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Two-environment selection with inbreeding in *Schizophyllum commune*

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SUMMARY

In two-way selection for growth rate in the fungus *Schizophyllum commune* in two environments (20 °C and 30 °C) response for low growth rate was always greater than for high. This asymmetry was due to inbreeding depression combined with a greater selection differential associated with the low lines in the high temperature environment. Despite the high level of inbreeding applied, two low selection lines derived from the same source (parental isolate 2) showed no decrease in genetic variance during selection. This maintenance of genetic variance was associated with a decrease in stability of development relative to the corresponding high selection lines.

1. INTRODUCTION

Previous work by Simchen & Jinks (1964) and Simchen (1966*a*, *b*) has demonstrated the suitability of *Schizophyllum commune* for the study of continuous variation and selection response. Because each genotype can be clonally propagated, measurements may be made on the same individual in several environments. In addition, selected parents or lines may be stored so that generations or progeny groups can be reproduced at a later date if required. This organism is especially suitable for investigating selection response in different environments.

The objective of the present work was to develop inbred lines adapted to specific environments which could later be used to study the consequences of such adaptation with respect to behaviour of such lines in a range of environments. The effects of inbreeding and direct response to selection for high and low growth rate in two temperature environments are reported here.

2. MATERIALS AND METHODS

(i) *Environments*

Two temperatures, 20 and 30 °C, were chosen as environments in which selection was performed. Incubators were used in which temperature was controlled to within $\pm 0{\cdot}5$ °C of that required.

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(ii) *Media and growth rate determination*

Growth tubes approximately 12 mm diameter and 15 cm long, containing 6 ml of 2 per cent malt (MT) medium were used (these growth tubes were a modified version of those described by Ryan, Beadle & Tatum, 1943). SF and SC media were used respectively for fruiting and random pair matings of isolated spores. Details of media, fruiting, spore isolation and measurement of growth rate are given by Simchen & Jinks (1964). Total growth (in mm) over a period of 9 days was used as the measure of growth rate in these experiments.

(iii) *Base populations*

Two dikaryons numbered 2 and 6 from the collection described by Simchen (1966*a*) were used to obtain two base populations. These two dikaryons differed in origin: number 2 was collected in Buckinghamshire, England, and number 6 in Massachussets, U.S.A. (Details of the method of isolation of each single dikaryotic mycelium are given by Simchen, 1966*a*.) The degree of heterozygosity and type of genetic variation stored within each has been investigated by Simchen (1966*a*, *b*). The genetic variation controlling growth rate in the progeny of both dikaryons was adequately accounted for in terms of additive and dominance gene effects. Progeny of dikaryon 6 showed a much higher error variance component than that of dikaryon 2.

Dikaryons 2 and 6 were fruited on SF medium. Two hundred haploid basidiospores of each dikaryon were mated in pairs at random. This is equivalent to selfing in a diploid organism. Because control of mating behaviour is of the bifactorial or tetrapolar type only 25% of these matings between full-sib haploids are expected to be compatible and form dikaryons. These dikaryotic progeny (51 from dikaryon 2 and 57 from dikaryon 6) formed the two base populations = generation 0, on which selection was initiated.

In the text dikaryons 2 and 6 will be referred to as parental isolates 2 and 6. This is to avoid confusion when reference is made to dikaryons which are selected as parents of successive generations.

(iv) *Selection lines*

All selection was at the dikaryotic phase of the life-cycle (equivalent to a diploid organism). The genotypes of generation 0 of each parental isolate were assessed for growth rate at both 20 and 30 °C. The two fastest- and two slowest-growing dikaryons (full-sibs) at each temperature were selected from the progeny of each isolate. Each subsequent generation was obtained by intermating the haploid spores from two full-sister dikaryons selected as parents in the preceding generation in each selection line.

Ten selection lines were initiated in the following manner: eight by two-way selection for growth rate at each temperature within the progeny of parental isolates 2 and 6, and two by two-way selection within the progeny of isolate 2 on the basis of combined performance at both temperatures (i.e. each genotype was

grown at both temperatures). The latter two lines are referred to as the combined selection lines.

