VARIATION IN WILD POPULATIONS OF *PAPAVER DUBIUM*

I. VARIATION WITHIN POPULATIONS; DIALLEL CROSSES

M. J. LAWRENCE
Department of Genetics, The University, Birmingham, 15

Received 9.xi.64

1. INTRODUCTION

An increasing amount of attention has been directed in recent years towards variation in wild populations of animals and plants. Dobzhansky and Ford together with their colleagues have repeatedly demonstrated the importance of polymorphisms in populations with respect to characters as diverse as those of the nuclear, the physiological and the morphological phenotype (cf. Dobzhansky, 1950 and Ford, 1964). Most studies, however, have been concerned with discrete characters. Less attention has been paid to genetic variance in populations with respect to continuously variable characters. Yet since inspection of the differences distinguishing closely related forms reveals that they are far more often of a quantitative nature (Mather, 1943) it is clear that our understanding of the way in which evolution causes speciation will remain incomplete until our knowledge of the genetic basis of this variation in populations becomes more complete than it is at present.

It was chiefly for this reason that a series of experiments were begun in 1958 with the long-head poppy, *Papaver dubium*. This species was chosen for study because it has a number of technically convenient attributes, among which are that it is easy to grow, that it produces large quantities of seed, that it can be readily self-pollinated and that it is common both in this country and the European continent.

The purpose of the investigation was to ascertain the amount and kind of genetic variance with respect to a quantitative character within and between populations. Five populations of *P. dubium* were sampled by obtaining seed from each and raising the resulting progenies in a randomised block experiment. These progenies clearly differed in respect of several characters, among them being the time at which the first flowers opened (tables 1 and 2). This character, referred to as flowering time, was chosen for further study.

Plants taken at random from each sample were used as parents in several breeding programmes including diallel crosses and random mating. In the present account we shall be concerned with results from diallel crosses carried out within samples, that is, within populations.

TABLE 1

Mean flowering times (in days) of the population samples (upper figures) and of the four parents chosen at random from them (lower figures). The standard error of the sample means is 0·5

Population sample				
S_1	S_2	S_3	S_4	S_5
29·3	16·3	14·9	15·8	19·8
26·0	15·0	14·5	15·5	19·5

TABLE 2

Analysis of variance of flowering time. Each population sample was represented in each of three blocks by a single plot of five plants (the average flowering times of these fifteen plants are shown in table 1

Item	N	MS
Populations	4	105·44***
Blocks	2	3·57
Populations × Blocks	8	
Total	14	

*** = $P<0·001$.

2. MATERIALS AND METHODS

The place from which each sample was obtained is shown in table 3. In each locality samples of seed were taken from ten randomly chosen plants by obtaining a single ripe capsule from each. The seed from each capsule was then mixed with that from the others and sown in 1958 in the experimental field in Birmingham. The seed obtained from Poznan (S_5) came from plants in the botanic garden there.

TABLE 3

Location of the material

Population sample	Location
S_1	Pittenweem, Fife, Scotland
S_2	Burntisland, Fife, Scotland
S_3	Birmingham, Warks, England
S_4	Lea Marston, Warks, England
S_5	Poznan, Poland

Four plants chosen at random from each population sample were then crossed in all possible ways to raise a 4×4 set of diallel crosses except that reciprocal crosses were not included. The F_1 progenies of these diallel crosses were grown in 1959 and 1960 and were used to raise F_2 progeny which were grown in 1961.

Although all experiments were of the randomised block design those grown in 1959 and 1960 differed from those grown thereafter. Originally each family in an experiment were represented in each of two blocks by a pair of plots containing five plants each, the plots being independently randomised within each block. In 1961 the same number (20) of plants were distributed individually at random within each of the two blocks.

The original procedure, which is standard to many agricultural experiments, suffers from the disadvantage that there is considerable correlation between the phenotypic values of members of the same plot because they enjoy a common environment. In consequence, it is necessary to partition total environmental variance into within plot and between plot items. Single plant randomisation on the other hand, is more efficient because it allows all the information relating to environmental variance in an experiment to be used to estimate a single component. This distinction between the designs of the experiments must of course be borne in mind when considering the analysis of their results.

3. RESULTS

(a) *Family means*

The mean flowering times of the F_1 and F_2 families are shown in table 4. (It should be made clear that the F_1 and F_2 terminology used here is not strictly correct since the parents being derived from wild populations are not necessarily inbred.) There is little doubt that overall the differences between populations with respect to flowering time are no less than they were between the original samples (table 5). Nevertheless, it is also apparent that the F_1 and F_2 material flowered on average later than the parents and that the ranking of the samples changed considerably over years.

The first point is of small consequence for although 31st May has been used in all seasons as the arbitrary origin of the scale used to measure flowering time, cultural operations varied from one year to the next. The second point is of greater importance to our enquiry for if the performance of the population samples changes differentially over seasons, confidence in being able to predict the genetic situation obtaining in wild populations from a knowledge of that obtaining in the breeding material must obviously be impaired. When the sample means are plotted against seasons, however, it becomes apparent that S_5 is largely responsible, the remaining samples behaving in a reasonably consistent manner (fig. 1). We must defer discussion on this point for a later section of this paper.

