

THE POPULATION GENETICS OF THE  
SELF-INCOMPATIBILITY POLYMORPHISM IN  
*PAPAVER RHOEAS*.  
III. THE NUMBER AND FREQUENCY OF S-ALLELES  
IN TWO FURTHER NATURAL POPULATIONS  
(R102 AND R104)

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SUMMARY

A sample of 36 plants drawn at random from each of two further natural populations (R102 and R104) contained between them 30 and 26 different *S*-alleles respectively. In both samples, the frequencies of these alleles were significantly unequal. These results are compared with those presented in a previous paper (the R106 sample; Campbell and Lawrence, 1981*b*) and with those obtained by Emerson (1939) from the *Oenothera* population. Unequal *S*-allele frequencies appear to be characteristic of natural populations of *P. rhoeas* in the West Midlands and probably also elsewhere in the British Isles. The number of *S*-alleles found in these samples suggest that the number of alleles in the natural populations from which they were obtained is unlikely to be less than the number that Emerson eventually found in the *Oenothera* population, namely, 45. However, if this is indeed the case, it is no longer possible to regard *P. rhoeas* as a colonising species; on the contrary, on the evidence obtained from these three populations, it appears to be a permanent member of the flora of wayside places.

1. INTRODUCTION

ACCORDING to the theory of the self-incompatibility polymorphism developed by Wright (1939), the frequencies of each of  $k$  alleles in a population are expected to be approximately  $1/k$ , provided that the population is in equilibrium and that the effect of selection on the gene is limited to that associated with incompatibility. We have shown that this expectation holds in the case of Emerson's (1939) *Oenothera* population (Campbell and Lawrence, 1981*b*). However, the frequencies of 31 different *S*-alleles in a randomly-drawn sample of 51 plants taken from a natural population (R106) of *Papaver rhoeas* turned out to be significantly unequal (Campbell and Lawrence, loc. cit.). Two, alternative hypotheses were proposed to account for this observation. Firstly, that the alleles in this population of poppies are subject to natural selection over and above the selection associated with the incompatibility effect, either directly, or *via* close linkage with other genes; secondly, that the unequal frequencies are due to chance effects of a kind to be particularly expected in populations of a colonising species like *P. rhoeas*. While both hypotheses predict that allele frequencies will be unequal in other natural populations of poppies also, the first predicts that the same alleles will occur at a relatively high frequency in different populations, whereas on the second hypothesis, different alleles

are expected to occur at a relatively high frequency in different populations. Furthermore, while in principle any allele could occur at a high frequency on the second hypothesis, only a few are likely to be favoured by the extra effect of selection envisaged by the first.

Now it is possible that, for some reason or other unknown to us, we had inadvertently chosen an atypical natural population of poppies in the experiment described in the previous paper. It is important, therefore, to ascertain that the frequencies of *S*-alleles are significantly unequal in other natural populations also before embarking upon the lengthy task of attempting to distinguish between the two hypotheses by cross-classifying the alleles of one population against those of another. The purpose of the experiments described in the present paper was to test the prediction that allele frequencies would be unequal in other populations of the species by examining a sample of 36 plants from each of two further natural populations.

## 2. MATERIALS AND METHODS

The seed from which these plants were raised was obtained from roadside populations found near Broad Oak in Herefordshire (R104) and Wellesbourne in Warwickshire (R102). The distance between these locations is 53 miles and they are 42 and 26 miles distant respectively from the location of the population analysed in the previous paper. Seed was collected from 70 wild plants at Broad Oak and 62 from Wellesbourne, using in each case the same sampling procedure as that employed with the first population. In 1978, four plants were raised from the seed of each of the R104 plants in a randomised block experiment. As these plants came into flower, one was chosen at random from each of the 70 half-sib families, the other three being discarded. A random sample of 36 of these plants was then classified (by M.J.L.) with respect to their incompatibility phenotype by means of a  $36 \times 36$  half-diallel crossing scheme. The same procedure was followed in 1979 when the analysis of 36 plants from the R102 population was carried out (by S.O.). All other details of these experiments were the same as those given in the previous paper (Campbell and Lawrence, loc. cit.).

## 3. RESULTS

### (i) *The R104 sample*

The number of crosses made between the 36 plants was 568 of which 2 were initially classified as incompatible, 103 as half-compatible and 463 as fully compatible. However, as the result of an experiment carried out on the progeny of these plants in 1980 (details of which will be published later), it became clear that two of these 568 pollinations had been misclassified. One of these was an incompatible mating that had been classified as half-compatible and the other a half-compatible pollination that had been misclassified as being fully compatible. The corrected number of pollinations falling into each of the three categories is thus 3, 103 and 462 respectively.

