HEREDITY VOLUME 24 PART 3 AUGUST 1969

VARIATION IN WILD POPULATIONS OF *PAPAVER DUBIUM*

II. VARIATION BETWEEN POPULATIONS

M. J. LAWRENCE

Department of Genetics, University of Birmingham

Received 24.vii.68

1. Introduction

In a previous paper (Lawrence, 1965), the results from five sets of *intrapopulation* diallel crosses showed that this species varies in respect of both additive and dominance effects of the genes determining the quantitative character, flowering time. The populations sampled also varied in respect of their mean flowering time, so that it is clear that selection has caused both differentiation and divergence in this material. Yet because these crosses concerned plants drawn from the same population, we are unable to examine the genetic basis of the observed divergence between populations. In the present paper, the results from four sets of *interpopulation* diallel crosses are discussed.

2. Materials and methods

The original population samples, grown in a randomised block design in 1958, provided the parental material of the progenies discussed here and in the previous paper. Four plants were chosen at random from each of five population samples. These 20 parent individuals were then crossed in the manner shown in table 1, which is a balanced partial 20×20 diallel design. This design has the convenient property that the five sets of intrapopulation 4×4 diallel crosses (of the previous paper) can be " detached " from the four sets of interpopulation 5×5 diallel crosses discussed here. All diallel progenies shown in table 1 were grown in 1959. In 1960, only sets 1 and 3 of the interpopulation diallels were grown, these being raised from the same batch of seed used in the previous year. In both years the experiments were of the randomised block design in which the 20 sibs of each cross were distributed evenly between 2 blocks each containing a pair of independently randomised plots of 5 sibs per plot.

3. Results

It is convenient to present the results in four parts:

(*a*) the 1959 experiment,
(*b*) the 1960 experiment,
(*c*) the joint analysis of sets 1 and 3 grown in 1959 and 1960, and
(*d*) the interpretation of non-additive variance.

TABLE 1

The balanced partial 20 × 20 diallel

Population		S1				S2				S3				S4				S5			
Plant	♂	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	♀																				
S1	1	w	w	w	w	b				b				b				b			
	2		w	w	w		b				b				b				b		
	3			w	w			b				b				b				b	
	4				w				b				b				b				b
S2	1					w	w	w	w	b				b				b			
	2						w	w	w		b				b				b		
	3							w	w			b				b				b	
	4								w				b				b				b
S3	1									w	w	w	w	b				b			
	2										w	w	w		b				b		
	3											w	w			b				b	
	4												w				b				b
S4	1													w	w	w	w	b			
	2														w	w	w		b		
	3															w	w			b	
	4																w			b	
S5	1																	w	w	w	w
	2																		w	w	w
	3																			w	w
	4																				w

w = Crosses *within* and b = crosses *between* populations.

(a) *The 1959 experiment*

The sets of diallel crosses referred to here are genetic replicates in the sense that each population is represented in each set by a different parent plant. The mean flowering times of each plot in each family in the experiment were analysed by partitioning their total variation into additive (a) and non-additive items (b_1, b_2 and b_3) after Hayman (1954) and Jones (1965). We have in addition to these main effects a series of interactions concerning differences between sets, blocks and sets and blocks simultaneously. The duplicates mean square, which is calculated from the sum of squares of differences between duplicate plots within blocks, sets and crosses, provides an estimate of sampling error. The mean flowering times of the crosses grown in 1959 are shown in table 2 and the analysis of variance of these data in table 3.

The performance of crosses turned out to be consistent over blocks and over sets and blocks so that the mean squares measuring these effects have been pooled to provide the block interactions mean square of the analysis with 59 degrees of freedom.

The significance of all main effects (column (i) of table 3) shows quite clearly that differences between crosses are determined by genes with both additive and non-additive effects. Since, however, the sets $\times a$ and sets $\times b_3$ items are also significant it is equally clear that these main effects are not expressed consistently over sets. Now differences between sets can arise only if the parent plants sampled from the same population are genetically different. In other words, these interaction mean squares provide a method of detecting the presence of genetic variation *within* populations. Indeed, since we have previously established that each of the five populations are

genetically variable, we expect to find that the sets × main effects items to be significant here.

TABLE 2

Mean flowering time of families averaged over duplicate plots and blocks of the 1959 experiment.