(b) *Analysis of variance: assumptions*

The assumptions we need to make concerning the inheritance of genes determining flowering time are those customary with the analysis used. It is important, however, to re-examine their validity here in

TABLE 4

*Mean flowering time (days) of F_1 and F_2 families of the sets of diallel crosses. The grand mean for each 4 × 4 set is shown in the bottom left-hand corner of each square. The F_1 family means are averaged over two seasons. † = cross failed; entries are means of array means. * = averaged over three blocks only (see text)*

Population sample		S_1				S_2				S_3				S_4*				S_5*			
Generation	Parent	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
F_1	1	40·7	36·4	41·0	39·1	20·5	27·1	24·1	24·5	35·7	42·9	35·6	36·6	39·8	41·3	30·9	36·2	19·1	17·9	19·4	16·9
	2		38·4	38·7	40·0		38·1	31·2	36·7		51·7	41·0†	41·3†		39·3	34·9	41·2		28·6	26·7	20·1
	3			39·7	34·8			26·0	30·1			33·1	35·1			26·9	30·2			26·3	18·1
	4	39·0			41·2	29·6			38·0	38·7			34·1	36·4			43·4	21·3			20·1
F_2	1	33·3	33·4	35·1	29·8	21·0	28·0	25·1	24·2	35·6	38·9	41·0	34·6	40·5	34·7	25·4	33·6	16·3	20·4	17·7	16·8
	2		28·6	25·5	27·4		38·4	35·3	38·7		53·1	40·7†	40·1†		30·9	26·3	33·6		25·1	23·8	23·0
	3			33·7	36·5			24·8	23·3			27·2	37·5			22·2	28·4			21·5	19·8
	4	33·2			49·1	29·7			38·3	38·4			35·5	30·7			30·9	20·3			18·8

view of our lack of knowledge about the genetic system and gene-action in this species.

The assumptions that have been adopted are:—

1. no genotype-environment interaction;
2. homozygous parents;
3. diploid segregation;
4. no reciprocal differences;
5. no epistasis;
6. no multiple alleles; and
7. uncorrelated gene distributions among parents.

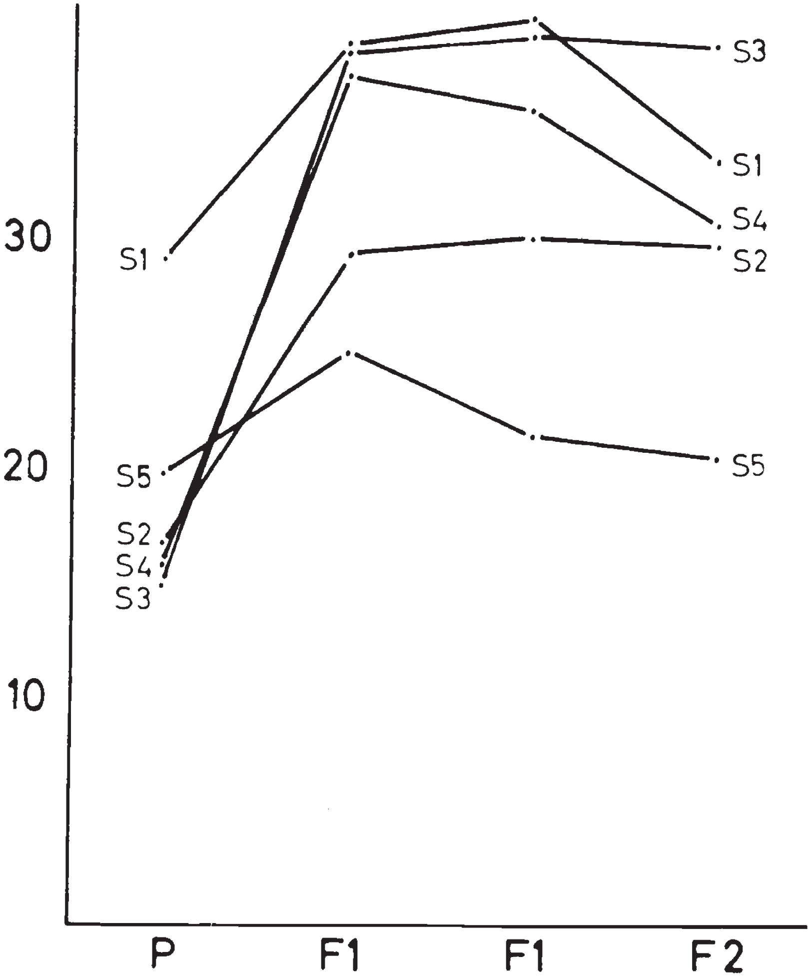

Fig. 1.—The variation of average flowering time over seasons.

Only the sixth is untestable. Since the main effects of the analysis of variance are with one exception (S_5 at F_2) consistent over blocks and years, there is little evidence of genotype-environment interaction. Again, although no reciprocal crosses have been raised in the present experiments, my colleague Dr M. J. Kearsey, who has investigated S_3 in detail, has found no evidence of maternal differences in respect of flowering time (Kearsey, 1965).

The assumption of diploid inheritance rests more on circumstantial than direct evidence. The chromosome numbers of those species in the same section of the genus as *P. dubium* indicate that it is a hexaploid. But my colleagues Dr B. John and Dr M. D. Hayward who have very kindly examined the chromosomes at P.M.C. meiosis find that they always pair to form bivalents. Furthermore, on general grounds it is likely that the species arose via amphiploidy. It is unlikely, therefore, that we need to consider the possibility of polysomic inheritance.

The remaining assumptions (2, 5 and 7) can be tested with respect to some of the populations by means of the covariance-variance analysis (W_r/V_r graph) of Jinks (1954, 1956) and Dickinson and Jinks (1956). Because this analysis is concerned mainly with dominance variation it can be applied only where we have prior evidence (from the analysis of variance of family means) of dominance. Given these circumstances, where the parents are homozygous and have uncorrelated gene distributions and where there is no epistasis the points of the W_r/V_r graph are expected to lie on a straight line of unit slope. Failure of any of these assumptions causes a characteristic disturbance of this relationship.

