THE EFFECTS OF INBREEDING ON RATE OF DEVELOPMENT AND ON FERTILITY IN *DROSOPHILA SUBOBSCURA*

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CONTENTS

1. Introduction

Haldane (1949) showed that in a random mating population in equilibrium under the influence of selection, no correlation is to be expected between the fitness of parents and offspring, whereas there will be a positive correlation between the fitness of sibs. The same conclusions would hold for characters closely correlated with fitness. These conclusions arise because, for an equilibrium to be stable, heterozygotes must be fitter than either homozygote. Now in *Drosophila subobscura*, Maynard Smith & Maynard Smith (1954) found that flies heterozygous for chromosome 5 developed more rapidly than did homozygotes, as measured by the time taken from egg-laying to eclosion. There was a similar difference in rate of development between flies heterozygous and homozygous for chromosomes other than 5. It therefore seemed possible that rate of development might be sufficiently correlated with fitness to illustrate the type of inheritance predicted by Haldane.

Accordingly, the effects of selection on rate of development were studied in the descendants of a female caught in the wild at Oxford. The results were only in partial agreement with expectation. After a few generations of inbreeding a high degree of infertility appeared in the descendants of this female, and it seemed possible that this might account for the differences between the results obtained and those to be expected on Haldane's assumptions.

A second line was therefore started from a female caught in the New Forest. In this second line the development of infertility was much less rapid, and the agreement between the observed and expected results for rate of development was close.

The infertility which was first observed in the Oxford line, and which in fact appears to be the normal result of inbreeding in this species, seemed worthy of further study. Thus the second part of this paper describes an investigation into the causes of infertility in inbred lines of *D. subobscura*.

The inbred lines used, and the symbolism adopted to describe them, are as follows:

O line. Derived from a fertilized female caught at Oxford in May 1953.

NF line. Derived from a fertilized female caught in the New Forest in November 1953. Divided into two sublines:

NFF line. Selected for fast development from the F_2–F_8.

NFS line. Selected for slow development from the F_2–F_8.

B line. Derived from a stock kept in mass culture for 15 years, originally descended from flies caught in the New Forest. Homozygous for the mutant *cherry*.

K line. Derived from a wild-type stock kept in mass culture for 5 years, originally descended from a female caught at Küsnacht in Switzerland.

All lines were kept by brother-sister mating. The **B** and **K** lines were at the F_{12}–F_{20} during the investigation; the stocks from which they were derived were structurally homozygous, but for different chromosome orders for three of the four long autosomes.

The notation **B/K** is used for F_1 hybrids with **B** mothers and **K** fathers, the reciprocal hybrids being denoted by **K/B**.

2. The O line

All flies have been kept at 68–70° F. However, during the summer of 1953 the temperature occasionally rose for a day or two as high as 75° F., and there were two occasions on which the temperature fell for a few hours as low as 60° F. as a result of electrical failures. Consequently rates of development have been compared only for sets of flies all raised at the same time.

The parents of each generation were kept in mating vials for from 7 to 14 days after eclosion, and then transferred on two successive days to two half-pint milk bottles containing a food medium of maize meal, agar and molasses, with a few drops of yeast suspension added. On the third day the parents were removed from the second culture bottle. Thus each of the two culture bottles contained a single day's lay of eggs from a single female. All the parents of a given generation laid eggs on the same 2 days, and all cultures were kept on the same shelf in order to minimize differences in temperature. Adult flies eclosing were counted daily, so that the time taken from egg-laying to eclosion for each fly was known to the nearest day.

Departures from this procedure were as follows. The original female was transferred to a new culture bottle on 7 successive days to increase the number of F_1 progeny available for selection. In the F_{12} and subsequent generations eclosion time was not recorded; but since it was desired to keep a record of the productivity of the line in these generations, each female occupied a single culture bottle for 2 successive days, and the number of flies eclosing was counted.

The first flies to eclose in each generation are referred to as FAST, or F, and the last to eclose as SLOW, or S. Pairs of F and of S sibs were mated together in each generation, as shown in the pedigree (Fig. 1).

