

## SELF-INCOMPATIBILITY IN RYEGRASS

### II. THE JOINT SEGREGATION OF *S* AND *Z* IN *LOLIUM PERENNE* L.

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#### SUMMARY

An analysis of the data from seven families of full-sibs reveals no evidence that the incompatibility genes, *S* and *Z*, are linked. In the absence of a knowledge of the parental genotypes it is necessary to adopt a special procedure of classifying the progenies in order to be able to perform tests for linkage. The application of this procedure to situations concerning traits other than self-incompatibility is discussed and a simple crossing-scheme which avoids this problem is given.

#### 1. INTRODUCTION

In a previous paper, we showed that the pattern of pollinations obtained from crosses within seven full-sib families was consistent with the hypothesis that self-incompatibility in diploid perennial ryegrass (*Lolium perenne*) is controlled by a pair of multi-allelic genes, *S* and *Z*, whose effect in the pollen is gametophytic (Cornish, Hayward and Lawrence, 1979). We have yet to consider, however, the possibility that these genes may be linked. On general grounds this would appear to be rather unlikely because it would reduce any advantage that the two-locus system of self-incompatibility might have over the more familiar one-locus system. More particularly, Lundqvist (1961) failed to find any evidence of linkage between *S* and *Z* in a self-progeny of *Festuca pratensis* that contained 71 plants. Since *F. pratensis* and *L. perenne* are closely related species, we do not expect the incompatibility genes of the latter species to be linked in their inheritance either.

The purpose of the present paper is to give an analysis of the data from the seven families previously mentioned in order to test this hypothesis of independent inheritance of the incompatibility genes in *L. perenne*.

#### 2. PROCEDURE

The results from these seven families are shown in summary form in table 1. Families D and E, F and G, and H and I are the progeny of three different crosses where each pair of parents was used in turn as male and as female. The pattern of pollinations obtained in families D and E, H and I, and in P indicated that their parents had no alleles in common at either the *S* or the *Z* locus, whereas the pattern obtained in families F and G showed

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that their parents had an allele in common at one locus. Since there is no dominance at either locus, we expect a total of 16 different phenotypic classes in both kinds of progeny (table 2).

Now we wish to test for linkage between *S* and *Z* in each of these seven families taken separately and on the pooled data of the three pairs of reciprocals. In principle this is a straightforward task because Lawrence, Cornish and Hayward (1979) have shown that it is possible to test for and to obtain independent estimates of both male and female linkage in a family of this type, which is a completely classified intercross. The tests of linkage

TABLE 1

*Summary of the results obtained from crosses within seven full-sib families. It should be emphasised that while the assignation of plants to classes is consistent over reciprocal crosses, the designation of genotypes between different families is arbitrary (see the text and Cornish et al., 1979, for further details)*

<i>S S Z Z</i>	D				H				F		
	D	E	E +	H +	I +	I +	P	<i>S S Z Z</i>	F	G	G +
1 3 1 3	0	5	5	4	0	4	2	1 1 1 3	5	2	7
1 3 1 4	0	4	4	3	1	4	1	1 1 1 4	5	3	8
1 3 2 3	2	2	4	1	5	6	1	1 1 2 3	0	0	0
1 3 2 4	2	3	5	0	4	4	3	1 1 2 4	0	2	2
1 4 1 3	1	0	1	5	5	10	0	1 2 1 3	4	2	6
1 4 1 4	1	1	2	1	2	3	3	1 2 1 4	5	0	5
1 4 2 3	1	2	3	2	2	4	3	1 2 2 3	0	0	0
1 4 2 4	1	3	4	0	0	0	0	1 2 2 4	0	1	1
2 3 1 3	1	1	2	3	3	6	2	1 3 1 3	7	3	10
2 3 1 4	5	3	8	0	0	0	2	1 3 1 4	3	1	4
2 3 2 3	1	1	2	2	1	3	0	1 3 2 3	1	2	3
2 3 2 4	5	1	6	3	3	6	1	1 3 2 4	2	4	6
2 4 1 3	1	3	4	1	5	6	3	2 3 1 3	1	2	3
2 4 1 4	1	4	5	3	1	4	4	2 3 1 4	3	1	4
2 4 2 3	3	1	4	1	2	3	2	2 3 2 3	1	4	5
2 4 2 4	4	0	4	2	3	5	3	2 3 2 4	0	3	3
Totals	29	34	63	31	37	68	30		37	30	67

