

## The inheritance in wheat of crossability with rye

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### 1. INTRODUCTION

Varieties of common wheat, *Triticum aestivum* ( $2n = 6x = 42$ ), differ in the ease with which they hybridize with rye, *Secale cereale* ( $2n = 14$ ). Following emasculation and hand pollination, more than 50% of the pollinated florets usually set seeds in readily crossable varieties. By contrast less than 5% of florets set seeds in poorly crossable forms. These differences between varieties have long been known to be inherited in a relatively simple manner (Backhouse, 1916; Taylor & Quisenberry, 1935).

The most detailed analysis of the character was carried out by Lein (1943), using the readily crossable wheat variety Chinese 466 and the poorly crossable varieties Marquis and Peragis. Ready crossability is recessive and, by pollinating with rye  $F_2$  plants from intervarietal crosses, Lein showed that allelic differences at two loci were responsible for the contrasted parental behaviours. On this basis it was suggested that Chinese 466 was genotypically  $kr_1 kr_1 kr_2 kr_2$  while Marquis and Peragis were  $Kr_1 Kr_1 Kr_2 Kr_2$ . Moreover, the variety Blausamtiger Kolben, which had an intermediate level of crossability and which was also included in the analysis, was thought to be genotypically  $Kr_1 Kr_1 kr_2 kr_2$ . From this work Lein concluded that the presence of  $Kr_1$  resulted in a more marked reduction in crossability than the presence of  $Kr_2$ .

Despite the significance of wheat-rye crossability in evolution and in agriculture little attention has been given to the character since the work of Lein. However, during the intervening period much has been learned about the cytogenetic structure of *T. aestivum* and new methods of genetic analysis have been developed. The purpose of the present work was to extend our knowledge of the genetics of the systems involved in relation to our present understanding of the structure of wheat and in particular to ascertain which chromosomes carry the crossability genes.

### 2. MATERIAL

The two parental varieties of *T. aestivum* L. emend. Thell. ssp. *vulgare* MacKey ( $2n = 6x = 42$ ) used in this work were Chinese Spring, which crosses readily with rye,

and Hope, which crosses poorly with rye. In addition twenty-one distinct inter-varietal chromosome substitution lines were used in which each pair of chromosomes of Chinese Spring was, in turn, replaced by its homologue from Hope.

The substitution lines were produced by E. R. Sears (Loegering, Sears & Rodenhiser, 1957) by backcross procedures initiated from 41-chromosome monosomic hybrids between the twenty-one distinct monosomics of Chinese Spring and euploid individuals of Hope. In each backcross generation, monosomic derivatives of the previous hybridization were used to pollinate corresponding monosomics of the recurrent parent, Chinese Spring. In this way intact chromosomes from Hope were substituted for the homologous Chinese Spring chromosomes. The substituted chromosomes were made disomic by selfing the monosomic derivatives of the final backcross.

The twenty-one pairs of chromosomes of wheat can be classified into three sets each of seven pairs, which represent the constituent A, B and D genomes. In the allopolyploid evolution of *T. aestivum* each genome was derived from a different diploid parental species. The chromosome complement can also be classified into seven homoeologous groups, each of three pairs. Homoeologous chromosomes have similar genetic activities but, in normal genotypes, do not pair and recombine at meiosis so that inheritance is disomic.

Every genome has one pair of chromosomes in every homoeologous group and every homoeologous group has one pair in every genome. This leads to the presumption that the genetic relationships of homoeologues stem from their evolutionary derivation from common chromosomes of the diploid progenitor of all three diploid parents of hexaploid wheat.

The designations used for wheat chromosomes indicate the homoeologous group and genome to which they belong. Thus chromosome 1A is in homoeologous group 1 and the A genome and the twenty-one pairs of chromosomes are designated 1A, 1B, 1D, . . . , 7D. In the present work the substitution lines, in which Hope chromosomes replace Chinese Spring chromosomes, will be designated CS/Hope 1A . . . CS/Hope 7D, indicating the chromosome substituted.

All the pollinations with rye made used of *S. cereale* L. var. King II ( $2n = 14$ ).

