Asymmetrical response to selection for rate of development in *Drosophila subobscura*

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1. INTRODUCTION

It is a common experience in selection experiments to find that the rates of response in the two directions are unequal. This phenomenon is observed in an extreme form in 'plateaued' populations, which, although they have ceased to respond to selection in a forward direction, respond readily to back-selection.

In this paper, an attempt is made to elucidate the genetic situation responsible for the asymmetrical response of *Drosophila subobscura* populations to selection for fast and for slow development. This is a character which in this species appears to have reached a 'plateau' under the influence of natural selection. Earlier work (Maynard Smith & Maynard Smith, 1954; Hollingsworth & Maynard Smith, 1955; Clarke, unpublished) has shown that genetic heterozygotes develop faster than homozygotes, and that it is difficult to increase development rate by selection, although the rate can readily be reduced by inbreeding.

Selection for fast and for slow development has been practised without inbreeding. In order to create conditions which might favour progress towards faster development, we started from a population formed by crossing a number of geographical populations, and selected on a diet with an unusually high level of protein. Selection was also practised on a very low protein diet, but irregular fluctuations in development rate occurred from generation to generation. Their presence made it impossible to detect the relatively small changes to be expected from selection, and therefore the line was discontinued. Despite the unusual environment and hybrid foundation population, very little progress was made towards faster development. But a comparison of this experiment with the earlier experiments by Hollingsworth & Maynard Smith (1955) has made it possible to give a picture of the genetics of development rate in this species which, although complex, may prove to be typical of characters which have in the past been exposed to directional selection for an appreciable time in a reasonably large population.

2. MATERIALS AND METHODS

A geographically hybrid foundation population was obtained by crossing together four wild-type stocks in such a way that each contributed equally to the foundation population. Of the four stocks, two were derived from groups of wild females caught in Edinburgh, Scotland and in Galilee, Israel, respectively, and two were inbred

lines, the structurally homozygous **K** line derived from a female caught in Switzerland, and the **M** line derived from a female caught in Edinburgh. From this foundation population, two lines, F and S, were started, selected respectively for fast and for slow development from egg to adult emergence.

To collect eggs, single fertilized females were transferred to a dark medium consisting of agar and molasses with live yeast suspension added, for a number of successive 6-hour periods. Batches of eight eggs laid in a single period were then collected on paper spoons and transferred to vials containing a food medium of agar, molasses and maize meal, with 12% by weight of dried and killed yeast, and with one drop of dilute live yeast suspension added to the surface. Clarke (unpublished) has found that the optimum concentration of dead yeast both for survival and for fast development is about 4%. As mentioned above, an experiment on a low protein diet, with 0·1% of dead yeast, was also started, but there were large fluctuations in development rate from generation to generation and the experiment was discontinued. During the selected generations, two to three cultures of eight eggs each from about twenty-five females of each line were set up. Emergence of adult flies occurs almost entirely in the morning, and so the number of adults emerging was recorded every afternoon, the development time being taken as the interval between 12 a.m. on the day of emergence and the mid-point of the 6-hour interval during which the eggs were known to have been laid. There is little difference between the emergence times of males and of females, and so all results are given for the two sexes combined. Vials in which fewer than four adults emerged were not included in the results.

The experiment was carried out in a room controlled at $20 \pm 0.5\%°$C. But even small changes in temperature, and perhaps differences between the food in successive generations, can have large effects on development time. Therefore all calculations have been based, not on the absolute development times, but on the differences between the development times of the selected lines and that of a control population set up synchronously with each selected generation. This control population was obtained by crossing the structurally homozygous **B** and **K** inbred lines, and mating together the F_1 hybrids to obtain ten to fifteen cultures of eight F_2 eggs each. The F_2 rather than the F_1 was chosen as a control because it is much easier to obtain an adequate number of eggs from F_1 females than from inbred females, and because it is only slightly more variable phenotypically.

After setting up the selected lines, further samples of eggs were collected from the parents, to measure the percentage egg hatch; this was found in most generations to be over 90%.

3. RESULTS

A. Heritability estimates on the foundation population

A half-sib analysis of the foundation population was performed by mating a number of males each to two females, the parents being selected at random from the foundation population. Two batches of six eggs each were collected from each female. The analysis of variance and estimates of heritability are shown in Table 1.

