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SELF-INCOMPATIBILITY IN RYEGRASS

I. GENETIC CONTROL IN DIPLOID *LOLIUM PERENNE* L.

M. A. CORNISH,*‡ M. D. HAYWARD† and M. J. LAWRENCE*

**Department of Genetics, University of Birmingham, Birmingham B15 2TT; †Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, SY23 3EB*

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Summary

Evidence is presented which shows that self-incompatibility in diploid *Lolium perenne* is determined, as in other self-incompatible grasses that have been investigated, by two multi-allelic genes and that control of the pollen phenotype is gametophytic.

The present results are compared with those of others which have led to a different conclusion concerning the genetical control or expression of self-incompatibility in this species. The importance of the materials and methods used in investigations of this kind are discussed.

1. Introduction

With one exception, all of the self-incompatible grasses that have been investigated in sufficient detail have been shown to possess a two-locus system in which control of the incompatibility phenotype of the pollen is gametophytic (table 1). The exception is perennial ryegrass (*Lolium perenne*).

Table 1

Grasses known to have a two-gene, multi-allelic gametophytic system of self-incompatibility

Species	Reference
Secale cereale	Lundqvist (1954, 1956)
Festuca pratensis	Lundqvist (1955, 1961a)
Phalaris coerulescens	Hayman (1956)
Hordeum bulbosum	Lundqvist (1962)
Dactylis aschersoniana	Lundqvist (1965)
Briza media	Murray (1974)

Two attempts have been made to determine the genetical control of self-incompatibility in this species. Hayward and Wright (1971), on the evidence obtained from three small families of full-sibs, whose parents originated from a long-established, natural population, were unable to arrive at any definite conclusion other than that no precise one- or two-locus system appeared to be present in the species. Spoor (1976), who examined a single, larger full sibship obtained by crossing two unrelated

‡ Present address: School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.

plants of botanical garden origin, came to the conclusion that self-compatibility was " controlled by a complicated multifactorial system with at least three loci ".

This conclusion is surprising since, with very few properly investigated exceptions, familial species possess the same type of self-incompatibility system. In particular, *L. perenne* is sufficiently closely related to *Festuca pratensis* to yield hybrid progeny both spontaneously and by hand (Jenkin, 1933, 1955) and the latter species is among those that are known to have a two-locus system of self-incompatibility (table 1). It would be of considerable theoretical interest if the ryegrasses turned out to have a different system from that of the other self-incompatible grasses. Furthermore, because perennial ryegrass is a crop of major economic importance, it is desirable for practical reasons that the genetical basis of its self-incompatibility should be properly understood before any attempt is made by breeders to exploit the mechanism in their improvement programmes (England, 1974).

The chief purpose of this paper is to present evidence which puts the answer to this question of the genetics of self-incompatibility in ryegrass, beyond doubt.

2. Materials and methods

Seven families of full-sibs have been examined (table 2). Six (D and E; F and G; H and I) are the progeny of reciprocal crosses between three plants that originated from material collected from a natural population in northern Italy and three from the Aberystwyth strain S24. They are,

Table 2

Parentage and provenance of families D–P

Family	Number of plants	Parents	Provenance
D	34	S24/19 × 8622/7	Northern Italy (8622/7, 8596/3/2 and 8590/4/1) and S24 (W.P.B.S.)
E	34	8622/7 × S24/19	
F	37	8596/3/2 × S24/26	
G	30	S24/26 × 8596/3/2	
H	31	8590/4/1 × S24/45	
I	37	S24/45 × 8590/4/1	
P	30	100/1/4/18 × 1/1/4/24	Monmouthshire Moors, Wales, U.K.

thus, the products of wide crosses between unrelated parents. Both the Italian material and S24 are of a relatively short-lived, early flowering hay type. The seventh family, P, on the other hand, is of very long-lived, late flowering material, being the product of a cross between two plants from different natural populations from the Monmouthshire Moors in Wales. The source of the latter family is the same as that used by Hayward and Wright (1971).

Families examined out of season in the autumn or winter were first vernalised for 12 weeks before being brought into the glasshouse and grown

on to flowering in a 16-hour day, Supplementary lighting was provided by a combination of 400-watt mercury fluorescent and 100-watt tungsten lamps. The latter are important if a reasonable level of male fertility is to be obtained.

Pollinations were performed *in vitro* using Lundqvist's (1961b) technique in which pistils are transferred to a recorded position on agar in a Petri dish. In this way pistils from up to 40 plants can be pollinated by a single male parent in a 50 mm dish, each plant being represented by two pistils and hence four stigmas. Each dish, therefore, is equivalent to a single male array of the diallel table that can be constructed by pollinating each plant in a family by every other. Stigmas were pollinated either immediately after extraction or on the following day.