take a simple form because a completely classified intercross is equivalent to a pair of double backcrosses in one. Thus the test for linkage on the female side of the cross is

$$\chi^2_{(1)} = (n_{1.} - n_{2.} - n_{3.} + n_{4.})^2/n_{..} = C_R^2/n \tag{1}$$

where  $C_R$  is written for  $(n_{1.} - n_{2.} - n_{3.} + n_{4.})$ , the subscript, *R*, indicating that the comparison involves the *row* totals of table 2 and  $n = n_{..}$ ; while that for linkage on the male side is

$$\chi^2_{(1)} = (n_{.1} - n_{.2} - n_{.3} + n_{.4})^2/n_{..} = C_C^2/n \tag{2}$$

where  $C_C$  is written for  $(n_{.1} - n_{.2} - n_{.3} + n_{.4})$ , the subscript, *C*, indicating that the comparison involves the *column* totals of table 2. The sum of these  $\chi^2$ 's may be partitioned to give a joint or overall test for linkage which is

$$\begin{aligned} \chi^2_{(1)} &= [(n_{1.} + n_{.1}) - (n_{2.} + n_{.2}) - (n_{3.} + n_{.3}) + (n_{4.} + n_{.4})]^2/2n_{..} \\ &= (C_R + C_C)^2/2n \end{aligned} \tag{3}$$

TABLE 2

The expected composition of a progeny produced by crossing an  $S_1Z_1/S_2Z_2$  plant used as female with an  $S_3Z_3/S_4Z_4$  plant used as a male parent. The frequency of recombination between S and Z is  $p_f$  on the female and  $p_m$  on the male side of the cross. The  $n_{ij}$ 's are the numbers observed for each genotype. In the text  $n_{..}$  is written as  $n$  ( $q_f = 1 - p_f$  and  $q_m = 1 - p_m$ )

♀	♂	$S_3Z_3$ $q_m/2$	$S_3Z_4$ $p_m/2$	$S_4Z_3$ $p_m/2$	$S_4Z_4$ $q_m/2$	Row totals
$S_1Z_1$ $q_f/2$	$S_1Z_1/S_3Z_3$ $q_fq_m/4$ $n_{11}$	$S_1Z_1/S_3Z_4$ $q_fp_m/4$ $n_{12}$	$S_1Z_1/S_4Z_3$ $q_fp_m/4$ $n_{13}$	$S_1Z_1/S_4Z_4$ $q_fq_m/4$ $n_{14}$	$q_f/2$ $n_{1.}$	
$S_1Z_2$ $p_f/2$	$S_1Z_2/S_3Z_3$ $p_fq_m/4$ $n_{21}$	$S_1Z_2/S_3Z_4$ $p_fp_m/4$ $n_{22}$	$S_1Z_2/S_4Z_3$ $p_fp_m/4$ $n_{23}$	$S_1Z_2/S_4Z_4$ $p_fq_m/4$ $n_{24}$	$p_f/2$ $n_{2.}$	
$S_2Z_1$ $p_f/2$	$S_2Z_1/S_3Z_3$ $p_fq_m/4$ $n_{31}$	$S_2Z_1/S_3Z_4$ $p_fp_m/4$ $n_{32}$	$S_2Z_1/S_4Z_3$ $p_fp_m/4$ $n_{33}$	$S_2Z_1/S_4Z_4$ $p_fq_m/4$ $n_{34}$	$p_f/2$ $n_{3.}$	
$S_2Z_2$ $q_f/2$	$S_2Z_2/S_3Z_3$ $q_fq_m/4$ $n_{41}$	$S_2Z_2/S_3Z_4$ $q_fp_m/4$ $n_{42}$	$S_2Z_2/S_4Z_3$ $p_fp_m/4$ $n_{43}$	$S_2Z_2/S_4Z_4$ $p_fq_m/4$ $n_{44}$	$q_f/2$ $n_{4.}$	
Column totals	$q_m/2$ $n_{.1}$	$p_m/2$ $n_{.2}$	$p_m/2$ $n_{.3}$	$q_m/2$ $n_{.4}$	1 $n_{..}$	