A diagrammatic outline of the selection procedure is given in Figs. 1 and 2. The origin and reference numbers used throughout the text is given in Table 1.

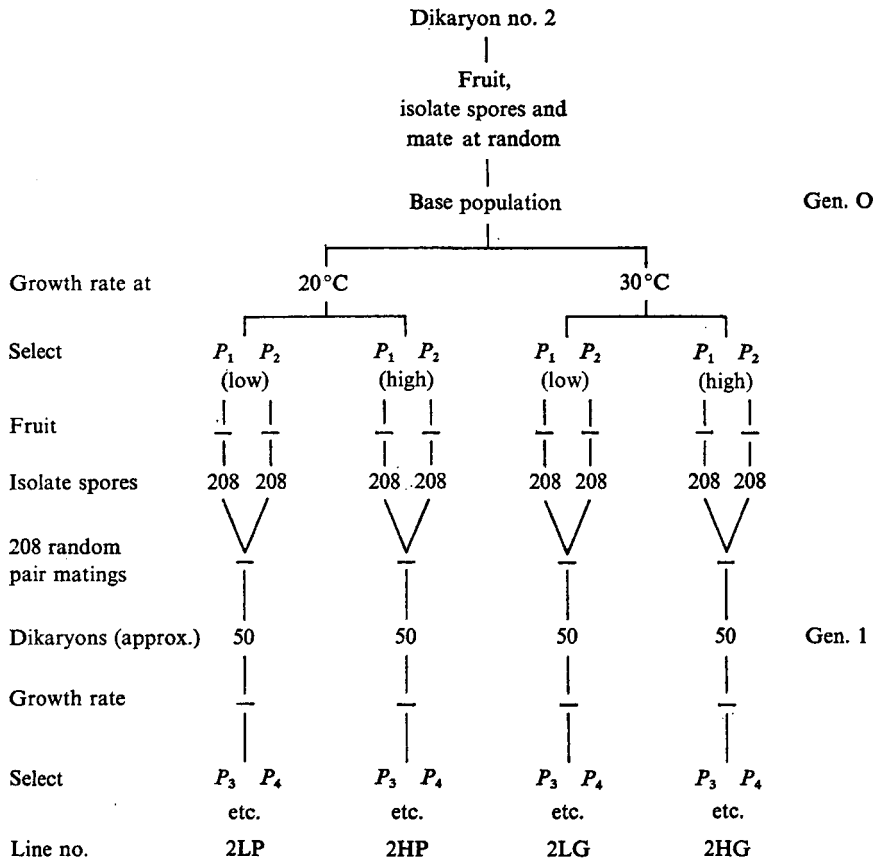

Fig. 1. Outline of the selection procedure for lines selected in one environment. The corresponding four lines derived from dikaryon 6 were obtained in a similar manner.

Because growth rate at 30 °C (good environment) was about double that at 20 °C (poor environment) it was necessary to re-scale the data before selection in the combined lines. The growth rate of each genotype was divided by the generation mean in each environment. The rescaled values were averaged over both environments to give a final index of combined performance on which selection was based. This procedure avoided the situation where a dikaryon with a very high (or low) growth rate at 30 °C but only a mediocre growth rate at 20 °C might be selected because it had the highest (or lowest) overall value on a straight average.

