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Selection for rate of larval development using *Drosophila melanogaster* cultured axenically on deficient diets

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1. INTRODUCTION

Drosophila melanogaster normally lives in an environment providing a transient food supply, and even in laboratory cultures the successive larvae which hatch quickly find the amount of available food limiting to their growth (Sang, McDonald & Gordon, 1949). Consequently, it is reasonable to assume that natural selection has favoured rapid larval development and it is not surprising that *Drosophila* is 'one of the fastest growing metazoa' (Gordon, 1959). This would seem to be the reason why attempts to select for rapid larval growth have been unsuccessful (Sang & Clayton, 1957; Sokal & Hunter, 1958; Clarke, Maynard Smith & Sondhi, 1961), even though larval development rate has an estimated heritability of 20–25%. What evidence there is, suggests this heritability is realized only by selection for slow development, but even this is not always achieved to the extent which the heritability estimate would imply (Sokal & Hunter, 1958). A possible reason for this second anomaly is the ability of larvae to prolong development under adverse nutritional conditions (Sang, 1959) so that selective effort would then be confounded, especially as different genotypes appear to respond variously to minor nutritional deficiencies (Robertson, 1959). The question then arises: can development rate be altered by selection in an abnormal environment to which the organism is not yet adapted? This is the first concern of this report.

If development rate can be altered by selection in an atypical nutritional environment, it is also important to know if the modification is adaptive and how far the normal biochemical processes of the organism have been changed in consequence. There is reason, therefore to alter the environment in a strictly defined way and not simply by adjusting the total food supply. In the following experiments either the supply of casein or the supply of pyridoxine, the vitamin most involved in amino-acid metabolism, has been restricted. These nutrients were chosen from the many possible, since there is no evidence (Sang, 1959) that deficiencies of other nutrients are likely to be limiting to *Drosophila* in nature (cf. Sang & King, 1961), although this has not yet been properly studied. The second concern of this work is therefore with an examination of pyridoxine and casein response curves of the selected larvae.

Although it is known that there are differences of nutritional requirements between strains of various organisms (Williams, 1956) there is no direct evidence

that these are consequences of selection: the requirements of such strains may be a fortuitous result of the procedures used in breeding them. Such 'abnormal' needs are usually higher than average, but this may merely reflect the fact that extra requirements are more likely to be identified. From the economic point of view, development of strains with lower requirements (higher efficiency) is of greater importance, particularly with respect to protein. The situation found by selecting *Drosophila* on low protein cannot be taken as a model of what might happen with other organisms, but it may indicate the kinds of biochemical situation underlying improvement of efficiency of protein utilization. Unfortunately, circumstances prevented the full nutritional and genetical analyses first planned for the selected lines, so that the information relevant to this point is less complete than intended.

Finally, the uses of 'abnormal' environments for selection have not been widely explored since most experiments are deliberately carried out under putatively optimal conditions. It does not follow that these conditions are still the best after selective changes have occurred, and an imbalance between environment and the new genotypes may be one reason why progress under selection is then limited. Conversely, sub-optimal environments might be expected to expose different arrays of genes to the action of selection, and it is of further interest to see how genotypes so selected then behave under normal conditions.

2. MATERIALS AND METHODS

Selection was started from a four-way cross of highly inbred lines of different origin: Oregon S, Oregon R, Nettlebed and Crianlarich (S, R, Nb and C hereafter). This permitted the reconstruction of the foundation stock when control series were required and provided half the genetic variance expected from a 'wild' stock (Falconer & Latyszewski, 1952).

Germ-free larvae were obtained and cultured using procedures previously described (Sang, 1956). Forty 2–4-hour-old larvae were inoculated into 6 × 1 inch tubes containing 5 ml. of the sterile, synthetic food medium and fifty tubes were inoculated for each group every generation, in so far as this was possible. That is, about 2,000 larvae were used each generation, for both the fast- and slow-growing lines of each of the two experimental series, and all were hatched from eggs laid during a 4-hour period. Eggs could not be set up since their viability depended on age and condition of the mother. Forty larvae per culture was near the optimal density for normal development (Sang, 1956).

Development rate of the larvae was measured by counting the numbers of males and females hatching each half-day. The first 500 or so females and the first 250–300 males to emerge became the selected parents for the fast-developing line and similar numbers of the last-emerging flies were used for the slow-developing line. Both fast and slow lines of a series were set up at the same time and on the same culture medium, previously prepared tubes being selected at random for each line. All cultures were kept together on the same incubator shelf at 25° C. The selected parents were kept on live yeast for 7–10 days after emergence to ensure that no deficiencies were passed through the egg. For obvious reasons, it was not always

possible to collect the exact number of parents required, nor was it always possible to avoid infection of cultures. Consequently, the selection differentials vary from generation to generation. Further, as it was essential to obtain an adequate number of parents to provide the necessary eggs during the short egg-collecting period defined, infertility of the late-hatching flies (slow line) tended to result in a lower selection pressure being exerted on these than on the fast lines. The large numbers of parents used eliminated any significant effects of inbreeding.

The synthetic-food medium used was Sang's (1956) medium C, except that the pyridoxine was reduced to 0·6 μg. per 5 ml. (the optimum being 3 μg. or more) in the low-pyridoxine series, and to 2·5% casein (optimum 5–5·5%) in the low-casein series. All other constituents were in optimal supply, or provided in excess where excess had no detrimental effect on growth. Consequently, growth was slowed on both media only as a result of the imposed dietary restriction. However, in the case of casein this restriction may represent a deficiency of an essential amino acid rather than an overall protein shortage (Sang, unpublished).

One technical difficulty was not properly appreciated when the work started, namely, that very minor differences in the autoclaving procedure affected the adequacy of the culture medium. High external temperatures, differences due to the amount of water in the autoclave and so forth, all appear to affect the final nutritive value of the medium after autoclaving, presumably by altering the degree of combination of sugar and protein (Malliard reaction) in the mixture. This results in a variation from generation to generation which has been minimized by finding the difference between fast and slow lines at each generation. For reasons previously given (Sang, 1956), development has been measured as the logarithm of the larval period ($\times$ 100) and all data are presented in terms of this scale. Data from males and from females have been averaged since there was no change in the sex difference in development time throughout the experiments.

