids, *AaBb*, are sterile because of the complementary action of *A* and *B*. Since the mutant genes, *A* and *B*, are concerned with sterility in the hybrid, it does not seem unreasonable to revise this model in the following way. Assume additive action for the genes effecting sterility. The genotypes, *Aabb* and *aaBb* would be partially sterile; *AAbb* and *aaBB*, slightly more sterile, etc. In the transition of *aabb* to *AAbb* and to *aaBB*, the first species would include the genotypes *aabb*, *Aabb*, and *AAbb*; while the second would include *aabb*, *aaBb*, and *aaBB*. The two species would exhibit variable partial sterility, and the fertility of hybrids between them would depend upon the individuals used in crossing. A scheme such as this, but built upon more loci, could explain the variable fertility in *Secale cereale*, in *S. montanum*, and in their F_1 hybrids.

SUMMARY

Secale cereale L. var. Merced and two strains of *S. montanum* were crossed using both X-rayed and untreated pollen. Meiosis in interspecific control hybrids did not differ depending on the strain of *S. montanum* used. At first metaphase the maximum association was a translocation chain of six chromosomes plus four bivalents. Univalents, which occurred in low frequencies, were usually chromosomes that were not included in the translocation configuration. Inversion bridges occurred at frequencies no greater than in the parental species. Lagging univalents divided in the first or in the second division. Meiotic irregularities were no more frequent in pollen sterile control hybrids than in the representative sample of control hybrids. Mean pollen and seed fertilities in control hybrids were 18.6 and 20.4 percent respectively.

Of 26 r-hybrids in which meiosis was studied, seventeen contained detectable induced translocations. In sixteen of these, induced translocations involved chromosomes of the original chain of six. Two or perhaps three of the plants with induced translocations also contained induced inversions. Very small inversions were induced in at least some of the nine plants which lacked gross structural changes. One r-hybrid exhibited fragmentation in meiosis. Another contained a small centric ring fragment and had lost chromosomal material. The frequency of induced gross structural rearrangements was no higher in the pollen sterile r-hybrids than in the sample as a whole. The r-hybrids were 12.2 percent pollen fertile and set seed in 10.7 percent of their florets.

The type of chromosome alterations induced in the r-hybrids were the same as those responsible for rearranging the genes in diverging species. This suggests the possibility of inducing chromosomal sterility barriers to prevent interbreeding between a plant breeder's improved varieties and the parental material. Such a barrier would be advantageous if improved varieties are to be grown in the proximity of parental strains.

The percentage of seed set in the r-hybrids was significantly lower than in the controls. The data suggest a similar reduction in pollen fertility, but the difference

was not significant. The correlation between pollen fertility and seed set was significant in r-hybrids but was not in controls. Correlations between fertility and meiotic irregularities were not significant in controls or in r-hybrids. The original as well as the induced chromosomal sterility was largely obscured in these hybrids by genic and perhaps environmental variation in fertility. That much of this variation was genic was indicated by fertility differences between families and the lack of correlation between chromosome behavior and fertility. Part of the genic variability in pollen fertility appeared to be independent of genic variability in seed set.

It is suggested that the variable partial sterility of the control hybrids represents the incipient stage of development of a sterility barrier between *Secale cereale* and *S. montanum*. Such a barrier could have the same genetic basis as the partial sterility that exists within the species.

ACKNOWLEDGMENTS

The writer wishes to express appreciation to the following persons: DR. G. L. STEBBINS, JR., for suggesting the problem and for advice throughout the investigation as well as for reading the manuscript and suggesting improvements; DR. ELIZABETH L. SCOTT and DR. E. R. DEMPSTER for suggestions regarding statistical treatment of the data.

ADDENDA

Brief accounts of the morphology and fertility of *Secale cereale* × *montanum* hybrids are given in the following papers which came to the attention of the author after the present manuscript was submitted for publication:

- ANTONOFF, S., 1936 Beitrag zum zytogenetischen Studium der Artbastarde *Triticum turgidum* × *Triticum durum*, *Triticum durum* × *Triticum monococcum* und *Secale cereale* × *Secale montanum*. Der Züchter **8**: 240-243.
- NAKAJIMA, G., 1954 Cytogenetic studies on the interspecific hybrids among genus *Secale*. I. Results of hybridization and external characters of F₁ plants. (In Japanese with English summary.) Japan. J. Breeding **4**: 132-134.