A few plants became too old to yield classifiable pollinations during the later stages of the experiment. For this reason, 62 of the total of 630

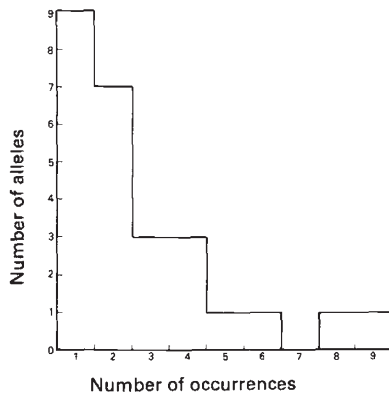


FIG. 1.—Distribution of S-Alleles in the R104 population ( $n = 26$ ,  $m = 72$ ,  $R = 0.67$ ,  $\chi^2_{(26)} = 44.618$ ,  $P = 0.0092$ ).

crosses that can be made between 36 plants were not classified. However, in each case, sufficient information was available from the crosses that had been made between these plants and others earlier in the programme to leave little doubt about their identity. Thus 12 of these 62 crosses would have been classified as half-compatible and 50 as fully compatible. Overall, therefore, of the grand total of 630 crosses, 3 (0.48 per cent) were incompatible, 115 (18.25 per cent) were half-compatible and 512 (81.27 per cent) were fully compatible. The proportion of crosses falling into each of these three categories in this experiment is thus very similar to the corresponding proportions (0.16 per cent, 16.86 per cent and 81.27 per cent, respectively) of the previous one (Campbell and Lawrence, loc. cit.).

The genotypes that were assigned to each of the 36 plants as a result of these crosses are shown in table 1. The total number of *S*-alleles

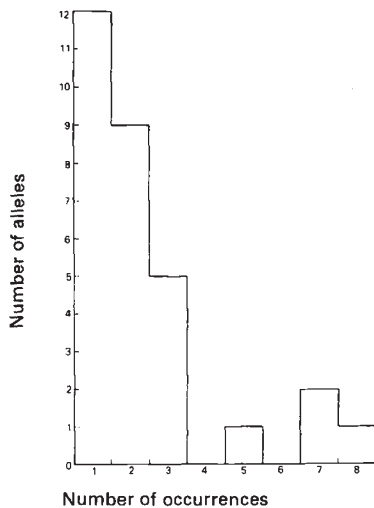


FIG. 2.—Distribution of *S*-alleles in the R102 population ( $n = 30$ ,  $m = 72$ ,  $R = 0.61$ ,  $\chi^2_{(29)} = 46.262$ ,  $P = 0.0221$ ).

TABLE 1

*Genotypes of the 36 plants classified in the R104 sample (note that there is no known correspondence between the labelling of the alleles in the present experiment and either of the previous ones)*

Plant No.	Genotype $S_i S_j$	Plant No.	Genotype $S_i S_j$
9	1, 2	38	5, 22
10	3, 4	39	9, 20
11	5, 6	43	3, 23
14	4, 7	44	10, 24
15	7, 8	45	7, 10
16	9, 10	46	3, 20
17	11, 12	47	7, 10
18	13, 14	49	4, 11
20	8, 9	50	10, 22
22	8, 16	51	7, 25
23	8, 9	53	7, 25
26	3, 13	54	3, 26
29	7, 17	55	8, 11
30	3, 18	56	7, 19
31	17, 18	61	3, 26
32	11, 19	65	6, 19
34	10, 20	68	22, 27
36	20, 21	70	3, 7

examined in the experiment was  $2 \times 36 = 72$ , of which 26 turned out to be different, giving a repeatability of  $R = 0.67$  (Campbell and Lawrence, 1981a). Once again, however, the most striking feature of these data is that the frequencies of these alleles are significantly unequal (table 2 and fig. 1;  $\chi^2_{(25)} = 44.618$ ,  $P = 0.0092$ ). Lastly, a conservative estimate of the number of alleles in the population from which this sample of 36 plants has been drawn is  $\hat{N} = 27.92 \pm 1.34$ .

(ii) *The R102 sample*

Of the grand total of 630 crosses, 4 (0.63 per cent) were incompatible, 94 (14.92 per cent) were half-compatible and 532 (84.44 per cent) were

TABLE 2

*The frequency of occurrence of the 26 S-alleles in the R104 sample of 36 plants*

$S_i$	Frequency	$S_i$	Frequency
1	1	14	1
2	1	16	1
3	8	17	2
4	3	18	2
5	2	19	3
6	2	20	4
7	9	21	1
8	5	22	3
9	4	23	1
10	6	24	1
11	4	25	2
12	1	26	2
13	2	27	1

TABLE 3

*Genotypes of the 36 plants classified in the R102 sample (note that there is no known correspondence between the labelling of the alleles in the present experiment and any of the previous ones)*