Table 1. *Analysis of the foundation population*

	Degrees of freedom	Variance	Components
Within cultures	506	3·18	Q
Between cultures	62	4·82	$Q + zC$
Between dams	31	7·97	$Q + zC + 2zD$
Between sires	30	6·94	$Q + zC + 2zD + 4zS$

Mean number of flies per culture, $z = 5·08$

$$\text{Total heritability, } h_{\text{DAM}}^2 = \frac{4D}{Q+C+D+S} = +0·33$$

$$\text{Additive heritability, } h_{\text{SIRE}}^2 = \frac{4S}{Q+C+D+S} = -0·054$$

Since the true value of the additive heritability cannot be negative, a better estimate of the total heritability is obtained by assuming $S = 0$, when $h_{\text{TOTAL}}^2 = 0·277$.

Note: In this Table, and in Tables 2 and 3, the unit is a 6-hour period, not a day.

The results show that, even with the precautions taken to reduce environmental variance, the total heritability of development time is low (approximately 0·3), and the estimate based on the sires suggests that there is little or no additive genetic variance.

However, it is worth pointing out that heritability estimates based on half-sib analyses are subject to large sampling errors unless very large numbers of parents are used, and that a more accurate estimate of additive heritability can be obtained with the same amount of labour by performing a single generation of selection.

B. Responses to selection

Selection for fast and for slow development was practised for eleven generations. The results, based on the differences between the selected lines and the controls, are shown in Fig. 1. The emergence time of the controls had a mean of 17·4 days, and a range from 16·8 to 18·1 days.

In the figure, the response to selection has been plotted against the cumulative selection differential, as suggested by Falconer (1955). The linear regression lines show realized heritabilities of $+0·186 \pm 0·031$ in the S line, and $+0·063 \pm 0·029$ in the F line. In calculating these regression lines, each point has been weighted by the reciprocal of its error variance, which is proportional to $(n_1 + n_2)/(n_1 n_2)$, where n_1 and n_2 are the number of flies in the selected line and in the controls respectively.

The main conclusion to be drawn is that more rapid progress was made in the S than in the F line; indeed, progress towards faster development was barely significant statistically.

The negative responses to selection in the eighth, ninth and tenth generations of the S line call for an explanation. During the early development of the eighth generation the temperature control broke down, the temperature rose to 25° C. for a few days, and in consequence there was a heavy mortality, particularly in the S line. The fall in the development time in the eighth generation of the S line, relative

to the controls, probably occurred because there was in this line a higher mortality among the more homozygous and more slowly-developing genotypes. Since relatively few flies emerged in the eighth generation, little selection could be practised on it. There was also a heavy mortality during the development of the ninth generation of the S line, for reasons which are not clear; consequently little selection could be practised on this generation either. But progress towards slow development was resumed in the eleventh generation. The irregular response to selection during the last few generations therefore does not prove that a plateau had been reached
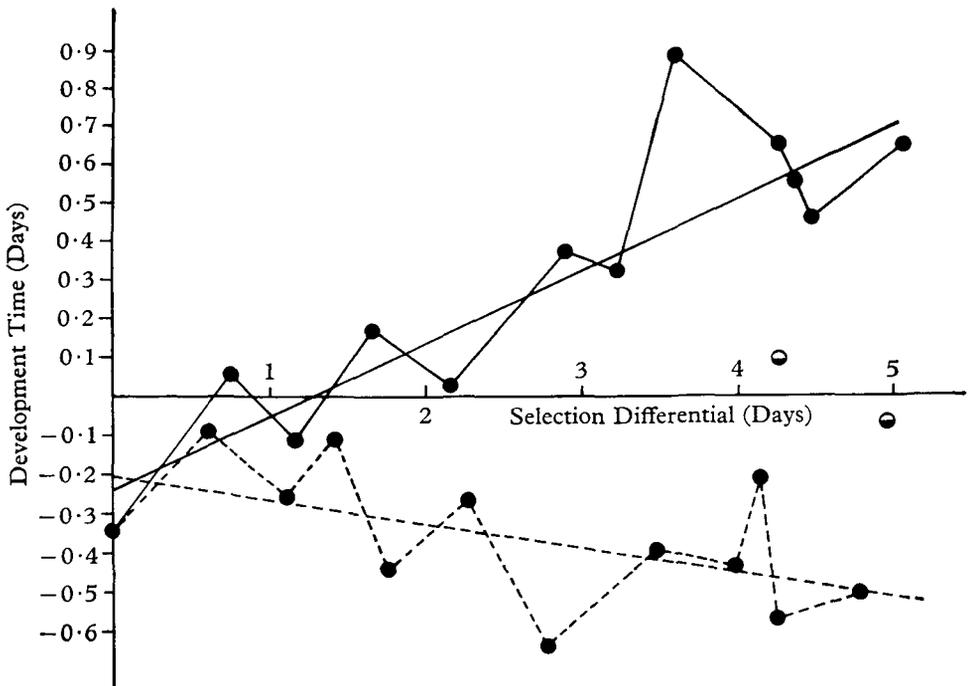
Fig. 1. Response to selection. Full line, S line; broken line, F line. ●, selected lines; ◒, F_1 hybrids between selected lines. The development times are the differences between the measured populations and synchronous **B/K** F_2 controls.