Pollinations were made by releasing a cloud of fresh, bright yellow, free-flowing pollen over the stigmas which thereafter were incubated at room temperature for 24 hours. Stigmas were then stained in a drop of aniline blue for about 1 min., mounted in glycerol under a coverslip and examined by ultraviolet light under the microscope (Martin, 1959).

The procedure used to classify the members of a family with respect to their incompatibility phenotype was as follows. Six to eight plants chosen at random as pollinators are crossed with every other member of the family as well as among themselves. The crosses among the six pollinators constitute a complete 6×6 diallel set of crosses. Taken together, however, each set of crosses to the same pollinator corresponds to a male array of the complete $N \times N$ diallel set that could be made among the N plants of the family. We are thus concerned with just six such male arrays chosen at random from the complete set of N. This is an obvious and convenient procedure when using Lundqvist's *in vitro* pollination technique, since each male array is contained in a single Petri dish.

The results obtained from crosses among the six pollinators are examined first. A pollinator can be replaced at this stage if it turns out to belong to the same class as another or if the quality of its pollen is too poor to allow unambiguous classification. Once these problems have been overcome, genotypes may be provisionally assigned to the original parents of the cross and to each of the progeny used as a pollinator. The results of crosses between the pollinators and the remaining (N-6) plants of the family are then used to classify the latter. This completes the first round of pollinations.

A second round of pollinations can now be carried out in order to confirm the classification of the first though this is not obligatory. Plants assigned to the same class are intercrossed and the expectation that they are incompatible is thereby put to the test. Plants from single-entry classes are crossed with others with which they are expected to be fully compatible.

A similar two-stage crossing scheme was used to cross-classify reciprocal families, except that three appropriately chosen pollinators from one reciprocal were used on a representative from each of the classes of the other reciprocal.

3. Results

(i) *Analysis of families*

It soon became apparent that it was necessary to recognise only four kinds of pollination; namely, fully incompatible (–), half compatible (H),

		Pollinators																												
S	Z	1	3	2	4	1	3	2	4	2	3	1	3	2	3	1	4	2	3	2	4	1	3	2	4	1	3	2	4	
S	Z	1	4	1	4	2	3	2	3	1	4	2	3	2	4	1	4	1	3	1	4	2	3	1	4	2	3	2	4	
S	Z	2	1	4	2	3	1	3	4	3	1	4	2	3	4	2	3	1	4	2	3	4	1	3	2	4	2	3	4	
S	Z	No.	4	22	37	11	19	31	5	12	17	23	25	2	10	28	33	21	15	16	20	26	30							
1	3	1	3																											
1	3	1	4																											
1	3	2	3	5	T°	T	H°	+	H	+	-	-																+	H	
				12	H	T	T	+	H	+	-	-																+	H	
1	3	2	4	4	-	H	+	T	T	+			-															+	H	
				17	-	H	+	T	T	+			-															+	H	
1	4	1	3	23	+	+	+	+	+	+				-														+	H	
1	4	1	4	25	T	H	+	+	+	H°					-	+												+	H	
1	4	2	3	2	H°	H	+	+	+	H°					+	-												+	T°	
1	4	2	4	22	H	-	+	+	+	H																		+	H	
2	3	1	3	37	+	+	-	T°	T°	+								H										T°	+	
2	3	1	4	1	T	+	H	-	+	T						-												H	+	
				10	T	+	H	-	+	T						-													+	
				11	T	+	H	-	+	T						-												H	+	
				14	H°	+	H	-	+	H°						-												H	+	
				36	T	+	H	-	+	T						-												H	+	
2	3	2	3	19	T	+	H	+	-	T																		H	+	
2	3	2	4	27	H	+	+	H	H	H							-	-										T°	+	
				28	H	+	+	H	H	H							-	-										T°	+	
				32	H	+	+	H	H	H							-	-										H	+	
				33	H	+	+	H	T°	H							-	-										H	+	
				34	H	+	+	H	H	T°							-	-										H	+	
2	4	1	3	21	+	+	H	T	T	+									-									T°	+	
2	4	1	4	29	+	T	T	H	+	H																		H	+	
2	4	2	3	6	+	T	T	+	H	H										-	-							H	+	
				15	+	T	T	+	H	H										-	-							H	+	
				16	+	H°	H°	+	T°	H										-	-							H	+	
2	4	2	4	13	+	H	+	T	T	-												-						H	+	
				18	+	H	+	T	T	-												-						H	+	
				20	+	H	+	T	T	-												-							+	
				31	+	H	+	H°	T	-												-						T°	+	
				35	+	+	+	+	+	+					+													+	+	
				38	+	+		+	+	+					+													H	+	
				8	+	+		T		T																		H	+	
				26	+	+	T	T	T	T																		-	+	
				30	T	T	+	+	+	+																		+	-	

