VARIATION IN WILD POPULATIONS OF *PAPAVER DUBIUM*

IV. A SURVEY OF VARIATION

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1. INTRODUCTION

IN earlier papers in this series (Lawrence, 1965, 1969, 1972), it was demonstrated that natural populations of *Papaver dubium* showed genetic variation within populations for the characters flowering time, stigmatic ray number and final height. A fourth character, capsule number, did not exhibit such intra-population variation. Since these characters were chosen entirely for ease of measurement, without any prior knowledge of their population genetics, the results would suggest that genetic variation within populations is common in this species. Accordingly, it was decided to conduct a wider survey of characters, in order to obtain a more quantitative check on the proportion of characters which might show such variation.

Ten new characters reflecting the general morphology and rate of growth of the plant were chosen for study. Where appropriate, these characters were measured at different stages of development. In view of the large number of measurements, some of which were time-consuming, it was decided to measure only a restricted number of plants (72) and grow the plants throughout in the glasshouse. However, subsequently, plants having the same parentage as those raised in the glasshouse were grown under experimental field conditions and some of the characters remeasured. A comparison between results obtained under these two regimes would give information about genotype-environmental interaction. A well-known difficulty found in working with metrical characters is that of inferring the performance of a genotype under one set of conditions (*e.g.* the natural habitat) from the performance under quite different conditions (*e.g.* the experimental field). Thus, it is easiest to work with characters showing little genotype-environmental interaction. Our results should give some evidence on this.

We propose to use the term polymorphism for cases where characters are controlled by genes which show variation within populations, although, strictly speaking, metrical characters are excluded from the definition of polymorphism given by Ford (*e.g.* Ford, 1964). It seems best at the present stage to assume that variation within populations for major genes and for genes controlling metrical characters are different aspects of the same phenomenon, and hence to use the same term for both. However, given that we have demonstrated that a number of characters (n , say) are showing such variation, it does not necessarily follow that there are n separate polymorphisms to be accounted for, since several characters may represent pleiotropic effects of one set of genes. By calculating correlations between

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characters, we shall attempt to obtain a minimum estimate of the number of polymorphisms present.

2. MATERIALS AND METHODS

The plants used derive ultimately from seed collected from six local (Warwickshire or Worcestershire) natural populations in 1965. Plants were raised from this seed, in the experimental field, in 1966. From every population, four pairs of plants were chosen at random. Plants comprising a pair were then crossed to one another to give a single family. Thus it was intended to raise four such biparental families per population. Germination rate was substantially increased by pretreatment of seed with concentrated sulphuric acid (Rana, personal communication). However, seed from six crosses failed to germinate, leaving 18 families with origin as follows: Henley (2), Wellesbourne (3), Luddington (2), Welford (4), Blakedown (4), University campus (3).

Four progeny were raised from every cross, giving 72 plants in all. These were grown, in the glasshouse, over the winter 1966-67.

Our crossing programme described above did not include reciprocal crosses since, as will be shown in a later paper, maternal effects are absent, or almost absent, in this species, at least in these six populations.

Characters measured were as follows. Time of scoring is given in parentheses; a number indicates weeks after sowing and F indicates flowering time (time of opening of first flower on the plant being measured).

1. Leaf number LN (3, 3½, 4, 7, 8, F)
2. Height H (7, 8, F)
3. Diameter D (7, 8, F)
4. Elevation E *i.e.* angle between leaf blade and horizontal (outermost leaf at 7, mean value for three outermost leaves at 8)
5. Number of flower buds B (F)
6. Number of anthers in first flower A (F)
- 7-10. Length L, Width W, Number of points P, Number of leaflets LF of stem leaf (all F)
11. Flowering time FT

In general, characters were chosen mainly for ease of measurement. However, earlier casual inspection of plants suggested that morphological distinction between plants is compounded mainly of differences of "shape" and "leafiness". It was thought that "shape" would be measured by characters 2, 3 and 4 and "leafiness" by 1 and to a lesser extent by 7-10. For the latter, the stem leaf (*i.e.* the leaf subtending the first flower) seems the obvious choice; basal leaves are difficult to standardize and tend to rot once they reach maturity. The number of buds, at flowering time, gives an indication of simultaneity of flowering of different flowers on the same plant. Flowering time was included to facilitate comparison with earlier work.