Set 1

1	2	3	4	5	$\bar{r}$
38·2	26·8	28·4	43·8	25·2	32·5
	19·9	32·8	23·5	20·10	24·6
		36·6	37·1	24·4	31·8
			40·6	24·1	33·8
				21·6	23·1

Set 2

1	2	3	4	5	$\bar{r}$
36·6	38·4	42·3	43·0	31·6	38·4
	39·6	41·9	40·6	27·5	37·6
		50·5	42·4	34·1	42·2
			38·9	33·8	39·7
				31·7	31·7

Set 3

1	2	3	4	5	$\bar{r}$
35·9	41·9	33·6	34·4	24·9	34·1
	24·8	28·0	28·6	25·3	29·7
		32·7	35·4	31·3	32·2
			30·9	23·9	30·6
				28·0	26·6

Set 4

1	2	3	4	5	$\bar{r}$
40·6	35·6	39·9	43·4	25·2	36·9
	34·5	34·9	37·3	23·5	33·1
		34·3	33·6	25·2	33·6
			41·5	25·1	36·1
				24·2	24·6

r = Array mean.

TABLE 3

The analysis of variance of the 1959 experiment

Source	d.f.	M.S.	(i)	(ii)
a	4	1388·64	***	***
b_1	1	166·50	**	—
b_2	4	132·99	***	**
b_3	5	66·07	**	—
Total (between crosses)	14	470·24	—	***
Sets	3	892·47	***	—
Blocks	1	241·00	***	—
Sets × a	12	132·85	***	—
Sets × b_1	3	26·70	—	—
Sets × b_2	12	20·62	—	—
Sets × b_3	15	69·43	***	—
Sets × Total (between crosses)	42	70·55	***	—
Block interactions	59	14·34	—	—
Duplicate plots	119+	22·98	—	—
Total	238	67·42	—	—

+ = One missing plot.

(i) and (ii) refer to tests of significance using the pool of non-significant items in the table and the appropriate interaction with sets item respectively.

Now we regard the sets as constituting a random sample of all possible sets from the populations. Hence the mean squares of the main effects of the analysis are expected to contain a component relating to differences within as well as the more obvious component relating to differences between populations. The significance of these main effects could therefore be due solely to the differences within populations just noted. If, therefore, we wish to establish that there are differences between populations in respect of flowering time here, we must test each main effect mean square against its

respective set interaction item (column (ii) of table 3)). When this is done, both the a and b_2 items turn out to be significantly greater than their corresponding interactions with sets. Thus we can conclude that differences between populations here are determined by genes with additive and non-additive effects.

(b) *The 1960 experiment*

The mean flowering times and the analysis of variance of these means are shown in tables 4 and 5. In the analysis, one high order interaction, namely, sets × block × b_3 was significant: otherwise, the main effects turned out to be consistently expressed over blocks and sets and blocks simultaneously. This

TABLE 4

Mean flowering time of families averaged over duplicate plots and blocks of the 1960 experiment.

Set 1						Set 3					
1	2	3	4	5	$\bar{r}$	1	2	3	4	5	$\bar{r}$
43·2	28·6	33·5	38·4	21·2	33·0	43·6	26·4	40·1	30·8	24·4	33·1
	21·0	28·4	28·7	19·0	25·1		27·1	28·0	28·3	25·2	27·0
		34·8	34·9	20·1	30·3			33·5	27·3	25·2	30·8
			38·8	25·9	33·3				27·2	25·5	27·8
				21·3	21·5					25·3	25·1

$\bar{r}$ = Array mean.

TABLE 5

The analysis of variance of the 1960 F_1 experiment

Source	d.f.	M.S.	(i)	(ii)
a	4	868·43	***	—
b_1	1	345·84	***	—
b_2	4	67·82	*	—
b_3	5	51·28	—	—
Total (between crosses)	14	310·52	—	**
Sets	1	0·03	—	—
Blocks	1	3446·48	***	—
Sets × a	4	162·54	***	—
Sets × b_1	1	3·48	—	—
Sets × b_2	4	11·84	—	—
Sets × b_3	5	32·06	—	—
Sets × Total (between crosses)	14	61·52	**	—
Block interactions	28+	30·69	—	—
Duplicate plots	59+	21·65	—	—
Total	117	92·24	—	—

+ = One missing family in Block II.

and the remaining interaction items have been pooled, therefore, as in the analysis of the 1959 experiment. As with the latter, there is clear evidence of both additive and non-additive effects concerning differences between crosses. The individual tests of main effects against their interaction with sets on the other hand turn out to be non-significant, although the total mean square is significantly greater than the corresponding sets × total item. However the degrees of freedom available for these comparisons are less than those of the previous analysis and the a, b_1 and b_2 mean squares are in

fact only just less than significantly greater than their respective interaction mean squares. The 1960 experiment does not therefore appear to be inconsistent with that of the previous year.