beyond which progress could not be made, since the irregularities were at least in part due to the temporary rise in temperature. But the results do suggest that natural selection, particularly when the temperature rose, was counteracting the effects of artificial selection for slower development. It seems likely that if a uniform temperature of 20° C. had been maintained, the value of the realized heritability in the S line would have been somewhat higher than 0·186; but there is no reason to think that the response in the F line would have had any greater.

C. Genetic variance of the selected lines

Analyses of variance of the combined first and second generations, and of the combined tenth and eleventh generations, of the two lines are given in Tables 2 and

Table 2. *Analyses of variance of the combined first and second selected generations*

	Degrees of freedom		Variance		
	F line	S line	F line	S line	Components
Within cultures	407	341	3·09	2·90	Q
Between cultures	34	32	4·16	4·90	$Q + zC$
Between families, within generations	42	30	7·63	8·20	$Q + zC + yF$

	F line	S line
Mean number of flies per culture (z)	6·22	6·32
Mean number of flies per family (y)	11·02	12·64
Total heritability, $h^2 = 2F/(Q + C + F)$	0·176	0·150

3. These show that there was significant genetic variance in both lines at the start of the experiment, but that in the last two generations there was no significant genetic variance in the F line, although the genetic variance of the S line had if anything increased.

Table 3. *Analyses of variance of the combined tenth and eleventh generations*

	Degrees of freedom		Variance		
	F line	S line	F line	S line	Components
Within cultures	544	428	3·15	3·57	Q
Between cultures	61	50	5·00	4·52	$Q + zC$
Between families, within generations	36	29	6·09	14·50	$Q + zC + yF$

	F line	S line
z	6·49	6·29
y	16·92	16·42
h^2	0·037	0·280

D. Crosses between lines

In parallel with the ninth and eleventh generations, the development rates of hybrids between the two lines were measured. The results are given in Table 4. They

Table 4. *Mean development times, in days, of the selected lines and of the F_1 hybrids between them*

Generation	F	S	F♀ × S♂	S♀ × F♂
9	16·81	17·57	17·03	17·20
11	16·56	17·71	17·00	16·99

show that the hybrids were approximately intermediate between the two parental lines, and that there was no significant difference between the reciprocal hybrids.

4. DISCUSSION

The results of the present experiment which call for an explanation are as follows:

(i) The response to selection was asymmetrical; the realized heritabilities were $+0\cdot186$ in the S line and $+0\cdot063$ in the F line, the latter being barely significant.

(ii) Hybrids between the F and S lines were approximately intermediate between their parents.

(iii) Analysis of the foundation population suggested that all or most of the genetic variance was non-additive.

(iv) Analysis of the last two generations of the selected populations suggested appreciable genetic variance in the S line, but little or no genetic variance in the F line.

These results can be compared to the results obtained by Hollingsworth & Maynard Smith (1955). This earlier experiment concerned the same character in the same species, but the procedure differed in the following ways:

(i) Selection was combined with brother–sister mating.

(ii) The foundation population consisted of the offspring of a single wild female.