### 3. METHODS

Wheat parents, of the full range of genotypes to be tested for their crossability with rye, were grown in the field in Cambridge. Spikes to be pollinated with rye were selected when the anthers of the most mature florets were just beginning to turn from dark to pale green. Such anthers were about 2 days from pollen maturity, and dehiscence, under field conditions in early summer in Cambridge. Several apical and basal spikelets were removed from the spike in order only to leave spikelets at somewhat similar stages of maturity. All except the two basal florets were removed from these remaining spikelets so that all the florets to be pollinated were at about

the same stage of development. There were between twenty and thirty florets per spike left for emasculation.

Approximately 2 mm. of the tips of the lemmas and paleas of these florets were cut off in order to give easier access for emasculation and pollination. The anthers were then removed from every floret, using forceps. Care was taken to avoid damage to the ovary and stigma.

The emasculated spikes were covered with cellophane bags to prevent accidental out-crossing. When the stigmas of most of the emasculated florets were feathery and receptive, which was usually 2 days after emasculation, the spikes were pollinated. Spikes of rye in which anther dehiscence was taking place were picked and taken to the emasculated and receptive wheat spikes. Rye anthers that were about to dehisce were removed individually from the rye spikes and brushed on to the stigma of each emasculated floret, care being taken that a liberal deposit of pollen was left on the stigma.

The wheat spikes were rebagged after pollination and were allowed to ripen. After harvest the numbers of florets with and without seeds were determined separately for every spike included in the experiment. However, in this presentation, the data have been summed over genotypes separately for both the workers participating (Riley and Chapman) and expressed as the percentage of successful crosses over the total florets pollinated. Clearly, in all estimates of artificial crossability, it is necessary to attempt to remove the variation caused by the idiosyncrasies of technique of different workers.

#### 4. RESULTS

##### (i) *The parental varieties*

The wheat varieties, Chinese Spring and Hope, were pollinated with rye and the success of the crosses observed (Table 1). The contrast between the varieties was very marked; 74.31% of the pollinated florets of Chinese Spring—but none of those of Hope—were fertile. Clearly, with this level of distinction, it was worthwhile testing the substitution lines, in which pairs of chromosomes of Chinese Spring were separately replaced by pairs from Hope, in an attempt to ascertain which chromosomes were active in determining crossability with rye.

Table 1. *The fertility of two varieties of wheat when pollinated with rye*

Variety	Worker				Total	
	Riley		Chapman			
	Florets pollinated	% fertile	Florets pollinated	% fertile	Florets pollinated	% fertile
Chinese Spring	84	76	98	72	182	74.3
Hope	46	0	58	0	104	0.0

(ii) *CS/Hope substitution lines*

Plants of the substitution lines, in which every pair of chromosomes of Chinese Spring was replaced in turn by its homologue from Hope, were pollinated by rye. The results of these pollinations are shown in Table 2 expressed as the percentage of the pollinated florets in which seeds were set. Only line CS/Hope 5B, in which the chromosome 5B pair was from Hope, displayed a pronounced reduction in fertility, although there was also some reduction in CS/Hope 5A. The combination of both these effects with the more normal behaviour of CS/Hope 5D resulted in the lines with substitutions for chromosomes of homoeologous group 5 displaying a distinctly lower overall fertility than the lines representing all the other groups.

Table 2. *The fertility, following pollination by rye, of wheat intervarietal substitution lines involving the replacement of pairs of Chinese Spring by pairs of Hope chromosomes*