FIG. 1.—Family D. Pollination relationships among the 34 plants examined. Key to pollinations: incompatible = —; half-compatible = H; three-quarters compatible = T; and fully compatible = +. A superscript dot indicates those pollinations which were misclassified. (See text for further details.)

three-quarters compatible (T) or fully compatible (+) (plate 1). On this evidence alone *L. perenne* appears to possess a two-locus, gametophytic system similar to that of the other self-incompatible grasses that have been investigated. This conclusion is fully confirmed by the results obtained from each of the seven families examined (fig. 1–3; table 3) which in every case may be most simply interpreted on the assumption of a two-locus model.

		Pollinators																													
S S Z Z		1 3 1 3	1 3 1 4	1 3 2 3	1 4 2 3	2 3 1 3	2 4 2 3	1 3 1 3		1 3 1 4		1 3 2 3	1 3 2 4		1 4 1 4	1 4 2 3	1 4 2 4		2 3 1 4		2 3 2 4	2 4 1 3		2 4 1 4	
S S Z Z	No.	35	4	2	11	8	14	5	19	28	30	18	9	23	22	16	29	31	25	27	13	10	21	6	32						
1 3 1 3	3	–	H	H	T	H	+	–	–																						
	5	–	H	H	T	H	+	–	–												+										
	7	–	H	H	T	H	+	–	–												+										
	19	–	H	H	T	H	+	–	–																						
	35	–	H	H	T	H	+	–	–																						
1 3 1 4	4	H	–	+	+	T	+			–	–																				
	12	T°	–	+	+	H°	+			–	–																				
	28	H	–	+	+	T	+			–	–																				
	30	H	–	+	+	T	+			–	–																				
1 3 2 3	2	H	+	–	H	T	+					–																			
	18	H	+	–	H	T	+					–			+																
1 3 2 4	9	+	H	H	T	+	+						–	–																	
	15	+	H	H	T	+	+						–	–																	
	23	+	H	H	H°	+	+						–	–																	
1 4 1 3																															
1 4 1 4	22	T	T°	+	+	+	+									+			+	+	+										
1 4 2 3	11	T	+	T°	–	+	H									–															
	16	T	+	H	–	+	H								+	–					+										
1 4 2 4	1	+	T	T	H	+	T										–	–													
	29	+	T	H°	H	+	T										–	–													
	31	+	T	T	H	+	H°										–	–			+										
2 3 1 3	8	H	T	T	+	–	T														+										
2 3 1 4	25	T	H°	+	+	H	+												–	–											
	26	T	T	+	+	H	+												–	–											
	27	T	T	+	+	H	+								+				–	–											
2 3 2 3	20	T	+	H	+	H	H			+	+				+	+	+	+	+	+					+	+					
2 3 2 4	13	+	T	T	+	+	T	+	+													+	+								
2 4 1 3	10	+	+	+	T	H	H														+	–	–								
	17	+	+	+	H°	H	H														+	–	–								
	21	+	+	+	T	H	T°															–	–								
2 4 1 4	6	+	+	+	+	T	+																	–	–						
	24	+	+	+	+	T	+																	–	–						
	32	+	+	+	+	T	+																	–	–						
	34	+	+	+	+	H°	+																	–	–						
2 4 2 3	14	+	+	+	T°	T	–																								
2 4 2 4																															

FIG. 2.—Family E.