(iii) Selection was carried out on a low protein diet, and less care was taken to standardize environmental conditions.

The results resembled the present ones in their main feature. The response was asymmetrical, considerable progress being made towards slower development but none towards faster development. But there were the following important differences:

(i) The response in the S line was more rapid (4 days after seven generations, instead of 1 day after eleven generations). This was partly due to the fact that the difference between two genotypes is greater on diets with less protein. But this is not the whole explanation. Even on 12% dead yeast the S line obtained in the earlier experiment takes $3\cdot5$ days longer to develop than most outbred populations.

(ii) Hybrids between the F and S lines were slightly faster than the F parents.

(iii) There was a striking decline in fertility and viability in both lines: in a replicate experiment from a different wild female, all the S lines were lost due to infertility. There was no comparable decline in fertility in either line in the present experiment.

The results of the earlier experiment can be explained if it is assumed that all the genetic variance of development rate is due to genes with heterotic effects, heterozygotes developing fast and homozygotes slowly. This assumption was confirmed by the observation that the S line was structurally homozygous, whereas the F line continued to segregate for inversions on three of the four long autosomes.

But it is far from certain that the present experiment is to be explained by a similar assumption. There are in fact two types of genetic situation which can give rise to asymmetrical responses to selection; first, non-additive interactions between alleles at the same locus (dominance and overdominance), and second, interactions between alleles at different loci (epistasis). For reasons which will now be discussed, the latter seems the more likely explanation in the present case.

Considering first dominance interactions at single loci, if the gene frequencies are such as to maximize the value of the character being measured, then the genetic variance as estimated by a half-sib analysis will be entirely non-additive, and there will be no response to selection in either direction, unless selection is combined with inbreeding, in which case progress will be made in a downward direction only; this is the hypothesis invoked to explain the earlier experiment by Hollingsworth & Maynard Smith (1955). But if the gene frequencies are not such as to maximize the value of the selected character, a half-sib analysis should reveal some additive genetic variance, and progress should be made in both directions. Yet the rates of progress in the two directions may in some cases be different, although the mean value of the two realized heritabilities should be approximately equal to the esti-mated additive heritability.

Such situations have been discussed by Falconer (1953, 1955); they are of two kinds. First, if the frequency of alleles for, say, slow development is much lower than that of alleles for fast development, then there is more room for progress towards slow development, and in a long-term selection experiment the response will be asymmetrical. Such a model seems inappropriate to explain the response of a population formed by mixing four geographical populations in about equal pro-portions. But asymmetrical responses can arise even if the frequencies of the two alleles at a single locus are approximately equal, due to directional dominance: that is, if alleles for fast development tend to be dominant over genes for slow development, or if the heterozygote is faster than either homozygote, but one homozygote is slower than the other. In either case progress towards slow develop-ment will be more rapid than towards fast development.

The degree of asymmetry to be expected from directional dominance will be greater the higher the intensity of selection, the higher the total heritability of the character, and the smaller the number of loci involved. It is easy to show that as the number of independent loci involved tends to infinity, the degree of asymmetry tends to zero. An attempt has been made to find a genetic model which will explain the present experiment in terms of directional dominance. It has been assumed that:

(a) Development rate is influenced by genes at 10 loci, segregating independently.

(b) For each locus, the effects on development time are as follows:

$$a_1 a_1, 1 + k; \ a_1 a_2, 1; \ a_2 a_2, 1 + \tfrac{1}{4}k.$$

(c) In the foundation population, all gene frequencies are 0·5.

(d) The environmental variance remains constant in the selected lines, and is such that the total heritability in the foundation population is 0·25.

(e) The selected parents consist of all individuals one standard deviation or more away from the mean; the population is assumed large, so that there is no inbreeding.

In this model, the selection intensity and total heritability have been chosen to agree with those obtaining in the actual experiment, and the other features to give a reasonable agreement with the responses to selection actually observed.