3. EXPERIMENTAL RESULTS

(*a*) *Selection on pyridoxine-deficient diets*

As already noted, the parent population was a four-way cross of inbred lines of different origin, and selection of its fast- and slow-growing members (called PF or PS respectively) at once produced a significant divergence of lines (Table 1). However, the trends of subsequent selection responses are partly a consequence of culture differences from generation to generation, and this makes it uncertain if progress continues at the same rate, and for the same time, in the slow- and fast-developing groups. This difficulty, resulting from environmentally caused fluctuations, has also been noted for live yeast cultures (Sokal & Hunter, 1958) and caused the abandonment of Clarke, Maynard Smith & Sondis' (1961) experiments using a food low in yeast content.

The starting population was recreated at generations 11, 13 and 14, and grown concurrently on the same media as the selected lines. The differences between these controls and the initial population (Table 1) gives a measure of the difficulty of

Table 1. *Summary of numbers set up, development times and survivals on low-pyridoxine media*

Generation	Nos. set up		Development time		% survival	
	Fast	Slow	Fast	Slow	Fast	Slow
P 1	2160		75·6		62·8	
F 1	1000	1000	73·4	77·5	73·3	59·3
F 2	1840	1280	73·2	77·0	67·7	54·8
F 3	2080	1880	72·8	70·4	70·7	76·0
F 4	1960	1800	70·9	76·2	66·2	66·6
F 5	1360	760	71·5	80·7	77·2	60·8
F 6	1840	1320	67·4	77·0	76·4	69·7
F 7	1760	1120	74·8	81·6	56·5	57·2
F 8	1960	1520	72·8	82·8	61·7	53·0
F 9	1960	1680	71·9	82·6	61·2	49·5
F10	2320	1600	73·2	83·2	73·6	54·2
F11	1600	1520	74·1	86·5	75·3	57·0
Control	400		81·7		61·0	
F12	1840	1040	71·9	83·1	79·6	66·0
F13	2000	2160	68·9	80·8	74·8	60·2
Control	360		76·6		84·0	
F14	1320	1760	73·6	84·4	72·8	60·6
Control	560		80·2		61·5	

Development time is given as 100 × log-days throughout.

preparing standard media on separate occasions, and indicates the need for eliminating this variation when considering the results. In so far as the fast- and slow-developing lines do not respond dissimilarly to this range of culture conditions, the differences between them can be taken as a measure of the response to the combined selection pressure applied to the two lines (Fig. 1). This overall response has continued for about nine generations, after which no further progress has been made. At this final level, about one-third of the response is due to a slowing, and about two-thirds to a speeding up, of development rate. Under these conditions, it is therefore possible to select genotypes favouring fast growth which are apparently not open to selection under normal culture conditions (Sang & Clayton, 1957). The data are inadequate to show if the asymmetry of response is a consequence of the fast and slow lines reaching their limits at different levels of total selection pressure or if the fast line responds twice as well as the slow line throughout: the data of Table 1 suggest that this may be the case.

Two further points need comment. While the general trend of the combined response during the first eight to nine generations suggests that the character has its expected heritability of about 20%, the response of the first generation greatly exceeds this, and that of the seventh generation falls well below the 20% line (Fig. 1). The only apparent difference in the first generation relates to survival, which is significantly lower than average in the slow line (Table 1). This difference is also found in subsequent generations and tends to be a permanent feature of the

data after the eighth generation, apparently because the faster-developing line survives better than even the foundation population.

The culture conditions of the seventh were probably worse than those of any other generation: survival was low, total variability was high and development

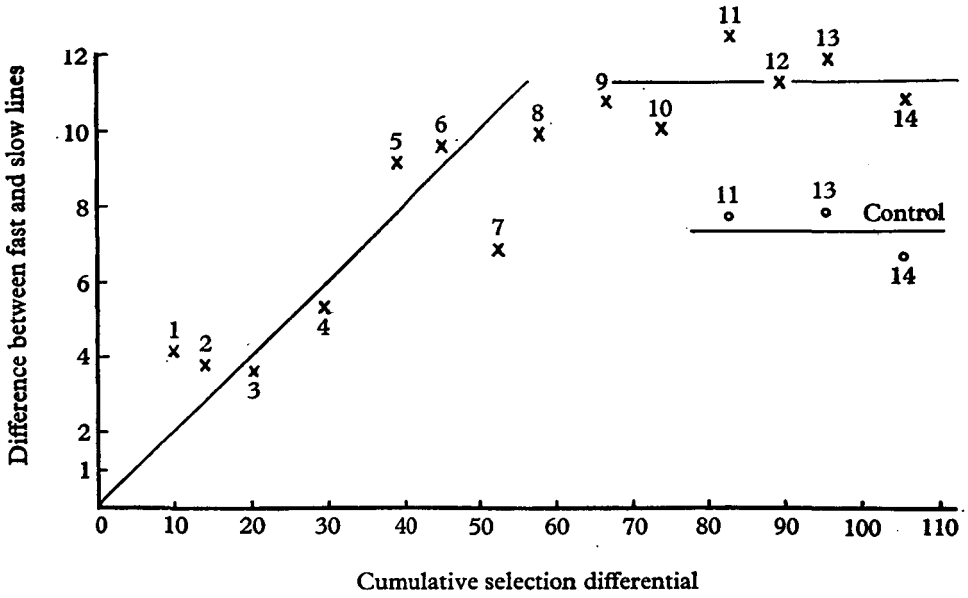

Fig. 1. The difference between PS and PF lines plotted against the combined cumulative selection differential. The generations are shown by the numbers against the points, and the line extending through the first eight generations is the 20% heritability level. The maximum response line has been averaged through generations 10 to 14, and the control (SRNbC) line through the points for generations 11, 13 and 14, after finding its position relative to the PF and PS lines. The difference above the control represents a slowing of development, the difference below the control, a speeding up of development.

was prolonged (Table 1). Under these circumstances it appears that the selected lines react differently to their environment, the potential rate of development of the fast line being then restricted. During the fourteen generations there was no other change of variability which merits comment.

(*b*) *Selection on casein-deficient diets*

Media low in casein proved to be even more difficult to duplicate than those short of pyridoxine, due to their greater sensitivity to the variations in the autoclaving procedure. Detailed examination of the response to selection is therefore limited to the difference between the fast and slow lines (called CF and CS, respectively) which minimizes these environmental effects (Fig. 2). The selection practised was very similar for both lines, which makes this procedure more meaningful. Taken over the fifteen generations, the response to selection is surprisingly regular, and continuous progress is made up to the termination of the experiment.