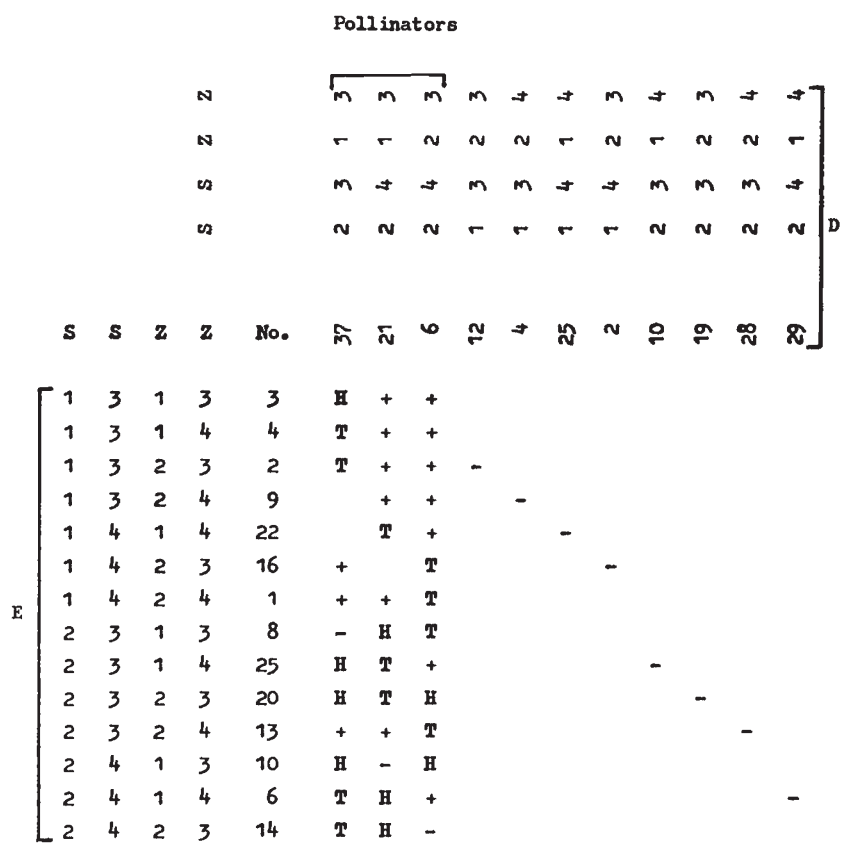

FIG. 3.—Cross-classification of families D and E.

It must be emphasised that while the assignation of plants to classes is consistent over reciprocal families, it is not over unrelated families, because no pollinations have been made between plants from these. Thus S_1, say, in families D and E may not and probably is not the same as the allele labelled S_1 in any other family; nor do we know whether the gene labelled S in one family is the same as that labelled S in another, for S and Z are interchangeable in these circumstances.

A proportion of the pollinations in each of the seven families are inconsistent with the overall classification of the plants into the indicated genotypic classes, though in no case was the frequency of such misclassifications higher than 9 per cent. With two exceptions only, misclassification is confined to the two types of partially compatible pollinations, H and T (shown in figs. 1 and 2 as T' and H' respectively). This outcome is hardly surprising, because the difference between these kinds of pollinations is quantitative rather than qualitative. Had counts been made of pollen grains and tubes, the percentage of misclassification could doubtless have been reduced still further. This, however, would have considerably reduced the total number of pollinations that could have been examined and hence would have reduced both the power of the analysis and the range of material that could have been examined.

TABLE 3

Summary of the results obtained from seven full-sib families. It should be emphasised that while the assignation of plants to classes is consistent over reciprocal families, the designation of genotypes over different families is arbitrary

$S_1S_3 \times S_1S_3$	D	E	D+E	H	I	H+I	P	$S_1S_3 \times S_1S_3$	F	G	F+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**

(ii) *Number of genotypic classes per family*

For reasons to be given later, 16 classes are expected in each of these families. Yet in none of these families have we found more than 14 and even where reciprocal crosses are pooled, in only one case, D and E, have we recovered the full number of 16 (table 4).

These observations raise the question of how many plants it is necessary to examine in each family to be certain, at a given probability level, of

TABLE 4

Number of plants and classes in each of the seven full-sib families and in those obtained by pooling over reciprocal crosses

Family	No. of plants	No. of classes	P_0*
D	29	14	0·0354
E	34	14	0·1076
D+E	63	16	0·7511
F	37	11	0·1703
G	30	13	0·0463
F+G	67	14	0·8032
H	31	13	0·0590
I	37	13	0·1703
H+I	68	14	0·8146
P	30	13	0·0463

* Probability of no empty classes given an expectation of 16 classes for families containing the indicated number of plants.

obtaining 16 classes. Following the procedure given in Lawrence, Afzal and Kenrick (1978), the numbers of individuals required to be 95 per cent and 99 per cent certain of obtaining at least one plant in each of the expected 16 classes are 90 and 115 respectively. These numbers are much larger than any of the individual ryegrass families that either we or others have examined. Since the probability of no empty classes (P_0) is never greater than 17 per cent (last column of table 4) for any of our individual families it is clear that the results we have obtained are in good agreement with expectation. Only when family size is doubled, as it is when reciprocals are pooled, does P_0 rise to around 80 per cent so that we are hardly surprised to have recovered all 16 classes in one of these (D+E). However, the chief point here is that the method of analysis we have used does not depend on being able to recover all of the expected number of classes.