LITERATURE CITED

- BRADLEY, M. V., 1948 A method of making aceto-carmines permanent without removal of the cover slip. Stain Technol. **23**: 41-44.
- DOBZHANSKY, TH., 1951 *Genetics and the Origin of Species*, 3rd ed. Revised, 364 pp. New York: Columbia Univ. Press.
- DUKA, S. K., 1935 Cytogenetic research on the interspecific hybrids *Secale vulgare* × *Secale montanum*. Bull. Appl. Bot., Genet., and Plant Breed. Ser. A. **14**: 233-238 (Plant Breed. Abst. **6**: 869).
- GERASSIMOVA, H., 1939 Chromosome alterations as a factor of divergence of forms. I. New experimentally produced strains of *C. tectorum* which are physiologically isolated from the original forms owing to reciprocal translocation. Compt. Rend. Acad. Sci. U.R.S.S. **25**: 148-154.
- GILES, N., 1940 Spontaneous chromosome aberrations in Tradescantia. Genetics **25**: 69-87.
- HEMELRIJK, J., 1952 Note on Wilcoxon's two-sample test when ties are present. Ann. Math. Stat. **23**: 133-135.
- KENDALL, M. G., 1948 *Rank Correlation Methods*. 160 pp. London: Chas. Griffin Co.
- KLINGSTEDT, H., 1939 Taxonomic and cytological studies on grasshopper hybrids. I. Morphology and spermatogenesis of *Chorthippus bicolor* Carp. × *Ch. biguttulus* L. J. Genet. **37**: 389-420.

- KOSTOFF, D., 1932 Pollen abortion in species hybrids. *Cytologia* **3**: 337-339.
- 1937 Interspecific hybrids in Secale (rye). I. *Secale cereale* $\times$ *Secale ancestrale*, *Secale cereale* $\times$ *Secale vavilovii*, *Secale cereale* $\times$ *Secale montanum*, and *Secale ancestrale* $\times$ *Secale vavilovii* hybrids. *Current Sci.* **5**: 583-584, also *Compt. Rend. Acad. Sci. U.R.S.S.* **14**: 213-214.
- LAMM, R., 1936 Cytological studies on inbred rye. *Hereditas* **22**: 217-240.
- LONGLEY, A. E., and W. J. SANDO, 1930 Nuclear divisions in the pollen mother cells of Triticum, Aegilops, and Secale and their hybrids. *J. Agr. Research* **40**: 638-719.
- MANN, H. B., and D. R. WHITNEY, 1947 On a test of whether one or two random variables is stochastically larger than the other. *Ann. Math. Stat.* **18**: 50-60.
- MÜNTZING, A., 1946 Sterility in rye populations. *Hereditas* **32**: 521-549.
- MÜNTZING, A., and S. AKDIK, 1948 Cytological disturbances in the first inbred generations of rye. *Hereditas* **34**: 485-509.
- ONO, H., 1950 Intergeneric hybridization in Chichorieae. VIII. Morphology and fertility of F₁ individuals of *Paraixeris denticulata* $\times$ *Crepidiastrum platyphyllum*. *Japan. J. Genet.* **25**: 167-176.
- PUTT, E. D., 1954 Cytogenetic studies in rye. *Can. J. Agr. Sci.* **34**: 81-119.
- SCHIEHMANN, E., and U. NÜRNBERG-KRÜGER, 1952 Neue Untersuchungen an *Secale africanum* Stapf. II. *Secale africanum* und seine Bastarde mit *Secale montanum* und *Secale cereale*. *Naturwissenschaften* **39**: 136-137.
- SHERMAN, M., 1946 Karyotype evolution: A cytogenetic study of seven species and six interspecific hybrids in *Crepis*. *Univ. Calif. Publ. Botany* **18**: 369-408.
- SMITH, D. C., 1942 Intergeneric hybridization of cereals and other grasses. *J. Agr. Research* **64**: 33-47.
- SMITH, L., 1939 Mutants and linkage studies in *Triticum monococcum* and *T. aegilopoides*. *Missouri Agr. Expt. Sta. Research Bull.* **298**.
- STEBBINS, G. L., JR., 1945 The cytological analysis of species hybrids. *Botan. Rev.* **11**: 463-486.
- 1950 *Variation and Evolution in Plants*, 643 pp. New York: Columbia Univ. Press.
- STEBBINS, G. L., JR., and H. A. TOBGY, 1944 The cytogenetics of hybrids in Bromus. I. Hybrids within the section Ceratochloa. *Am. J. Botany* **31**: 1-11.
- STEBBINS, G. L., JR., and A. VAARAMA, 1954 Artificial and natural hybrids in the Gramineae, tribe Hordeae. VII. Hybrids and allopolyploids between *Elymus glaucus* and *Sitanion* spp. *Genetics* **39**: 378-395.
- TOBGY, H. A., 1943 A cytological study of *Crepis fuliginosa*, *C. neglecta*, and their F₁ hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. *J. Genet.* **45**: 67-111.
- TSCHERMAK, E., 1927 Über seltene Getreide- und Rübenbastarde. *Zeit. Ind. Abst. Ver.* **2**: 1495-1498.
- WALTERS, M. S., 1950 Spontaneous breakage and reunion of meiotic chromosomes in the hybrid *Bromus Trinnii* $\times$ *B. maritimus*. *Genetics* **35**: 11-37.
- 1952 Spontaneous chromosome breakage and atypical chromosome movement in meiosis of the hybrid *Bromus marginatus* $\times$ *B. pseudolaevipes*. *Genetics* **37**: 8-25.
- WILCOXON, F., 1945 Individual comparisons by ranking methods. *Biometrics* **1**: 80-83.