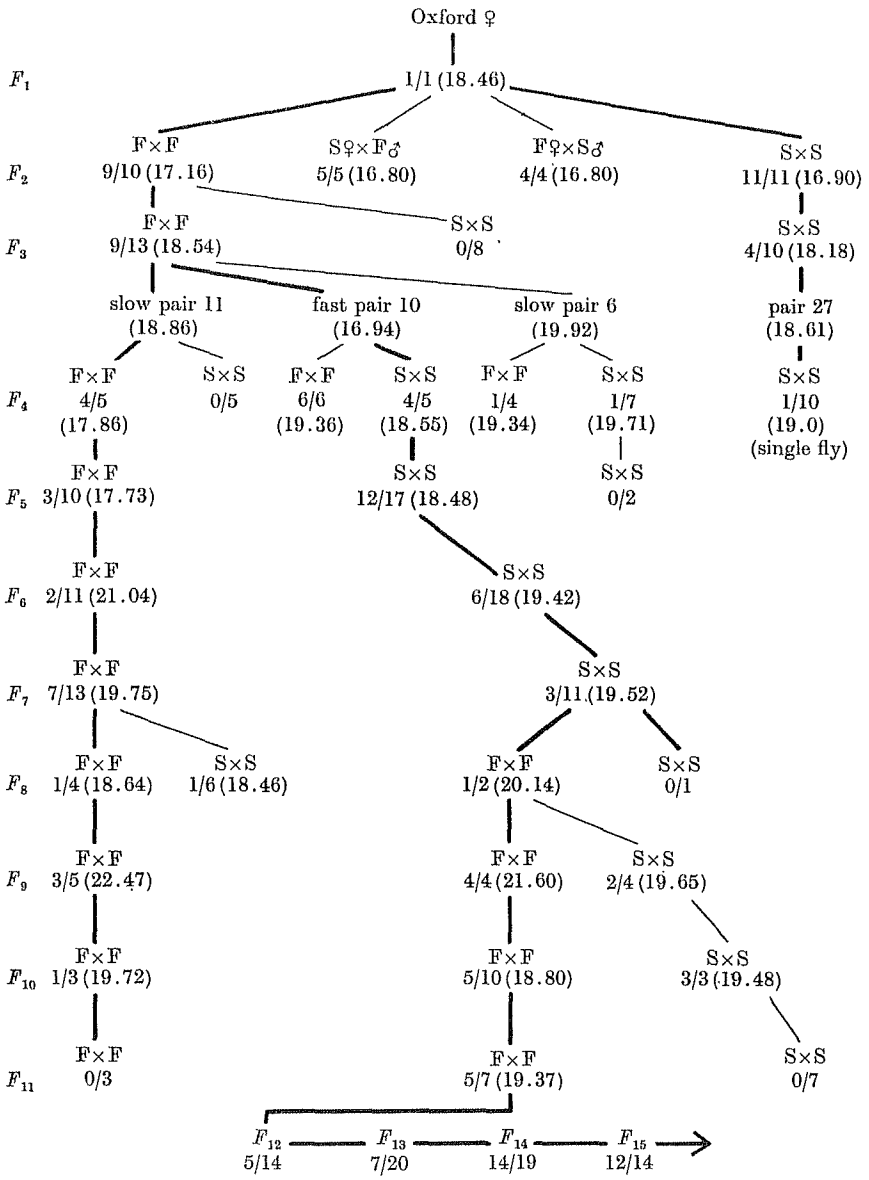


Fig. 1. The descendants of the Oxford female. For each set of matings, the proportion of pairs leaving progeny is shown as a fraction, and the mean eclosion time in days of the progeny is given in brackets.

The fraction below each set of matings gives the number of pairs which left adult progeny, divided by the total number of pairs which were set up, and which survived to be transferred to culture bottles. The occasional pair of which one or both members died or escaped before transfer to the bottles have been omitted.

The number in brackets gives the mean time in days from egg-laying to eclosion for the progeny of the set of matings.

The remarkable features of this pedigree are:

(i) the absence of any difference in rate of development between the fast and slow selected lines, and

(ii) the rapid decline in fertility, particularly in the slow-selected lines, as measured by the proportion of pairs leaving adult progeny.

3. The **NFF** and **NFS** lines

To see whether or not these features were due to some peculiarity of the Oxford female, a second line was started from a female caught in the New Forest. It was arranged that this female laid eggs in culture bottles on the same 2 days as the parents of the **O** F_8, and generations in the two lines were subsequently kept synchronous. The pedigree is shown in Fig. 2. The notation is the same as for the **O** line.

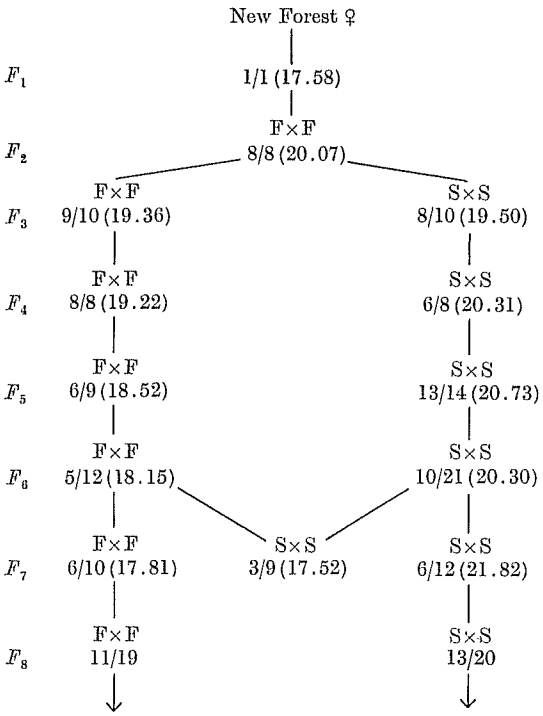


Fig. 2. The descendants of the New Forest female. Notation as in Fig. 1.

The New Forest female was, from her appearance, old when captured, and laid relatively few eggs. All her progeny were F (17 or 18 days), so that it was impossible to select both F and S flies from the F_1, and selection was therefore not started till the F_2.

In the F_6 S flies were selected from the **NFF** and **NFS** sublines and mated together.

As in the **O** line, there is little difference in rate of development of the progeny after the first generation of selection. In subsequent generations, however, there is an increasing difference in rate of development between the **NFF** and **NFS** lines.

The decline in the proportion of pairs leaving adult progeny is less rapid than in the **O** line, and there is no difference between the proportion of fertile cultures in the two lines.

4. THE RESPONSE TO SELECTION FOR RATE OF DEVELOPMENT

Owing to the fluctuations in temperature mentioned above, comparisons of rates of development can only be made between members of the same generation, or between members of synchronous **O** and **NF** generations. The heritability of rate of development can be measured as

$$h^2 = \frac{\bar{x}_S - \bar{x}_F}{D},$$

where $\bar{x}_S$ = mean eclosion time of the progeny of S pairs, $\bar{x}_F$ = mean eclosion time of the progeny of F pairs, and D = difference in mean eclosion time of S and F parents.

D has been calculated differently for the parents of the $\mathbf{O}F_2$, since these flies developed from eggs laid on 7 successive days, and there were appreciable fluctuations in temperature during their development. The effect of these fluctuations on the estimate of D has been eliminated by calculating for each fly the difference between its eclosion time and the mean for the individual culture bottle in which it was raised. D is then the sum of the mean differences for the F and S parents.

The intensity of selection is $i = D/\sigma$, where σ is the standard deviation of the population from which the parents were selected.

In calculating the standard error of h^2, allowance must be made for the fact that there are significant differences between the mean eclosion times of different families.

If there are k S families, containing $n_a \ldots n_i \ldots n_k$ progeny, the total number of progeny from S pairs is

$$N = \Sigma n_i.$$

The sum of squares for the progeny of S pairs can be analysed into two parts:

S_w = sum of squares within families, and

S_b = sum of squares between families.

An estimate of the variance of the means of the different families is

$$V_b = \left(\frac{S_b}{k-1} + \frac{S_w}{n-k}\right)\frac{N(k-1)}{N^2 - \Sigma n_i^2},$$

and the error variance of $\bar{x}_S$ is

$$V_{\bar{x}_S} = \frac{S_w}{N(n-k)} + \frac{\Sigma n_i^2}{N^2} V_b,$$

so that the standard error of h^2 is

$$\sigma_{h^2} = \frac{1}{D}(V_{\bar{x}_S} + V_{\bar{x}_F})^{\frac{1}{2}}.$$

The values of h^2 obtained are given in Table 1.

In order that a reasonable estimate can be made of V_b, only those comparisons are made where there are three or more families in each class.