and a test for heterogeneity between linkage on the male and female side of the cross which is

$$\chi^2_{(1)} = [(n_{1.} - n_{.1}) - (n_{2.} - n_{.2}) - (n_{3.} - n_{.3}) + (n_{4.} - n_{.4})]^2 / 2n_{..}$$

$$= (C_R - C_C)^2 / 2n \tag{4}$$

In addition, it is possible to carry out tests of the segregation of  $S_4 : S_3$ ,  $Z_1 : Z_2$ ,  $S_3 : S_4$  and of  $Z_3 : Z_4$  on the row and column totals of table 2, though the linkage  $\chi^2$ 's given above require no amendment if only one ratio is disturbed in one or both parents.

In practice, however, we need to know the genotypes of both parents of the cross, including the phase of linkage, if any, between S and Z in each, in order to be able to arrange the data from a family in a 4 x 4 table of the kind shown in table 2. In the present circumstances we have no direct knowledge of the parental genotypes, only of the composition of their progeny. Thus although we know that the allelic pairs  $S_1$  and  $S_2$ ,  $S_3$  and  $S_4$ ,  $Z_1$  and  $Z_2$ , and  $Z_3$  and  $Z_4$  must each have come from the same parent, because this is a matter of definition, we do not know whether the  $S_1$ ,  $S_2$  pair came from the same parent as the  $Z_1$ ,  $Z_2$  pair or from the same parent as the  $Z_3$ ,  $Z_4$  pair of alleles, or do we know in general which pairs of alleles came from the female or the male parent, so that the use of the terms male and female in the analyses which follow is necessarily arbitrary. However, as it happens, the occurrence of selfs among the legitimate offspring of family D provides unambiguous evidence that  $S_1$  and  $S_2$  came from the female parent of this family and hence from the male parent of the reciprocal family E. The terms male and female can therefore be used in accordance with this evidence in this pair of families.

In general, however, our ignorance of the ancestry of the incompatibility alleles in these families means that we have initially to consider a total of eight (not counting reciprocals) different matings, any one of which could have given rise to the families we have scored (table 3). It is not necessary, however, to consider all eight matings when testing for linkage. This can be seen by reference to table 4 which shows the row and column totals and the numerators of the four  $\chi^2$ 's for each of the eight possible arrangements of the data from family D.

TABLE 3  
*The eight different ways (ignoring reciprocals) of writing a cross between two double heterozygotes with no alleles in common*

1	$S_1Z_1/S_2Z_2 \times S_3Z_3/S_4Z_4$	Type A
2	$S_1Z_1/S_2Z_2 \times S_3Z_4/S_4Z_3$	
3	$S_1Z_2/S_2Z_1 \times S_3Z_3/S_4Z_4$	
4	$S_1Z_2/S_2Z_1 \times S_3Z_4/S_4Z_3$	
5	$S_1Z_3/S_2Z_4 \times S_3Z_1/S_4Z_2$	Type B
6	$S_1Z_3/S_2Z_4 \times S_3Z_2/S_4Z_1$	
7	$S_1Z_4/S_2Z_3 \times S_3Z_1/S_4Z_2$	
8	$S_1Z_4/S_2Z_3 \times S_3Z_2/S_4Z_1$	

The first four crosses (type A) have the same intergenic pairing of the alleles but differ in the linkage phase of the genes in the parents. This means that they give numerically the same row and column totals but in different orders depending on which gametes in the parents are of the parental and which are of the recombinant kind. As can be seen, the absolute values of  $C_R$  and  $C_C$  are unaffected by this change of order, though they differ in sign as we move from one cross to another. It follows, therefore, that the tests of linkage on the female and male side are independent of the phase of linkage because the numerators of the corresponding  $\chi^2$ 's are the squares of the linear comparisons, being  $(C_R)^2$  and  $(C_C)^2$  respectively. Thus crosses 1-4 give the same value for each of these two  $\chi^2$ 's.