Chromosome substituted	Worker						% fertility* (over homoeologous groups and workers)
	Riley		Chapman		Total		
	Florets pollinated	% fertile	Florets pollinated	% fertile	Florets pollinated	% fertile*	
CS/Hope 1A	38	32	52	52	90	41.7	
CS/Hope 1B	44	75	50	74	94	74.5	59.6
CS/Hope 1D	40	80	55	45	95	62.5	
CS/Hope 2A	38	42	46	74	84	58.0	
CS/Hope 2B	40	47	54	63	94	55.2	58.0
CS/Hope 2D	40	62	56	59	96	60.7	
CS/Hope 3A	42	79	56	64	98	71.4	
CS/Hope 3B	36	64	43	60	79	62.2	64.3
CS/Hope 3D	82	66	50	54	130	59.9	
CS/Hope 4A	40	65	52	81	92	72.9	
CS/Hope 4B	42	76	52	44	94	60.2	62.4
CS/Hope 4D	42	40	56	68	98	54.2	
CS/Hope 5A	44	43	98	9	142	26.2	
CS/Hope 5B	138	7	182	5	320	6.4	28.3
CS/Hope 5D	46	48	30	57	76	52.2	
CS/Hope 6A	38	82	50	70	88	75.8	
CS/Hope 6B	40	57	49	59	89	58.3	66.1
CS/Hope 6D	38	58	58	71	96	64.3	
CS/Hope 7A	44	54	52	60	96	57.1	
CS/Hope 7B	38	84	46	67	84	75.8	61.8
CS/Hope 7D	44	57	56	48	100	52.5	

\* unweighted averages.

In order to determine the significance of these observations the percentages of fertile florets, of the total pollinated by each worker in each substitution line, were converted to angles and an analysis of variance was carried out (Table 3).

This showed that there was no significant difference between the fertilities of the pollinations carried out by the two workers but that there were significant differences between the substitution lines (V.R. = 3.38,  $P < 0.01$ ). Further analysis of the variation due to lines showed that those with substitutions for chromosomes 5A and 5B were significantly different from all the others (V.R. = 47.9,  $P = < 0.001$ )

Table 3. *Analysis of variance of the percentage fertility, converted to angles, of the CS/Hope chromosome substitution lines when pollinated by rye*

Item	Mean square	Degrees of freedom	Variance ratio
Workers	19.2	1	< 1
Lines	229.5	20	3.38**
5A and 5B versus rest	3257.3	1	47.92**
5A versus 5B	218.8	1	3.22*
Remaining line variation	61.9	18	< 1
Error	68.0	20	

\*  $P$  between 0.1 and 0.05.

\*\*  $P < 0.01$ .

but that these two lines were not different from each other (V.R. = 3.22,  $P = 0.1-0.05$ ) nor was there any significant variation among the remaining lines.

The genetic difference in crossability between Hope and Chinese Spring is, therefore, almost entirely due to the activities of chromosomes 5A and 5B. Moreover, although no statistically significant difference between them was detectable, the data suggested the possibility that chromosome 5B of Hope causes a greater reduction in crossability than does chromosome 5A.

##### 5. CHROMOSOMAL LOCATION OF $Kr_1$ AND $Kr_2$

This evidence concerning the chromosomes that participate in the determination of the resistance to crossability of Hope is of interest in relation to the origins of the variety. Hope was isolated in a breeding programme which had the object of introducing rust resistance into the hard red spring wheats of the American and Canadian mid-west. In this programme the tetraploid Yaroslav emmer (*T. dicoccum*) was hybridized with the hexaploid variety Marquis (McFadden, 1930). After the initial hybridization, self-pollination was allowed to occur in subsequent generations and selection was practised for segregants with the general characteristics of hexaploid wheats but the disease resistance of the tetraploid parent. Hope was a hexaploid, disease resistant, derivative of the programme.

Marquis, the hexaploid parent of Hope, has poor fertility when pollinated by rye and Lein (1943) considered that this was due to its  $Kr_1 Kr_1 Kr_2 Kr_2$  genotype. By contrast most tetraploid wheats cross readily with rye and, although no data are available, it can be assumed that this applies to Yaroslav emmer, the tetraploid

parent of Hope. This would imply that the poor crossability of Hope was derived from the Marquis parent and that it is homozygous for one or both of the unlinked dominants  $Kr_1$  and  $Kr_2$ . It is reasonable to conclude that both genes are present in Hope and that they are on chromosomes 5A and B5, since there was markedly lower fertility in the lines with substitutions of either of these two chromosomes from Hope into Chinese Spring.