Heritability is close to 10%, and is half that found in the preceeding experiment. However, some generations behave in an anomalous fashion, each falling below the general trend by about the same amount. How far this was due to differences in the culture media was tested by measuring generations 10 and 14 on separate occasions (Table 2). These repeat experiments show that it is possible to account for the anomalous generations since the repeat of generation 10 falls below the

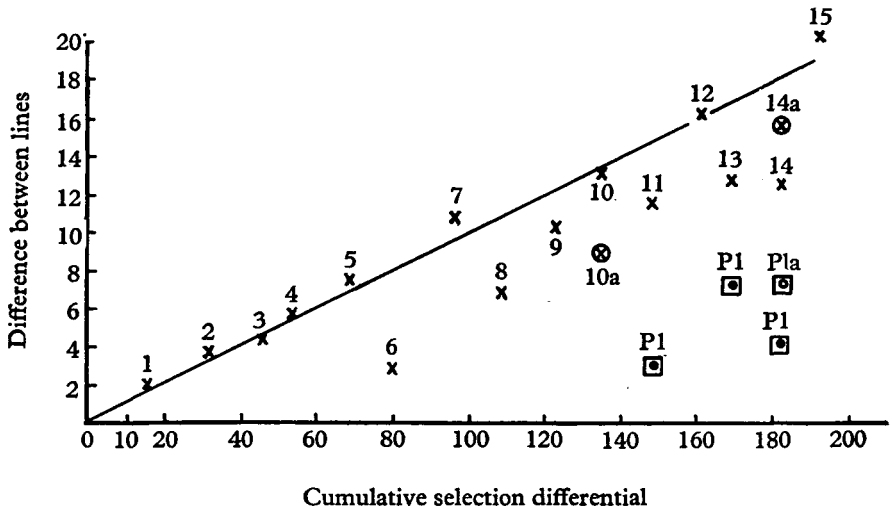

Fig. 2. Differences between CS and CF lines plotted against cumulative selection differentials. The generations are shown by the numbers beside the points and the line running through them is the 10% heritability level. Repeat experiments are shown by a circle around the point, and the control by □.

original by about the amount found for the others (Fig. 2). Generation 14 is particularly instructive since it also demonstrates that the measured response falls short of the general trend only for the fast selected line; the two slow-developing lines differing from the control by the same amount. This explanation also appears to fit generation 11 in which the difference of the fast strain from the control is less than expected from the general trend; but not generation 13, in which survival of the slow line is very poor (Table 2).

Table 2. *Development time and survival for aberrant generations and in repeat tests*

	Nos. set up			Development time			Difference from control		% survival		
Generation	Fast	Slow	Control	Fast	Slow	Control	Fast	Slow	Fast	Slow	Control
P1	—	—	2400	—	—	83·1	—	—	—	—	84·0
10	1840	1960	—	85·5	98·6	—	—	—	71·7	65·0	—
10 repeat	1360	2080	—	80·7	89·8	—	—	—	72·5	62·0	—
11	1600	1720	520	84·1	95·7	87·0	−4·0	+8·6	75·2	63·6	67·3
13	1760	1840	400	83·4	96·3	90·7	−7·3	+5·5	70·1	53·0	68·5
14	1760	1640	480	83·2	95·7	87·3	−4·1	+8·5	86·0	75·0	83·5
14 repeat	520	480	560	82·4	98·1	89·7	−7·4	+8·3	92·0	75·0	85·0

(c) *Comparison of selected populations*

If one judges the two culture media by the time taken for larval development, the low-pyridoxine medium is superior and variability is significantly less among larvae reared on it. However, survival is better on the low-casein medium (Tables 1 and 2), indicating that the two ways of interfering with protein metabolism act differently on the larvae. This difference is reflected in the responses, which continue on the casein medium at least to the point where the total selection pressure is twice that required to reach the maximum response (plateau populations) obtained on the low-pyridoxine medium. Further, the kinds of response are different in the two environments: on the low-casein medium the response is more or less equal in the two directions, whereas it is greater towards fast development on the low-pyridoxine medium, and the rate of progress (realized heritability) differs in the two cases. In neither situation is variability much affected by selec-

Table 3. *Characteristics of tenth-generation selection larvae, cultured on their respective media*

	Pyridoxine selected					Casein selected				
			Development time					Development time		
	n	% Survival	Mean	Variance	♂ weight	*n*	% Survival	Mean	Variance	♂ weight
Fast line	104	86·6	74·6	19·3	0·924 mg.	108	90·5	74·7	34·4	0·917 mg.
Slow line	71	69·2	87·1	12·8	0·902	89	74·0	83·4	43·1	0·850

The weights are the mean of a sample of twenty-five males in each instance.

tion, so that it is difficult to assume that the lack of progress in the last five selection generations on the low-pyridoxine medium is a consequence of the elimination of suitable genotypes: although this might account for limited progress in the slow lines, it seems improbable for the fast lines which both survive somewhat better than the original population (Tables 1 and 2). Further, data on the size of males (Table 3) show that the PS and CS have also been selected for small size, when compared with the fast lines (but see p. 104). As Robertson (1960*b*) has noted, selection may produce the same kind of result by different routes and the type of environment in which it is practised has a determining influence in this connexion. It is also worth noting that in both experiments an exceptionally poor environment affects the expression of the fast-developing lines more than it does the slow lines. This is also found to apply to the dose-response curves (Fig. 6).

(d) *Some nutritional characteristics of the selected lines*

Dose-response graphs were prepared for the selected lines at generations 8, 10 and 14, and a control series was run with the last. Due to the technical difficulties already noted, detailed comparisons cannot be made between these tests performed

at different times. Interpretation of the data is further complicated by problems involving their statistical treatment. The conventions which have been adopted are (i) that all response are linear on the scales employed, since this seems justified by previously published data (Sang, 1956, 1959), and (ii) the calculated regression slopes exclude points at, or close to, the inflexions when inclusion of such points would markedly alter the regression. The course of the two response differ. As measured by development time the response to pyridoxine is proportional to the supply until sufficient is provided to give optimal growth; more than this does not affect development time. With casein, an excess as well as a deficiency slows growth (Sang, 1956). The inflexion points represent, therefore, the minimum necessary for optimal growth with pyridoxine, and the true optimal supply of casein.