The comparisons in Table 1 are of two kinds:

(i) first selected generation, where the selected F and S parents were all members of the same family and

(ii) second or later selected generation, where the F and S parents were themselves the progeny of F and S flies respectively.

In the **O** line h^2 is never significantly different from zero. Two possible reasons for this absence of a response to selection will be discussed.

Table 1. *Heritability of rate of development in the* **O** *and* **NF** *lines*

Selected generation			N	k	$\bar{x}$	V_w	V_b	$V_{\bar{x}}$	D	$\bar{t}$	h^2
					Oxford line						
1st	F_2	F × F	481	9	17·16	1·31	0·142	0·020	3·3	2·06	−0·08±0·06
		S × S	624	11	16·90	0·79	0·119	0·015			
1st	F_4 from pair 10	F × F	679	6	19·36	2·49	0·886	0·162	2·3	2·84	−0·35±0·18
		S × S	221	4	18·55	2·76	0	0·012			
2nd	F_3	F × F	464	9	18·54	2·01	0·530	0·075	3·2	3·02	−0·11±0·14
		S × S	100	4	18·18	3·40	0·254	0·123			
2nd	F_5	F × F	45	3	17·73	0·94	0·796	0·515	3·4	1·89	+0·22±0·24
		S × S	408	12	18·48	1·93	1·150	0·138			
					New forest line						
1st	F_3	F × F	453	9	19·36	2·16	0·471	0·049	5·9	3·60	+0·02±0·07
		S × S	293	8	19·50	2·15	0·747	0·138			
2nd	F_4	F × F	363	8	19·22	2·76	0·718	0·120	4·86	2·98	+0·22±0·09
		S × S	198	6	20·31	2·87	0·257	0·072			
3rd	F_5	F × F	84	6	18·52	1·92	0	0·023	4·25	2·24	+0·52±0·07
		S × S	344	13	20·73	2·56	0·452	0·066			
4th	F_6	F × F	72	5	18·15	0·85	0·054	0·026	5·00	2·66	+0·43±0·11
		S × S	270	10	20·30	1·00	2·570	0·291			
5th	F_7	F × F	112	6	17·81	1·74	0·137	0·048	4·5	2·42	+0·89±0·07
		S × S	114	6	21·82	1·18	0·208	0·052			
		S from **NFF** × S from **NFS**	59	3	17·52	0·45	0·008	0·010	—	—	

A. *All variance of eclosion time is environmentally caused*

If this were so, no response to selection would be expected. However, this explanation is unlikely to be correct for the following reasons:

(i) It is known that large genetically determined differences in eclosion time do exist. Maynard Smith & Maynard Smith (1954) found differences of from 1 to 2 days in the mean eclosion time of two classes of sibs, respectively homozygous and heterozygous for chromosome 5. In these experiments both environmental and maternal effects could be ruled out.

(ii) If all differences in eclosion time were environmentally caused, the differences between members of the same family raised in different culture bottles would be as great as the differences between members of different families. This is not the case, as is shown in Table 2.

The variance due to differences between the two cultures comprising a single family is significantly greater than the within-culture variance. Therefore some differences in eclosion time were caused by environmental differences between culture bottles.

However, the between-family variance is more than twice the between-culture within-family variance. It follows that members of a family resemble one another because they have the same parents, and not only because they were raised in the same culture bottles. Unfortunately, the analysis cannot distinguish between genetic and maternal effects.

One other possibility remains. It could be that large cultures tend to be slow because of the competition between larvae. Since the two cultures comprising a family tend to be the same size, this could also account for the correlation between sibs which has been demonstrated in Table 2.

There is in fact a slight association between large numbers and slow development in a culture. Considering the first six generations of the **O** line, in forty-eight out of seventy-one cases the larger of the two cultures comprising a family had the greater mean eclosion

time. However, it does not follow that large families were usually slow ones. During the same six generations, of thirty-five families which were larger than the mean for their generation, sixteen, or about half, had a mean eclosion time shorter than the mean for their generation. These data suggest that the slight increase in eclosion time caused by competition in large cultures was just counterbalanced by a genetical association between large family size and fast development.

Table 2. *Analysis of variance of eclosion time*

	Sum of squares	Degrees of freedom	V	P
	Oxford, F_{2-6}			
Within cultures	6169	4164	1·48	
Between cultures, within families	1016	82	12·4	$<10^{-6}$
Between families	2480	88	28·2	10^{-4}
Total (within generations)	9665	4334		
	New Forest, F_{2-7}			
Within cultures	4973	2572	1·94	
Between cultures, within families	679	75	9·1	$<10^{-6}$
Between families	3515	79	44·5	$<10^{-6}$
Total (within generations)	9167	2726		

Table 3. *Comparison of eclosion times of* **O** F_9 *and* **NF** F_2

	Flies per fertile culture	$\bar{x}$	V_w
O F_9	11·4	21·64	3·99
NF F_2	26·7	20·07	2·19

Genetically determined differences can sometimes easily override environmentally determined differences due to culture size. This can be shown by comparing synchronous **O** and **NF** generations. Table 3 compares the relatively outbred **NF**F_2 with the synchronous **O**F_9. The less inbred flies developed faster, and were less variable, in spite of the larger size of the cultures. This observation confirms that of Maynard Smith & Maynard Smith (1954), and suggests a better explanation of the absence of advance under selection.

B. *All genetic variance of eclosion time is due to genes or to chromosome regions with heterotic effects*

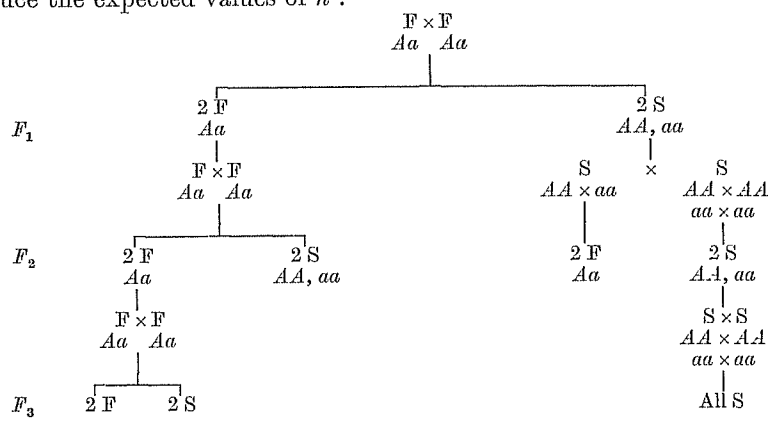
For simplicity, consider the case where all variance of eclosion time is genetic, and is due to a single pair of alleles A, a, such that AA and aa are S, and Aa is F. Starting with a pair of heterozygotes, and combining selection with brother-sister mating, the results shown on p. 302 would be obtained.