Lein (1943) also indicated that homozygosity for  $Kr_1$  and  $kr_2$  resulted in lower fertility in rye crosses than homozygosity for  $kr_1$  and  $Kr_2$ . Consequently  $kr_2$  could be described as a more efficient promoter of crossability than  $kr_1$ ; or  $Kr_1$  could be described as a more effective inhibitor of crossability than  $Kr_2$ . Substitution line CS/Hope 5B had a lower fertility than CS/Hope 5A in crosses with rye. From this it may be assumed that in Hope, chromosome 5B carries  $Kr_1$  and chromosome 5A carries  $Kr_2$ , while the alternative alleles are present on these chromosomes in Chinese Spring.

#### 6. ACTIVITY OF THE CROSSABILITY GENES

In considering the functional means by which different levels of crossability with rye are determined in wheat, it may first be asked whether the recessive alleles actively promote fertility or alternatively whether the dominant alleles inhibit crossability. To test this plants of the readily crossable variety Chinese Spring, that were nullisomic for chromosome 5B and simultaneously tetrasomic for the homoeologous chromosome 5D (nulli-5B tetra-5D), were pollinated with rye. The advantage of this genotype was that the complete absence of a chromosome could be tolerated, because of the compensating capacity of the tetrasomic condition of its homoeologue, without disruption of the ability to set seeds. In nulli-5B tetra-5D,  $kr_1$ —normally carried on chromosome 5B—was absent. Chromosome 5D—in extra dosage—displayed no effect on crossability in studies of the CS/Hope substitution lines. Nulli-5B tetra-5D differed from CS/Hope 5B in that chromosome 5B of Chinese Spring was replaced by extra dosage of a homoeologue rather than by a substituted homologue from another variety.

A total of 116 florets of nulli-5B tetra-5D was pollinated by rye and of these sixty-seven, or 57.7%, set seeds. Therefore the absence of chromosome 5B and the  $kr_1$  allele caused no disturbance of the ready crossability of Chinese Spring. From this it can be concluded that the  $kr_1$  allele does not actively promote crossability, and it must be inferred instead that  $Kr_1$  of Hope is an inhibitor of crossability. In view of the similar dominance relationship at the homoeologous locus on chromosome 5A it seems reasonable to assume that  $Kr_2$  is also an inhibitor of crossability.

This view of the operation of the system is confirmed by the dominance relationships of the crossability alleles. If the  $kr_1$  and  $kr_2$  alleles, which when homozygous result in high levels of crossability, were responsible for the supply of a product necessary for crossing to occur then this product would also be present in the heterozygote. However, although the  $Kr_1$  and  $Kr_2$  alleles are not completely dominant, crossability is greatly reduced in heterozygotes (Lein, 1943). Moreover the recessi-

vity of  $kr_1$  does not simply derive from a threshold level of activity that is not attained with the allele in single dose. This is displayed by the high crossability of plants of Chinese Spring monosomic for chromosome 5B and therefore with only a single  $kr_1$  allele. In the present work 261 florets of this genotype were pollinated and these set 184 seeds, giving 70.5% success. The presence of the products of the  $Kr$  alleles, therefore, reduces the probability of wheat-rye hybridization through active inhibition.

Neither the CS/Hope 5A nor the CS/Hope 5B substitution line was as infertile, in crosses with rye, as was the variety Hope. Consequently the effects of the inhibitors of crossability,  $Kr_1$  and  $Kr_2$ , are either complementary or additive.

These inferences about the control of crossability are relevant to modifications of the character reported to be produced by grafting. Pissarev & Vinogradova (1944) claimed that, when embryos from seeds of varieties of wheat that crossed poorly with rye were transplanted on to the endosperm of rye seeds, the resulting plants had levels of crossability that were higher than untreated plants of the same variety. This work was repeated in Sweden by Hall (1954) with similar results. Subsequently Hall (1956) transplanted embryos from poorly crossable varieties on to the endosperm of seeds of readily crossable varieties of wheat. The resulting plants crossed more readily with rye than did untreated plants of the same variety.

These results might be interpreted as implying that crossability is enhanced by the transfer to the transplanted embryo of some persisting factor from the recipient endosperm. If, however, as can be argued from the present results, crossability is actively inhibited by gene products, the results of the embryo transplantation experiments are more difficult to understand. It is now necessary to envisage the removal of the inhibition in the transplanted embryo even though, in a heterozygote, the activities of the crossability alleles in the recipient endosperm would be recessive to those of the embryo.