Now since we are concerned with five populations each of which is represented by an F_1 and an F_2 generation we may expect to obtain a maximum of ten graphs. As we shall see, however, the analyses of variance associated with three of these (S_1 and S_3 at F_1; S_5 at F_2) failed to indicate any significant dominance effects. The graphs of the remaining seven sets of families are shown in fig. 2. The regressions of three of these (S_4 at F_1; S_2 and S_3 at F_2) although not quite significant are clearly similar to the significant regressions of S_2 and S_5 at F_1, and S_1 and S_4 at F_2 and in none of the graphs is there any suggestion of disturbances characteristic of epistasis or correlated gene distribution. There is, however, a suggestion that the parents are not complete homozygotes for there is a tendency for intermediate points to lie above a line connecting the extreme points. Although we have no test of significance of these deviations it does suggest a mild degree of the triangulation typical of that produced by heterozygous parents (Dickinson and Jinks, 1956). Be this as it may, the differences between family means sufficiently exceed those obtaining within families to allow the assumption of homozygosity of the parent individuals to be carried out not merely through the analysis of variance but also, as we shall see, through the analysis of second degree statistics also.

(c) *Analyses of variance: results*

The total variance within each set of diallel crosses has been partitioned into the two main effects of Yates (1947) and Hayman (1954*a*) which are the *a* and *b* mean squares (due allowance having been

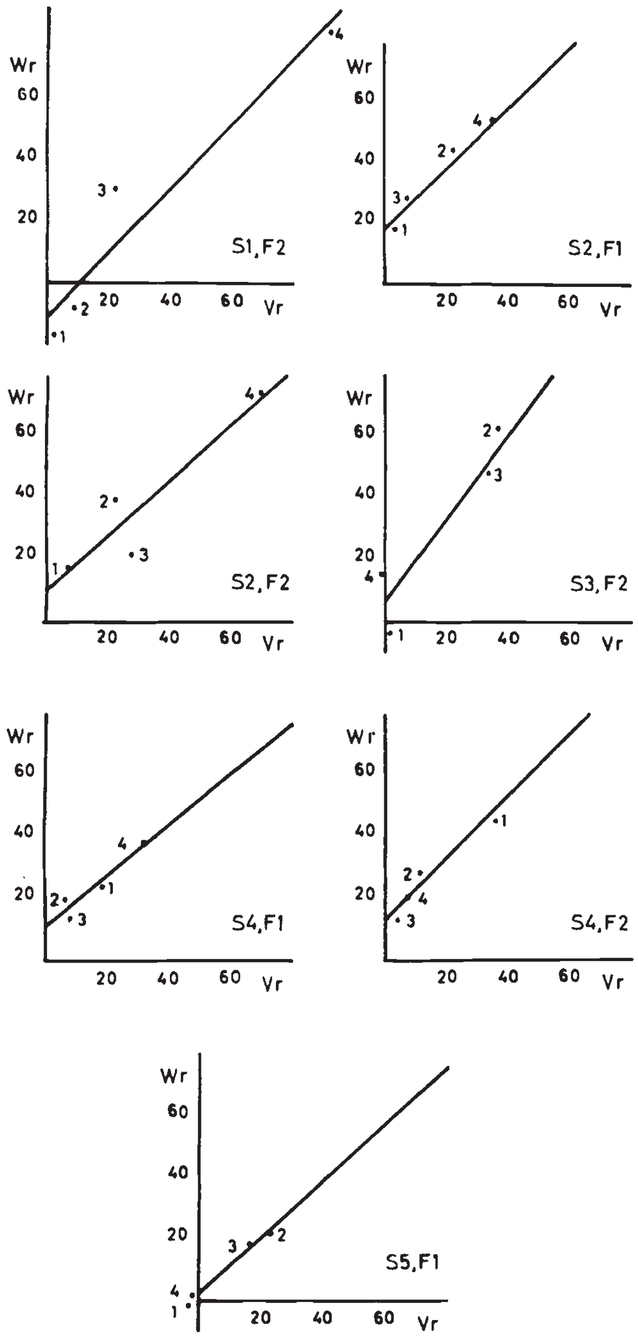

Fig. 2.—The regression of array covariance (W_r) on array variance (V_r) in respect of flowering time. Those of S_2 and S_5 at F_1 and those of S_1 and S_4 at F_2 are significant and none departs significantly from unity. The numbering of the points indicates the common parent of the array (see table 4). All statistics have been adjusted in respect of environmental variance.

made for the absence of reciprocal crosses in the present design (Morley Jones, 1965)). These items estimate variation in respect of the average effects of each parent on its progeny (*a*) and variation not ascribable to this cause (*b*). In a statistical sense the mean squares measure additive and non-additive variance respectively. In breeding parlance this amounts to *a* estimating general combining ability and *b* specific combining ability. Their genetic interpretation is that *a* estimates (primarily, though not exclusively) additive, fixable variance while *b* estimates dominance or unfixable variance.

The *b* mean square can be partitioned into an item measuring mean dominance (b_1), another measuring dominance deviations attributable to particular parents (b_2) and a third measuring deviations not ascribable to b_1 or b_2 (b_3).

Hayman (1954*a*) has given the genetic expectations of these mean squares in the case of a full diallel with homozygous parents. In general irrespective of the breeding system obtaining among the parents, the *a* mean square is proportional to twice the variance of array means ($2\sigma^2_{Br}$) while the *b* means square is proportional to the difference between the mean variance of arrays and the variance of array means ($\sigma^2_{Wr}-\sigma^2_{Br}$). The total variance is thus $\sigma^2_{Br}+\sigma^2_{Wr}$. The expectations of these statistics in terms of Jinks's (1954, 1956) and Haymans's (1954*b*) components are given in the next section.

The model of the analysis of variance appropriate in the present circumstances is of course Eisenhart's (1947) Model II. For this reason and because it is hoped to make the analysis more widely intelligible the expectations of the *a* and *b* mean squares are shown in table 5 in conventional notation also.

