

## SELF-INCOMPATIBILITY IN RYEGRASS

### IV. SEED-SET IN DIPLOID *LOLIUM PERENNE* L.

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

\* *Department of Genetics, University of Birmingham, Birmingham B15 2TT*; † *The Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, SY23 3EB*; ‡ *Present Address: School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY*

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#### SUMMARY

Crosses were made between 58 pairs of plants which included pairs that were expected, on the basis of pollen-stigma reactions, to be incompatible, half-compatible, three-quarters compatible and fully compatible. The seed-set obtained from these crosses completely confirmed this classification.

#### 1. INTRODUCTION

THE conclusion that self-incompatibility in *Lolium perenne* is controlled by a pair of independently inherited, multi-allelic genes whose effect on the pollen is gametophytic (Cornish, Hayward and Lawrence, 1979*a, b*) was based on the observation of pollen development on the stigma by fluorescence microscopy (Martin, 1959; Lalouette, 1967). While this is the most rapid and accurate method of assessing incompatibility relationships between plants, the final effect of incompatibility is to prevent the formation of seed when a plant is self-pollinated or crossed with another of similar or identical genotype. In most species, seed-set is a much less useful criterion for genetical analysis than the more immediate effect of incompatibility on pollen development. Thus not only is it usually not possible to distinguish partially from fully compatible pollinations with seed-set data, but misclassification of pollinations is also more likely both because the formation of seed can be arrested by factors other than incompatibility and because there is rarely sufficient time in which to repeat doubtful crosses in the same season. Seed-set is still used as a classificatory criterion, even in the investigations of complex systems (Larsen, 1977; Østerbye, 1977), despite the fact that the disadvantages of this method were first pointed out some forty years ago (Emerson, 1938). However, conclusions based on the observation of pollen behaviour alone cannot be regarded as wholly trustworthy until it has been shown that the expected relationship between this and seed-set holds for the species in question. The present paper examines this relationship in *Lolium perenne*.

#### 2. MATERIALS AND METHODS

One hundred and eighteen plants selected from the first six families analysed by Cornish *loc. cit.* were crossed reciprocally in pairs giving a total of 59 pairs in all. Twenty-nine of these pairs involved plants that were expected to be incompatible, seven that were expected to be half, nine that

were expected to be three-quarters and 10 that were expected to be fully compatible. The remaining four crosses, involving plants from families F and G, were expected to be half-compatible in one direction and incompatible in the other. Each of the 58 plants of the incompatible pairings were also selfed.

Crosses were made without emasculation (Jenkin, 1931*a*) by enclosing inflorescences of pairs of plants in a single "Cellophane" bag. The plants were then left to flower in the glasshouse, the bags being agitated daily to distribute the pollen inside. When flowering had finished, the inflorescences of each plant were bagged separately. Two months later, the number of seeds contained within each were counted. At the same time an attempt was made to estimate the number of florets in each unit by counting the number of inflorescences, the number of spikelets on an inflorescence of average size and the number of florets in a central spikelet of this inflorescence. The estimated number of florets in a unit was obtained as the product of these three numbers. Seed-set for each unit was then calculated as the percentage of florets which yielded seed.

The number of inflorescences in a unit varied from five to over 20. Most of the compatible crosses, however, were made with only six or seven inflorescences, the selfs and incompatible crosses involving rather more. Inflorescences bore between 16 and 22 spikelets and most of the central spikelets had from four to eight florets.

### 3. RESULTS

#### (i) *Selfs and incompatible crosses*

One of the crosses in the set of 29 that were expected to be incompatible, set a lot of seed and will be dealt with later. The average seed set obtained by self-pollinating each of the remaining 56 plants was 0.257 per cent. Over half of the plants set no seed at all and none gave a seed-set of more than 2 per cent. These results leave little doubt that perennial ryegrass is a highly, though not completely, self-incompatible species.

A similar result was obtained when each of these plants was reciprocally crossed with another which, on the evidence of pollen behaviour, had the same incompatibility phenotype. Thus the average seed-set of these crosses was 0.362 per cent; just over 40 per cent set no seed at all and none gave a seed-set of more than 3.5 per cent. Though the seed-set of the crosses is a little higher than that of the selfs, the analysis of variance of these data shows that this difference is not significant (table 1(*a*)). There is little doubt, therefore, that the species is also highly cross-incompatible and that these results confirm our classification of these plants into 28 incompatible pairs on the basis of the observation of pollen behaviour.