With these assumptions, the additive heritability estimated by a half-sib analysis

of the foundation population would be $+0.104$. The responses to selection for fast and slow development are shown in Fig. 2. The agreement between Fig. 2 and the responses actually obtained is reasonably good. But the model leads to two pre-
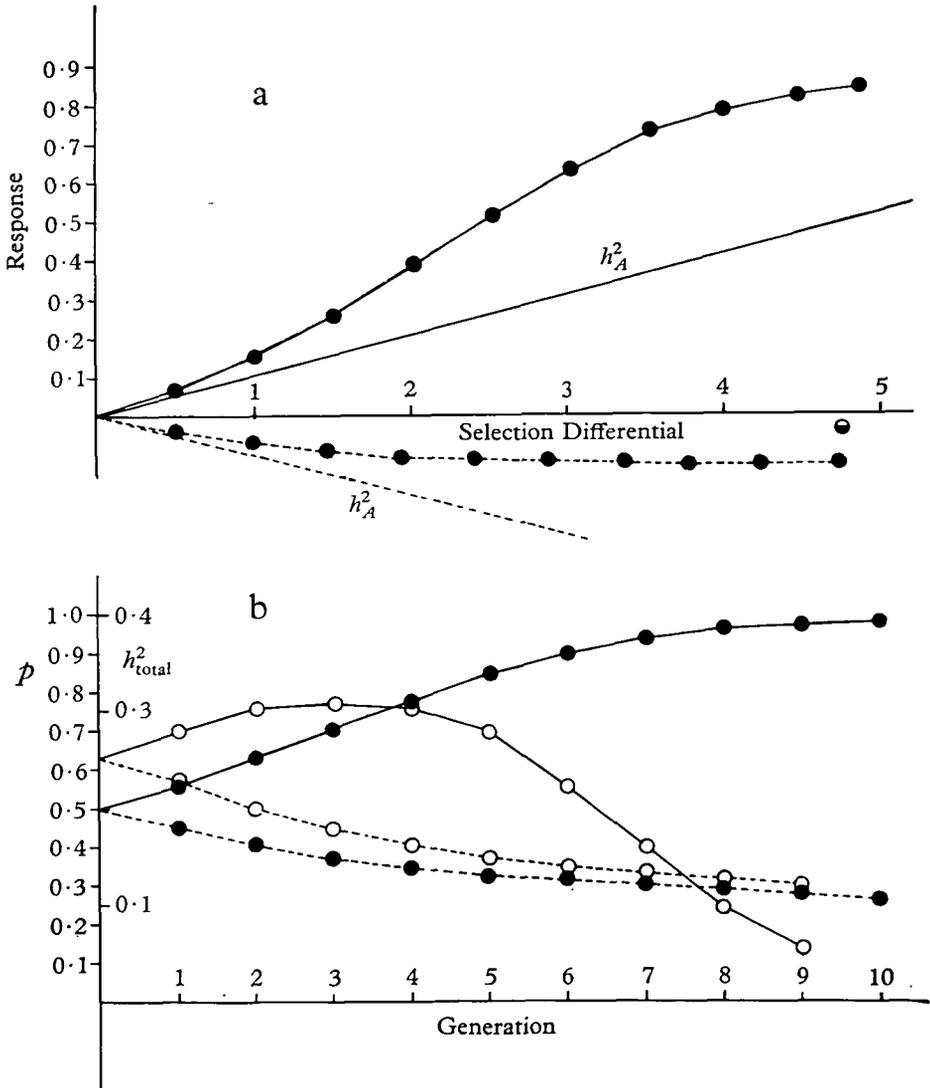
Fig. 2. Theoretical results of selection on a population showing directional dominance.
Full lines, S line; broken lines, F line.

a: responses to selection, and responses predicted from a half-sib analysis of the foundation population. ◒, F_1 hybrids between selected lines.

b: ●, frequency p of alleles for slow development; ○, total heritability.

dictions which are contradicted by the experimental results. First, in the model the total heritability would decline to 0.05 in the slow line, but remain reasonably high at 0.12 in the fast line; this is the exact opposite of what actually happened. Second

and more decisive, the model predicts that hybrids between the fast and slow line should be almost as fast as the fast line, whereas in fact the hybrids were roughly intermediate between the two lines. Any attempt to explain the results by a model based on directional dominance would run into the second difficulty. In fact, it seems impossible to devise a model based on interactions between the alleles at single loci which will account adequately for all the features of the present experiment.