First, there would be no response to selection in the first selected generation (F_2). This expectation has been realized in both the **O** and **NF** lines.

Secondly, the F_2 from F pairs would be more variable within cultures, whereas the F_2 from S pairs would show less variance within cultures and greater variance between cultures. This expectation was not realized in either line. This effect would be masked by the presence of environmentally caused variations, and would also be reduced as the number of loci involved increased. However, the complete absence of the effect is probably to be explained by the fact that the environmental variance in eclosion time

will be greater in homozygous than in heterozygous families (cf. Table 3, and Maynard Smith & Maynard Smith, 1954).

Finally, there would be a positive heritability of 0·5 in the second and subsequent generations. If alleles at more than one locus affect rate of development, the value of h^2 expected in the second selected generation would still be 0·5, but h^2 would then be expected to increase further in subsequent generations, and, in the absence of environmental causes of variation, to approach unity. The presence of environmental variation would reduce the expected values of h^2.



The **NF** line followed this expected behaviour closely (Table 1). h^2 was 0·22 in the second selected generation, and reached 0·89 after five generations of selection. This high value of h^2 suggests that non-genetic causes of variation in rate of development were of little importance.

Table 4 shows the eclosion times of the F_7 progeny of the **NFF** and **NFS** lines, and of a cross between S flies from the two lines. There is little difference between the cross and the **NFF** line, although the variance of the **NFF** line is greater, due to the greater proportion of 'stragglers' eclosing in 19 or more days. These data agree with expectation if all genetic variance is heterotic, but they could also be explained by the complete dominance of alleles for fast development. However, if the latter were the case, it would be difficult to explain the absence of a response to selection in the first selected generation, either in the **O** or **NF** lines.

Table 4. *Eclosion time of* **NF** F_7

	Eclosion time in days											
	16	17	18	19	20	21	22	23	24	25	26	Total
NFF	7	51	35	7	3	6	2	1	—	—	—	112
NFS	—	—	—	—	10	39	39	18	4	3	1	114
S from **NFF** × S from **NFS**	1	30	25	2	1	—	—	—	—	—	—	59

The **O** line resembles the **NF** line in the absence of a response to selection in the first selected generation (Table 1). However, in the **O** line there was no response in subsequent generations either. This is probably to be explained by the inviability or infertility of S flies in this line, i.e. to the opposing effects of natural selection. The pedigree (Fig. 1) shows that the original slow-selected line was lost in the F_4, and that three other slow-selected lines were lost during the first five generations.

The results obtained for the two lines are therefore consistent with the following hypotheses:

(i) Most variance in eclosion time is genetic.

(ii) Most genetic variance of eclosion time is due to genes or to chromosome regions with heterotic effects.

(iii) In the **O** line, S flies tended to be either inviable or infertile.

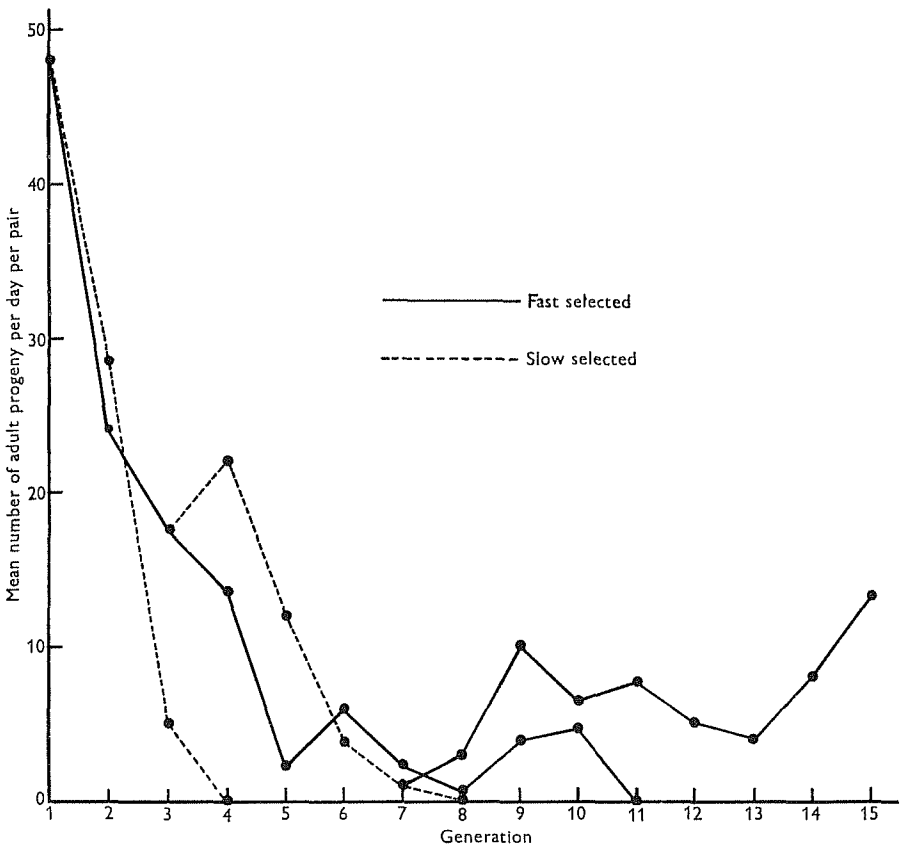


Fig. 3. The overall productivity of the **O** line.

5. THE FERTILITY OF INBRED LINES

(*a*) *The overall productivity of the* **O** *line*

Fig. 3 shows the mean number of adult progeny obtained per day per pair for the following sublines derived from the Oxford female:

(i) The original F line, lost in the F_{11}.

(ii) The original S line, lost in the F_4.

(iii) A slow-selected line, derived from the original F line in the F_3, and lost in the F_8.

(iv) The only surviving line, derived from line (iii) in the F_7, and subsequently selected for fast development.

These four sublines are indicated in Fig. 1 by bold lines; they include the majority of the descendants of the Oxford female.