The remaining items in the analysis are those customary to experiments which have been replicated over blocks and over years. The significance of these interaction items together with that of the main effects was tested in the case of the F_1 diallel crosses by the duplicates item, this being the average variance between duplicate plot means within families, blocks and years. The degrees of freedom carried by this item vary from one population to the next because of missing plots (S_1 and S_3) and because of excluded data (S_4 and S_5). The missing plots of (S_3) were caused by the failure of two of the initial crosses and are responsible for the loss of eight degrees of freedom (two from each of four blocks). The missing values were estimated as the means of the appropriate array means. This procedure, may of course, deflate the variance within the affected arrays and should be accompanied by an adjustment of the degrees of freedom in the analysis. No adjustment has in fact been attempted because of the difficulty of doing so in this type of analysis (Morley Jones, personal communication).

The results from the second block of S_4 in 1959 and that of S_5 in 1960 do not appear in either table 4 or 5 because preliminary analysis showed that they provided no evidence of genetic variance in respect

TABLE 5*a*

Item	N		S_1	S_2	S_3	S_4	S_5
a	3		0·92	892·71***	748·19***	473·05***	218·99***
b	6		52·49	27·82	10·69	40·58**	50·54**
b_1		1	57·20	57·13*	0·31	36·93	202·65***
b_2		3	17·11	17·59	20·32	68·37**	4·21
b_3		2	103·19*	28·51	1·45	0·72	43·99
total	9		35·30	316·11	256·44	184·74	106·69
Years	1		89·40	17·42	32·04	496·65***	886·72***
Y*a*	3		67·27	43·58*	3·94	25·92	25·11
Y*b*	6		33·22	19·01	4·20	4·29	7·19
Y*t*	9		44·57	27·20	4·11	8·62	10·83
Blocks	2		3449·51***	1934·13***	1783·62***	—	—
Ba	6		25·39	12·94	13·64	—	—
Bb	12		20·37	7·42	11·56	—	—
Bt	18		22·05	9·26	12·25	—	—
Total	39		119·35	183·13	112·36	117·73(19)	102·34(19)
Duplicates			31·27(38)	17·30(40)	17·83(32)	10·94(30)	16·20(30)

TABLE 5*b*

Analyses of variance of F_1 5(a) and F_2 5(b) diallels. Entries are mean squares. The degrees of freedom of the duplicates, the within families and the total M.S.'s of the S_4 and S_5 F_1 analyses are shown in brackets. The expectations of the a *and* b *mean squares of the main components of variance are:*

$$F_1\ \mathrm{a} = \sigma^2 + 2\sigma_s^2 + 12\sigma_g^2 \text{ and } F_2\ \mathrm{a} = \sigma^2 + \sigma_s^2 + 6\sigma_g^2$$

$$\mathrm{b} = \sigma^2 + 2\sigma_s^2 \qquad \mathrm{b} = \sigma^2 + \sigma_s^2$$

where σ^2 = sampling component; σ_s^2 = specific combining ability and σ_g^2 = general combining ability.

Item	N	S_1	S_2	S_3	S_4	S_5
a	3	131·62***	245·37***	178·62***	163·66***	53·33***
b	6	65·28***	29·77***	40·05***	11·18**	0·76
b_1	1	115·25***	11·22*	4·14	20·34*	0·12
b_2	3	56·62***	28·75***	72·34***	12·55**	1·43
b_3	2	53·30***	40·59***	9·55	4·56	0·06
total	9	87·40	101·64	86·24	62·01	18·28
Blocks	1	80·00***	127·01***	118·58***	195·23***	562·86***
Ba	3	6·49	5·00	23·68*	3·83	17·32***
Bb	6	3·17	3·42	4·96	1·06	0·88
Bt	9	4·27	3·95	11·20	1·98	6·36
Total	19	47·63	56·70	52·40	50·69	41·30***
Within families		2·80(157)	2·80(162)	7·47(114)	3·10(171)	1·62(170)

* $P = 0{\cdot}05$-$0{\cdot}01$; ** $P = 0{\cdot}01$-$0{\cdot}0001$; *** $P < 0{\cdot}0001$.

of flowering time. Nor were they consistent with their sister blocks which did. The results from these blocks were therefore excluded from further analyses and their exclusion is responsible for the absence of any blocks × main effects items in the S_4 and S_5 analyses.

The corresponding sampling variance of the F_2 analyses is the within families item. Remembering that the F_2 experiments were grown under the single plant randomisation design we may validly compute the sampling variance appropriate to family means from that relating to variation between individual plants within families and blocks. As before, the individual within families items carry different numbers of degrees of freedom, but since in all analyses these are many we may use the χ^2-test of significance.

Occasionally the sampling variances of both the F_1 and F_2 analyses turned out to be significantly heterogeneous over blocks or years or both. They were nevertheless pooled as it is unlikely that we shall be misled into serious errors of interpretation by this practice in the present context.

Lastly, it should be made clear that these sampling variances are not pure estimates of environmental variance. Insofar that the material under investigation is not inbred, all sampling variances must be expected to be inflated by a genetic component whose size will naturally depend on the amount of segregation occurring in the material.

Turning now to the results of the analysis of the F_1 generation, we notice that four populations (S_2, S_3, S_4, S_5) display large and highly significant amounts of additive variance with respect to flowering time (the a item). There is in addition some evidence in S_2 that this additive variance is expressed differently in different years. Since, however, a is significantly greater than the Ya item there is little doubt that interaction is a relatively trivial source of variance here.

