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VARIATION IN WILD POPULATIONS OF *PAPAVER DUBIUM*

VIII. THE MATING SYSTEM

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SUMMARY

Previous workers have obtained evidence suggesting that, under natural conditions, *Papaver dubium* reproduces by mixed selfing and random mating, the contribution of selfing being substantial. In order to obtain a more quantitative estimate of the amount of selfing, a small number of plants, homozygous or heterozygous for the recessive (flower colour) mutant, magenta, were raised among a large number of wild-type homozygotes and allowed to open pollinate. Progenies raised from these recessive homozygotes and from the heterozygotes, provided estimates of the degree of selfing. Results showed that the degree of selfing varies significantly, both between plants and between different flowers on the same plant. The estimates of average selfing obtained lay between 71 and 81 per cent, the estimates being about the same in two different years. In view of these and earlier results, it was concluded that a high degree of selfing obtains in natural populations of *P. dubium*; the degree of selfing being sufficiently large to have an important effect on the genetical structure of such populations.

1. INTRODUCTION

GENETICAL variation, within natural populations, has been found for 14 out of 18 metrical characters studied in *Papaver dubium* (Lawrence, 1965, 1972; Gale and Arthur, 1972; Gale and Eaves, 1972). Such observations draw attention to a recurring problem in present-day population genetics. Namely, how does selection act so that genetical variation is maintained within populations? Several possibilities, arising both from experimental observations and theoretical considerations have been put forward in answer to this problem. For example, disruptive selection, heterozygous advantage and frequency dependent selection may all, in certain circumstances, maintain genetical variation within populations.

However, any hypothesis concerning the maintenance of variation in natural populations must take account of the distribution of variation between the individuals comprising a given population. In other words, it is important to consider the genetical structure of populations. For example, theories involving heterozygous advantage are very unlikely to be applicable if the individuals in a population are mainly homozygous.

The genetical structure of a population depends largely upon the breeding system in that population. Thus inbreeders are expected to be more homozygous than outbreeders. The purpose of the study described in this paper was to obtain information on the mating system in *P. dubium*, which would help in trying to understand how the genetical variation observed is maintained in populations of this species.

Clapham, Tutin and Warburg (1962) describe *P. dubium* as being " homogamous and self-compatible but often outcrossed ". Previous work

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carried out by others, while not providing quantitative information, showed indirectly that *P. dubium* probably inbreeds to a large extent but that individuals in natural populations are by no means completely inbred (Gale, Rana and Lawrence, 1974).

In this study, an attempt was made to obtain some quantitative estimate of the amount of selfing occurring in *P. dubium*. If we assume that the mating system is a mixture of selfing and random mating, then (Fyfe and Bailey, 1951), the empirical inbreeding coefficient may be obtained as:

$$f = \frac{s}{2-s}$$

where s is the proportion of seed produced by selfing.

The above assumption is likely to be fairly realistic in the case of *P. dubium*. Thus although possible in principle, assortative mating has been shown to be unlikely in *P. dubium*, at least for flowering time (Gale *et al.*, 1974).

It is also possible that, owing to the relatively poor method of seed dispersal in *P. dubium*, mating between close relatives may occur to give some inbreeding. The situation is complicated by the high dormancy of the seeds in *P. dubium* (Harper and McNaughton, 1962) and also the behaviour of the pollinating insects, about which little is known. However it seems likely, as will be shown later, that the effects of mating between close relatives, on the amount of inbreeding that occurs, are trivial compared to the effects of selfing.

2. MATERIALS AND METHODS

Estimates of selfing were obtained in 2 years using a recessive major gene marker. The marker used was a rare flower colour mutant called magenta, first found by Drs M. J. Lawrence and A. E. Arthur. This mutant produces flowers with red-coloured petals, instead of the lighter, orange-red wild-type petals. In crosses with wild-type plants, it was found that the magenta mutant is inherited in a simple diploid manner as shown by the results presented in table 1.

It should be noted that these results differ significantly from those expected on any simple model of polyploid inheritance. Thus despite the presumed hexaploid origin of the species, inheritance is diploid, at least at this locus.

It is of interest to note that a peroxidase isoenzyme, found in root extracts of *P. dubium*, was also found to be inherited in a simple diploid manner.