(iii) *Genotypes of parents of families*

If the number of alleles at each of the two incompatibility loci is large, most pairs of plants chosen at random should have no alleles in common at either locus. If this is the case then the cross between two such plants is of the type $S_{1.2}\ \mathcal{Z}_{1.2} \times S_{3.4}\ \mathcal{Z}_{3.4}$ and we should not expect to find any differences between reciprocal matings among the progeny of such a cross. Three of the families are of this kind, namely, D and E, H and I and P. Where the parents have one (or more) alleles in common, however, a quarter of their offspring will be homozygous for that allele (or those alleles) in which case we expect to encounter differences between reciprocal matings which involve such homozygotes. Since reciprocal differences were found in the fourth family, F and G, we deduce that the cross giving rise to this progeny was of the type $S_{1.2}\ \mathcal{Z}_{1.2} \times S_{1.3}\ \mathcal{Z}_{3.4}$. Bearing in mind that the parents of this progeny had a quite different origin, this is a rather unexpected outcome which, if taken at face value, suggests that the number of alleles at one of the incompatibility loci may not be all that large.

(iv) *Segregation ratios of* S *and* Z *loci*

In general, tests on the segregation of $S_1 : S_2$, of $S_3 : S_4$ ($S_1 : S_3$ in families F and G), of $\mathcal{Z}_1 : \mathcal{Z}_2$ and of $\mathcal{Z}_3 : \mathcal{Z}_4$ show good agreement with the expected 1:1 ratio. In family D, the ratio of $S_1 : S_2$ turned out to be 8 : 21 which gives $\chi^2_{(1)} = 5{\cdot}828$ with $P = 0{\cdot}02 - 0{\cdot}01$. Since we have carried out four independent tests of segregation in each of seven families, giving a grand total of 28 such tests in all, the occurrence of a single χ^2 of this magnitude is to be expected and need not therefore be taken very seriously. The only instance of what appears to be a genuine case of disturbed segregation in these data is that concerning $\mathcal{Z}_1 : \mathcal{Z}_2$ in family F which at 33 : 4 is a highly significant departure from the expected 1:1 ratio ($\chi^2_{(1)} = 22{\cdot}730$, $P < 0{\cdot}001$). In the reciprocal cross, however, (family G) $\mathcal{Z}_1 : \mathcal{Z}_2$ is as 14 : 16. It appears therefore that this disturbance is confined to one of the two parents of this cross. However, since it is not possible to deduce from which parent the alleles $\mathcal{Z}_1$ and $\mathcal{Z}_2$ are descended, we are unable to decide whether the disturbance occurs in the pollen or the ovules, though on general grounds, the former is perhaps the more likely.

(v) *Analysis of anomalous members of family D*

The fifth and final point worth making about these results concerns the five plants in family D (fig. 1) which cannot, as matters stand, be accommodated with the others. We note that plant no. 35 is fully compatible with each of the seven classified plants of family D with which it has been pollinated. Furthermore, each of these seven plants is of a different genotype. Though it is possible for a member of an intercross to be fully compatible with as many as seven other genetically different members of that family, there are constraints on the genotypes of the latter which are not met in this case. We therefore conclude that this plant cannot belong to the sibship. Its most probable origin is that of a seed contaminant.

Two of the remaining four plants, nos. 26 and 30, were included in the original set of pollinators before their identity was recognised and they were discarded for this purpose. There are two features of their pollination relationships with the classified plants of family D which reveal this identity. Firstly, since each of these plants is either half or three-quarters compatible with a member of D, they must have at least two alleles in common with the latter and are unlikely, for this reason alone, to be seed contaminants like plant no. 35. Secondly, when used as female, no. 26 is three-quarters compatible with the four pollinators containing S_2, nos. 37, 11, 19 and 31, whereas when used as male it is half-compatible with three of these pollinators, either directly (11 and 19) or indirectly (31). The occurrence of these reciprocal differences indicates that 26 is homozygous at one of the two incompatibility loci. Since this non-reciprocity is confined to crosses with plants carrying S_2, plant 26 must be homozygous for this particular allele, a conclusion which is confirmed by the fact that when used as male, 26 is always fully compatible with plants carrying S_1 and always partially compatible, mostly half-compatible, with those carrying S_2. By a similar argument, plant no. 30 must be homozygous for the S_1 allele.