While characters 1 to 10 show obvious variation between plants, we did not know at the time when the characters were chosen whether such phenotypic variation concealed any genotypic variation. In this restricted sense we can regard the characters as a "typical" sample of characters that might have been chosen.

3. TYPE OF VARIATION

Estimated components of variation, in percentages, together with their significance levels, are given in table 1. More detailed results are available from the authors on request. Between population and between family components measure variation which is entirely genetical in origin. Variation within families is part genetical, part environmental.

TABLE 1
Components of variation (percentages) for plants raised in the glasshouse

Character		$\hat{\sigma}^2$	$\hat{\sigma}_F^2$	$\hat{\sigma}_P^2$
Leaf number	3	85.90	14.10	0.00
	3½	64.04	31.95**	4.01
	4	70.41	29.59**	0.00
	7	35.68	38.67***	25.65
	8	39.47	54.52***	6.01
	F	18.73	16.59***	64.68***
Height	7	36.70	16.60**	26.70**
	8	33.03	30.20***	36.77*
	F	54.31	31.14**	14.55
Diameter	7	36.32	33.80***	29.88*
	8	36.70	12.72**	50.58*
	F	51.97	19.62*	28.41*
Elevation	7	52.00	32.00*	16.00
	8	70.61	10.70	18.69
Bud number	F	74.17	0.00	25.83**
Anther number	F	40.38	19.03***	40.59***
Stem leaf				
Length	F	78.20	17.80	4.00
Width	F	74.52	25.48*	0.00
Points	F	67.73	23.09*	9.18
Leaflets	F	65.28	7.28	27.44*
Flowering time		18.51	25.51***	55.98**

$\hat{\sigma}^2$ = Estimated variance within families.

$\hat{\sigma}_F^2$ = Estimated variance between families within populations.

$\hat{\sigma}_P^2$ = Estimated variance between populations.

*** $P < 0.1\%$. ** $P = 1\%-0.1\%$. * $P = 5\%-1\%$.

It will be seen that, out of the 11 characters studied, eight show significant differences between families within populations. Hence the genes controlling these eight characters vary within at least some populations.

Significant differences between populations were found for seven characters (leaf number at flowering time only, height at the seedling stage only). However, since, in the analysis of variance, the between populations mean square had to be tested against the between families, within populations mean square (in cases where the latter was significant), we are more likely, in the present experiment, to detect within population variation than between population variation of comparable size.

In cases where measurements were made of the same character at different seedling stages, results within populations are consistent for height and diameter and also for leaf number, apart from one very early measurement in the latter case. It is noticeable that the relative contribution of the within family variation for leaf number falls as the plants develop. An

apparent anomaly is given by the results on elevation. However, as explained above, the character was not measured in the same way on the two occasions of measurement and probably we should regard these two measurements as representing distinct characters.

Measurements at the seedling stage and at flowering time are probably also best regarded as separate characters. For both height and diameter, estimated correlations between family means for a character at seedling stage and flowering time turned out to be negative, as will be discussed below. For both leaf number and height, the percentage of total variation attributable to between population variation seems very different at maturity from that at the juvenile stage, although the differences in value may be due, at least in part, to sampling error.

Thus, counting elevation, leaf number, height and diameter as two characters each, we have (including flowering time) 15 characters in all; adding the other characters investigated by Lawrence gives us 18. Of these, 13 show significant genetic variation within at least some of the populations. Clearly, such variation is the norm in this species.

4. CORRELATIONS BETWEEN CHARACTERS

We shall now attempt to make a minimum estimate of the number of independent sets of loci controlling these characters.

If two characters found to be under some genetic control also show significantly high genetic correlation *inter se*, then it is likely that these characters are influenced by the same gene system; in other words, they are pleiotropic effects of the same set of genes. If high correlations, say 0.9, were observed, this would indicate pleiotropy (or perhaps linkage—see below). Very low correlations indicate two separate sets of genes controlling the characters at some stage. Intermediate correlations may indicate one set of genes affecting both characters, in conjunction with two other independent sets of genes, one for each character. Alternatively, intermediate correlations would arise with only one set of genes if different loci in the set affected the two characters unequally, some loci in the set having more effect on the first and other loci on the second character. We can, of course, make no inference about the effects of loci which are identical in all plants studied. In view of the difficulty in assessing intermediate correlations, we shall attempt to obtain only a minimum estimate of the number of systems, characters being regarded as distinct only when the correlations are low.