(1) *Low-pyridoxine selected lines*

At the eighth generation there was no evidence that the inflexion points of the pyridoxine responses differed between the slow- and fast-growing lines. The difference between the lines was not constant, being greater when the smallest

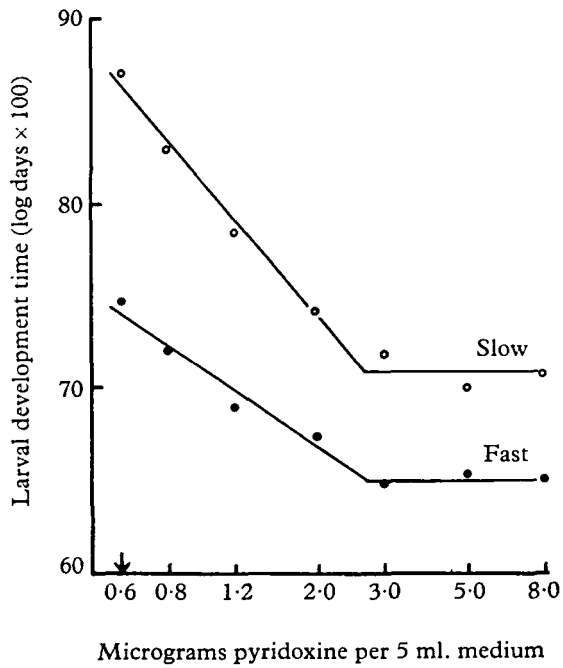

Fig. 3. Response of the tenth generation, PS and PF lines, to pyridoxine. Selection was carried out at the 0·6 mg. pyridoxine level.

amounts of pyridoxine were provided. This difference between the lines was more clearly seen by the tenth generation (Fig. 3) when it was more than twice as great at the lowest level of pyridoxine, as when adequate amounts (*ca.* 3 μg. or more) were provided. At this generation there was again no evidence that the inflexions

of the two responses differed; about 2·7 μg. pyridoxine per culture was sufficient to permit the best growth-rate possible in both cases. The situation had changed by the fourteenth generation (Fig. 4*a*), although there was no measurable response

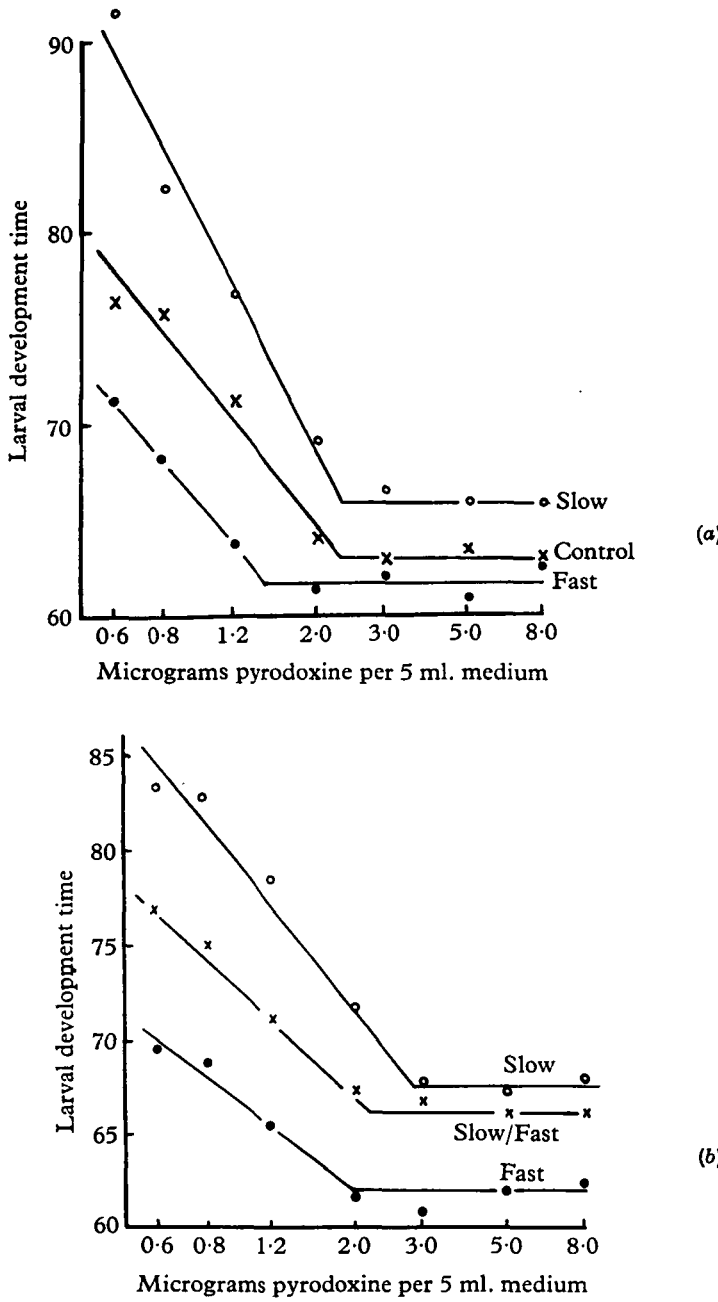

Fig. 4. (*a*) Response of PS and PF lines of the fourteenth generation and of the concurrently tested control (SRNbC) population to pyridoxine.

(*b*) Responses to pyridoxine of PS, PF and PS/PF lines of the fifteenth generation.