As has been previously mentioned, there is no evidence from elsewhere in family D that the parents had any alleles in common. Now the seed used in this investigation was obtained by using Jenkin's (1931) automatic cross-pollination without emasculation method. Homozygotes, such as plants no. 26 and 30, must have arisen because of a breakdown in the self-incompatibility of the female parent, a risk to which this technique is obviously vulnerable. The presence of these selfs in family D does, however, allow us to deduce that the genotype of their female parent at the first locus must be $S_{1.2}$. The genotype of plant no. 26 is, thus, $S_{2.2}\ Z_{1.2}$ (or $S_{2.2}\ Z_{3.4}$) and that of plant no. 30 is $S_{1.1}\ Z_{1.2}$ ($S_{1.1}\ Z_{3.4}$).

Much less information is available about the identities of the remaining pair of plants, nos. 38 and 8. Both were rather weak plants that came into flower later than the rest of the family. In so far as each is partially compatible with at least one other plant in the family, both appear to have at least two alleles in common with the latter. It is, therefore, possible that these are two further selfs. Despite these anomalies, all of the results that we have obtained are explicable in terms of a simple, two locus hypothesis.

4. Discussion

In this analysis, we have attempted to fit the simplest model to the data. There are, of course, dangers in this and we take Larsen's (1977) point " that the choice of working hypothesis, material and methods makes one find what is looked for and within certain limits only that ". We have deliberately used wide crosses so as to minimise the chance of working with families in which less than the full number of loci are segregating and have examined a wide range of agronomic types from relatively short-lived hay types to long-lived pasture types.

Our working hypothesis was, for reasons given earlier, that self-incompatibility in ryegrass was controlled by the multiple alleles at just two independently inherited loci. We had no difficulty in fitting the data from six of the seven families examined to this hypothesis. In the seventh, however, family D, in which five exceptional plants were found, it was necessary to propose that four of these were offspring arising from selfing in the female parent of the cross and that the fifth was a seed contaminant. We have attempted to obtain independent evidence of the status of these five exceptional plants by scoring the parents, their legitimate offspring and the exceptional plants with respect to their phosphoglucoisomerase (PGI) phenotype. Four alleles, *a*, *b*, *c* and *d*, are known at the PGI-2 locus in ryegrass which differ in their electrophoretic mobility (Hayward and McAdam, 1977). The female parent of family D turned out to be a *bc* heterozygote and the male parent, a *bb* homozygote. Their legitimate offspring must therefore be either *bb* or *bc*; and illegitimate selfs of the female parent could be either *bb*, *bc* or *cc*, only the third of which is peculiar to the self progeny.

All of the legitimate offspring were either *bb* or *bc*, as expected. The suspected seed contaminant, plant no. 35, turned out to be *ab* which puts its status beyond doubt. The origin of two of the four suspected selfs (nos. 26 and 38) was confirmed by their *cc* genotype. On the present evidence alone, the remaining pair (nos. 8 and 30) could be either selfs or crosses for both were *bc*. However, since plant no. 30 was one of the pair used as pollinators we had a great deal of information about its pollination relationships with legitimate members of family D which led us to the conclusion that it was in fact a self. Thus of the five exceptional plants, we have independent evidence which confirms the status of three; a fourth for which the incompatibility evidence is strong, but whose status cannot be confirmed by the PGI data, and a fifth for which neither kind of evidence is conclusive. Taken as a whole, however, the evidence confirms the status of these exceptional plants, and there is no evidence from any part of the data which contradicts the two-locus hypothesis.

The chief reason why our results and conclusions differ from those of Hayward and Wright (1971) and Spoor (1976) is that we have been able to fully exploit the two chief advantages of the aniline-blue fluorescence technique (Martin, 1959; Lalouette, 1967). These advantages are that viable pollen can be distinguished from non-viable or non-reactive pollen, since only the former will take up dye to form a fluorochrome with callose; and that incompatible pollen can be clearly distinguised from compatible pollen, because the tubes of both are visible under the microscope. The incompatibility reaction takes place on the stigmatic surface in ryegrass