Since we are making minimum estimates, our conclusions will not be upset by linkage between loci controlling the various characters, even if loci are not in linkage equilibrium. In the absence of such equilibrium, the effects of linkage and pleiotropy will be, to some extent, confounded. However, the effect of this will be to reduce the estimate of the number of sets of loci.

Hence, correlation coefficients were calculated for most of the characters, taken in pairs. In order to minimise environmentally caused correlation, the correlations were calculated on family means. However, in the case of leaf measurements, the within family component of variation was over 65 per cent. for all four characters. Indeed, two of the characters showed no significant heritable variation. We shall not, therefore, consider these characters further.

Correlation coefficients for the seedling measurements at 7 and 8 weeks are given in table 2 and for flowering time measurements in table 3. For convenience, flowering time has been included in both tables. In cases where coefficients were small, the data were checked for possible non-linear relationships, but none was detected.

We can form a general picture of plant development, at least for early and late flowering types. Early flowering plants tend to be large at 7 and 8 weeks, with few upright leaves; the interval between opening of successive flowers on the same plant is relatively large. On the other hand, late-flowering types are flat leaved, with many leaves at flowering time, and tend to have successive flowers close in time.

TABLE 2

Correlations between family means for seedling characters and for flowering time

	H7	H8	D7	D8	LN7	LN8	E7	E8	FT
H7		0.9							
H8			0.6	0.8	-0.5	-0.4	0.6	0.4	-0.9
D7				0.7	-0.6	-0.4	0.7	0.5	-0.9
D8					0.9	0.2	-0.1	-0.1	-0.4
LN7						-0.3	0.2	0.0	-0.7
LN8							0.6	-0.3	0.5
E7								-0.8	0.6
E8									-0.8
FT									-0.7

Correlations above 0.47 in absolute value are significant at the 5 per cent. level.

TABLE 3

Correlations between family means for characters measured at flowering time

	HF	AF	DF	LNF	BF	FT
HF		0.8	0.5	0.2	0.4	0.4
AF			0.6	0.0	0.3	0.2
DF				0.5	0.8	0.6
LNF					0.7	0.9
BF						0.7
FT						

5 per cent. significance level as in table 2.

Leaf number, height and elevation (all at 7 or 8 weeks) show correlation *inter se* and thus are under the control of one or more sets of genes. We count this as one set since we are able to make only minimum estimates. On the other hand, diameter (at 7 or 8 weeks) shows no or very little correlation with elevation or leaf number and this must represent a separate system. In view, however, of the correlation between height and diameter, we must regard the two systems as follows:

- (1) Height H and diameter D.
- (2) Height H, elevation E and leaf number LN.

We may now consider characters scored at flowering time. These again fall into two groups. The characters leaf number, bud number and flowering time form one group. Another group is made up of height and anther

number. The diameter shows correlation with both groups. Thus the two systems at flowering time are constituted:

- (3) Leaf number LNF, bud number BF, flowering time FT and diameter DF.
- (4) Height HF, anther number AF and diameter DF.

However, it is clear that system (3) is intimately connected with both (1) and (2), as may be seen from the complete table of correlation coefficients, as given in table 4, where we have averaged values for 7 and 8 weeks. For example, height at 7 or 8 weeks and flowering time are closely associated, the plants which grow quicker flowering earlier. However, the following points emerge:

1. AF and HF are isolated from all characters, except DF and perhaps H. Hence AF and HF must be in a group which includes no other characters apart from DF and possibly H.
2. E and LN cannot be in the same group as D.