In three of the populations there is also clear evidence of dominance with respect to flowering time, though this is expressed in different ways in different populations. Thus the significance of the b_1 item in the S_2 and S_5 analyses indicates that in these populations dominance is manifested as a simple difference between the average flowering times of F_1 progenies and those of their parents selfed. A dominance difference of this type is perhaps better described as potence (Wigan, 1949). The significance of the b_2 item in the S_4 analysis on the other hand indicates a more complex situation, for here there are differences between arrays in respect of dominance. This in turn implies that the parents have made different contributions to their progenies in respect of genes which display dominance. However, as Hayman (1954*a*) points out, when the average gene frequencies of those alleles determining flowering time and displaying dominance are equal in the parents, these parental contributions balance out and there are no detectable dominance differences between arrays. Under these circumstances the expectation of b_2 is zero. Thus the non-significance of the b_2 mean squares in the S_2 and S_5 analyses does not necessarily imply that in these populations the parents make no differential contribution to their progenies in respect of dominant genes. We can now extend our conclusions concerning dominance in S_4 for at the loci displaying dominance in respect of flowering time the frequencies of increasing and decreasing alleles must be unequal. Reference to table 4 shows that it is the increasing alleles which are in excess for only one of the parent plants were early-flowering, the others being late-flowering. In S_2 and S_5 on the other hand, there are equal numbers of early- and late-flowering parents. We cannot, therefore, expect the b_2 item of their analyses to be significant.

Despite this equality of gene frequencies there is in fact good evidence in these populations of differential parental contributions in respect of dominant alleles. We have seen in the previous section that it was possible to fit significant linear regression lines to the W_r/V_r graphs of the F_1 generation in both of these populations (fig. 1). But this is possible only if the parents do in fact make differential contributions to their progenies in respect of dominant alleles. Thus although the b_2 item of the analysis of variance and the scatter of points of the W_r/V_r graph both measure the same kind of dominance variation, the latter is insensitive to variation of gene frequency, whilst the former is not. Taking into account, therefore, the evidence from both these analyses, there is little doubt that in all populations which display dominance (S_2, S_4 and S_5) that this takes the form of differential contributions from the parents in this respect.

The remaining significant mean squares of the F_1 analyses of variance (years in S_4 and S_5, blocks in S_1, S_2 and S_3) require no comment for they derive their significance from seasonal and cultural differences which as mentioned previously are of no consequence for our present enquiry.

The results yielded by the analysis of the F_2 generation agree on the whole with those of the previous generation. Thus S_2, S_3, S_4 and S_5 exhibit as before considerable additive variance with respect to flowering time. There appears, however, to be much more variation due to dominance at F_2, both in respect of quantity and nature, than there was in the F_1 material. This outcome is of course unexpected. Insofar as a generation of inbreeding by selfing is expected to halve the proportion of heterozygotes it should decrease the contribution which dominance makes to the total genetic variance by a half. We thus expect to find less evidence of dominance at F_2 than in the F_1 generation.

Part of the explanation of this apparent paradox lies in the difference between the generations in respect of the precision of tests of significance. The sampling variances of the F_1 generation must be scaled down to one-quarter of their size in table 5 if they are to be compared with those of the F_2 generation (due to differences in the analysis of the experiment). When this has been done, however, those from the F_2 generation of S_1, S_2 and S_5 are smaller than the corresponding items from the previous generation and in all cases the degrees of freedom attaching to the latter are much less than those of the former. In short, the precision of comparisons in the F_2 analyses exceeds those of the F_1 generation. This increase in precisions, which has resulted in the appearance of dominance at F_2, is of course a consequence of the change in the design of the experiments mentioned earlier.

In S_1 this difference in precision is particularly marked and has resulted in the appearance of both significant additive and dominant variation at F_2. We note also, that in this population b_3 is significant, as it is in S_2 also. This implies that there are dominance differences within arrays as well as those between arrays and between the F_2 progenies and those of their parents selfed.

The analysis of the F_2 generation of S_5 deserves comment in respect of two points, the first of these concerning the significance of the *Ba* item. Since the *a*/*Ba* ratio is not significant there is no doubt that additive variance is manifested in different ways in different environments. This, however, is the only instance we have encountered in the analyses of genotype-environment interaction and is doubtless due to the early-flowering habit of this population which could render it particularly sensitive to the viscitudes of the environment.

The second point concerns the absence of significant dominance variation at F_2 despite its presence in the F_1 generation of this population. This state of affairs is, of course, the converse of that for other populations. While the absence of detectable dominance could be due to a generation of inbreeding it is worth noting that because of the change of the structure of the experiments previously mentioned the power of the F_2 analyses with respect to specific combining ability is only half that of the F_1 analyses. Thus both the proportion of heterozygotes and the power of the analysis are reduced by one-half as we pass from the F_1 to the F_2 generation in the present circumstances. Nevertheless, since the precision of comparisons in this population at the same time increases considerably, we cannot be certain that the reductions in power or heterozygosity (or both) are a sufficient explanation of the absence of dominance at F_2.

(d) *Components of second-degree statistics*

The results from the analyses of variance leave no doubt that both additive and dominance variation are of importance in respect of flowering time in *P. dubium*. We now wish to know, however, whether populations differ in respect of amounts of genetic variance. The analysis of variance cannot conveniently be used to answer this question since there is no cross-classification obtaining between populations.

We can, however, approach this question in another way. Thus we can obtain estimates of genetic components from certain second-degree statistics from each population sample and compare the estimates from one sample with those of another. The methods and models used are those of Mather (1949), Jinks (1954, 1956) and Hayman (1954*b*). As before, a homozygous model is fitted by the method of unweighted least squares to the statistics in question. The latter, together with the model that has been fitted to them are shown in table 6. The expectations of the mean variance of arrays (σ^2_{Wr}), the variance of array means (σ^2_{Br}) and the mean variance of arrays ($\text{cov}^2_{r/P}$) change with generation. The expectation of the variance of parents (σ^2_{P}) does not of course change on a homozygous model. Nevertheless, it was thought prudent to keep separate the F_1 and F_2 estimates of this variance because of possible difficulties due to the change in the design of the experiment. For the same reason the F_1 and F_2 estimates of " environmental " variance (σ^2_{E}) also have been kept separate.