Since the seed-set of selfs and incompatible crosses is similar, we may pool the data to give the combined distribution shown in fig. 1(*a*) (the complete seed-set data are given in Cornish, 1979). An inspection of the data reveals, however, that seed-set of both selfs and crosses in families F and G is a little higher than that in either of the two pairs of families. Thus while over half the units produced no seed at all and none gave more than a 1 per cent seed set in families D, E, H and I (fig. 1(*b*) and (*d*)), less than a quarter of the units from plants of families F and G contained no seed and

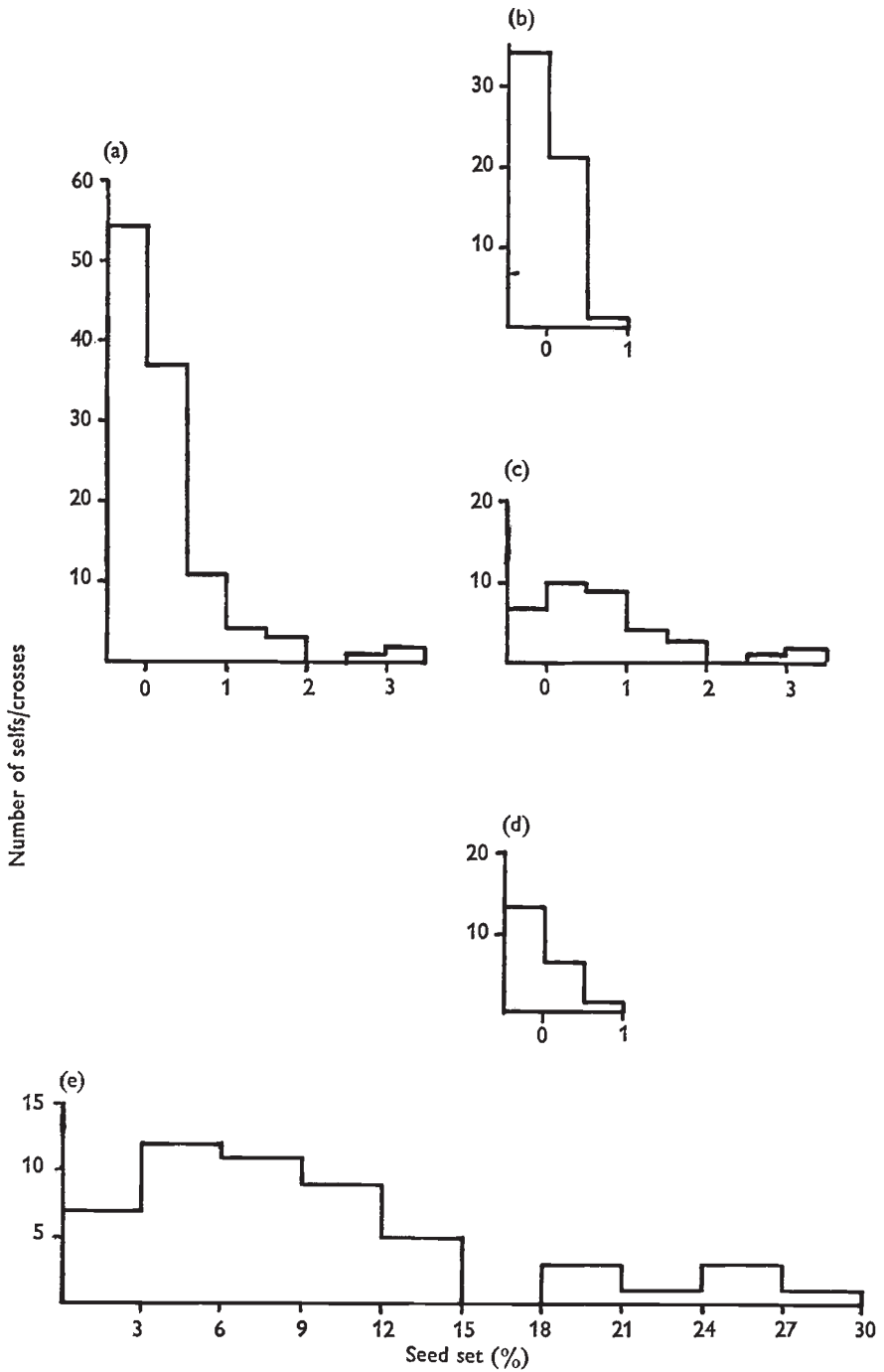


FIG. 1.—The distribution of seed-set in (a) the selfs and incompatible crosses pooled over families; (b) the selfs and incompatible crosses of families D and E; (c) those of families F and G; (d) those of families H and I; and (e) the compatible crosses pooled over families.