The most striking feature is the rapid fall in overall productivity from forty-eight adult progeny per day from the original wild-caught female (averaged over 7 days) to from one to three progeny per day in the F_7 and F_8. This was followed by a rise in productivity in the surviving line.

A fall in overall productivity could be due to failure of the parents to mate, to a reduction in the number of eggs laid per female, to a failure of the eggs laid by fertilized females to hatch, or to mortality in the larval and pupal stages.

Failure to mate was not a major cause of the decline. Of seventy-seven females which were parents of the F_{6-10}, only nine proved on dissection to be virgins, or possibly to have mated with males lacking testes, or with the testes unattached to their ducts; one male of each of these kinds was found among about fifty **O** males dissected for spermatogenesis preparations.

We have no reason to suspect a high larval or pupal mortality, although in inbred lines there are always some pupae which darken but do not give rise to imagines.

It will be shown that the failure of eggs laid by fertilized females to hatch will account for a fall in overall productivity to about 25% of its initial value. The reduction in the number of eggs laid has probably also contributed to the decline, but has not been measured independently of other factors.

(*b*) *Methods and terminology*

Attention has been concentrated on the hatchability of the eggs. Eggs were collected on a dark brown food medium of agar and molasses. A drop of this medium was poured on to a slip of balsa wood, and yeast suspension added with a paint brush. The slips of balsa wood were made a 'push fit' in 1 in. diameter vials, thus facilitating the transfer of flies. The females to be tested were placed in such vials and left for 24 hr. If they had laid eggs, they were removed, and the eggs examined for hatching 48 hr. later. In some experiments they were re-examined after 72 hr., and it was found that eggs which had not hatched after 48 hr. did not hatch in the next 24 hr. Females which had not laid eggs were returned to the test vials for a further period of 24 hr.

The age of the females when tested varied from 7 to 16 days. They were separated from the males immediately before testing. Flies were etherized on the day of eclosion but not subsequently. The test vials were kept throughout at 70° F., and covered with a damp cloth.

Females which laid no eggs in the test period, or which laid eggs none of which hatched, were dissected to discover whether they had been fertilized, from the presence of sperm in the ventral receptacle. The reliability of this technique was confirmed by dissecting a series of known virgin and fertilized females, identified by a code unknown to the dissector.

There was only a 28·9% hatch of the eggs laid by fertilized females from the $\mathbf{O}F_{6-10}$ (Table 7). This failure of a large proportion of the eggs laid by fertilized females to hatch may be due to a variety of causes. It is convenient to classify these causes as follows:

(i) Female infertility, i.e. the female would have produced many eggs which did not hatch, irrespective of the male to which she was mated.

(ii) Male infertility, i.e. any female mated to the male parent would have produced a high proportion of eggs which did not hatch.

(iii) The failure of eggs to hatch, although neither parent was infertile as defined above. This may be the result of:

(*a*) Parental incompatibility, i.e. the failure of egg and sperm to fuse, although both were capable of fusion with different partners. This phenomenon, which occurs in species and other distant crosses (e.g. Patterson, Stone & Griffin 1942) has not been met with in our inbred lines. Or

(*b*) Zygotic inviability, i.e. the death of a number of zygotes formed by the fusion of eggs and sperm, either of which could have given rise to viable zygotes had they fused with different partners. This would be expected when mating close relatives, due to the formation of zygotes homozygous for recessive lethals; it would also be expected in distant crosses.

The value of such a classification is that it suggests experiments. The first requirement for such experiments is a supply of flies known to be fully fertile. To demonstrate, for example, male infertility in an inbred line, it has been considered sufficient to show that matings between inbred males and unrelated, fully fertile females gave eggs a large proportion of which did not hatch. It is possible that mating the same males to a different type of fertile female might have given fully viable eggs. However, no inconsistencies have as yet arisen from assuming that one type of fertile female is equivalent to another for the purpose of such test matings.

Table 5. *Fertility of flies used for test matings*

Mating	No. of females	No. of eggs		% Hatch
		Laid	Hatched	
NF F_1♀ × **NF** F_1♂	6	71	70	98·6
NF F_2♀ × **NF** F_2♂	9	140	123	87·8
B/K♀ × **B/K**♂	9	344	343	99·7

We shall describe first the evidence for male and for female infertility in the **O**, **NF**, **B** and **K** lines in turn, and then discuss the evidence for zygotic inviability.

The first test matings of flies from the **O** line were made to F_1 and F_2 progeny of the wild-caught New Forest female. However, the use of the progeny of wild females for test matings is inconvenient, and the remainder of the test matings have therefore been made to **B/K** hybrids. Table 5 gives egg hatchability data for the various types of flies used for test matings. The table shows that both sexes of the **NF** F_1 and of the **B/K** hybrids are fully fertile, and that the **NF** F_2 flies have a high fertility, although a few eggs (12%) did not hatch.

Table 6 shows the results of mating **O** F_{12} males each to two **B/K** females. The table includes only those cases where both females were fertilized. These data illustrate the following points:

(i) There is good agreement between the percentage hatch of eggs laid by the two females mated to the same male.

(ii) No male is completely sterile. For example, one of the two females mated to **O**♂2 laid sixty-eight eggs of which none hatched. This might be regarded as evidence that the male was wholly sterile, were it not for the fact that the second female laid two eggs, out of seventy-three, which did hatch.

(iii) There are large differences between the fertilities of different males. This has been the case in all our experiments, except those in which all the flies were fully fertile. It

follows that a significant difference between two groups of flies cannot be demonstrated by comparing the total numbers of eggs laid and hatched from the two groups. We have therefore classified matings into those giving >90%, 20–90% and <20% egg hatch. This classification is arbitrary; it is not intended to indicate segregation into sharply defined classes, but to show the range of variation present. The significance of a difference

Table 6. *Results of mating nine* **O** F_{12} *males each to two* **B/K** *females*

		No. of eggs	
O♂	B/K♀	Laid	Hatched
1	*a*	57	51
	b	30	23
2	*a*	68	0
	b	73	2
3	*a*	54	39
	b	61	27
4	*a*	50	41
	b	37	25
5	*a*	29	24
	b	4	4
6	*a*	6	5
	b	9	9
7	*a*	18	2
	b	52	7
8	*a*	22	14
	b	6	5
9	*a*	42	29
	b	47	35

between two groups of matings can be calculated from the numbers of matings in the two groups which fall into these three classes. Values of P quoted in the text have been calculated in this way; they are for departures from equality in either direction as great as that observed. However, in considering the evidence for zygotic inviability (Table 10), it was necessary to calculate the standard error of the estimates of percentage egg hatch; this has been done by the method given by A. Robertson (1951).