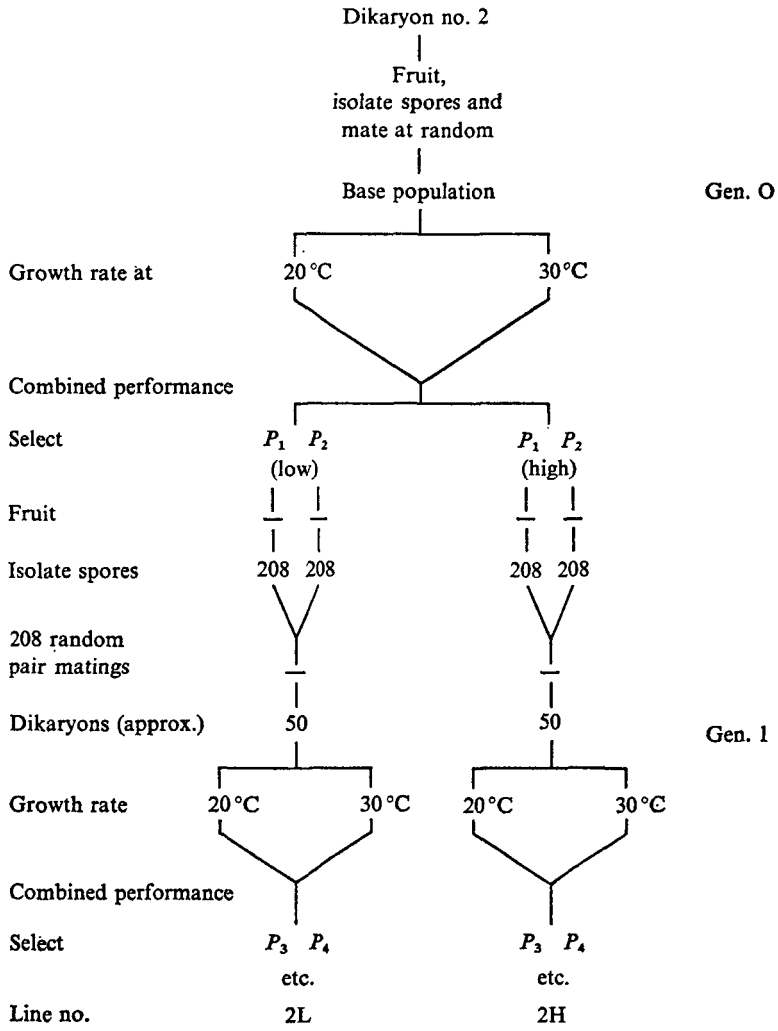

Fig. 2. Outline of the selection procedure for the two combined selection lines derived from dikaryon 2. Selection is based on performance in both environments.

(v) *Experimental design*

In each generation the progeny of the corresponding pair of high and low lines, the parents of that generation (two for each line) plus three standard dikaryons were randomized and grown together in the same experiment. Two randomized blocks were used in each experiment. One replicate of each progeny genotype and two replicates of the four parental and three control dikaryons were included in *each* block. In each generation approximately 50 dikaryons of each line were measured.

At the end of the selection programme all the parents and controls, which were stored in stock bottles at 5 °C, were grown in a single experiment, one in each environment. These data were used to overcome the problem of different generations being assessed under slightly different experimental conditions arising from

Table 1. *Origin and reference numbers of the ten selection lines*

(Symbols H and L refer to high and low selections respectively. G = good = 30 °C environment. P = poor = 20 °C environment. The numerals 2 and 6 refer to the parental isolate from which the lines were derived.)

Parental isolate	Environment of selection	Direction of selection	Reference number
2	20 °C	High	2HP
		Low	2LP
	30 °C	High	2HG
		Low	2LG
6	20 °C	High	6HP
		Low	6LP
	30 °C	High	6HG
		Low	6LG
2	20 and 30 °C = combined selection lines	High	2H
		Low	2L

Table 2. *Difference in response (accumulated over seven generations) to selection for high and for low growth rate, and estimates of the effects due to inbreeding depression*

Selection lines	Asymmetry (mm) (L–H)	Estimated inbreeding depression effect
Poor environment	(mm)	(mm)
2LP–2HP	1·3	2·0
2L–2H	4·8	2·0
6LP–6HP	1·8	3·2
Good environment		
2LG–2HG	16·5	4·3
2L–2H	12·8	4·3
6LG–6HG	11·4	2·6

variation between batches of medium, micro-environmental control within the incubator, etc. These fluctuations were small and in no case exceeded 10 % of the growth rate of the parental isolates (averaged over all generations within each environment) which were used as one of the three controls in each experiment.

3. RESULTS

(i) *Generation means*

The response to selection for all lines is shown in Fig. 3. Response was greater for low than for high growth rate. This asymmetry, as measured by the difference in accumulated response between high and low lines (Table 2), was large for all lines selected in the good environment (30 °C) and small but consistent for those selected in the poor environment (20 °C).