(c) *The joint analysis of the 1959 and 1960 experiments*

The chief purpose of this joint analysis is to investigate the possibility that flowering time is a character subject to genotype-environment interaction. Inspection of the analysis (table 6) shows that genotype-environment interaction appears to be restricted to non-additive effects (*e.g.* Years $\times b_3$). The

TABLE 6

The analysis of variance of the 2 sets grown in 1959 and 1960

Source	d.f.	M.S.	(i)	(ii)
a	4	1554·49	***	—
b_1	1	313·15	***	—
b_2	4	99·85	***	**
b_3	5	54·99	*	—
Total (between crosses)	14	514·68	—	**
Sets	1	18·70	—	—
Years	1	46·82	—	—
Sets × Years	1	16·64	—	—
Blocks	4	3550·61	***	—
Sets × a	4	296·56	***	—
Sets × b_1	1	47·31	—	—
Sets × b_2	4	2·85	—	—
Sets × b_3	5	92·93	***	—
Sets × Total (between crosses)	14	122·11	***	—
Years × a	4	23·68	—	—
Years × b_1	1	74·03	—	—
Years × b_2	4	29·35	—	—
Years × b_3	5	115·69	***	—
Years × Total (between crosses)	14	61·76	***	—
Sets × Years × Total (between crosses)	14	29·57	—	—
Block interactions	55+	24·96	—	—
Duplicates	118+	18·81	—	—
Total	236	73·81	—	—

+ = One missing family in Block II, 1960 and 1 missing plot in 1959.

analysis of the intrapopulation diallels, on the other hand, provided little or no evidence of this source of variation. Here, however, the Years $\times b_3$ mean square is of the same magnitude as that of the Sets $\times b_3$ mean square, indicating the relative importance of genotype-environment interaction in respect of interpopulation crosses.

(d) *The analysis of non-additive variance*

In both seasons, part of the genetic variance between crosses is due to non-additive effects of the genes determining flowering time. In the case of the intra-population diallels, this non-additive variance turned out to be due solely to dominance. Is this the situation here?

The Wr/Vr analysis provides a method enabling us to answer this question (Jinks, 1954). Now, the analysis of variance of all sets, except that of set 2 in 1959, indicate the presence of non-additive gene effects. The Wr/Vr graphs of these five sets are shown in fig. 1. With the exception of set 3,

which will be discussed later, the regression of Wr on Vr is significantly different from zero, yet not significantly different from unity. The interpretation of the non-additive variance in these diallel sets is thus clear, namely that it is due solely to dominance.