TABLE 4
Complete table of correlations between family means

	H	FT	LNF	BF	D	E	LN	AF	HF	DF
H		-0.9	-0.8	-0.7	0.7	0.5	-0.5	-0.4	-0.5	-0.7
FT			0.9	0.7	-0.6	-0.8	0.6	0.2	0.4	0.6
LNF				0.7	-0.4	-0.8	0.7	0.0	0.2	0.5
BF					-0.5	-0.5	0.3	0.3	0.4	0.8
D						0.0	-0.1	-0.1	0.0	-0.4
E							-0.6	-0.2	-0.4	-0.5
LN								0.2	0.3	0.3
AF									0.8	0.6
HF										0.5
DF										

Thus at least three separate sets of loci must be involved in the variation of these characters. In other words, at least three polymorphisms have been detected in these populations.

A tentative identification of the groups is:

- (A) AF, HF, DF, H (?)
- (B) E, LN, H, FT, LNF, DF (?), BF (?)
- (C) D, H, FT, BF, LNF(?), DF(?)

Any polymorphism presumably represents variation at several loci.

5. GENOTYPE-ENVIRONMENTAL INTERACTIONS

Progeny from the crosses which had given rise to the plants used in the glasshouse investigations were also grown in the experimental field (in summer 1967) as part of a different investigation. Two of the 18 families failed to establish and will not be considered further. Plants were raised in two randomised blocks sown 1 week apart; four plants from every family were grown in each block. Thus comparisons can be made between family means for the performance of plants growing in two quite different environ-

ments. This would give some indication of the existence of any genotype-environmental interactions for the characters measured.

As this was part of a large-scale experiment, the characters measured were restricted to five, namely the juvenile characters leaf number, height, diameter and elevation, measured at about the same stage as they were measured in the glasshouse, and also flowering time. It might be expected that some differences in performance would emerge, but it might also be reasonably expected that the order of performance of the different families would remain approximately the same. For example, families which, on average, flower early in the glasshouse would be expected to flower early in the field. This would be true if there were no strong genotype-environmental interactions operating.

This is important when considering plants growing under experimental conditions, where plants are grown as spaced plants in soil with a high nutrient status. If plants show strong genotype-environmental interactions, any result obtained from such experimental plants can be interpreted only with much caution, since performance could be very different in the natural environment.

Some genotype-environmental interaction is almost inevitable. For our present purpose, however, genotype-environmental interaction can be regarded as trivial, for the two environments chosen, if the overall performance of families is essentially the same in the two environments, in particular if the ranking of family means in the two environments is similar.

Accordingly, family means were ranked according to performance in glasshouse and field separately. For example, in the case of height, the tallest family was given a rank number 1, the next tallest number 2 and so on, for the two environments separately. Rank correlation coefficients between the rankings in the two environments for a given character were then calculated using Spearman's rank correlation coefficient ρ (see *e.g.* Yule and Kendall, 1953), according to the formula

$$\rho = \frac{\frac{1}{6}(n^3 - n) - (T_x + T_y) - \Sigma(d^2)}{\frac{1}{6}\{(n^3 - n) - 2T_x\}^{\frac{1}{2}}\{\frac{1}{6}(n^3 - n) - 2T_y\}^{\frac{1}{2}}}$$

where

n = number of pairs ranked

d = difference in rank value for any one pair

T_x, T_y = allowance for tied ranks for the first and second ranks respectively, calculated from

$T_x = \Sigma \frac{1}{12}(t^3 - t)$, t being the number in a particular tie and similarly for T_y .

This coefficient was calculated for the four juvenile morphological measurements made and also for flowering time. Results are given in table 5.

In addition, analyses of variance were carried out on the data in order to check whether a character showing heritable variation under glasshouse conditions was also doing so under field conditions. The components of variation expressed as percentages are presented in table 6. It will be seen that leaf number gave no heritable variation under field conditions.

Turning now to table 5, we note that fairly high positive correlations are found between performance in the two environments for the characters

height, diameter and flowering time. This indicates relatively little genotype-environment interaction for these characters. Of course, we should have to survey performance over a much more extensive series of environments before attempting to extend this statement to environments in general.