The response of the combined selection lines in each environment was similar to that of the individual direct selection lines. As indicated in Fig. 3, the parents of

the 1st and 2nd cycles of selection of the high line in the good environment (2HG) were also the best genotypes on average over both environments. Hence these dikaryons were also the parents of generation 1 and 2 of the high growth rate combined selection line. Similarly the parents of the 1st generation of the low line (2LG) and the combined low line are the same.

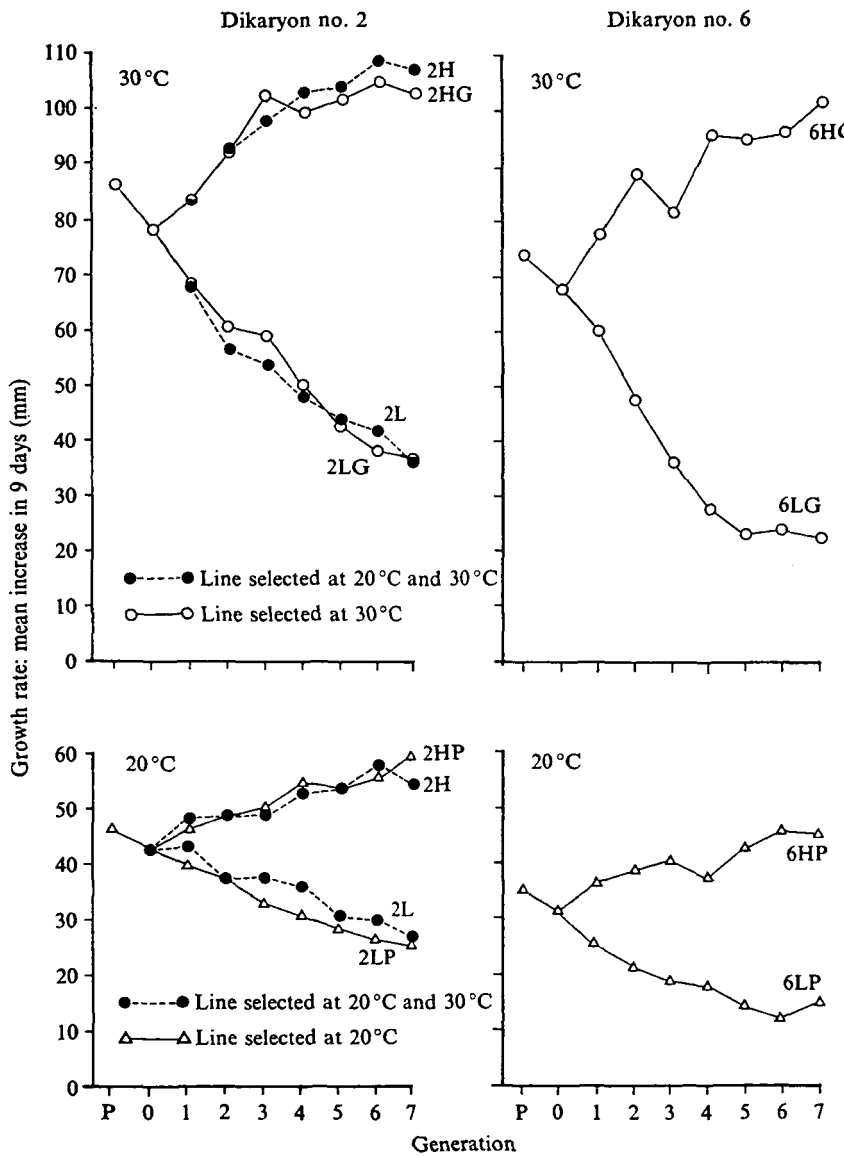

Fig. 3. Response to selection. Generation means of all ten selection lines. P, Original dikaryotic isolate.