Now the expectations appearing in table 6 are those appropriate to an infinitely large statistical population. Since, as mentioned earlier, we regard the four parent individuals of each set of diallel crosses as random samples obtained from their respective wild populations it is clear that the estimates of the statistics must be adjusted for bias due to sampling variance. Any statistic (all except σ^2_{Br}) which measures variation between family means must be adjusted by removing an appropriate portion of within family variance. This in fact is carried out automatically because all these statistics contain an environmental component which is computed from within family variance. Only the variance of array means therefore requires special consideration and the appropriate adjustment is carried out by subtracting $1/n$th of the mean variance of arrays from it. All estimates shown in table 6 have been adjusted in this way.

The first model fitted to these statistics was the six-component model shown in table 6. This model proved to be adequate in respect of four of the population samples, the exception being S_1 where since only the F_2 information is relevant we have a perfect fit and no test of significance was therefore available. In the other populations, since ten statistics may be estimated, four degrees of freedom remain for testing the goodness of fit of the model to these statistics. The mean square associated with these four degrees of freedom (the remainder item) was compared with a replicates item obtained from a preliminary analysis of variance of the statistics which measures the consistency of the estimates over replicates (blocks and years). Where this comparison showed that the mean squares were homogeneous the model was considered adequate. Under these circumstances the standard errors of the estimates of the component can be obtained in the usual way.

The result of fitting this model to each population showed that many of the components were not significantly different from zero

TABLE 6

Estimates of second-degree statistics and their expectations in terms of the genetic components of Jinks (1954). $D = 4\Sigma uvd^2$; $H_1 = 4\Sigma uvh^2$; $H_2 = 16\Sigma u^2v^2h^2$; *and* $F = 8\Sigma uv(u-v)dh$

Generation	Statistic	Components						Estimates				
		D	H_1	H_2	F	E_2'	E_2''	S_1	S_2	S_3	S_4	S_5
F_1	σ^2_P	1·0000	—	—	—	1·0000	—	—	85·39	84·16	56·88	16·85
	σ^2_{Wr}	0·2500	0·2500	—	−0·2500	1·0000	—	—	25·99	18·59	30·24	15·67
	σ^2_{Br}	0·2500	0·2500	−0·2500	−0·2500	0·2500	—	—	13·32	9·01	7·72	3·82
	$\text{cov}_{r/P}$	0·5000	—	—	−0·2500	0·2500	—	—	39·88	37·25	26·31	6·40
	σ^2_E	—	—	—	—	1·0000	—	—	4·33	4·46	2·73	4·05
F_2	σ^2_P	1·0000	—	—	—	—	1·0000	80·47	84·00	132·20	62·66	16·24
	σ^2_{Wr}	0·2500	0·0625	−0·0625	−0·1250	—	1·0000	35·90	35·93	28·61	17·19	7·15
	σ^2_{Br}	0·2500	0·0625	—	−0·1250	—	0·2500	0·14	12·81	4·08	8·50	5·22
	$\text{cov}_{r/P}$	0·5000	—	—	−0·1250	—	0·2500	23·51	39·78	36·88	27·95	11·04
	σ^2_E	—	—	—	—	—	1·0000	2·80	2·82	7·48	3·10	1·63

and therefore redundant. The next stage in the analysis was therefore to attempt to fit a simpler model with the proviso that it must be common to all five population samples. This is necessary because we wish to compare populations in respect of values of the components of variation.

The appropriate model turned out to be one consisting of the four genetic components only and the values of the components obtained from each population are shown in table 7. Evidently in all five populations it is not necessary to take environmental variation in respect of flowering time into account. This does not mean of course that this character is not subject to this course of variance but only that within the context of a somewhat crude unweighted analysis and in the presence of very considerable amounts of heritable variance the environmental component is of no importance.

TABLE 7

Values of estimates of the four components (common model) obtained from each population sample

Component	S_1	S_2	S_3	S_4	S_5
D . . .	80·47 ± 2·67 ***	85·20 ± 3·32 ***	93·55 ± 6·71 ***	58·60 ± 2·55 ***	16·94 ± 1·93 ***
H_1 . .	520·02 ± 61·75 ***	31·71 ± 21·11 NS	24·33 ± 31·77 NS	77·49 ± 17·26 ***	50·63 ± 13·06 ***
H_2 . .	572·16 ± 60·82 ***	55·58 ± 20·99 *	43·79 ± 31·60 NS	93·27 ± 17·28 ***	46·95 ± 13·08 **
F . . .	133·78 ± 24·04 **	9·64 ± 15·98 NS	41·43 ± 24·05 NS	13·40 ± 12·82 NS	4·70 ± 9·70 NS

As regards this genetic variance, it is quite clear that this varies from one population to the next, both in respect of amount and of kind. In terms of amount, the additive variance of the population samples falls into three groups, where S_1, S_2 and S_3 comprise the first, S_4 the second and S_5 the third.

It will be recalled that S_5 originated from the botanic garden at Poznan in Poland so it is not perhaps surprising that this population has the lowest additive genetic variance, this being a consequence of its domestication. There is much variation between populations in respect of dominance variation also and again they fall into three, though different groups. Thus S_1 falls into the first, S_4 and S_5 into the second and S_2 and S_3, in which there is little, if any, dominance, into the third. Furthermore, where dominance is a significant source of variance, its level is high. Indeed, in S_1 and S_5, H, is significantly greater than D though in neither population have we encountered any previous evidence of significant overdominance. This result is therefore unexpected and no explanation of its cause can be advanced at present.