TABLE 5
Rank correlations between performance in two environments

Leaf number			Height		
	BK II	GH7	GH8		
BK I	0.31	-0.01	0.07	BK I	0.62*
BK II		0.20	0.56*	BK II	0.61*
GH7			0.54*	GH7	0.75*
					0.60*
					0.84*
Diameter			Elevation		
	BK II	GH7	GH8		
BK I	0.83*	0.38	0.58*	BK I	0.69*
BK II		0.63*	0.75*	BK II	0.36
GH7			0.89*	GH7	0.61*
					0.23
					0.55*
					0.48
Flowering time					
	BK II	GH			
BK I	0.92*	0.88*			
BK II		0.88*			

BK I, BK II—Blocks of field experiment.

GH7, GH8—Measurements in the glasshouse at 7 and 8 weeks respectively.

* $P < 0.05$

TABLE 6
Components of variation (percentage) under field conditions

BLOCK I			
Character	σ^2	σ_F^2	σ_P^2
Leaf number	83.67	4.08	12.24
Height	25.46	49.38***	25.16
Diameter	25.69	34.08***	40.23*
Elevation	52.21	44.83***	2.96
Flowering time	16.28	28.67***	55.05*
BLOCK II			
Character	σ^2	σ_F^2	σ_P^2
Leaf number	69.73	11.50	18.71
Height	20.08	37.57***	42.34*
Diameter	46.14	6.88	46.98**
Elevation	54.36	35.44***	10.20
Flowering time	21.22	29.30***	49.48*

Notation as in table 1.

On the other hand, elevation showed less, and leaf number very little agreement. This is as expected for leaf number, since variation in the field for this character was entirely (or almost entirely) environmental. This is reflected in the non-significant correlation between performance in the two

blocks. The correlation for leaf number between performance in block II and in the glasshouse at 8 weeks is, however, very puzzling and does not seem to admit of any explanation, apart from sampling error.

With regard to height, the plants measured in the field were considerably more advanced in development (*i.e.* greater in overall mean) than when measured in the glasshouse. Plants in block II were, on average, taller than those in block I. Thus, in terms of development, the sequence is

GH7, GH8, BKI, BKII.

Good agreement was found between the performance of families in the two blocks. In accordance with the time sequence just given, field data agreed more with GH8 than with GH7, BKI giving rather better agreement than BKII. These results indicate that, provided measurements can be made at corresponding stages of development, results in the two environments will be in good agreement, *i.e.* genotype-environmental interaction need not be a serious problem when working with this character.

A very similar picture is found for diameter, except that, for some obscure environmental reason, the sequence was found to be

GH7, GH8, BKII, BKI.

On the whole, correlations between glasshouse and field performance are rather lower for diameter than for height.

Observations subsequent to those described here made it clear that elevation tends to fall as the plants develop. Plants were, on average, less elevated in block I than in block II, so that for this character the sequence is

GH7, BKII, BKI.

As mentioned previously, GH8 measurements are not comparable with the GH7 measurements, as they were made on a different basis. The field data correspond to the GH7 type of measurement; BKII but not BKI data are in good agreement with GH7, as expected.

The flowering time data show excellent agreement between performances in the two environments.

Thus, for the two environments studied, genotype-environmental interaction would probably be small for the characters height, diameter, elevation and flowering time, if a method could be found for standardising the stage at which the first three characters were measured. If these results apply to environments in general, it follows that results obtained in the experimental field could be related to those which would be obtained under natural conditions, provided the time-table of events in these two environments could be related to one another.

A critical test of this notion would be to measure the heights say, of a number of plants under near-natural conditions at a number of stages of development. If these plants were then selfed, progenies raised on the experimental field and these progenies also measured at various stages, correlations between offspring and parent could be estimated and tested for significance.

6. SUMMARY

1. Previous work has shown that three out of four characters studied show genetic variation within natural populations of *Papaver dubium*. Fourteen

more characters have now been investigated. Out of a total of 18 characters, 13 show such genetic variation within populations, which must therefore be a very general phenomenon in this species.

2. In some cases two or more characters are the expression of the same set of genes. However, correlations between family means for the different characters taken in pairs indicate the presence of at least three different polymorphisms.

3. For at least some characters and particularly for flowering time, results so far indicate that genotype-environmental interaction is small. If this result holds over environments in general, it will be possible for such characters to infer the relative performance of different families under natural conditions from their performance in the experimental field.

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