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(ii) *Heritability*

The high rate of inbreeding during selection (full-sib mating) might be expected to cause a progressive decrease in heritability, but the relationship of response to accumulated selection differential, plotted for each line in Figs. 4 and 5, showed

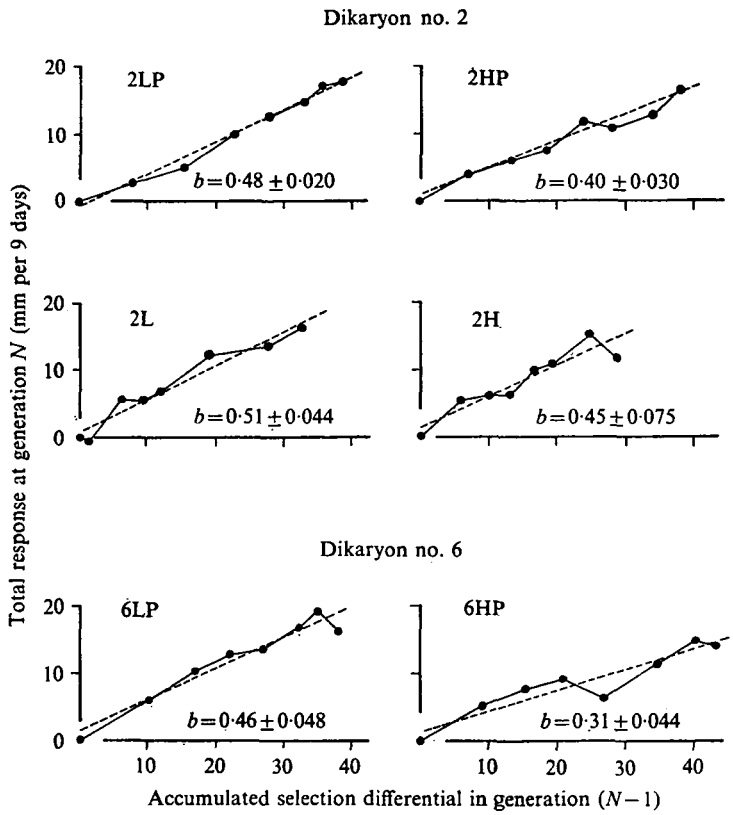

Fig. 4. Realized heritability for each line in the 20 °C (poor) environment.

little sign of the expected curvilinearity except for line 2HG. The realized heritability, estimated by the linear regression coefficient (b), is given with its standard error on each graph. The heritability measured in this way was about 50 % for up and down selection in the good environment (30 °C) and for down selection in the poor environment (20 °C) from both dikaryons, but progress was slower during up selection in the poor environment (40 % and 31 % for 2HP and 6HP). The combined selection line 2H also showed relatively faster progress in the good than in the poor environment.

From an analysis of variance of clonal replicates over blocks estimates of total genetic (V_G) and error variances (V_E = genotypes × blocks mean square) were obtained for each generation of each line. These estimates are shown in Figs. 6 and 7. A square-root scale was used since this was considered the most appropriate for

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the representation of these '2nd degree' statistics. With the exception of the low lines derived from parental isolate 2 (i.e. lines 2L and 2LG) there was a progressive decrease in both the genetic and error components of variance. Lines 2L and 2LG (Fig. 7) maintained a high level of both V_G and V_E. This compensating change in the heritable and non-heritable components was the main reason why the heritability remained more or less constant throughout the period of selection.