It is however possible to suggest a model which will explain the present experiment, if it is assumed that much of the genetic variance is epistatic. Suppose that the
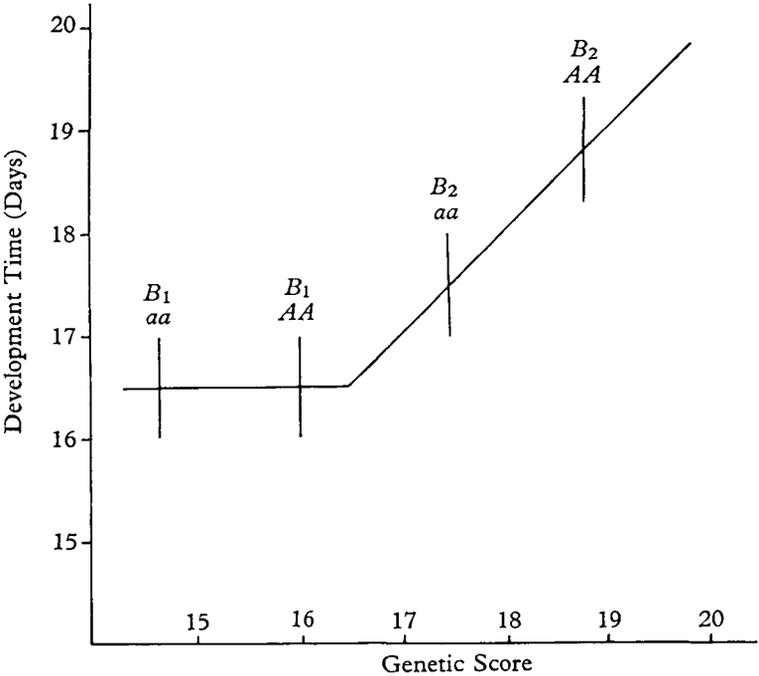
Fig. 3. Relationship between phenotype and genetic score; for explanation see text.

minimum possible period of development for this species in the given environment is 16·5 days. In populations all of whose members take longer than 16·5 days to develop, it is assumed that there is no epistatic genetic variance; i.e. a given gene substitution has the same phenotypic effect whatever the genetic background at other loci. The genetic variance of such a population may be due to genes with additive or with heterotic effects. It would be possible to select from such a population a number of genotypes which, on the assumption of no epistatic variance, would have development times of less than 16·5 days. These development times, predicted on the assumption of no inter-locus interaction, will be called the 'genetic scores' of the individuals. The existence of a developmental barrier at a minimum of 16·5 days implies that all individuals with genetic scores of 16·5 or less will have actual development times of 16·5 days. The relationship between genetic score and phenotype is shown in Fig. 3. The phenotypic effect of a given gene substitution,

say AA for aa, will then be zero on genetic background B_1 (many alleles for fast development) but appreciable on background B (many alleles for slow development).

Situations of this kind, involving a non-linear relationship between genetic score or dosage and phenotype, have been met with in selection experiments on the expression of mutant characters, a partial or absolute barrier existing at the wild-type phenotype (Rendel, 1959; Sondhi, 1960). There is no reason why similar barriers should not influence a character such as development rate, although neither the existence nor the position of the barrier could be predicted *a priori*.

A model of this kind will explain the present results. From previous work, it was to be expected that much of the genetic variance of development time would be heterotic, and consequently that the highly heterozygous foundation population would be close to the minimum possible development time. If so, an appreciable response to selection would be possible only in the S line. But some additive genetic variance would be expected, since geographical populations with different development rates were crossed to obtain the foundation population. Therefore a response would be expected in the S line, although a less rapid one than with inbreeding, since only the additive component of the genetic variance could be utilized. Since most of the response would be due to changes in the frequency of genes with additive effects, the hybrids between the F and S lines would be intermediate in development rate. Finally, the F line would approach still closer to the developmental limit, and as it did so the genetic component of the variance would decrease, although there would still be plenty of variation in the 'genetic score'.

There remains only the fact that the half-sib analysis of the foundation population failed to reveal any additive genetic variance. There were probably two reasons for this. First, analyses of this kind are necessarily inaccurate because of the relatively few degrees of freedom available. Second, since the foundation population was close to the developmental limit, not all differences in the 'genetic score' could be recognized phenotypically.