The joint linkage and heterogeneity  $\chi^2$ 's, on the other hand, being functions of  $(C_R + C_C)^2$  and  $(C_R - C_C)^2$  respectively are affected by the sign of  $C_R$  and  $C_C$ . Thus the joint linkage  $\chi^2$ 's of crosses 1 and 4 have the same value as the heterogeneity  $\chi^2$ 's of crosses 2 and 3 and vice versa. This is because a change in the linkage phase of one of the parents changes the sign of either  $C_R$  or  $C_C$  which, in turn, causes an interchange of the values of these two  $\chi^2$ 's. In the absence of information about the phase of linkage in the parents, therefore, the designation of these items as a joint linkage and a heterogeneity  $\chi^2$  is arbitrary. This ambiguity, however, causes no real difficulty in practice (see later), so that crosses 1-4 can be handled by the same analysis.

The four type B crosses (5-8) differ from 1-4 in the intergenic pairings of the parents. This results in new sets of row and column totals quite different from those of the type A matings. However, the arguments used above with the latter apply also to crosses 5-8, so that these too can also be handled by a single analysis, though one which is different from that appropriate to type A matings.

TABLE 4

The row and column totals, linear comparisons between these totals ( $C_R$  and  $C_C$ ) and the numerators of the four linkage  $\chi^2$ 's for each of the eight possible arrangements of the data from family D

	$n_{1.}$	$n_{2.}$	$n_{3.}$	$n_{4.}$	$C_R$	$n_{.1}$	$n_{.2}$	$n_{.3}$	$n_{.4}$	$C_C$	$(C_R)^2$	$(C_C)^2$	$(C_R + C_C)^2$	$(C_R - C_C)^2$
Type A														
1	2	6	8	13	1	4	12	6	7	-7	1	49	36	64
2	2	6	8	13	1	12	4	7	6	7	1	49	64	36
3	6	2	13	8	-1	4	12	6	7	-7	1	49	64	36
4	6	2	13	8	-1	12	4	7	6	7	1	49	36	64
Type B														
5	4	4	6	15	9	6	10	4	9	1	81	1	100	64
6	4	4	6	15	9	10	6	9	4	-1	81	1	64	100
7	4	4	15	6	-9	6	10	4	9	1	81	1	64	100
8	4	4	15	6	-9	10	6	9	4	-1	81	1	100	64

It turns out, therefore, that it is necessary to consider only two types of mating when testing for linkage in each family, each of which leads to a unique analysis. These are:

$$\begin{array}{l} \text{Type A} \quad S_1 S_2 \zeta_1 \zeta_2 \times S_3 S_4 \zeta_3 \zeta_4 \\ \text{Type B} \quad S_1 S_2 \zeta_3 \zeta_4 \times S_3 S_4 \zeta_1 \zeta_2 \end{array}$$

in the case of families D and E, H and I, and P; and

$$\begin{array}{l} \text{Type A} \quad S_1 S_2 \zeta_1 \zeta_2 \times S_1 S_3 \zeta_3 \zeta_4 \\ \text{Type B} \quad S_1 S_2 \zeta_3 \zeta_4 \times S_1 S_3 \zeta_1 \zeta_2 \end{array}$$

in the case of families F and G where the  $S_1$  allele is common to both parents. In the absence of linkage between  $S$  and  $\zeta$ , none of the four  $\chi^2$ 's in either of the two sets are expected to be significant. If linkage is present, however, one or more of the  $\chi^2$ 's in one set (either those of the type A or those of the type B analysis) are expected to be significant, but none in the other set (that is, either those of the type B or those of the type A analysis, respectively) because in the latter case the pairs of alleles have come from different, rather than the same parents. Furthermore, once linkage has been detected, it should be possible, by an inspection of the row and column totals of the  $4 \times 4$  table in which the data have been arranged to deduce the phase of linkage in each parent and hence to decide which of the  $\chi^2$ 's that have been calculated according to equations 3 and 4 is the joint linkage item or which is the heterogeneity  $\chi^2$ .

However, because we need to carry out *two* independent sets of tests for linkage (one on the assumption that the mating is of type A and the other that it is of type B), the probability of an error of the first kind is twice the corresponding probability for a single test. Hence in testing the null hypothesis of no linkage between  $S$  and  $\zeta$  it is appropriate to choose  $P = 0.025$  and  $P = 0.005$  as the levels of significance rather than the conventional 5 per cent and 1 per cent levels.

We can now consider the analysis of the data from each of the seven families taken separately as well as that obtained by pooling the data of the three pairs of reciprocal crosses.