If the recipient endosperm were indeed to affect the transplanted embryo, the relationship expected would be the reciprocal of that reported. That is, with the active inhibition of crossability, the transplantation of the embryo of a crossable variety on to the endosperm of a poorly crossable variety should lead to poor crossability. Such an experiment has apparently never been carried out. However, it would be interesting not only to determine whether the relationships are as predicted, but also to ascertain whether the crossability of plants grown from transplanted embryos is indeed affected by the recipient endosperm.

## 7. EVOLUTIONARY SIGNIFICANCE

The totally independent cultivation of wheat and rye is of comparatively recent origin. For much of their agricultural histories rye and wheat have grown together either as components of a mixed crop or with rye present as a weed of wheat. Indeed, weed ryes are still common in parts of eastern Europe and western Asia.

Wheat has been cultivated for approximately 11,000 years, but for much of this period tetraploid rather than hexaploid species were used. The first wheats taken

in to cultivation were tetraploids, but their persisting use has varied a great deal—depending upon climatic conditions.

Tetraploid wheats set seeds readily when pollinated with rye, in the present work ninety-five florets were pollinated and thirty-nine, or 43%, were fertile. However these seeds rarely germinate. Consequently, in a tetraploid crop, spontaneous hybrid seeds could be produced and would contribute to yield, but if sown they would merely add to the proportion that failed to germinate for many other reasons. Outcrossing with rye would therefore be of no agricultural significance in crops of tetraploid wheat.

Hybrid seeds from crosses between hexaploid wheat and rye germinate freely and form vigorous and aggressive  $F_1$  plants which are however completely sterile. Spontaneous hybrid seeds could therefore be produced easily in a crop of hexaploid wheat that had the readily crossable genotype. The hybrid seeds—as in a tetraploid crop—would contribute to yield but a new situation would arise when these seeds were sown in the next crop. The wheat-rye seeds would germinate and vegetatively vigorous hybrid plants would establish. These would compete aggressively with the crop but, because of their sterility, they would not contribute to the yield of the crop.

The hybrids would therefore occupy the role of weeds—depriving the crop of space, light, nutrients and water, but returning nothing at harvest. Under conditions of free hybridization with rye, hexaploid wheat is thus capable of generating its own weeds.

The ready crossability of tetraploid wheats, and the failure to recognize any genetic impediment to crossability in the D genome, suggest that the first hexaploids were probably able to hybridize freely with rye. The dominant inhibitors of crossability  $Kr_1$  and  $Kr_2$  probably arose by mutation and were favoured by natural selection, and by unconscious agricultural selection, because of the escape they provided from the production of wheat-rye 'weeds'.

Almost all the hexaploid wheats of Europe, where rye is common, have poor crossability. Indeed, apparently the only region where readily crossable forms are common is in central China—a region into which rye was only recently introduced. The character of ready crossability has thus been preserved, on a large scale, where it was of no evolutionary disadvantage but it is rare where rye is common. In a rather impressive manner this emphasizes the evolutionary significance of the character and the strength of the selection favouring the inhibition of crossability in Europe and western Asia.

#### SUMMARY

1. By the use of intervarietal chromosome substitution lines, the poor crossability with rye (*S. cereale*,  $2n = 14$ ) of the wheat (*T. aestivum*,  $2n = 42$ ) variety Hope was shown to be determined by chromosomes 5A and 5B. The genes  $Kr_1$  and  $Kr_2$ , which are responsible for poor crossability (Lein, 1943), are probably located on chromosomes 5B and 5A respectively.



2. Crossability is actively inhibited by the dominant alleles,  $Kr_1$  and  $Kr_2$ , of Hope and is apparently not enhanced by the recessive alleles,  $kr_1$  and  $kr_2$ , of the readily crossable variety Chinese Spring.

3. The inhibition of crossability with rye conferred evolutionary and agricultural advantage upon wheat by preventing the production of sterile wheat-rye hybrids which could be regarded as weeds generated from within the crop.

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