Taking both additive and dominance components into account the populations fall into four groups for only S_2 and S_3 appear to be similar in both respects. Before we go on to discuss the implications of these results, however, we ought not to overlook the fact that with the exception of S_1 some of the components in table 7 are redundant. In particular, we note that in the case of S_4 and S_5 that within the limits of sampling error $H_1 = H_2$ and $F = 0$. This situation implies that the average frequency of the increasing alleles which display dominance equals that of the decreasing alleles ($u = v$). Under these circumstances it should prove possible to fit a single dominance component, H together with the usual additive component to these populations. In other words, we need to take the analysis one step further by finding the simplest model capable of accounting for the variation between the statistics of each population in turn. The results of this operation are shown in table 8 from which it is clear that our prediction is correct.

TABLE 8

Values of estimates of components obtained by fitting the simplest model to the statistics yielded by each population sample

Component	S_1	S_2	S_3	S_4	S_5
D . . .	80·47±2·67 ***	83·97±2·57 ***	91·68±3·62 ***	56·33±2·26 ***	16·48±1·62 ***
H . . .	—	—	—	66·08±12·57 ***	46·24±9·01 ***
H_1 . .	520·02±61·76 ***	—	—	—	—
H_2 . .	572·16±60·82 ***	—	—	—	—
F . . .	133·78±24·04 **	—	—	—	—

4. DISCUSSION

We have seen that the population samples investigated in these experiments provide clear evidence that flowering time in *P. dubium* is determined by genes, probably many genes, which display additive and can display dominance properties. We have seen also that the population samples differ in respect of their quantities of both these sources of genetic variance. The question that we must now turn to concerns the extent to which the genetic properties of these samples represent the situation obtaining in the wild populations from which they were obtained.

Now the relationship between the experimental material and the wild population will depend of course on the adequacy of sampling with respect to:—

1. the individual plants visited in the wild;
2. the seed obtained from those plants;

3. the individual plants raised in the experimental field from that seed; and
4. the individuals chosen as parents of the breeding programme designed to investigate the genetical situation obtaining in the population.

At each stage, sampling should be random and the samples taken be of adequate size. Yet, in practice, these objectives are not easily achieved. For example, adequacy in respect of sample size will depend on the variability of the material under investigation. But since the estimation and assessment of the nature of this variability is the main purpose of this investigation, it is clear that we cannot obtain this information in advance. Again, it is difficult to define what is meant by the term population in the case of an annual weed species. *Papaver dubium* can be found only on recently disturbed ground where it may, in the first year, become the near dominant species. In the second year, however, it has become virtually absent. These observations imply that in this and similar species it is the reservoir of seed present in a loosely defined locality which constitutes the population. Yet, since it has been shown repeatedly that the variance in respect of many morphological characters of seedling progenies greatly exceeds that of their parents, it is unlikely that the adult plants that we visit constitute a random sample drawn from a population of seed.

On the other hand, because the populations have been in fact sampled by taking seed rather than plants, this difficulty may not be too serious in practice. However, the way in which this seed is obtained can obviously affect sampling. Thus poppies shed their seed quite rapidly once the pores of any particular capsule have opened. On sampling a locality, therefore, seed can be obtained only from those capsules which have ripened recently. If only a single visit is made to a site, as in the present investigation, we may easily obtain a biased sample of seed. An investigation of the effect of stratified sampling is, however, in hand and it is hoped to report its results in a later publication.

The third and fourth stages of sampling are more amenable to investigation since they concern the fate of the material in the experimental field. At present, we have no information on variation between repeated samples of progeny obtained from a single sample of seed, though here again, this is now being investigated. On the other hand, it is clear from table 1 that the mean flowering times of each set of the four parents of the breeding programme correspond fairly closely with their population sample means with perhaps some bias in favour of early flowering.

All these points concerning sampling deserve emphasis not merely because they have an obvious bearing on the interpretation of the genetic information presented here, but also because there is some reason to doubt whether the population samples are in fact representative of the populations from which they were obtained. Thus we saw earlier that in terms of mean flowering time the performance of the population samples is not consistent over seasons (fig. 1). The major cause of this inconsistency is that the flowering time of S_5 varies rather little over seasons, while for the other population samples it varies considerably, though more or less uniformly. This could of course be due to genotype-environment interaction. The fact that the analyses of variance failed to provide much evidence of genotype-environment interaction is not strictly relevant since in those we were concerned with variation within population samples, whereas here comparisons concern differences between populations. We must withhold judgment on this possibility until more evidence is available.

A more serious possibility is that selection has caused these changes in respect of flowering time. Selection is, of course, always present however many precautions are taken to minimise it. Thus the act of bringing wild material into cultivation must nearly always be accompanied by considerable amelioration of the environment and hence a lessening of some forces of selection to which plants growing in the wild are subject. At the same time it could lead to an intensification of other forces of selection. Thus, on the one hand, there is no doubt that the size and vigour of plants grown in the experimental field far exceed that of plants observed in the wild, while on the other hand, the experimental regime of necessity imposes a uniformity on the material which is unlikely to be encountered in natural populations.

There are three observations which lend support to the notion that selection may have caused the differential response in respect of sample means to seasonal changes. The first is that the largest response in this respect was observed between 1958 and 1959. If the amelioration of the environment is indeed responsible for these changes in population means, we should expect the largest effect in the early generations of cultivation.

The second observation concerns the capacity of a population to respond to relaxation of selection pressure which must clearly depend on the amount of genetic variance available. Now we have seen that S_5 has less additive genetic variance than other populations and it is this population which has changed least from one season and generation to the next. It is therefore tempting to attribute its lack of response to domestication to this cause alone. But we must also recall that S_5 was obtained from the botanic garden in Poznan and had therefore enjoyed a cultivated environment before it was grown in Birmingham. Indeed, it is more likely, as has been mentioned earlier, that its relatively small genetic variance is due to just this history of cultivation.