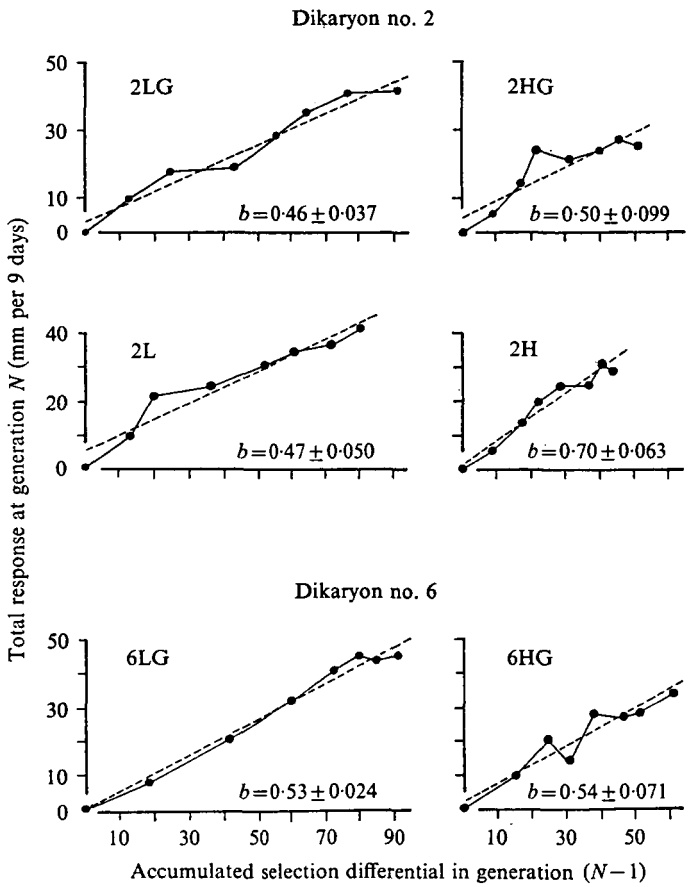

Fig. 5. Realised heritability for each line in the 30 °C (good) environment.

(iii) *Dominance, inbreeding depression and asymmetry of response*

The unselected progeny mean (generation 0) was significantly less than that of the parental isolates in each environment. This indicates that directional dominance or other non-additive gene effects were present (Wright, 1951). The results of Simchen (1966*a*, *b*) indicate that dominance could account for all the non-additivity. Selection with inbreeding in the presence of directional dominance should lead to asymmetry of response.

The parental isolates are assumed to be non-inbred (which is reasonable since this organism has a mating system which effectively promotes outbreeding) and

the inbreeding coefficient of generation 0 is 0·5. The difference between the parental isolates and their selfed progeny can be used to estimate the level of depression due to inbreeding (Falconer, 1953). The coefficient of inbreeding at generation 7 is estimated as 0·87 – an increase of 37 % during selection. The expected depression due to this increase in inbreeding is given in Table 2. The data indicate that the