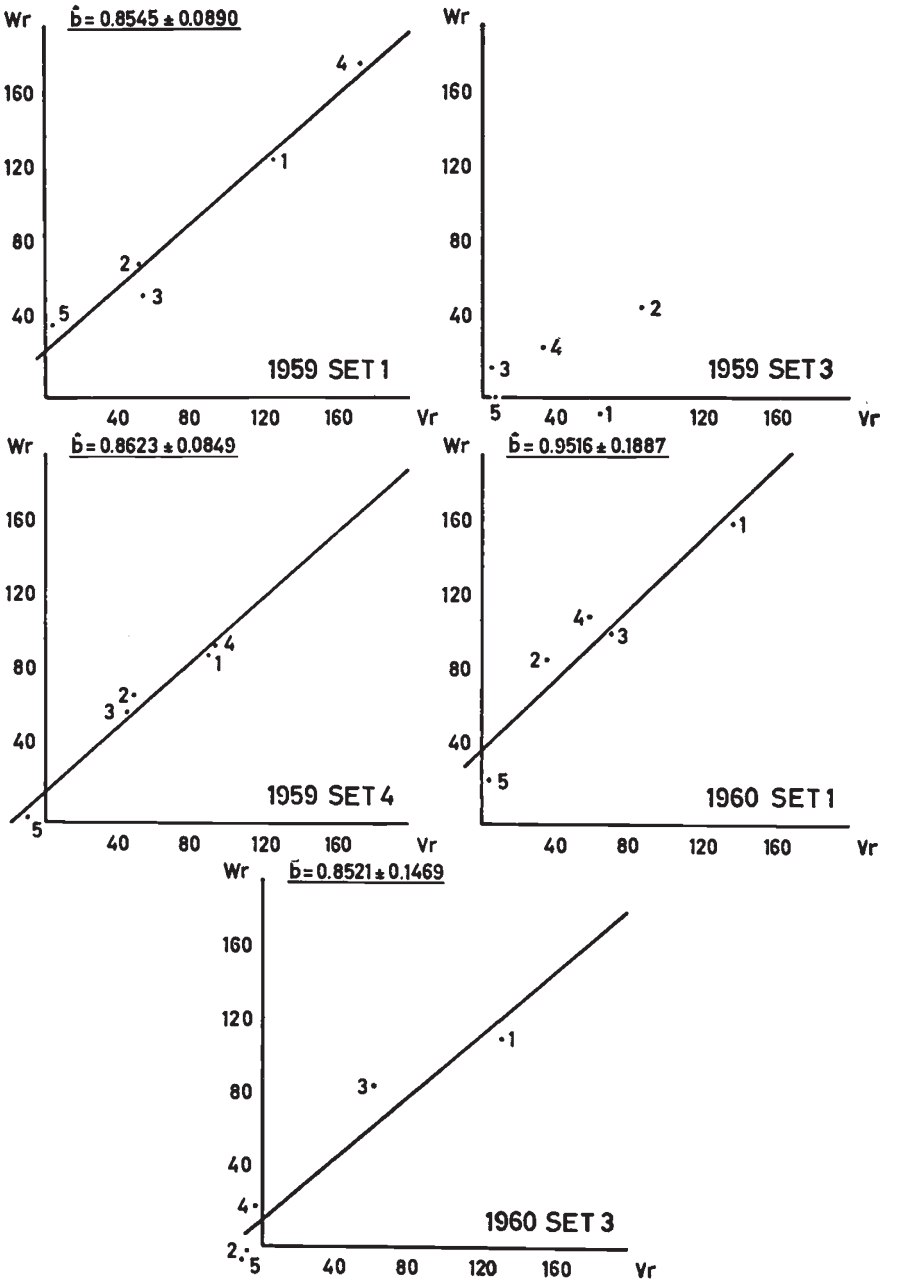

FIG. 1.—The regression of array covariance (Wr) on to array variance (Vr) for those sets in which non-additive effects have been detected in their analysis of variance.

We need next to inquire whether this dominance is expressed consistently over sets. Firstly, the joint regression analysis of Wr and Vr shows that the slopes of the graphs are the same and the joint estimate is $b = 0{\cdot}8738 \pm 0{\cdot}0599$. We wish, secondly, to find out whether the order of the points on the graphs is consistent from one set to another; for this purpose we may carry out an analysis of the metric Wr + Vr over sets and seasons and it turns out that the order is in fact quite consistent (Allard, 1954). This implies, therefore, that although dominance effects are detectable within populations, these here must be a property of the genes differentiating populations, rather than plants within them.

The final point worth mention here concerns the relationship between the order of the points on the graph and the flowering times of the parent common to that array. This comparison is shown in table 7, and it is clear

TABLE 7

Relationship between flowering time and order of points on the Wr/Vr graph. Flowering times are averages of selfs over block, sets and years (sets 1 and 4 in 1959; sets 1 and 3 in 1960)

Population	Mean flowering time (days)	Wr/Vr rank
5	23·1	1
2	25·7	2
3	34·8	3
4	37·0	4
1	41·4	5

that there is a straightforward linear relationship between this pair of variables. This implies that parent individuals from population 5 contribute proportionately more dominant alleles to their progeny than the individuals from any other population; and that those from population 1 contribute proportionately fewer, the other populations being intermediate in this respect.

We turn lastly to the graph of set 3 in the 1959 experiment, in which there is no significant regression of Wr on Vr. The distribution of the points here suggests the presence of non-allelic interaction of the complimentary type and that array 1 contains the interacting cross or crosses (*cf.* fig. 2, p. 779 of Jinks (1954); and Jinks (1956)). Re-analysis of the data from this set, excluding the first array, removes all evidence of non-additive variance, *i.e.* its presence in the complete set must be due to array 1 only. Indeed, inspection of the mean flowering times of this set (table 2) shows that the cross 1×2 is the cause of the non-allelic interaction detected.