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to selection during the four intervening generations (p. 93). The PS line and the control (SRNbC) population inflected at very similar levels (*ca.* 2·5 μg. pyridoxine per culture) whereas the PF line required less (*ca.* 1·5 μg. pyridoxine). As before, the difference between the lines was greater at the low concentrations of the vitamin. A fifteenth generation, which was bred without further selection, was tested to confirm this unexpected difference, and a cross between the lines replaced the control. Practically the same difference of inflexion points was again found for the PF and PS lines (Fig. 4*b*) although the absolute requirement of both was higher than in the previous test. The cross of the lines behaved more or less as an intermediate, but its inflexion point was very similar to that of the PF line, in

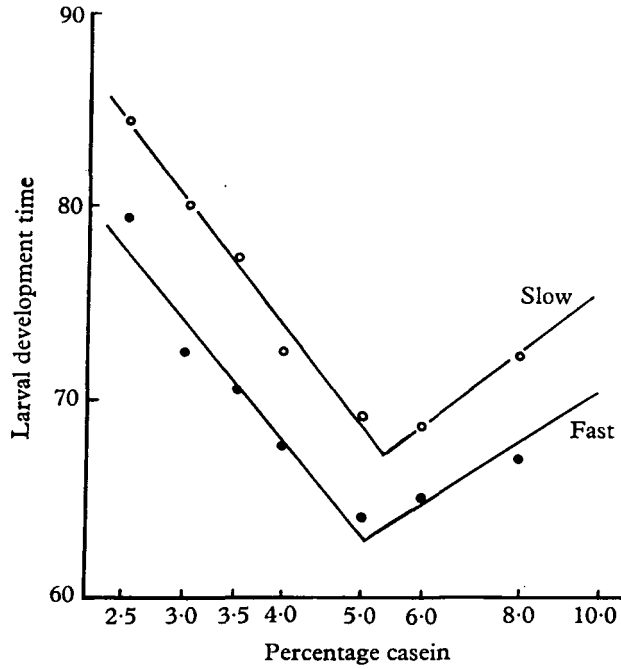

Fig. 5. Responses of the tenth-generation PS and PF lines to varying casein.

contradistinction to that of the control (Fig. 4*a*). Consequently, the relationship of the PF/PS hybrid to the average of the parents changes with the provision of dietary pyridoxine.

The responses of the eighth and tenth PF and PS generations to varying casein showed no change of optimal requirements; the differences between the lines being more or less constant and the inflexion points very similar (Fig. 5). The fourteenth generation behaved in a like fashion, although there was some suggestion that the PF line required less protein than the PS line. What was surprising was that both lines needed less casein than the control for optimal growth (Fig. 6), and that both responded to a decrease of protein by a more marked slowing of development rate than did the control.

Taken together, these results suggest that selection on a low-pyridoxine medium affects development in three ways: non-specifically, by a general slowing of growth; specifically in the sense that reactions to a poor pyridoxine supply are more exaggerated than to an adequate supply, and eventually by an alteration of the

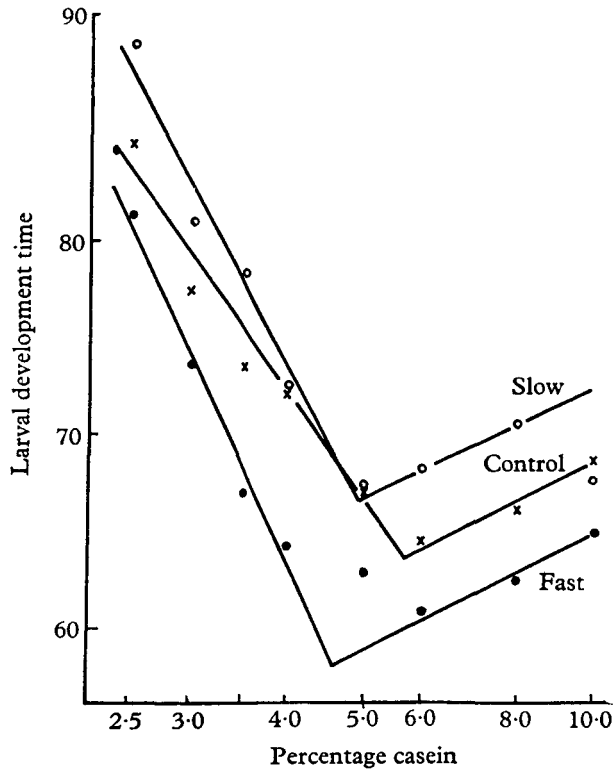

Fig. 6. Response of the fourteenth-generation PS and PF lines and of the control population to casein. Note that the 5% casein points are excluded from the regression calculations and that the 10% point was also excluded for the PS line. Selection was carried out at the 2·5% casein level.

level of requirement: these changes are reflected not so much in the use of pyridoxine in casein metabolism generally, but more particularly when casein is in relatively short supply. Each of the selected genotypes, the parent population and the cross between the selected lines would then be expected to have its own particular pattern of requirements and of responses to deficiencies of pyridoxine or of casein in their diet.

(2) *Low-casein selected lines*

At the eighth generation, the selected lines differed in their response to both low and high levels of protein, but not in their optimal requirements for rapid growth. These differences between the lines were again more obvious by the tenth generation (Fig. 7). The provision of an optimal supply of casein lessens the difference

between the development rates of the two selected lines and there is no clear-cut distinction between the optimal supplies for the two lines. The CS line is more affected than CF by excess supplies of casein as well as by any deficiency. Unfor-

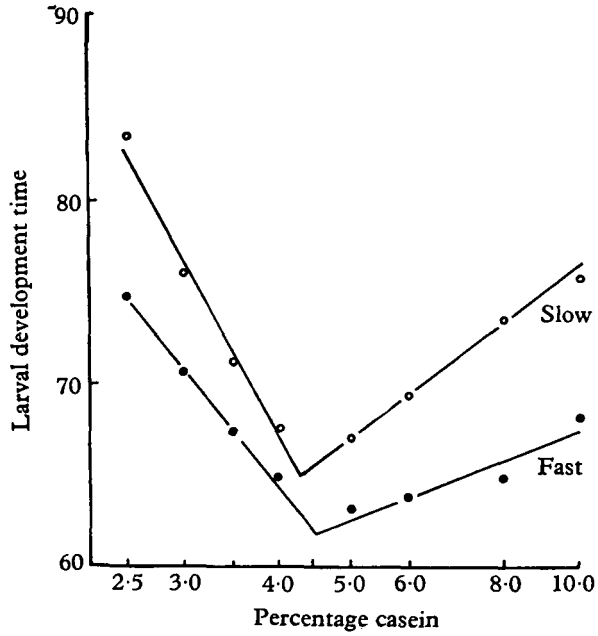

Fig. 7. Response of tenth-generation CS and CF lines to varying casein.