(*c*) *Fertility of the* **O** *line*

Table 7 gives the results of experiments on egg hatchability for the **O** line. The experiments were not started until the F_6, when it was realized from the proportion of cultures not taking that a high degree of infertility had developed. Since at this stage there was no longer any consistent difference between the various sublines, the data for different sublines have been combined.

The matings between **O** males and fertile females show a high degree of male infertility; approximately half the eggs from such matings failed to hatch. The **O** females, on the other hand, although inferior to **B/K** females, are fairly fertile. The only low percentage hatch was 63·5% from the F_{12} females, a value based on only nine females.

It seemed unlikely that the male infertility could be due to the segregation of a single recessive, but it was thought advisable to test this possibility. Infertility due to a single recessive should reappear in one-quarter of the F_2 males from a mating between an infertile male and a **B/K** female. Three highly infertile **O** F_{13} males were mated to **B/K** females; two of these matings gave adult progeny, and F_2 cultures were set up from these. From the first mating, of twenty-seven eggs collected in a test vial only one hatched.

Thirteen F_2 males from this mating were test mated to **B/K** females, and of 423 eggs laid only five failed to hatch. No female laid more than one egg which failed to hatch. From the second mating, of 36 eggs collected only three hatched. Fourteen F_2 males were mated to **B/K** females, and of 493 eggs laid only ten failed to hatch. Of these fourteen females, one laid thirty-eight eggs of which nine failed to hatch. Thus of twenty-seven F_2 males only one showed any sign of infertility, and that to a much less extent than his grandfather ($P=0{\cdot}0042$ if one infertile male in four is expected). It follows that male infertility is not due to the segregation of a single recessive.

Table 7. *Fertility tests on the* **O** *line*

Parents	No. of fertilized females whose % egg hatch was >90%	20–90%	<20%	Total	No. of eggs Laid	Hatched	% Hatch
O F_6	1	8	11	20	369	94	25·5
F_7	0	3	7	10	207	30	14·5
F_8	2	4	2	8	86	47	54·6
F_9	0	6	2	8	118	52	44·0
F_{10}	0	6	7	13	125	38	30·4
F_6–F_{10}	3	27	29	59	905	261	28·9
O F_{15}	0	4	11	15	341	129	38·8
NF F_1 and F_2♀♀ × **O** F_8 and F_9♂♂	2	10	4	16	336	164	48·8
B/K♀♀ × **O** F_{12}♂♂	2	14	4	20	727	392	53·9
B/K♀♀ × **O** F_{13}♂♂	12	8	4	24	908	619	68·1
B/K♀♀ × **O** F_{14}♂♂	6	17	0	23	883	641	72·6
B/K♀♀ × **O** F_{15}♂♂	0	13	1	14	383	182	47·5
O F_8 and F_9♀♀ × **NF** F_1 and F_2♂♂	13	5	0	18	188	172	91·5
O F_{12}♀♀ × **B/K**♂♂	3	6	0	9	104	66	63·5
O F_{14}♀♀ × **B/K**♂♂	8	13	0	21	416	250	84·2

(*d*) *Fertility of the* **NFF** *and* **NFS** *lines*

Eggs were collected from nine females caught in the New Forest. All were fully fertile, although from their appearance they were old when captured. One of these females was the ancestor of the **NF** line. The egg hatchability of both the **NFF** and **NFS** lines was followed from the F_{1-7}. Test matings of males and females from both lines to **B/K** mates were made in the F_6 and F_7. The results are given in Table 8 and Fig. 4.

There was a steady decline in hatchability in both lines. By the F_7 egg hatchability in the **NFF** line was 20·3%, as compared with 49·4% in the **NFS** line. The difference, however, is not significant ($P=0{\cdot}085$).

The test matings show that there is some degree of infertility in both sexes and in both lines. In both lines the males are less fertile than the females; the difference is not significant for either line taken independently, but is so for the combined data ($P=0{\cdot}11$ for the **NFF** line, $P=0{\cdot}073$ for the **NFS** line, and $P=0{\cdot}02$ for the two lines combined).

(*e*) *Fertility of the* **B** *and* **K** *lines*

Egg hatchability data for eggs from the **B** and **K** lines, and for F_1, F_2 and backcross eggs, are given in Table 9. Egg hatchability is low in both lines, but is lower in the **B** line. At the same time as these tests were made (after 12–14 generations of brother-sister mating), eggs were collected from fourteen females from the mass-cultured Küsnacht stock from which the **K** line was derived. Of 127 eggs, eighty-seven, or 68·5%, hatched: there had therefore been no further fall in egg hatchability due to the more intensive inbreeding since the origin of the **K** line.

About one-third of the F_1 eggs failed to hatch; this shows that the failure of eggs to hatch in these inbred lines cannot be wholly due to zygotic inviability. The backcrosses show that the males of both lines are fertile, whereas the females show some degree of infertility, slight in **K** females and marked in **B** females. The failure of backcross eggs

Table 8. *Fertility tests on the* **NFF** *and* **NFS** *lines*

Parents	No. of fertilized females whose % egg hatch was >90%	20–90%	<20%	Total	No. of eggs Laid	Hatched	% Hatch
Wild-caught New Forest ♀♀	9	0	0	9	59	59	100
NF F_1	6	0	0	6	71	70	98·6
Fast-selected line F_2	6	3	0	9	140	123	87·8
Fast-selected line F_3	8	4	2	14	285	245	85·9
Fast-selected line F_4	4	6	4	14	316	208	65·8
Fast-selected line F_5	4	1	3	8	177	95	53·7
Fast-selected line F_7	1	4	8	13	256	52	20·3
B/**K**♀♀ × fast-selected F_6 and F_7♂♂	5	9	2	16	254	154	60·6
Fast-selected F_6 and F_7♀♀ × **B**/**K**♂♂	10	4	3	17	571	437	76·5
Slow-selected line F_2	4	3	0	7	203	171	84·3
Slow-selected line F_3	5	6	2	13	336	214	63·7
Slow-selected line F_4	5	10	5	20	414	297	71·7
Slow-selected line F_5	6	3	4	13	245	168	68·5
Slow-selected line F_7	2	8	4	14	225	111	49·4
B/**K**♀♀ × slow-selected F_6 and F_7♂♂	13	15	4	32	1151	874	76·0
Slow-selected F_6 and F_7♀♀ × **B**/*K*♂♂	15	6	2	23	421	361	85·8