TABLE 1

The system of inheritance of the magenta mutant, as shown by the segregation observed in generations derived from a cross between magenta and wild-type

	Segregation observed		Goodness of fit of observed with that expected in a simple diploid system of inheritance		
Generation	Magenta	Wild-type	χ^2	d.f.	P (%)
F_2	83	290	1.50	1	30-20
Backcross to magenta with magenta as mother	31	28	0.15	1	70
Reciprocal backcross to magenta	22	32	1.85	1	20-10

Heterogeneity between backcrosses $\chi^2_1 = 1{\cdot}57$; P = 20 per cent.

This isoenzyme may be observed as a band near the anode after electrofocusing root extracts in thin layers of polyacrylamide gel (Awdeh, Williamson and Askonas, 1968) and staining with dianisidine and hydrogen peroxide (Brown and Allard, 1969). The isoenzyme is always present in F_1 progeny of crosses between plants having and not having the isoenzyme. Thus it behaves as a dominant character. In the F_2 generation, derived from such crosses, ratios are obtained which agree with the ratio 3 to 1 (enzyme present to enzyme absent) expected with simple diploid inheritance. The ratios differed significantly from those expected with simple polyploid systems of inheritance. It should be mentioned that no alternative isoenzyme could be observed in plants not possessing the isoenzyme discussed above.

A standard method was adopted to obtain selfing estimates using the magenta mutant. This involves growing a small number of homozygous recessive mutant plants (magenta in this case) among a large number of wild-type plants and allowing them to open pollinate. The proportion of magenta individuals in the progeny of magenta mothers provides a direct estimate of the amount of selfing that has occurred.

There is, however, a problem associated with the use of magenta plants in this way. It is possible that pollinating insects may distinguish between magenta and wild-type flowers and may preferentially visit a flower of the same colour as that visited previously. In this case, the estimates of selfing are inflated owing to frequent cross-pollinations between magenta plants. In practice this problem may be trivial, as our observations indicate that bees move between wild-type and magenta flowers quite freely. Similar behaviour has also been observed for different colour forms in other species (Darwin, 1878; Grant, 1950).

Also, bees are red-blind though they are able to perceive the ultraviolet light that poppy petals reflect (Von Frisch, 1966). It is likely that magenta and wild-type flowers reflect similar ultraviolet rays and thus appear identical to a bee.

In order to obtain further information about the importance of this problem, magenta × wild-type F_1 plants were also grown among wild-type plants and allowed to open pollinate. As magenta is recessive, the F_1's appear to have the normal wild-type flower colour. It is, therefore, very unlikely that they can be distinguished from wild-type plants by pollinating insects. The amount of selfing in the F_1 plants may be estimated from the segregation ratio observed in the progeny, as the expected proportion of magenta progeny is $s/4$, where, as above s is the proportion of selfing. However, in order to achieve reasonably precise estimates, a large number of progeny from F_1 parents must be screened.

Thus if n progeny are raised from a homozygous magenta parent, the variance of the estimated frequency of selfing is:

$$\frac{s(1-s)}{n}$$

whereas if k progeny are raised from an F_1 parent, the corresponding variance is:

$$\frac{16\frac{s}{4}\left(1-\frac{s}{4}\right)}{k} = \frac{s(4-s)}{k}.$$

Hence, for equal precision:

$$\frac{k}{n} = \frac{4-s}{1-s}$$

which, for example, is 7 for $s = \frac{1}{2}$, 13 for $s = \frac{3}{4}$.

3. The 1970-71 experiment

In the spring of 1970, 30 late-flowering, homozygous magenta plants (descended from a single magenta plant found in a population near Pillerton Priors, Warwickshire) and 30 slightly earlier flowering Pillerton Priors magenta × wild-type, F_1 plants were randomised among 2820 presumed homozygous wild-type plants (*i.e.* no magenta plants had been observed among their ancestors) grown for other purposes. Thus the frequency of magenta and F_1 plants was very low and in the analysis of the results it was decided to disregard the possibility of random cross-fertilisations between plants carrying the magenta mutant. This may be allowed for, but it complicates the final analysis (Fyfe and Bailey, 1951; Vasek, 1964, 1967).