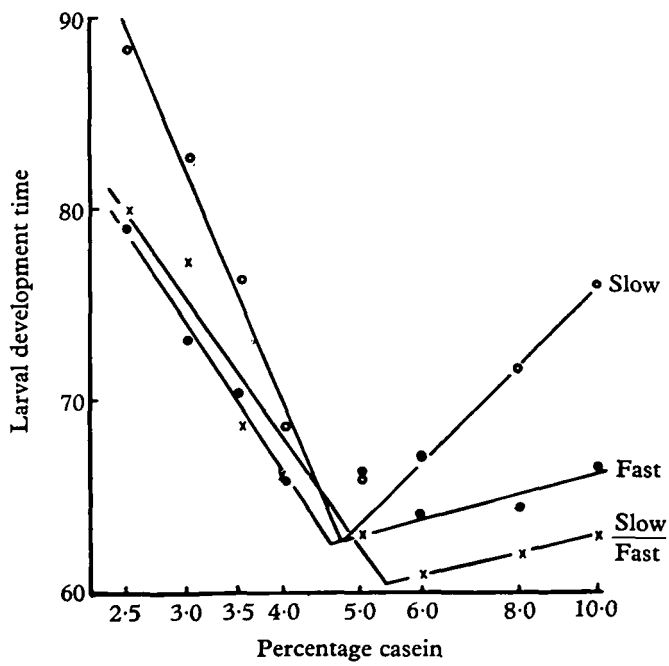

Fig. 8. Response of fifteenth-generation CS, CF and CS/CF lines to casein.

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tunately the test cultures of the fourteenth generation became infected, so that this experiment had to be checked using flies from the last (fifteenth) selected generation, and a cross between the lines was again included as a substitute for the original control population (Fig. 8). The result was the same, except that in this instance the optimal provision of casein apparently permitted both lines to grow at the same rate: the genetic differences resulting from selection at a low level of casein supply were completely compensated for by an adequate casein supply. The cross between the lines, on the other hand, required somewhat more protein at its optimum (cf. Fig. 4*b*), grew faster than either selected line when this amount or more supplied, but grew more slowly than the CF line at sub-optimal levels of casein. Its response throughout was more like the CF than like the CS line.

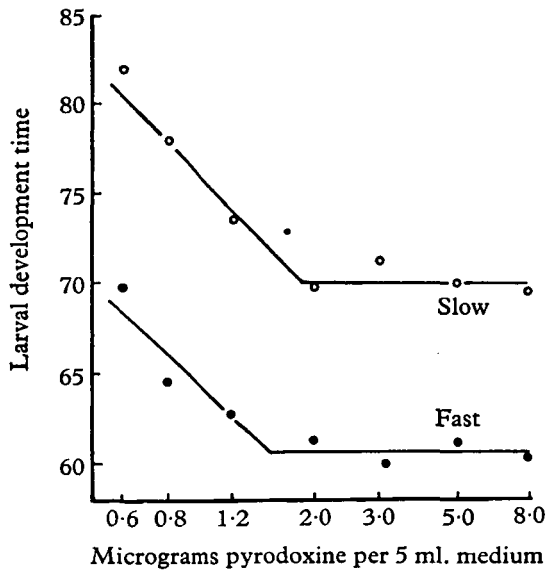

Fig. 9. Response of fifteenth-generation CS and CF lines to pyridoxine.

The CF and CS selected lines were tested for their responses to pyridoxine: at the eighth, tenth and fourteenth generations they showed similar responses indicating a constant difference of development rate throughout the range tested (Fig. 9). There was no evidence that the minimum requirements of pyridoxine, necessary for their fastest growth, had been altered by selection. The genetic changes resulting from selection on a low-casein diet had little or no effect on the utilization of pyridoxine by the lines.

(3) *Comparison of the selected lines*

It is evident that selection under both conditions has resulted in more or less specific responses, in the sense that the difference between the lines is greatest when they are grown in the poor environments under which selection was implemented. In both cases, better environments lessen the differences between the

lines. This was further checked by growing larvae of the tenth generation on live yeast media (i.e. the optimal, complete environment) and no statistically significant differences were found between any of the lines, nor did they differ from the foundation population. That is to say, the genetic changes induced by selection were completely counteracted in the optimum environment and could display themselves fully only in the relevant poor environments. It follows that only a minor part, if any, of the divergence between lines at low levels of pyridoxine or casein can be accounted for by deficiencies of the scale used. In fact, there is no good evidence that inadequacy of the development-time scale would distort the responses to give the results described (cf. Sang, 1956, 1959).

The second interesting point of comparison is provided by the reaction of the lines to an excess of casein. In so far as one can compare experiments done at separate times, it seems that the overdose response of the low-pyridoxine selected lines is the same as that of the foundation population, whereas the low-casein selected lines behave differently (Fig. 8); the CF line being less affected and the CS line more affected by excess casein. It follows that selection on a casein-deficient diet has altered the abilities of the larvae to metabolize excess casein whereas selection on the pyridoxine-deficient diet has not. There is no clear evidence that the responses of the low-casein lines to pyridoxine have been altered (Fig. 9).

(*e*) *Crosses amongst the lines*

Samples of flies from the tenth and then from the fourteenth generations were crossed and their progeny reared on the standard synthetic medium (5·5% casein and 50 μg. pyridoxine). The dose-response tests using flies of the fourteenth and fifteenth generations had not then been carried out, of course, and it was not appreciated that each genotype might have its own optimal nutritional environment, and that an arbitrary cross-section of this complex might conceal more than it revealed. However, some points of importance were disclosed by these tests.

The parent lines of the tenth generation behaved as already described (Figs. 3 and 7) and the fast lines emerged significantly earlier than the slow lines when grown on the standard medium. All the crosses, except that between the two lines selected on low casein, were indistinguishable from the average of their parents. The exceptional cross was significantly faster than this average but not as fast as the better (CF) parent.

The medium used for the fourteenth-generation test proved to be atypical and all the selected lines took longer to develop than in the corresponding dose-response tests (Figs. 4 and 8) but the difference between fast and slow lines was in agreement with these tests. All the crosses grew significantly faster than the average of their parents (Table 4) and three grew faster than the better parent, including the cross of the two slow-developing lines. This result may be due to the particular conditions of the experiment, and one cannot conclude that the genetic changes induced by selection between generations 10 and 14 have produced gene combinations not present at the tenth generation which result in hybrid vigour in the later

test, although this seems a possible explanation of the results. On the other hand, it is obvious from these and from the previous results (Figs. 4 and 8) that tests of the performance of crosses may give notably different results in different environments.