Plant No.	Genotype $S_iS_j$	Plant No.	Genotype $S_iS_j$
1	1, 2	30	1, 16
2	3, 4	31	15, 16
4	1, 5	32	21, 22
5	3, 6	35	15, 16
6	1, 7	36	16, 23
7	8, 9	37	12, 24
9	10, 11	41	16, 17
10	7, 12	48	15, 25
11	13, 14	49	5, 10
12	3, 14	50	1, 26
15	2, 7	52	27, 28
17	1, 7	53	2, 29
18	1, 15	55	15, 30
19	13, 15	56	15, 20
23	15, 16	58	10, 23
26	17, 18	60	6, 27
28	16, 19	61	8, 14
29	7, 20	62	19, 27

fully compatible, 619 of these crosses being classified directly and the balance of 11 indirectly. The results of experiments carried out on the progeny of these R102 plants confirm in every case the accuracy of the original classification. The proportion of crosses falling into each of these three categories in this experiment is thus again similar to the corresponding proportions of each of the previous experiments.

The genotypes assigned to each of the 36 plants as a result of these crosses are shown in table 3. The total number of *S*-alleles examined in the experiment was again 72, of which 30 were found to be different, giving a repeatability of  $R = 0.61$ . However, as in each of the two previous samples, the most important feature of these data is that the frequencies of the alleles are unequal (table 4 and fig. 2;  $\chi^2_{(29)} = 46.262$ ,  $P = 0.0221$ ).

Lastly, a conservative estimate of the number of alleles in the R102 population based on these data is  $\hat{N} = 33.74 \pm 1.36$ .

#### 4. DISCUSSION

In a preliminary survey of the self-incompatibility polymorphisms in *P. rhoeas* 32 different *S*-alleles were found in a sample of 61 obtained from eighteen families whose ancestors came from three natural populations in the West Midlands (Campbell and Lawrence, 1981*a*). Though two-thirds of these 32 alleles occurred in one or other of these populations only, an analysis of their distribution over localities failed to indicate any significant differentiation between populations in this respect. Because no more than 24 alleles were sampled from any of these populations, the power of the test of differentiation could not have been very high. At present, therefore, it is not possible to decide whether these populations contain essentially

TABLE 4

The frequency of occurrence of the 30 S-alleles in the R102 sample of 36 plants

$S_i$	Frequency	$S_i$	Frequency
1	7	16	7
2	3	17	2
3	3	18	1
4	1	19	2
5	2	20	2
6	2	21	1
7	5	22	1
8	2	23	2
9	1	24	1
10	3	25	1
11	1	26	1
12	2	27	3
13	2	28	1
14	3	29	1
15	8	30	1

the same complement of S-alleles or not. On this evidence alone, we cannot be sure even that we are dealing with three different populations, rather than with three independent samples taken from different parts of the same population. It is, however, known from other evidence that these populations are distinct, for Ooi (1970) has shown that they differ significantly in respect of five out of a total of eight metrical characters that he studied.

Having now completed a more thorough and extensive analysis of the polymorphism in each of these three natural populations we turn first to a comparison of the results obtained from this second phase of our investigation of the population genetics of self-incompatibility in *P. rhoeas*. A summary of these results is given in table 5 which also includes those from Emerson's (1939) investigation of the *Oenothera organensis* population.

TABLE 5

Summary of the results obtained from each of the three poppy populations and the *Oenothera* population. The number of alleles examined is twice the number of plants examined ( $m = 2r$ ) and dissimilarity was calculated as  $D = (2\chi^2)^{1/2} - (2n - 3)^{1/2}$ . See Campbell and Lawrence (1981a, b) for other details. The values of  $n$  and  $m$  shown for *Oenothera* were calculated from the data given in the column headed female totals of Emerson's (1939) table 2 (p. 532) which were obtained from cuttings taken from wild plants. The values of  $n = 34$  and  $m = 134$  for *Oenothera* given in the previous paper were calculated from the data shown in the final column of Emerson's table 2, which, being based on a mixture of half-sib families raised from seed taken from some wild plants and cuttings from others is less suitable for our present purposes than that obtained from the latter alone

Population	R102	R104	R106	<i>Oenothera</i>
No. of alleles found ( $n$ )	30	26	31	28
No. of alleles examined ( $m$ )	72	72	102	74
Repeatability ( $R$ )	0.61	0.67	0.72	0.65
Dissimilarity ( $D$ )	2.069	2.446	3.656	-1.240
$\chi^2$	46.262*	44.618**	64.260***	18.243
Number of alleles in the population ( $\bar{N}$ )	33.74	27.92	32.23	30.47
Confidence interval, 99 per cent	30-43	26-35	31-37	28-37

The first point to make about these results is that the frequencies of the alleles are apparently unequal in each of the three natural populations that we have studied. Though the magnitude of this inequality varies from  $D = 3.656$  in the R106 to  $D = 2.069$  in the R102 sample, there is little doubt that these poppy populations differ greatly in this respect from the *Oenothera* population for which  $D = -1.240$ . Thus unequal *S*-allele frequencies appear to be a characteristic of poppy populations in the West Midlands and since these are not obviously different in their ecology from those elsewhere in the British Isles, probably characteristic of wayside populations of the species in general. However, it is now clear that it is worth embarking upon the task of cross-classifying the alleles of one population against those of each of the others in order to find out whether the same alleles occur at a relatively high frequency in all three populations.