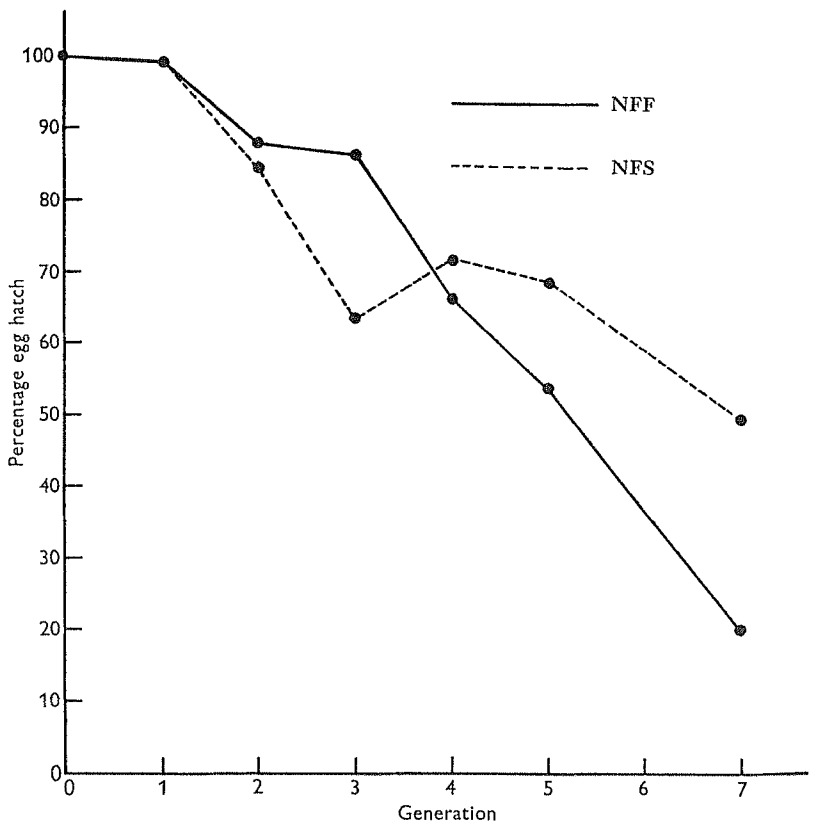


Fig. 4. The percentage egg hatch in the **NFF** and **NFS** lines.

from inbred females to hatch does not by itself establish female infertility, since the tested females are related to the F_1 males to which they were test mated, and the failure of eggs to hatch could therefore be due to zygotic inviability. However, the high percentage hatch in the reciprocal backcrosses of inbred males to F_1 females rules out this explanation.

Table 9. *Fertility tests on the* **B** *and* **K** *lines*

Parents	No. of fertilized females whose % egg hatch was >90%	20–90%	<20%	Total	No. of eggs Laid	Hatched	% Hatch
B♀×B♂	0	13	6	19	433	189	43·6
K♀×K♂	11	36	3	50	647	438	67·6
B♀×K♂	1	7	1	9	144	99	68·8
K♀×B♂	1	13	1	15	309	194	62·8
B/K♀×B/K♂	9	0	0	9	344	343	99·7
K/B♀×K/B♂	8	6	0	14	325	286	88·0
B♀×B/K♂	2	9	1	12	275	188	68·4
B♀×K/B♂	2	9	1	12	232	157	68·6
B/K♀×B♂	11	3	0	14	498	471	94·5
K/B♀×B♂	8	6	0	14	440	379	86·2
K♀×B/K♂	3	4	0	7	174	149	85·6
B/K♀×K♂	15	1	0	16	599	580	96·8

The hatchability of F_2 eggs is high, but there is a difference depending on the direction in which the original cross was made. There is a similar difference between the fertilities of the reciprocal F_1 hybrid females when backcrossed to **B** males. These differences suggest that **B/K** females are more fertile than are **K/B** females* ($P = 0{\cdot}02$ on the combined F_2 and backcross data). The difference is not very marked, but is associated with a slight difference in longevity and in continued fertility in old age which has been commented on by Clarke & Maynard Smith (1955).

(*f*) *Zygotic inviability*

Zygotic inviability is difficult to demonstrate in inbred lines showing a high degree of parental infertility. Ideally, males and females from a line should be test mated to fertile and unrelated flies, and those inbred flies which proved fully fertile in such tests could then be mated together to detect zygotic inviability. However, such a method is impractical because a female *D. subobscura* will not readily mate twice.

Some idea of the incidence of zygotic inviability can be obtained by comparing the egg hatchability of a line with that which would be expected from the parental fertilities in the absence of zygotic inviability, i.e. with the product of the parental fertilities. Such a comparison is made for the various lines in Table 10. In all except the **O** line, the observed hatchability is lower than the expected, although the difference is significant only in the **B** and **K** lines. It therefore seems likely that some eggs from inbred lines fail to hatch due to zygotic inviability. However, this does not mean that these lines are segregating for recessives which would be lethal in homozygous condition on any genetic background. The presence of such recessives would have prevented the high hatchabilities observed in some of the F_2 and backcross experiments. Thus zygotic inviability, like male infertility in the **O** line, is the result of inbreeding, but not of homozygosity at any particular locus.

* Footnote added in proof: later work has failed to confirm this difference.

Table 10. *Zygotic inviability*

	Male fertility	Female fertility	Overall hatchability		
			Expected	Observed	P*
B	94·5	68·4	64·7	43·6	0·03
K	96·8	85·6	82·8	67·6	0·009
NFF F_7	48·1	66·0	31·8	20·3	0·27
NFS F_7	76·5	92·0	70·5	49·4	0·11
O F_8 and F_9	48·8	91·5	44·6	48·5	—
O F_{15}	47·5	84·2†	40·0	38·8	—

* For a departure in the observed direction only.

† Based on **O** F_{14}.

(*g*) *Conclusions*

In the **O**, **NFF** and **NFS** lines, derived by brother-sister mating from wild-caught females, there was a rapid decline in the percentage egg hatch to from 20 to 50% after seven generations. In all three lines the major cause of the failure of eggs to hatch was the infertility of the males, i.e. the inadequacy of a proportion of the sperm produced by inbred males. Smaller contributions to the failure of eggs to hatch were made by female infertility and by zygotic inviability. Analysis of male infertility in the **O** line showed that it was not due to the segregation of a single recessive.