### 3. RESULTS

Tests on the segregation of  $S_1 : S_2$ , of  $S_3 : S_4$  (or  $S_1 : S_3$  in families F and G), of  $\zeta_1 : \zeta_2$  and of  $\zeta_3 : \zeta_4$  show that there is good overall agreement with the expected 1 : 1 ratio. Of a total of  $7 \times 4 = 28$  such tests only two gave a significant departure from expectation, one of these occurring in family D and the other in F (see Cornish *et al.*, *loc. cit.*, for further details). Therefore, the linkage  $\chi^2$ 's given in equations 1-4 may be validly used on our data.

Two sets of four linkage  $\chi^2$ 's have been calculated on the row and column totals of each of the seven families separately as well as on the pooled data of the three pairs of reciprocal families. One set of four has been obtained in each case on the assumption that the cross giving rise to the family in question was of type A and the other set of four  $\chi^2$ 's on the alternative assumption that the cross was of type B. The results of this analysis are shown in table 5.

TABLE 5  
*Tests for linkage on the data from each of the seven families taken separately and on the pooled data of the three pairs of reciprocal crosses. Each  $\chi^2$  has 1 degree of freedom;*  
*\*  $P = 0.025 - 0.005$  (see text for further details)*

Type of cross	Item	D	E	D+E	F	G	F+G	H	I	H+I	P
A	[Female	0.034	1.882	0.778	0.676	0.533	1.209	3.903	0.243	0.941	1.200
	[Male	1.690	0	0.778	0.243	0.533	0.731	0.032	1.324	0.529	0
	[Joint	0.621	0.941	1.556	0.054	0	0.030	2.323	1.351	0.029	0.600
	[Heterogeneity	1.103	0.941	0	0.865	1.067	1.910	1.612	0.216	1.441	0.600
B	[Female	2.793	0	1.286	0.676	1.200	0.015	2.613	0.027	1.471	0.133
	[Male	0.034	0.471	0.397	2.189	3.333	5.388*	0.032	6.081*	0.529	0
	[Joint	1.724	0.236	1.556	2.649	0.267	2.418	1.032	2.649	0.118	0.066
	[Heterogeneity	1.103	0.236	0.127	0.216	4.267	2.985	1.613	3.460	1.882	0.066

M.B.  $P(\chi^2 \geq 5.024) = 0.025$  and  $P(\chi^2 \geq 7.879) = 0.005$ .

It is at once apparent that there is very little evidence of linkage between  $S$  and  $Z$  in these data, for only two out of a total of 80  $\chi^2$ 's are significant. We conclude, therefore, that there is no evidence of linkage between the incompatibility genes,  $S$  and  $Z$ , in these full-sib families.

#### 4. DISCUSSION

There are three points worth making about this analysis of the joint segregation of  $S$  and  $Z$ . The first is that the result we have obtained with *L. perenne* is the same as that found by Lundqvist (1961) in *Festuca pratensis*, and it is very likely that  $S$  and  $Z$  assort independently in the other self-incompatible grasses also.

The second point concerns the analytical procedure we had to follow in order to test for linkage because we had no direct knowledge of the genotypes of the parents of our families. In most linkage experiments, the phenotypes of the parents will be known and, dominance apart, their genotypes will be known also. The progeny of such crosses can usually be classified without ambiguity, so that there is no need to resort to the analytical procedure that we have used here. Where, however, it is not possible to score the parents of a cross, as might be the case in a species with an annual habit, this procedure may be used to advantage. One of its more obvious applications is to the analysis of linkage between genes coding for enzyme variants (isozymes), whose alleles, like those which determine self-incompatibility in the grasses, display no dominance.

The third and final point is that the problem of not knowing the ancestry of the genes which are segregating in a family can be avoided by raising a few plants of a half-sib relationship to those of the family of interest. The pattern of reactions of pollen from a half-sib on the stigmas of plants in the main family will reveal those alleles in the latter which are also present in the former and hence those alleles which originated from the parent common to both families. In this way, it is possible to decide without any ambiguity whether the cross is of type A or of type B in advance of any analysis of the data from the main family. While in principle it should be possible to deduce the origins of the alleles in a family by using the pollen from a single half-sib, in practice it would be prudent to have several half-sibs available for this purpose.

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