4. DISCUSSION

The genetics of differences within populations is, of course, directly relevant to our concern with the cause of genetic variability within natural populations of this and indeed other species. In this context, a knowledge of the genetic basis of differences between such populations appears at first glance to be somewhat incidental to our main purpose, for such differences are

a by-product of selection acting on the genetic variation within populations. Some explanation is necessary therefore as to why the genetics of population differences is indeed relevant to our chief concern.

Now, as a working hypothesis we can assume that the genetic structure of a character, for which the individuals of a population vary, is adaptive; that is to say, that the particular array of genotypes we detect, together with the mode of action of their constituent genes, has arisen as a result of natural selection. It follows, therefore, that a knowledge of the genetic architecture of a character provides an insight into the type of selection acting in the past on that character (Mather, 1960, 1966).

No such hypothesis can be entertained, however, with respect to the genetics of differences *between* populations. While the properties of genes segregating in the progenies of interpopulation crosses might be held to indicate the type of selection acting on the species prior to its fragmentation into a number of populations, evidence of this kind must in general be much less important than a knowledge of contemporary gene action in populations. On the other hand, where selection has caused changes within populations, we expect to detect differences between the genetics of interpopulation and intrapopulation families, providing that the effects of selection have been different in different populations. In short, the comparison of inter- and intrapopulation crosses affords a historical perspective of evolution within the species.

With these considerations in mind, we can turn now to the interpretation of the results we have obtained from the interpopulation diallels. Firstly, we can be sure that the effects of selection have in fact differed in different populations because both the mean expression and the genetic architecture of the character varies from one population to another. Secondly, we have seen that part of the additive variance relating to differences between diallel crosses can be ascribed to differences between populations. This conclusion implies that the number of loci determining differences between interpopulation progenies must be greater than the number determining differences between individuals within a population. Indeed, this situation is, of course, to be expected for if selection has been effective, gene frequencies will be different in different populations. Furthermore, since the mean flowering times of the populations differ appreciably, it is likely that some loci have become fixed, these being different loci in different populations.

The third point that emerges from this comparison between inter- and intrapopulation crosses concerns gene action. The analysis of the interpopulation diallels has shown that gene action in these crosses is in general similar to that of the intrapopulation diallels. In both types of cross the genes determining flowering time manifest additive and dominance properties. Though there was no evidence of either genotype-environment or non-allelic interaction in the intrapopulation crosses, there is some evidence of both these effects in the interpopulation progenies. Yet the magnitude of these effects hardly implies that they are major sources of variance.

This conclusion is unexpected, for there is much evidence that the progenies of wide crosses are frequently quite extraordinarily variable (see Stebbins, 1950). Much of this hybrid variability is due to segregation at loci that have become differentially fixed in the parents. The frequent occurrence of extreme forms in such progenies, however, is almost certainly due to what can be described as the breakdown of co-adapted gene complexes

or as non-allelic interaction. Unfortunately it is not possible to analyse the data from much of the early evidence in the manner required for the unambiguous identification of non-allelic interaction. In a more recent investigation of the population genetics of *Schizophyllum commune*, however, Simchen (1967) has shown that the progenies from mating monokaryons from different wild isolates display considerable, though varying amounts of non-allelic interaction. Matings within isolates, on the other hand, never do. In another fungal species, *Aspergillus nidulans*, crosses between strains showed marked non-additive interaction compared with the progenies of strains which regularly undergo heterokaryosis (Jinks *et al.*, 1966) and Butcher (1968) has shown that this interaction is due to additive by additive gene effects.

The occurrence of non-allelic interaction in the progenies of crosses between different populations, therefore, may be taken as evidence of independent evolution of these populations. On this argument, our poppy populations appear to have had only a limited independent evolution; enough to enable us to recognise that their mean flowering times and their proportions of additive and dominance variance are different, but insufficient to disrupt the co-adaptation of the genes of the system. In other words, these populations are relatively recent. With the possible exception of some lepidoptera, little is known about the frequency of occurrence and distribution of any species of wild plant or animal. It is of some interest however that McNaughton and Harper (1964) mention that *P. dubium* was apparently comparatively rare in some areas of the country, where it is now common, until the early years of this century. The species is, of course, an annual, but in view of its colonising role and the very considerable dormancy and longevity of its seed, it is most unlikely to have a mean generation length of only one year. Thus the little historical evidence available is at least consistent with our genetic reconstruction of the recent evolution of the species.