The plants were left to open pollinate and 10 capsules per plant were sealed with " parafilm " just before the capsule pores opened. At the end of the season, seed was collected, the seed from capsules on the same plant being bulked. This was a necessity in the case of the F_1 plants, as many progeny were required. The same was done for the magenta plants in order to obtain a corresponding estimate of selfing for the plant as a whole. 100 progeny from each of the 10 magenta plants most isolated from other plants possessing a magenta allele and 1000 progeny from each of the five similarly most isolated F_1 parents were grown and were scored for flower colour. A few plants died before they could be scored. The results are presented in table 2.

In the case of the homozygous magenta parents, the proportion of selfing ($\hat{s}$) is estimated as the proportion of magenta plants in the progeny. For the

Table 2

Progenies obtained in 1970-71 from 10 open-pollinated magenta plants and five open-pollinated F_1 plants

Type of parent	Progeny		Total	Estimated proportion of selfing ($\hat{s}$) (%)	Standard error of $\hat{s}$ (%)
	Magenta	Wild-type			
Magenta	69	24	93	74·19	4·54
	77	17	94	81·91	3·97
	79	18	97	81·44	3·95
	84	11	95	88·42	3·28
	73	27	100	73·00	4·44
	72	25	97	74·23	4·44
	69	31	100	69·00	4·63
	87	13	100	87·00	3·36
	84	16	100	84·00	3·67
	81	18	99	81·82	3·88
F_1	140	855	995	56·28	4·41
	212	787	999	84·88	5·17
	175	825	1000	70·00	4·81
	162	837	999	64·86	4·67
	211	787	998	84·57	5·17

F_1 parents, given, as shown by the data presented in table 1, that magenta is a completely recessive mutation inherited in a simple diploid Mendelian manner, the proportion of selfing is estimated as four times the observed proportion of magenta in the progeny. Since we are dealing with perfect-fit situations, our estimates are maximum likelihood estimates.

The estimates of selfing shown in table 2 range from 69 to 88 per cent with a weighted mean of 81 per cent in the magenta plants and from 56 to 85 per cent with a weighted mean of 71 per cent in the F_1 plants. However, there is a significant heterogeneity among the estimates for both the magenta plants:

$$(\chi^2_9 = 22{\cdot}83, \quad P = 1\% - 0{\cdot}1\%)$$

and the F_1 plants

$$(\chi^2_4 = 26{\cdot}39, \quad P = <0{\cdot}1\%).$$

Because of this heterogeneity, it is difficult to determine whether the difference between the magenta and F_1 means is significant. If it is a true difference, it provides little evidence to support the idea that pollinating insects discriminate between magenta and wild-type flowers. In fact, any difference may well be attributable to a difference in the mean flowering time of the magenta and F_1 plants. Thus the magenta plants flowered so late that many of the surrounding wild-type plants had finished flowering, therefore reducing the opportunities for cross-pollination to occur. Hence an investigation of this possibility was included in the 1971-72 experiment.

4. The 1971-72 experiment

This experiment was carried out along the same lines as the 1970-71 experiment. Late flowering Pillerton Priors magenta plants were again used as parents. However, earlier flowering F_1 progeny of a cross between a very early flowering magenta plant, descended from a plant found near Blakedown, Worcestershire, and one of the Pillerton Priors magenta plants were also available. Dominance, if present at all, is towards early flowering in *P. dubium* (Lawrence, 1965, 1969).

In the summer of 1971, 10 Pillerton Priors magenta (denoted P) and 10 Pillerton Priors magenta × Blakedown (denoted PB) magenta F_1 plants were randomised among 2580 wild-type plants grown for another purpose. As in the previous experiment, the plants were left to open pollinate and capsules were sealed up with " parafilm " just before maturation.

It was decided to investigate the progeny of 3 capsules, taken at random, from each of 5 P magenta and each of 5 PB magenta plants. Parent plants were again chosen on the basis of their position in relation to other magenta plants in the experiment, *i.e.* the most isolated parent plants were chosen. 100 progeny were raised from the seed of each capsule chosen and their flower colour recorded. The results are shown in tables 3 and 4, the proportion of selfing ($\hat{s}$) being estimated as before.