To sum up, earlier work had shown that much of the genetic variance of development rate is due to genes or to chromosome regions with heterotic effects. The present experiment shows that, at least in the geographically hybrid population studied, there was also some additive genetic variance, which could be utilized in selection for slow development. But this variance could not be used in selecting for fast development. This can be explained by saying, either that there exists a 'developmental barrier' preventing development in less than a certain minimum time, or, what amounts to the same thing expressed in genetic terms, that gene substitutions which in one part of the range of phenotypes are additive as between loci, are epistatic over another part of the range; there is a law of diminishing returns as genes for rapid development are introduced into the genotype.

It seems likely that developmental barriers of this kind, and the associated epistatic genetic variance, will prove to be common, particularly in the study of physiologically important characters which have been exposed to directional selection for some time. It is important to emphasize that there is nothing in the

least mystical about the concept of a developmental barrier. A hypothetical example will make this clear. It is known that in *D. subobscura* egg-laying accelerates the rate of ageing of females (Maynard Smith, 1958): a female laying eggs rapidly may survive for 60 days, whereas a genetically similar female laying eggs slowly or not at all may survive for 100 days. Starting from a population with a mean life-span of 60 days, selection for increased female longevity might perhaps produce a response, at the expense of reducing the rate of egg-laying, until a mean female life-span of 100 days was reached. Further progress would probably require much more profound physiological changes; if so, selective progress would be halted by a 'developmental barrier'. There is no reason to regard such barriers as absolute; for example, *D. melanogaster* develop appreciably faster than *D. subobscura* at the same temperature. But their nature in any particular case will call for a physiological rather than a genetical investigation.

SUMMARY

Starting from a geographically hybrid foundation population of *Drosophila subobscura*, selection for fast and for slow development has been practised without inbreeding on a diet with an unusually high level of protein. Realized heritabilities in the fast and slow lines were $+0.063 \pm 0.029$ and $+0.186 \pm 0.031$ respectively. A half-sib analysis of the foundation population and full-sib analyses of the first two and the last two selected generations were carried out. Hybrids between the two lines were approximately intermediate between their parents.

Two types of genetic explanation of the asymmetrical response are discussed. The first assumes directional dominance of alleles for fast development. Such an assumption can explain the asymmetrical response, but runs into difficulties in explaining the nature of the genetic variance in the selected populations and the intermediacy of the hybrids between the two lines.

A second assumption, which appears to fit the facts better, is that there exists a 'developmental barrier' preventing development at a rate appreciably faster than that of the foundation population. In physiological terms this implies that more rapid development requires a more profound modification of the population than could be achieved by a few generations of selection. In genetic terms, it implies epistatic interactions between genes at different loci: gene substitutions at a given locus which increase development rate on a genetic background causing slow development have little or no effect on a genetic background causing rapid development. In other words, there is a law of diminishing returns as more and more alleles for fast development are accumulated in the genotype. It is suggested that genetic situations of this kind may be common in populations which have been exposed to directional selection for a long time in reasonably large populations, either in nature or in domestication.

REFERENCES

FALCONER, D. S. (1953). Selection for large and small size in mice. *J. Genet.* **51,** 470–501.

FALCONER, D. S. (1955). Patterns of response in selection experiments with mice. *Cold Spr. Harb. Symp. quant. Biol.* **20,** 178–196.

HOLLINGSWORTH, M. J. & MAYNARD SMITH, J. (1955). The effects of inbreeding on rate of development and on fertility in *Drosophila subobscura. J. Genet.* **53,** 295–314.

MAYNARD SMITH, J. (1958). The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura. J. exp. Biol.* **35,** 832–842.

MAYNARD SMITH, J. & MAYNARD SMITH, S. (1954). Genetics and cytology of *Drosophila subobscura.* VIII. Heterozygosity, viability and rate of development. *J. Genet.* **52,** 152–164.

RENDEL, J. M. (1959). Canalization of the acute phenotype of *Drosophila. Evolution,* **13,** 425–439.

SONDHI, K. C. (1960). Selection for a character with a bounded distribution of phenotypes in *Drosophila subobscura. J. Genet.* (in press).