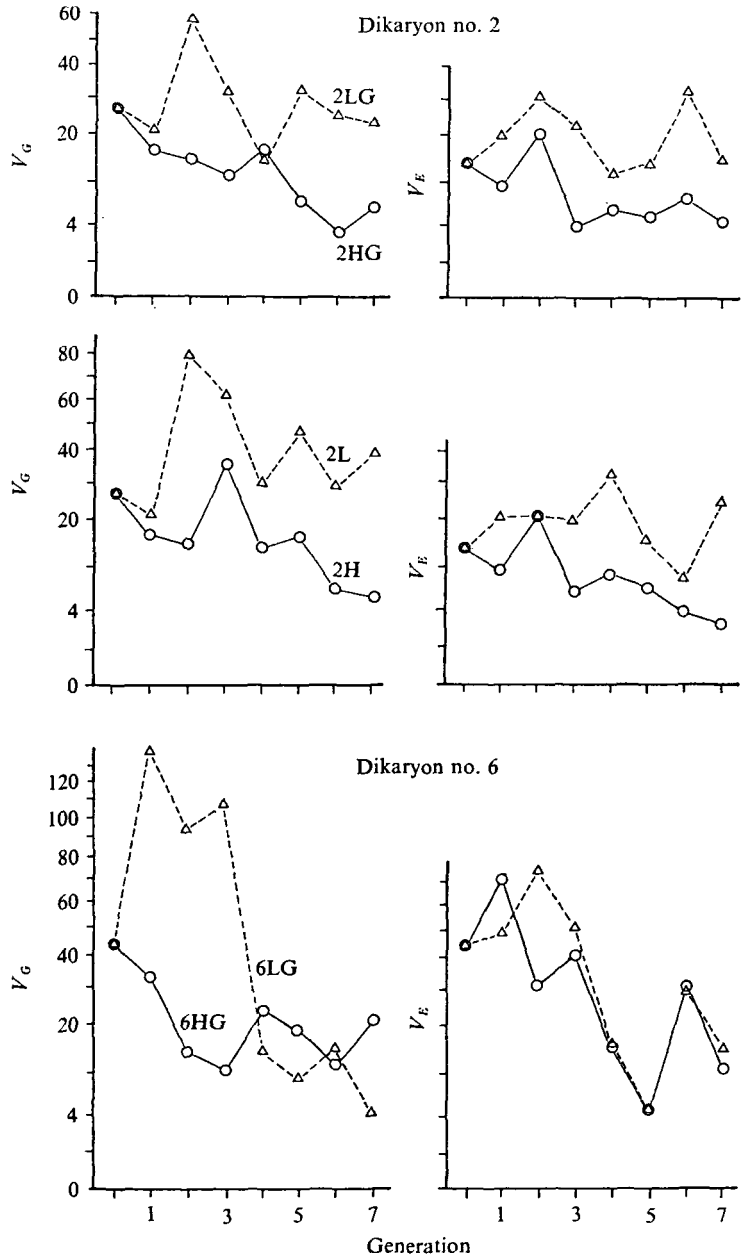

Fig. 6. Genetic (V_G) and error (V_E) components of variance for all lines grown in the poor (20 °C) environment. A square-root scale is used for the variances.

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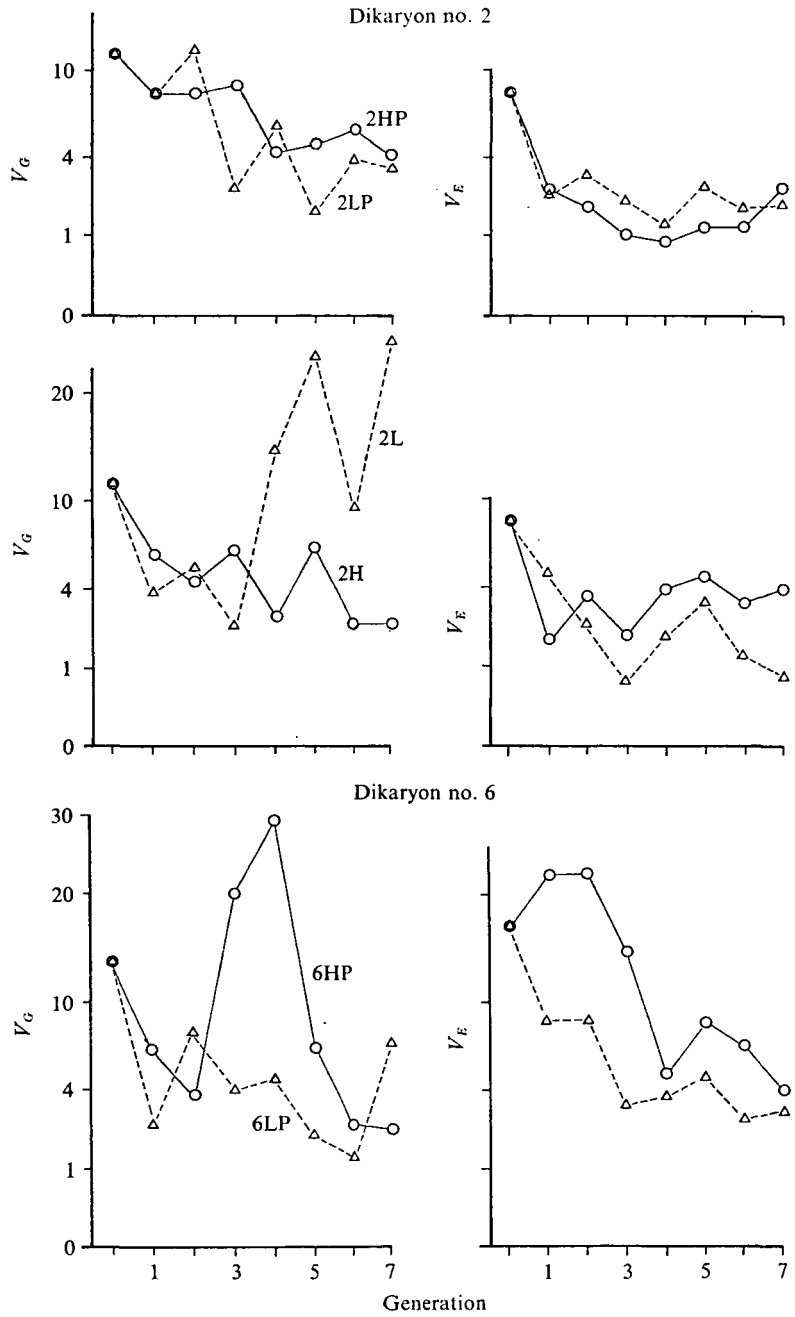