The estimated amount of selfing ranged from 40 to 93 per cent, with a weighted mean of 72 per cent, in the PB magenta plants and from 32 to 93 per cent, with a weighted mean of 80 per cent, in the P magenta plants. Thus the ranges are greater than in the 1970-71 experiment. However, it must be remembered that in the 1970-71 experiment, the results are based on progeny raised from a mixture of seed from several capsules of the same plant. On the other hand, in the 1971-72 experiment, the results are based on progeny raised from seed from individual capsules. If the estimates, obtained from different capsules of the same parent plant in the 1971-72 experiment are averaged, the range is from 51 to 80 per cent for the PB magenta plants and from 61 to 90 per cent for the P magenta plants. These are more like the ranges obtained in the 1970-71 experiment.

Table 3

Progenies of Pillerton Priors × Blakedown F_1 magenta plants (1971-72)

Plant number	Capsule number	Progeny		Total	$\hat{s}$ (%)	Standard error of $\hat{s}$ (%)
		Magenta	Wild-type			
1	1	83	17	100	83·00	3·76
	2	73	27	100	73·00	4·44
	3	77	23	100	77·00	4·21
2	1	51	49	100	51·00	5·00
	2	58	42	100	58·00	4·94
	3	51	48	99	51·52	5·02
3	1	85	15	100	85·00	3·57
	2	61	39	100	61·00	4·88
	3	65	34	99	65·66	4·77
4	1	47	53	100	47·00	4·99
	2	40	60	100	40·00	4·90
	3	65	35	100	65·00	4·77
5	1	75	25	100	75·00	4·33
	2	71	29	100	71·00	4·54
	3	93	7	100	93·00	2·55

Table 4

Progenies of pure Pillerton Priors magenta plants (1971-72)

Plant number	Capsule number	Progeny		Total	$\hat{s}$ (%)	Standard error of $\hat{s}$ (%)
		Magenta	Wild-type			
1	1	89	11	100	89·00	3·13
	2	73	27	100	73·00	4·44
	3	78	21	99	78·79	4·09
2	1	82	17	99	82·83	3·77
	2	76	24	100	76·00	4·27
	3	72	28	100	72·00	4·49
3	1	32	67	99	32·32	4.68
	2	87	13	100	87·00	3·36
	3	76	22	98	77·55	4·17
4	1	86	14	100	86·00	3·47
	2	92	7	99	92·93	2·56
	3	89	8	97	91·75	2·75
5	1	67	33	100	67·00	4·70
	2	48	49	97	49·48	5·00
	3	66	33	99	66·67	4·71

It can be seen from tables 3 and 4 that much variation exists, both between plants and between capsules from the same plant, with regard to the amount of selfing that occurred. Analyses of variance shown in table 5, carried out on the data transformed into angles, support this conclusion.

The between-plant variance is similar for both sets of parents. However, in the case of the P magenta parents, the within plant variance is rather inflated so that the between-plant variance is not significant when tested against it.

TABLE 5

Analyses of variance on the results (transformed into angles) obtained in the 1971-72 experiment

Item	d.f.	S.S.	M.S.	V.R.	P (%)
Pillerton Priors × Blakedown F_1 parents					
Between plants	4	873·17	218·29	4·71	2
Between capsules Within plants	10	463.07	46·31	5·64	<0·1
Total	14	1336.24			
Binomial error			8·21		
Pure Pillerton Priors magenta parents					
Between plants	4	766·96	191·74	2·26	20-10
Between capsules Within plants	10	848·26	84·83	10·33	<0·1
Total	14	1615·22			
Binomial error			8·21		

The weighted mean for the pure Pillerton Priors magenta plants in 1971-72 (80 per cent) agrees well with the 1970-71 figure (81 per cent) whilst the weighted mean for the PB magenta plants in 1971-72 (72 per cent) agrees with the mean obtained for the Pillerton Priors magenta × wild-type F_1 plants in 1970-71 (71 per cent). As the PB plants in 1971-72 flowered around the same time as the Pillerton Priors magenta × wild-type F_1 plants in 1970-71, there seems to be a tendency, in both years, for the later flowering set of parents to give the higher mean estimate of selfing. However, the difference are small and an analysis of variance on the combined 1971-72 data (an analysis of variance cannot be carried out on the combined 1970-71 data because of the different numbers of progeny raised) showed the difference not to be significant ($F = 1\cdot15$ for 1 and 8 degrees of freedom, $P > 25$ per cent).