One final point remains concerning the pattern of this evolution. The analysis of the dominance relations of the genes determining flowering time revealed a one to one relationship between the flowering times of the parents and the variance-covariance values of their progenies (table 7). This was interpreted as implying that the earliest flowering population (5) had the highest proportion of dominant alleles and that the latest (1) had the lowest proportion. The consistency of the order of the Wr/Vr points however indicates that the dominance relationships depicted in the graphs are a property of differences between rather than within populations. Taking these two observations together we arrive at the somewhat unexpected conclusion that the genes manifesting dominance here are, on the balance of probability, fixed in the populations. Thus they can make no dominance contribution towards variation within these populations, their dominance properties being revealed only in crosses between populations.

There can, of course, be no certainty as to the origin of this state of affairs. We may postulate, however, that in the " base " population from which these independent populations originated, flowering time was determined by genes in some of which dominance was increasing and in other decreasing the expression of the character. On average, therefore, dominance in the original population would thus be ambi-directional. In the localities in which our seed was sampled there is directional selection of variable intensity for early flowering time. The effect of selection therefore would be to increase the frequency of genes manifesting dominance for early

flowering, this selection being strongest in population 5 (from Poznan, Poland) and least in population 1 (from Pittenweem, Scotland, see table 3 in previous paper). Ultimately, these dominant alleles will have become fixed and their dominance properties which were advantageous during the transient, early stages of the differentiation, of these populations will become inconsequential. It is possible that the divergence of these populations occurred during a northward migration of the species from the Mediterranean area, into regions where, because of a relatively short season, early flowering would be at a premium. The advantage of this admittedly speculative hypothesis is that it is at least testable, for we predict that the proportion of dominant alleles fixed in a population would increase with latitude.

5. Summary

1. The results from an analysis of the inheritance of flowering time in interpopulation diallel progenies is compared with those reported in a previous paper concerning interpopulation diallel crosses.

2. In general the inheritance of this quantitative character is similar in both types of progeny in that flowering time is determined by genes with both additive and non-additive properties.

3. Though there is some evidence that these genes manifest both genotype-environment and non-allelic interaction only in interpopulation progenies, these effects are not major sources of variation.

4. These results suggest therefore, that the five populations studied have had only a brief history of independent evolution so far as this character is concerned.

Acknowledgments.—Much of the programme used to analyse the data from these experiments was written whilst the author was an N.I.H. Post-doctoral Research Fellow at North Carolina State University, Raleigh, N.C. I wish to thank Mr S. Mercer, Dr E. Scheinberg and the staff of the Computer Centre in Raleigh for their kind advice and guidance with this task. My thanks are due also to the staff of the S.R.C. Atlas Computer Laboratory at Chilton, where most of the data was processed.

6. References

ALLARD, R. W. 1956. The analysis of genetic-environmental interactions by means of diallel crosses. *Genetics, 41,* 305-318.

BUTCHER, A. C. 1968. Personal communication.

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

JINKS, J. L. 1954. The analysis of continuous variation in a diallel of *Nicotiana ructica* 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.

JINKS, J. L., CATEN, C. E., SIMCHEN, G., AND CROFT, J. H. 1966. Heterokaryon incompatibility and variation in wild populations of *Aspergillus nidulans. Heredity, 21,* 227-239.

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

LAWRENCE, M. J. 1965. Variation in wild populations of *Papaver dubium.* 1. Variation within populations; diallel crosses. *Heredity, 20,* 183-204.

MATHER, K. M. 1960. Evolution in polygenic systems. *Evoluzione e Genetica.* Academia Nazionale dei Lincei, Quad., *47,* 131-152.

MATHER, K. M. 1966. Variability and selection. *Proc. Roy. Soc., B., 164,* 328-340.

MCNAUGHTON, I. H., AND HARPER, J. L. 1964. Biological flora of the British Isles, No. 99, *Papaver L. J. Ecol., 52,* 767-793.

SIMCHEN, G. 1967. Independent evolution of a polygenic system in isolated populations of the fungus *Schizophyllum commune. Evolution, 21,* 310-315.

STEBBINS, G. L. 1950. *Variation and Evolution in Plants.* Columbia University Press, New York.