The variability of the hybrids behaves irregularly, lying in some instances close to the parental average, in others being significantly larger or smaller than this. There is no correlation between variability and rate of development or, in the crosses, with superiority over the better parent. The genetic control of interactions with environment must therefore depend on particular combinations of genes. There is a positive and significant relationship between development rate and survival, faster development being associated with improved viability. There seems to be no distinction between the lines and crosses in this respect.

Table 4. *Development on standard medium of F14 selected lines and their crosses*

		Development time (100 × log-days)					
Genotype	*n*	Mean	Mid-parent	Variance	Survival (%)	Mean male weight (mg.)	Growth rate
CF	158	67·3	—	38·6	79·5	0·87	0·184
CS	198	71·8	—	22·7	83·3	0·92	0·176
CF/CS	239	64·2*	69·6	31·7	85·4	0·86	0·196
PF	218	65·2	—	32·7	90·5	0·88	0·196
PS	61	71·4	—	17·5	76·3	0·93	0·179
PF/PS	249	64·4†	68·3	18·8	88·4	0·89	0·200
CF/PF	139	64·1†	66·2	18·3	86·9	0·91	0·206
CF/PS	264	63·5*	69·3	19·9	94·0	0·90	0·208
CS/PF	147	66·9†	68·5	25·1	88·2	0·92	0·197
CS/PS	200	67·9*	71·6	43·2	83·0	0·89	0·187

The variance is the within-culture variance, the male weight is the mean of a sample of 20–25, and the growth rate is the weight ÷ larval period in days.

* Significantly faster than the more rapidly developing parent.

† Significantly faster than the mid-parent, judged at the 1% level.

Fast development is associated with smaller size in the parent lines (Table 4); in fact, deficient media produce flies of almost the same size as the complete medium (cf. Table 3). The environment therefore does not set the same limits to the expression of size as it does to development time. Size of the crosses is not correlated with development time and some crosses are superior to (CF/PF and CS/PF) and others inferior to the mean size of their parents (CF/CS, PF/PS and CS/PS). It is interesting to note that the cross of the smallest lines (CF/PF) produced the greatest improvement of size in accordance with Robertson & Reeve's (1955) finding for crosses between lines selected for size; but the cross of the largest lines was considerably smaller than the parent mean and not approximating to it, as these workers found. How far this also depends on development rate, cannot be determined from these limited data. It is also interesting to note that weight gain per unit time has some regularity; the two casein-selected lines

are alike and less efficient than the corresponding pyridoxine-selected lines. The crosses are always more efficient than their parents by this measure, especially those involving the CF line.

4. DISCUSSION AND CONCLUSIONS

In their studies of selection for rate of larval development, Sokal & Hunter (1958) found the fluctuations from generation to generation to be so great on live yeast media that they failed in their objective of estimating the heritability of this 'character'. They directed attention specifically to this unexpected phenomenon, which they presumed to be due to a gene-environment interaction of peculiar sensitivity and of nutritional origin. Clarke, Maynard Smith & Sondhi (1961) overcame this difficulty by running a control population concurrently with each generation and by measuring progress as the difference of development times from this control. Even so, the responses obtained on their live yeast *D. subobscura* cultures also show some fluctuations from generation to generation. The data described above indicate that larval development rate is, indeed, particularly susceptible to small differences of diet. Surprisingly enough, both fast- and slow-growing strains respond to about the same extent to at least some of these variables so that the difference between fast and slow selected lines is generally regular and consistent when they are cultured on identical diets. Other conditions appear to affect the fast-growing lines most, apparently by setting a limit to their development rate. It does not follow that the same arrays of genes are exposed to selection when successive generations are grown on slightly different media, and this is presumably why estimates of heritability based on the usual one-generation tests are notably different from, and larger than, those based on selection responses (Sang & Clayton, 1957; Clarke, Maynard Smith & Sondhi, 1961).

When the environments in which development occurs are very different as in the two cases reported above, responses to the same selection pressure progress in a different way and to a different end. On the pyridoxine-deficient diet, the heritability found is about twice that on the low-casein diet, responses are mostly in the direction of fast development rather than of slow development, but reach a plateau (without loss of variability), whereas no such cessation of response occurs on low casein. Contrary to previous experience, it was possible to select for more rapid larval development on both deficient diets, although to a different extent on each. We can only conclude, as Robertson (1960*a*, *b*) has done from similar selection experiments for size of *Drosophila*, that different environments expose different gene arrays to selection. The statistical description of the results of selection are incomplete by themselves, therefore, and are an inadequate guide to our understanding of the processes affected by selection.

The most important differences between the two experiments are in the heritabilities and in the courses of the responses to selection. Since no genetic analyses were possible when the experiments had to terminate, we can note only that the phenotypic variance of the foundation population (SCRNb) was greater

in the low-casein medium (Table 5) and that this was partly accounted for by a larger environmental component (as measured by the inbred parents and their F1's). The realized heritabilities would thus be expected to have the relationship found (i.e. would be lower on the low-casein medium). The situation is not simple, since the regularity of the variances of the parents on the low-pyridoxine medium is matched by an equal variance in the four-way cross (Table 5). In the low-casein environment, the variances are heterogeneous (by Bartlett's test) even if the four-way cross is excluded, and it is obvious that each genotype tends to have its own reaction to the low-casein environment, possibly by having somewhat different requirements for essential amino acids. This difference of reaction is not evident in the low-pyridoxine series, except possibly for the C line and for the four-way cross which has a lower variance than expected (Table 5). This contrast of the reactions of pure-line parents and F1's to different environments emphasizes the difficulty inherent in any estimation of the environmental component of variance, a topic which will be considered in a subsequent publication.

Table 5. *Within-culture variances of development time for inbred lines and their crosses*

	Genotype						
Diet	S	C	S/C	R	Nb	R/Nb	SCRNb
Low-pyridoxine	28·4 (64)	— (4)	25·7 (63)	30·9 (20)	28·3 (63)	22·3 (120)	23·3 (100)
Low-casein	18·4 (112)	26·9 (39)	36·7 (91)	33·0 (89)	34·5 (83)	44·7 (108)	58·4 (114)

The numbers per sample are shown in brackets. The C line was very inviable on 0·6 μg. pyridoxine: when 0·8 μg. pyridoxine was supplied the variance was 46·6 (40).