The third observation here concerns the irregular germination of poppy seed in common with that of many wild species. Experimental requirements demand that we treat each plant as far as possible in the same way as every other and this in turn means that we inevitably select plants that have germinated at the same time. In practice, this procedure favours early germination. Yet it is unlikely that such uniformity is at a premium in the wild.

These observations about germination are relevant to the whole problem of selection, for although there were occasional losses of adult plants in the experimental field this was not of a scale sufficient to account for he effect of the presumed selection. The inference here is that po ulations possess genetic variance in respect of time of germination nd that through linkage or pleiotrophy this is correlated with flow ring time. The obvious advantage of this hypothesis is that it is testa le and once again an investigation of this is now in hand.

Doubt thoug there might be about the extent to which the genetic information obt ined from the experimental material is relevant to natural populat ns, it is reasonable to assume that differences which have survived u niform treatment reflect genuine differences in the wild material. What then may we infer about the genetical control of flowering tim in wild populations of *P. dubium*?

Firstly, there are considerable differences in respect of both additive and dominance variation between populations. The present data are, of course, n sufficient to indicate whether or not these differences reflect clinal va iation. Much more extensive sampling is needed before this type of evidence becomes available. Nor do we know the ecological circumstances of the localities sampled, so that we are unable to correlate the genetic differences observed with them. Nevertheless, it can hardly be doubted that these differences are caused by natural selection and their existence suggests that investigation along these lines would be worthwhile.

The second inference we can make concerns dominance. Although this was not recoverable by the component analysis in all populations, in those where it was (*e.g.* S_1, S_4 and S_5) it was found to have a high level. Yet comparisons of F_1 and F_2 family means with those of the parents showed that this dominance was balanced or ambidirectional in its expression. Mather (1960) has argued that such a situation implies a previous history of stabilising selection, for where selection is directional, dominance would be expected to be directional in its expression. While it is easy to see that it is undesirable for a plant to flower either very early or very late (thereby isolating itself) it is not obvious that response to stabilising selection need be accompanied by apparent overdominance, as it appears to be here.

Thirdly, the fact that the fitting of a homozygous model to the data has turned out to be successful suggests that the level of inbreeding within a population must be quite high. Though the species is easily selfed by hand observation suggests that outcrossing is common both because bees are frequent visitors to open flowers and because the level at which the anthers are borne is appreciably below the stigmatic disc.

Inbreeding, however, can result from assortative mating. Now clearly any particular plant can cross only with others that are flowering at the same time. While the total period of flowering of a population may well extend to 2 months or more that of its individual members is much less. Unfortunately, we have little information on flowering habit of individual plants in the wild, but Salisbury (1942) has shown that the modal number of capsules borne by wild plants is as low as two. Since it is a matter of observation that flowers open in rapid succession once a plant has come into flower, it is clear that individuals can hardly avoid mating assortatively. Thus a so-called population of poppies may well consist of an assemblage of small, partly isolated populations which together constitute a heterogeneous collection of partially inbred lines. Such an explanation would at least account for the apparent paradox of high genetic variance in the presence of conformity with the assumption of homozygous parents.

5. SUMMARY

1. Samples from five populations of *Papaver dubium* have been investigated in respect of a continuously variable character, flowering time.

2. Four plants from each sample were used to raise a set of F_1 and F_2 diallel crosses within each population.

3. The analysis of these crosses showed that flowering time is controlled both by additive and by dominance variation and that populations differ in respect of both these components of variance.

4. Despite this evidence of considerable genetic variance the assumption of homozygosity among parents of the crosses has in all populations proved to be reasonable. It is suggested, therefore, that populations of poppies comprise heterogeneous collections of relatively inbred lines, this being the expected outcome where mating in respect of flowering time is assortative.

Acknowledgments.—I am indebted to Dr J. L. Jinks for his advice and encouragement throughout the course of this work and to Professor K. Mather for his advice on the preparation of this publication.

6. REFERENCES

DICKINSON, A. G., AND JINKS, J. L. 1956. A generalised analysis of diallel crosses. *Genetics*, *41*, 65-78.

DOBZHANSKY, TH. 1951. *Genetics and the origin of species.* Columbia University Press, New York.

EISENHART, C. 1947. The assumptions underlying the analysis of variance. *Biometrics*, *3*, 1-21.

FORD, E. B. 1964. *Ecological genetics*, Methuen, London.

HAYMAN, B. I. 1954*a*. The analysis of variance of diallel tables. *Biometrics*, *10*, 235-244.

HAYMAN, B. I. 1954*b*. The theory and analysis of diallel crosses. *Genetics*, *39*, 789-809.

JINKS, J. L. 1954. The analysis of continuous variation in a diallel of *Nicotiana rustica* varieties. *Genetics*, *39*, 767-788.

JINKS, J. L. 1956. The F_2 and backcross generations from a set of diallel crosses. *Heredity*, *10*, 1-30.

KEARSEY, M. J. 1965. Biometrical analysis of a random mating population: a comparison of five experimental designs. *Heredity*, *20*, 205-235.

MATHER, K. 1943. Polygenic inheritance and natural selection. *Biol. Rev.*, *18*, 32-64.

MATHER, K. 1949. *Biometrical genetics*, Methuen, London.

MATHER, K. 1960. Evolution in polygenic systems. *Evolutzione e genetica*, *47*, 131-152.

MORLEY-JONES, R. 1965. The analysis of variance of the half-diallel table. *Heredity*, *20*, 117-121.

SALISBURY, E. J. 1942. *The reproductive capacity of plants*. Bell, London.

WIGAN, L. G. 1949. The distribution of polygenic activity on the x-chromosome of *Drosophila melanogaster*. *Heredity*, *3*, 53-66.

YATES, F. 1947. Analysis of data from all possible reciprocal crosses between a set of parental lines. *Heredity*, *1*, 287-301.