10 gave a seed-set of more than 1 per cent (fig. 1(c)). Furthermore, the analysis of variance shows that this difference between families is significant (table 1(b)), the mean seed-set of plants, summing over selfs and crosses being 0.085 per cent in families D, E, H and I and 0.784 per cent in families F and G. At first sight, the slightly higher level of pseudo-self-compatibility might appear to have a genetical cause, since it is confined to families F and G. However, it will be recalled that Cornish *et al.* (1979a) found four illegitimate selfs in family D, which suggests that the environment in which pollination takes place can also have an effect on the incidence of pseudo-self-compatibility.

TABLE 1

*Analysis of variance of the seed-set data of selfs and incompatible crosses; (a) analysis of the differences between selfs and crosses and (b) of differences between families and between plants within families. Data transformed from percentages to angles before analysis. The theoretical error is  $820.7/N$ , where  $N$  is the harmonic mean of the estimated numbers of florets per unit. (\*\*\*,  $P < 0.001$ )*

(a)		
Item	d.f.	M.S.
Selfs v. Crosses	1	7.892
Within Selfs and Crosses	110	6.269
(b)		
Item	d.f.	M.S.
Between Families	2	123.873***
Within Families	109	4.126***
Theoretical error	$\infty$	0.997

Lastly, since the Within Families mean square is significantly larger than the binomial error variance (table 1(b)), differences between plants within these three pairs of families are much greater than would be expected on the basis of random causes alone, though again whether these are due to genetical or environmental differences between individuals of these families is not clear.

#### (ii) *Compatible crosses*

The distribution of seed-set obtained from the 26 pairs of compatible crosses is shown in fig. 1(e). Seed-set in these crosses ranged from 0.35 per cent to 27.12 per cent. While there is a slight overlap between this distribution and that of the selfs and incompatible crosses (fig. 1(a)), all units from compatible crosses contained seed, and the difference between the means of these distributions, 9.56 per cent and 0.31 per cent respectively, is large. There is thus little doubt that, on average, these plants have also been correctly classified into 26 compatible pairs on the basis of our observations of the behaviour of the pollen in these crosses.

The average seed-set of these compatible crosses is low, which suggests that, despite attempts to avoid this, there was a shortage of viable pollen in the bags when the stigmas were receptive. In these circumstances we expect that half-compatible crosses might set less seed than three-quarters compatible crosses, which, in turn, might set less seed than fully compatible

crosses. An examination of the mean seed-set of the three categories of cross reveals that this expectation is realised in these data, the means being:

half-compatible	6.68 per cent
three-quarters compatible	10.33 per cent
fully compatible	10.88 per cent

Though the analysis of variance (table 2) shows that these differences are not significant (on a one-tailed test, because these means are in the expected

TABLE 2

*Analysis of variance of the seed-set data from compatible crosses. Details as for table 1*

Item	d.f.	M.S.
Between categories	2	90.012 0.10—0.05
Within categories	49	43.669***
Theoretical error	∞	1.201

rank-order), it is likely that this is due to the relatively small number of crosses examined, and that a larger experiment would confirm that these differences are genuine.

(iii) *One-way compatible crosses*

Since the parents of families F and G had an *S* allele in common (Cornish *et al.*, 1979a, c), a number of plants in them were homozygous for this allele. In these circumstances, crosses can be made which are half-compatible in one direction and incompatible in the other. The seed-set obtained from four such crosses is shown in table 3. Once again, these results are in complete accord with expectation.

TABLE 3

*Seed-set in four one-way compatible crosses between plants from families F and G*

Plant A	Plant B	Genotype of A	Genotype of B	Seed-set per cent	
				A × B	B × A
F40	F27	$S_{1.1}\bar{Z}_{1.3}$	$S_{1.2}\bar{Z}_{1.3}$	9.24	0.60
F37	F44	$S_{1.1}\bar{Z}_{1.4}$	$S_{1.2}\bar{Z}_{1.4}$	17.89	2.08
F53	G14	$S_{1.1}\bar{Z}_{1.3}$	$S_{1.2}\bar{Z}_{1.3}$	12.20	0.07
G15	G25	$S_{1.1}\bar{Z}_{1.3}$	$S_{1.3}\bar{Z}_{1.3}$	14.40	2.27