Two aspects of the course of the responses have to be considered: the asymmetry of the response on the low-pyridoxine diet, and the limit of response reached by the 8–9th generation. As already noted, directional dominance would be expected to apply to the genes determining larval development rate and this might account for an asymmetry of response. Clarke, Maynard Smith & Sondhi (1961) have examined a model of this situation which would lead to the prediction that more progress would be made in the slow rather than in the fast direction, and that hybrids between fast and slow lines would develop almost as rapidly as the faster parent. This model might explain the results found with the low-casein diet since the asymmetry of the response is as predicted (Fig. 2) and the hybrids of the tenth and fourteenth generations grow as fast, or faster than, the faster-growing parent. The model cannot explain the responses on low pyridoxine where the asymmetry favours fast development and in which the tenth-generation hybrid is intermediate and indistinguishable from the average of the parents. The only other possible explanation of asymmetry is that it involves epistatic interactions, and this is the explanation favoured by Clarke, Maynard Smith & Sondhi (1961) for their data on *D. subobscura*. The experiments described here provide no critical data which permit the certain categorization of the response as due only

to epistasis, and we can merely note that the asymmetry of the pyridoxine response is in the direction opposite to that found by Clarke *et al.* (1961). It is difficult to believe that two such different response patterns as those described could result from the operation of the same mechanism.

The 'plateau' in the response to selection on the low-pyridoxine diet is better considered in conjunction with the dose-response data.

On low casein, the alteration of growth-rate with respect to amount of casein in the diet is almost exclusively a change of reaction to sub-optimal supplies, since with an optimal supply the CF and CS lines grow at virtually the same rate. With excess casein the two lines separate again (Fig. 8). What has been altered is the biochemical mechanisms involved in dealing with casein when not provided in optimal amounts, i.e. one class of mechanism. On low pyridoxine, the situation is different since the selection response to low pyridoxine is not fully compensated for by adequate supplies of the vitamin; that is, the mechanisms modified by selection continue to affect development under optimal conditions and are therefore partly general and partly specific to the poor environment. Further, although development rate is not altered significantly by selection between the tenth and fourteenth generations the requirement of pyridoxine is, and the optimal provision of pyridoxine for the two lines then changes (Fig. 4). Although the analyses are insufficient to support the contention in detail, one can plausibly imply that at least three classes of reaction systems are being altered by selection on low pyridoxine.

One of the most significant findings is that the form of the dose-response to pyridoxine changes from the tenth to the fourteenth generations, when there is no apparent alteration of the 'character' being selected. That such a change may occur was foreshadowed by Schultz (1953), who found that 'cryptic gains' were made when selecting poultry for size, and that these gains were expressed in crosses. A similar expression in hybrids of a 'cryptic gain' seems to hold in the present case. The explanation of the failure to make progress after a certain level of selection on the low-pyridoxine diet would seem to be most simply interpreted, therefore, by assuming that the 'character' itself changes under selection, that a different combination of physiological properties, and hence a different set of genes, is presented to the selection force. This would seem to be an inevitable consequence of selection whenever this is operating on a complex character.

This contention is further supported by the descriptions of the dose-responses of the selected lines and their crosses, which show that each genotype may have its own particular optimal combination of nutritional supplies and may behave differently when more or less than this amount is provided. It was hoped by combining studies of casein and pyridoxine requirements of all the lines that some indication would be found of the changes which had occurred: for instance, the pyridoxine requirements of the casein selected lines might have been different, indicating, among other possibilities, an alteration of their transaminating systems. But no clear-cut differences were found, and it is impossible to specify what biochemical processes have been altered by selection.

Previous results of crossing lines of *Drosophila* selected for development rate have been contradictory. Where selection has been accompanied by inbreeding (Hollingsworth & Maynard Smith, 1955) the hybrid grew faster than either parent. However, it seems that crosses of inbred lines always behave in this way, even when not specifically selected for development rate. When selection was not accompanied by inbreeding the hybrid was intermediate (Clarke, Maynard Smith & Sondhi, 1961). The results described here show that non-inbred, selected stock may behave differently, but that this depends in some unspecified way on the kind of changes produced by selection and also on the environment in which the development of the lines and crosses is tested. In some environments, the crosses would behave intermediately, in others they would perform better than the superior parent. One anomalous result has to be considered in this context: the PF/PS hybrid would apparently not grow faster than the PF line (Fig. 4) throughout the pyridoxine range, whereas in the fourteenth-generation test it is found to do so. Other work (Sang, 1956 and unpublished) indicates that minor alterations of the amino-acid supply, such as those produced by autoclaving, affect pyridoxine requirements, and this may be why the two experiments differ.

Finally, we must note that the consequences of crossing the selected lines depend also on the changes produced by selection, since crosses of lines of the tenth generation did not give the clear-cut results found at the fourteenth generation (Table 4). This, and the further fact that selection under special circumstances has not produced alterations which manifest themselves under optimal conditions, indicates the need for further studies of the biochemical changes produced by selection.

SUMMARY

1. The problem of improving rate of larval development of *Drosophila* by selecting for this 'character' on deficient diets is examined by culturing larvae axenically on low-casein and low-pyridoxine media. Under these conditions it is possible to develop strains which grow faster than the parent population.

2. Selection for fast- and slow-growing larvae on a low-pyridoxine diet proceeds with a realized heritability of about 20%, but progress ceases after eight to nine generations. The selected larvae show no alteration of pyridoxine requirements up to the tenth generation, but the lines develop at different rates under optimal conditions. This difference is exaggerated when the diets are low in pyridoxine. By the fourteenth generation, requirements of the two lines for optimal growth have become distinct, the fast line requiring less pyridoxine than the control. Casein requirements show about the same optimum for the two lines but this is lower than that of the control, foundation population.

3. Selection for fast- and slow-development lines on a low-casein diet continues to be effective throughout the fifteen generations of the test. The realized heritability in this case is about 10%. The optimal requirements of the two lines are the same, and there is little difference in their development rates when reared on this optimal diet. The response is found only under sub-optimal conditions, both

of deficiency and of excess casein. Pyridoxine requirements do not seem to be altered in the two lines.

4. Crosses between the selected lines show that each genotype has its own optimal environment, as judged by pyridoxine and casein requirements. Crosses among the lines after fourteen generations show that all the hybrids are superior to the mid-parent and three grew faster than the better parent. Other environments would have given different results.

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