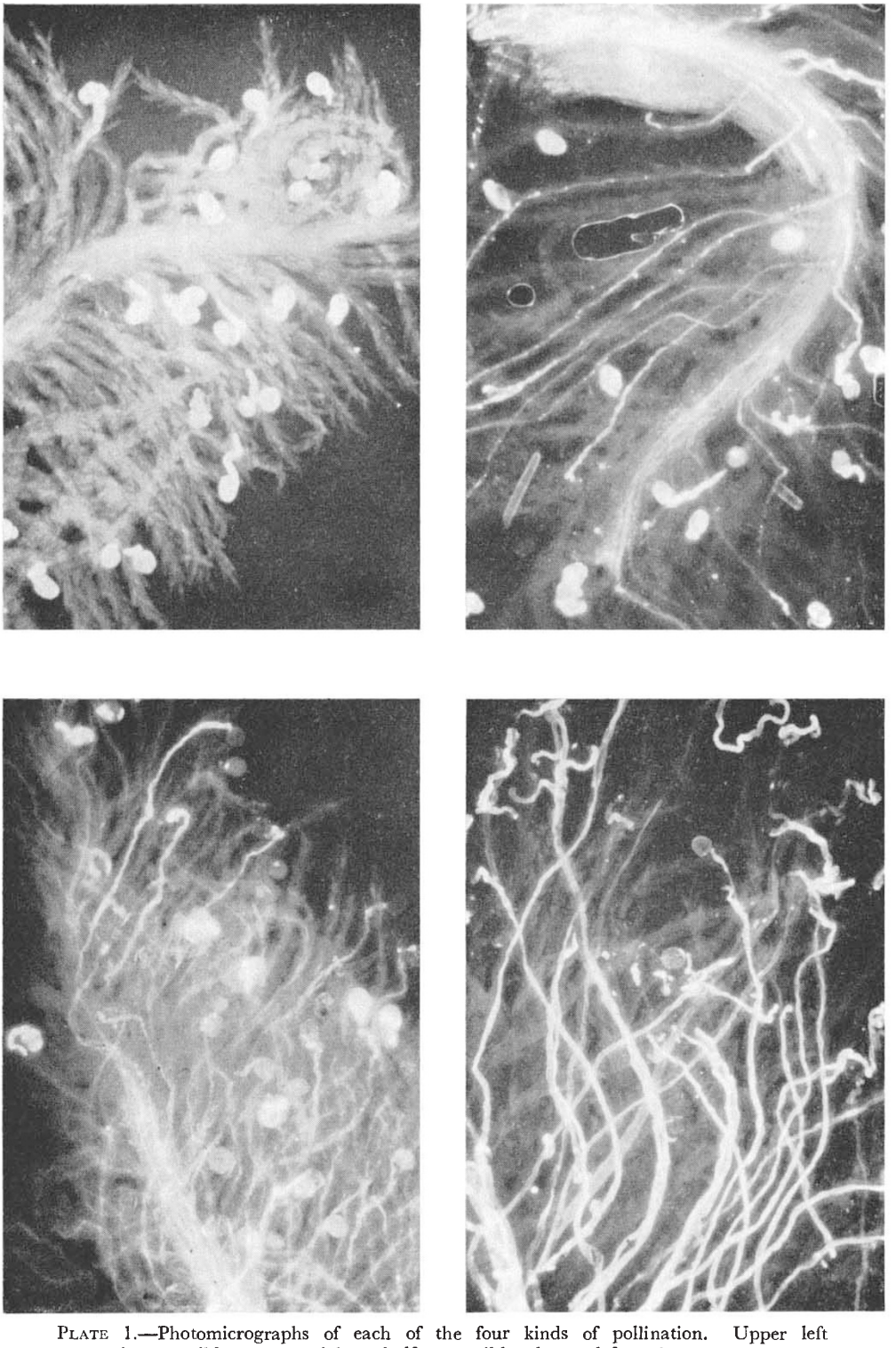

PLATE 1.—Photomicrographs of each of the four kinds of pollination. Upper left = incompatible; upper right = half-compatible; lower left = three-quarters compatible; lower right = fully compatible.

so that incompatible pollen produce only a short tube whose volume is filled with brightly fluorescing callose which is also present in the grain itself. In contrast, the long tubes of compatible pollen fluoresce much less brightly; also callose is not found within compatible pollen grains. It is thus possible to distinguish between half-compatible and three-quarters compatible pollinations (plate 1). Furthermore, because this technique is rapid, it is possible to set-up further rounds of pollination in order to check the classification of earlier rounds.

Hayward and Wright used material of very similar origin to that of family P, used Lundqvist's (1961b) *in vitro* method of pistil culture and also used the same stain, since cotton blue and aniline blue are synonyms. However, they mounted their stigmas in lactophenol and did not examine them with ultra-violet light. We have compared their technique with ours on a small number of pollinated pistils by applying cotton blue in lactophenol to one stigma and aniline blue with glycerol to the other stigma of each pistil and found it much more difficult to distinguish between compatible, incompatible and inviable pollen with the former treatment. Though Hayward and Wright attempted to overcome this problem by comparing the ratio of empty (assumed to be compatible) to full (assumed to be incompatible) pollen grains on cross-pollinated stigmas with that on self-pollinated stigmas, they were unable to either repeat doubtful pollinations or to check their provisional classifications by further rounds of pollination. Thus, although the cotton blue method has been used successfully in the past (Hayman, 1956), if the pollen used contains inviable or empty grains, misclassification is almost bound to occur.