(iv) *The anomalous cross*

As mentioned earlier, one cross among the 29 that were expected to be reciprocally incompatible set a lot of seed, the seed-set obtained by both selfing and crossing the plants concerned, no. 4 and no. 36 from family F, being as follows:

F4 selfed	0 per cent
F36 selfed	0.89 per cent
F4 × F36	19.20 per cent
F36 × F4	0.17 per cent

The cross clearly resembles the one-way crosses that we considered in the

previous section, though both plants were originally classified as  $S_{1.3}\tilde{Z}_{1.4}$ . On the seed-set data, however, plant no. 4 appears to be  $S_{1.1}\tilde{Z}_{1.4}$ . While it is, of course, possible that this plant has been misclassified, this is unlikely because it would have involved the misclassification of two half-compatible pollinations as incompatible (see Cornish, 1979, for further details). A more likely explanation of this anomaly is that a mistake has occurred during clonal replication of the stock plant subsequent to its classification, which is, of course, always a hazard when handling and maintaining a large collection of perennial plants, or that compatible pollen was allowed to reach the florets before the inflorescences of these plants were bagged up.

#### 4. DISCUSSION

The results of the present experiment clearly show that apart from the single exception just noted, the expected relationship between the pollen-stigma reaction and seed-set holds in this species. Thus, on average, compatible crosses gave more seed than incompatible crosses, the seed-set of the latter being similar to that of the selfs. Furthermore, these results also show that on average fully compatible crosses set a little more seed than three-quarters compatible crosses, which, in turn, set more seed than half-compatible crosses.

There are two points worth making about these results. The first of these is that they leave little doubt that perennial ryegrass is both a highly self- and a highly cross-incompatible species, the average seed-set of the selfs being 0.257 per cent, while that obtained by crossing plants of the same incompatibility genotype being 0.362 per cent. This finding has considerable practical implications in respect of both breeding and seed production, since a synthetic cultivar is generally based on a low number of plants, often selected from within a single pair cross family. In these circumstances, there is clearly a high probability that such basic plants will be of the same incompatibility genotype. Since such plants will be cross-incompatible, their presence will bring about a considerable reduction in seed-setting which could have serious economic consequences on seed production. It is clearly preferable to found a synthetic cultivar on plants which are known to be fully cross-compatible with every other plant.

The second point concerns the detection of differences in seed-set between the three types of compatible cross, which was, of course, quite unexpected in a species whose ovaries contained one ovule only. The amount of viable cross pollen that was available to, or able to function on, receptive stigmas must have been very small, a deduction which receives some support from the fact that even the fully compatible crosses gave a seed-set of only 10.88 per cent. Indeed, if we assume that a single compatible pollen grain on the stigma is sufficient to fertilise the ovule and that the ratios of compatible to incompatible pollen in the case of the three-quarters and half-compatible crosses are as expected, the seed-set that we have obtained from the three types of cross is not inconsistent with the assumptions that on average only two viable pollen grains were able to function on each receptive stigma. Though the seed-set that we have obtained with these compatible crosses is low, it is nevertheless similar to that obtained by others with this crossing technique (Jenkin, 1931*b*; Hayward and Breese, 1966; Foster and Wright, 1970).

Microscopic examination of stigmas taken from inflorescences enclosed in crossing bags involving fully compatible combinations show that there is, in fact, a great excess of pollen on these stigmas (C. Scarrott, unpub. obs.). However, half of this pollen had failed to react and most of the remainder, being clearly incompatible, must have been of self-origin. As predicted, only a very small proportion of the pollen on these stigmas had germinated to produce a tube. It is not possible to say on the present evidence whether this proportion was small because of poor dispersal of cross pollen in the bag or because the presence of so much pollen impedes access to or dehydrates the stigma (Heslop-Harrison, 1979) or, indeed, because of the generally poor environment known to occur within these crossing bags with respect to seed production (Foster, 1968). If, however, the amount of viable cross pollen delivered to the stigma could be increased by, for example, hand pollination, the amount of seed set should also increase and differences between the three categories of compatible pollination vanish. The fact that Beddows, Breese and Lewis (1962) obtained an average seed-set of 42.0 per cent when making crosses by hand emasculation and pollination suggests that this prediction is correct, though results from hand-crosses are not directly comparable to those obtained by Jenkin's method, both because with the former all but one or two of the basal florets are removed from each spikelet of the inflorescence and because the amount of pollen applied to the stigma is likely to be less than with the latter.

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