Fig. 7. Genetic (V_G) and error (V_E) components of variance for all lines grown in the good (30 °C) environment. A square-root scale is used for the variances.

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small degree of asymmetry observed in the poor environment could be accounted for in terms of inbreeding depression. In the good environment the observed asymmetry was much larger than the estimated effect due to inbreeding depression. Genetic and phenotypic variances were greater for the low than for the high selection lines in the good environment (Fig. 7). For the direct selection lines the average selection differential per generation in standard units was 1·87, 1·61, 1·39 and 1·13 for lines 2LG, 2HG, 6LG and 6HG respectively. The realized heritabilities for these high and low line pairs were similar (Fig. 5). The high degree of asymmetry in the good environment was due to the combined effects of inbreeding depression and greater selection differential in the low lines.

Because the increase in variance was associated with the decreasing mean values of the low lines, transformation of the data to a log or square-root scale would serve to exaggerate rather than decrease the asymmetry.

4. DISCUSSION

Effect of inbreeding and selection on the genetic and environmental components of variance

For all the lines derived from dikaryon 6 and all the high lines from dikaryon 2 *both* the genetic and error variances showed an overall decline with inbreeding and selection (Figs. 6, 7). In the course of further study a second estimate of V_E was obtained for generations 0, 1, 2, and 4 of the lines derived from dikaryon 2. In general, the coefficient of variation of the repeat experiments was lower in generations 0 and 1 than that obtained during selection. This would suggest that some of the decrease in V_E may have been due to improvement in experimental technique in the early stages of selection.

For the high and low lines derived from dikaryon 6 the estimates of V_E in the last 2 or 3 generations of selection were about 30% of that for generation 0, a reduction too great to be explained by improvement in experimental technique. It is concluded that a real increase in stability occurred during selection in these lines.

In the good environment the two low selection lines (2LG and 2L) derived from dikaryon 2 (Fig. 7) maintained throughout the period of inbreeding and selection a level of genetic variance as great or greater than that in generation 0. This was accompanied by a higher error variance than that associated with the corresponding high lines. The progeny of each pair of high and low lines were grown in the same experiment, hence these estimates of V_E may be used as measures of the relative stability of the lines concerned.

Following two-way selection at the haploid (monokaryotic) phase of the life-cycle among progeny derived from the same parental dikaryon (isolate 2), Simchen (1966*a*) found that the low lines also maintained a high level of genetic variance coupled with a marked decrease of stability despite the intense inbreeding (full-sib haploid mating) employed during selection. Some of this genetic variance was maintained by linkage of genes controlling growth rate to the mating type loci (Connolly & Simchen, 1968). Because of the similarity in behaviour of the haploid

lines and the dikaryotic low lines of the present experiments, all of which were derived from isolate 2, it is reasonable to suggest that linkage to the mating type loci was also a factor in maintaining genetic variance in the dikaryotic selections. No test to verify this hypothesis was performed on the dikaryotic low lines.

The fact that the decrease in stability (i.e. increase in V_E) always occurred in the low lines supports the conclusion of Simchen (1966*a*) that some form of selection for instability has occurred in these lines. The results of the present experiments indicate that the decrease in stability shown by some of the low lines was dependent on the direction of selection, on the environment and on the genetic constitution of the original population.

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