The second point to emerge from these results concerns the number of alleles in these populations. Although the number of alleles examined ( $m$ ) varies from 102 in the R106 sample to 72 in each of the others, the numbers of different alleles found in each ( $n$ ) are nevertheless similar and similar also to the number found in the *Oenothera* sample. This suggests that the total numbers of alleles in each of these natural populations ( $N$ ) are also similar.

Now while it is obvious that  $N \geq n$ , any attempt to estimate the total number of *S*-alleles in these poppy populations runs into the difficulty that the equation of estimation of this number

$$n = \hat{N}(1 - (1 - 2/\hat{N})^r)$$

where  $r = m/2$ , assumes that the genotypes in these populations are equally frequent (Paxman, 1963; appendix of Campbell and Lawrence, 1981*b*). But we have tested and have rejected the hypothesis that the alleles in these populations are equally frequent, so that it is unlikely that the incompatibility genotypes are equally frequent either. It is thus clear that the use of this equation in present circumstances is not strictly valid. It is, of course, obvious that those alleles which occur at a relatively high frequency in the population are more likely to turn up in a randomly drawn sample than those of a lower frequency. It follows that the estimates of the number of alleles ( $\hat{N}$ ) in these poppy populations shown in table 5 are conservative estimates that are biased downwards. Hence the actual number of alleles in these populations is likely to be rather higher than these estimates indicate.

At first sight, no such reservation attaches to the estimate of the number of alleles in the *Oenothera* population, for in this case there is no evidence in Emerson's 1939 sample that the allele frequencies are other than equal. Furthermore, we note that the upper bound of the confidence interval of the estimate of 30.47 alleles is equal to the number of alleles that Emerson had found at this time, namely 37. However, he eventually found 45 different *S*-alleles in the population (Emerson, 1940) which suggests that one or more of the assumptions underlying the equation of estimation of  $N$  may not be valid for the *Oenothera* population also. Nevertheless, since the number of alleles found in the *Oenothera* sample is similar to the numbers found in the poppy samples, it is likely that the number of alleles in the population from which the latter were drawn is at least 45.



This conclusion, if correct, forces us to change our ideas about the population biology of *P. rhoeas*. Thus, hitherto, we have supposed that the species is an opportunistic, colonising species whose populations became greatly reduced in size from time to time (Campbell and Lawrence, 1981*a*). But Wright (1939) has shown that the minimum size of the population required to maintain 45 *S*-alleles, given a mutation rate of  $1 \times 10^{-6}$  and that the population is in equilibrium, is of the order of 5000 individuals. This calculation, however, is based on the assumption that the effect of selection is limited to that associated with incompatibility. Since in our case, this may well not be true, the minimum size of population required to maintain the same number of alleles whose frequencies are unequal, due to an extra effect of selection with respect to a small number of alleles, would clearly have to be greater than 5000 in order to ensure that none of the alleles not favoured by this extra effect of selection were lost by drift from the population. Thus unless the number of *S*-alleles in the species is very much greater than the number in these populations, for which there is no evidence at present, these data are clearly not consistent with the notion that we are dealing with populations whose size becomes very small from time to time.

On this evidence, it is clearly misleading to regard *P. rhoeas* as a colonising species and it is, furthermore, opportunistic only in the special sense that seed will germinate to give a stand of flowering plants only when the soil has been disturbed. There is no difficulty with this species in supposing that a stand of flowering plants consists of upwards of 5000 individuals. Thus we have found that what appears on first sight to be a very small population frequently consists of several thousand plants and care was taken in the present investigation to sample only from large populations. In addition, the number of viable seeds in the seed bank from which the stand of flowering plants is recruited when the soil is disturbed and by which the populations persists between such episodes is likely to be very large (Brenchley and Warrington (1933) estimated that there were over 35,000 seeds  $m^{-2}$  of Papaver species in the topsoil of the Broadbalk field at Rothamsted). It follows, therefore, that the species should be regarded as a permanent member of the flora of wayside places, albeit one with an unusual reproductive biology, rather than as a colonising species.

These arguments, if correct, suggest that the dynamics of the self-incompatibility polymorphism in this species is complex. In particular, they force us to reconsider the second of the two hypotheses that have been proposed to account for the unequal allele frequencies in these populations, namely, that this is due to chance effects of a kind to be particularly expected in a population of a colonising species; this is a matter to be dealt with in a future paper.

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