Though Spoor used the same technique as ourselves he recognised only two kinds of pollination, namely, those which were compatible and those which were incompatible. We assume that his compatible class must have included both fully and partially compatible pollinations. Yet to fail to recognise the latter is to forgo critical information about the number of alleles which plants have in common and direct evidence that control of the pollen phenotype is gametophytic. Spoor also appears not to have checked any pollination.

There is thus some evidence from both of the previous attempts to interpret the genetics of self-incompatibility in ryegrass that a proportion of the pollinations have been misclassified. There are two points worth emphasising in this connection. Firstly, if misclassification occurs it is bound to lead to the conclusion that the genetical control of self-incompatibility is more complicated than it really is. Secondly, unless a determined attempt is made to fit a simple Mendelian model to the data, misclassified pollinations will remain undetected. Thus, although we agree with Larsen (*loc. cit.*) that choice of material and methods is critical to the success of an analysis of self-incompatibility we disagree with him about the consequences of choosing a simple working hypothesis on the outcome of that analysis. In our view, the danger of concluding that a self-incompatibility system is more complex than it really is is considerably greater than the converse.

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5. References

ENGLAND, F. J. W. 1974. The use of incompatibility for the production of F_1 hybrids in forage grasses. *Heredity, 32*, 183-188.

HAYMAN, D. L. 1956. The genetical control of incompatibility in *Phalaris coerulescens* Desf. *Aust. J. Biol. Sci., 9*, 321-331.

HAYWARD, M. D., AND MCADAM, N. J. 1977. Isozyme polymorphism as a measure of distinctiveness and stability in cultivars of *Lolium perenne*. *Z. Pflanzenzuchtg., 79*, 59-68.

HAYWARD, M. D., AND WRIGHT, A. J. 1971. The genetic control of incompatibility in *Lolium perenne* L. *Genetica, 42*, 414-421.

JENKIN, T. J. 1931. The breeding of herbage plants. Methods and techniques of selection, breeding and strain building in grasses. *Bull. Bur. Pl. Genet. Aberystwyth, 3*, 1-34.

JENKIN, T. J. 1933. Interspecific and intergeneric hybrids in herbage grasses. Initial crosses. *J. Genetics, 28*, 205-264.

JENKIN, T. J. 1955. Interspecific and intergeneric hybrids in herbage grasses. XVI. *Lolium perenne* and *Festuca pratensis* with reference to *Festuca loliacea*. *J. Genetics, 53*, 379-441.

LALOUETTE, J. A. 1967. Growth of pollen tubes exhibited by callose fluorochrome reaction. *Grana palynol., 7*, 601-603.

LARSEN, K. 1977. Self-incompatibility in *Beta vulgaris* L. I. Four gametophytic, complementary *S*-loci in sugar beet. *Hereditas, 85*, 227-248.

LAWRENCE, M. J., AFZAL, M., AND KENRICK, J. 1978. The genetical control of self-incompatibility in *Papaver rhoeas*. *Heredity, 40*, 239-253.

LUNDQVIST, A. 1954. Studies on self-sterility in rye, *Secale cereale* L. *Hereditas, 40*, 278-294.

LUNDQVIST, A. 1955. Genetics of self-incompatibility in *Festuca pratensis*. *Hereditas, 41*, 518-520.

LUNDQVIST, A. 1956. Self-incompatibility in rye. I. Genetic control in the diploid. *Hereditas, 42*, 293-348.

LUNDQVIST, A. 1961a. Self-incompatibility in *Festuca pratensis* Huds. *Hereditas, 47*, 542-562.

LUNDQVIST, A. 1961a. A rapid method for the analysis of incompatibility in grasses. *Hereditas, 47*, 705-707.

LUNDQVIST, A. 1962. Self-incompatibility in diploid *Hordeum bulbosum* L. *Hereditas, 48*, 138-152.

LUNDQVIST, A. 1965. Self-incompatibility in *Dactylis aschersoniana* Graebn. *Hereditas, 54*, 70-87.

MARTIN, F. W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technology, 34*, 125-128.

MURRAY, B. G. 1974. Breeding systems and floral biology in the genus *Briza*. *Heredity, 33*, 285-292.

SPOOR, W. 1976. Self-incompatibility in *Lolium perenne* L. *Heredity, 37*, 417-421.