Although these results are rather inconclusive as regards the effect of flowering time on selfing, they do show that the results are very unlikely to be biased by bees discriminating between magenta and wild-type flowers. Thus the difference between the two sets of parents was similar in both experiments, although wild-type flower colour F_1 parent were used in the 1970-71 experiment.

One other possible bias in the results lies with the fact that plants grown in the experimental field are generally larger and have more flowers than those growing in the wild. Thus a plant in the experimental field has more than one flower open at the same time more often than a plant in the wild. If there was a tendency for bees to move from flower to flower on the same plant, then more selfing would occur in the experimental field than in the wild. However, our observations have shown that, in poppies, bees very seldom visit two flowers on the same plant in succession. Thus it is very likely that the high proportion of selfing found under experimental conditions also occurs in the wild.

5. Discussion and conclusions

We will now consider the mechanism by which a high proportion of selfing may be brought about.

The anthers in *P. dubium* generally lie below the edge of the stigmatic disk and only rarely do they touch the edge of the stigmatic rays. However, release of some pollen normally occurs in the bud (Clapham, Tutin and Warburg, 1962; McNaughton and Harper, 1964). Rogers (1969) found the pollen released in the bud to be mature and able to germinate immediately on the stigma of the same flower. Thus self-pollen, provided it reaches the stigma, may be well on the way to fertilising many ovules before the flower opens to allow pollen from other sources to reach the stigma. Pollen may fall on to the edges of the stigmatic rays if the bud is in a drooping position at the time of anthesis. (Buds usually remain in a drooping position until the day before they flower.)

However, as Rogers (1969) points out, the pollen tubes, from pollen thus deposited, must grow along the whole length of the stigmatic rays before reaching the transmitting canal. Thus pollen tubes from foreign pollen, deposited later but nearer the centre of the stigma, may reach the ovules first.

Studies carried out by Rogers (1969) and McNaughton and Harper (1964) showed that the seed set from autodeposition of pollen in *P. dubium* is around 30-40 per cent of that obtained with open pollination. This indicates that 30-40 per cent selfing can occur in *P. dubium* through bud pollination, *in the absence of competition from other pollen*. In natural conditions, where competing foreign pollen is present, bud pollination may be responsible for a much lower degree of selfing, as explained above. Therefore, the high estimates of selfing (70-80 per cent) obtained in the present investigations require further explanation.

The answer probably lies in the activity of the pollinating insects which are mainly bees. We are indebted to Dr D. A. Jones for a colour film of this behaviour.

When bees visit a poppy flower, they may alight directly on the stigma or, more often than not, near the base of the petals. They then proceed to walk round the capsule, crawling through the anthers and collecting pollen as they go. In so doing, a bee may often place one or more legs on the stigmatic disk and sometimes it may even crawl right over the stigmatic disk. Thus it seems very likely that the amount of foreign pollen, deposited on a stigma by a bee, may well be small in comparison to the self-pollen deposited in the act of pollen collecting.

The variation within each of the groups of parent plants used in the investigations was found not to be correlated with flowering time, unlike the larger differences between the groups as discussed earlier. Neither were correlations found with the degree of isolation, by distance, of parent plants from neighbouring plants carrying the magenta allele. Also, it must be remembered that the 1971-72 results showed differences to occur between capsules from the same plant. It seems likely that the environmental conditions at the time of flowering may be important in this respect. The environment may exert a great deal of influence over such things as the time of anther dehiscence and also the behaviour of the pollinating insects. Also, the fact that the between-plant variation was significantly greater than the within-plant variation, for Pillerton Priors × Blakedown magenta plants in the 1971-72 experiment, suggests that genetical differences between plants (perhaps for characters such as anther number and stigmatic ray number which are known to show such variation) may also have an effect.

Although the experiments carried out in this investigation have provided estimates of selfing only under artificial (*i.e.* experimental field) conditions, they probably give a reasonable picture of the situation in natural populations. That is, although much variation exists in the amount of selfing in *P. dubium*, it is, on average, fairly large. Taking the results from the 1970-71 and the 1971-72 experiments as a whole, the average amount of selfing is around 75 per cent. Using this value, Fyfe and Bailey's formula, mentioned earlier, gives an empirical inbreeding coefficient of 0·6.

Thus any future hypothesis concerning the maintenance of variation within natural populations of *P. dubium* must take into account the high level of inbreeding in the populations.

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