As judged by the proportion of pairs leaving adult progeny, the decline in fertility in the **O** line was most rapid in the slow-selected lines; unfortunately, these lines died out before a detailed study could be started. In the **NF** line there was no significant difference between the fertilities of the fast- and slow-selected lines.

In the **B** and **K** lines, derived from structurally homozygous stocks which had been kept in the laboratory for many years, there was no spectacular decline in the proportion of cultures leaving progeny when brother-sister mating was started, and in the **K** line it is known that more intensive inbreeding has not produced a further decline in the proportion of eggs hatching. The males of both these lines are fully fertile, whereas the **B** females are markedly infertile and the **K** females slightly so. There is a slight but significant difference between the fertilities of reciprocal F_1 hybrid females. In both lines some eggs fail to hatch due to zygotic inviability, but this cannot be due to the segregation of recessives which would be lethal in homozygous condition on any genetic background.

Further discussion of the causes of infertility in these inbred lines must await the results of a cytological study of gametogenesis, which is at present in progress.

6. Inversions in the **O** line

O flies were outcrossed to the structurally homozygous **K** line in the F_4 and F_{17}, and the salivary glands of the F_1 larvae examined. The chromosome orders of the **K** line are taken as the standard, and are referred to as the α orders.

Seventy-four F_4 flies were outcrossed, but such flies could be classified only if analysable salivary preparations were obtained from at least six of their larval offspring. Only twenty-nine **O** F_4 flies were so classified; the results were:

Homozygous α order for all chromosomes	15
Heterozygous for Balbiani ring inversion, chromosome I	3
Heterozygous for double inversion, chromosome U	9
Heterozygous for inversions in chromosomes I and U	2
Total	29

There was no association between rate of development and structural type of the flies tested.

O flies were again outcrossed to **K** flies in the F_{17}, and salivary preparations made from a single larva from each mating. It was found that the line was still segregating for inversions on the I and U chromosomes.

Whatever the structural type of the original Oxford female, or of the male by which she was fertilized, not more than one-quarter of her progeny could have been structurally heterozygous for both the I and U chromosomes, and one-quarter must have been structurally homozygous for both. Nevertheless, there was little sign of infertility among these F_1 flies. 29/30 of the F_2 pairs left progeny, and productivity was high (mean of 26·2 adult offspring per day per pair). This suggests that structural homozygotes were not at any serious disadvantage in the early stages, before inbreeding had led to homozygosity for the uninverted chromosome regions.

Further, 15/29 of the adult F_4 flies analysed were structural homozygotes. Therefore, at this stage at least, some structural homozygotes were viable and were not sterile.

However, the maintenance of structural heterozygosity for both the I and U chromosomes for seventeen generations of brother-sister mating shows that flies which were structural homozygotes for either of these chromosomes (or for both) were at a severe selective disadvantage.

7. DISCUSSION

A major embarrassment in interpreting experiments on the inheritance of quantitative characters lies in the multiplicity of hypotheses available. Our data on rate of development are consistent with the hypothesis that most genetic variance for this character is due to chromosome regions with heterotic effects. Since this was the hypothesis which our experiments were originally designed to test, our results may be regarded as a partial confirmation of it. However, it is impossible to distinguish between heterosis due to alleles at single loci (*Aa* faster than *AA*, *aa*) or to dominant alleles at a number of linked loci (*Ab*/*aB* faster than *Ab*/*Ab*, *aB*/*aB*).

Similarly, we have found in the **O** line a marked correlation between slow development and infertility. This could be explained by linkage between polygenes, as invoked by Mather & Harrison (1949) to explain the sterility which appeared in lines of *D. melanogaster* selected for both high and low bristle number. They argued that since linkage between polygenes would be expected to produce the observed sterility, it was unnecessary to invoke pleiotropic gene effects to explain it. In the present case, however, it is simpler to regard the association between slow development and infertility as a form of pleiotropism. All five inbred lines studied show some degree of infertility, which appears to be an almost inevitable accompaniment of inbreeding, and presumably of homozygosity, in this species. There is also ample evidence that most homozygous genotypes develop slowly (cf. Maynard Smith & Maynard Smith (1954) for chromosome 5 homozygotes, and Table 3 for a comparison of the **O** F_9 and **NF** F_2; it is also known from unpublished data that both the **B** and **K** lines have an eclosion time 1–3 days longer than hybrids between them). Thus slow development and infertility can be regarded as pleiotropic effects of homozygosity at a number of loci. However, the **NFF** line, although developing almost as rapidly as hybrids between it and the **NFS** line, is highly infertile. This is the only indication we have that slow development and infertility may result from homozygosity for different alleles or at different loci.

Fortunately, it is often easier to predict the results of a selection experiment than to elucidate the genetic mechanisms underlying it. Falconer (1953) has pointed out that an asymmetrical response to selection is to be expected in two genetic situations, unequal gene frequencies and directional dominance. He gives as examples selection for body size and for lactation in mice; in the latter case the asymmetry was so great that selection in an upwards direction was ineffective. Rate of development resembles lactation in this respect (cf. Table 4), although Falconer's curves show a positive response to selection in a downwards direction in the first selected generation, whereas we found no response in the first generation. Our results are in sharp contrast to those of Mather & Harrison (1949) and of Scossiroli (1953), who, in selecting for bristle number in *D. melanogaster*, obtained an immediate and approximately symmetrical response. Selection for size in *D. melanogaster* (Robertson & Reeve, 1953; F. W. Robertson, 1954) gave results intermediate between those for bristle number and for rate of development; chromosomes from lines selected for large size usually showed some aggregate dominance over chromosomes from small-selected lines.

Falconer (1953) pointed out that characters which show an asymmetrical response to selection will be those which are subject to inbreeding depression. Clarke & Maynard Smith (1954) suggested that such characters may be recognizable *a priori*. They will be such as to confer fitness in a wide range of environments, and will be properties of the organism as a whole rather than of one of its parts.

The genetic variance of characters which confer fitness in many environments is likely to be of the kind discussed by Haldane (1949) and demonstrated in this paper for rate of development, i.e. it will be due to genes with heterotic effects. Genetic variance due to genes with additive effects, or showing simple dominance, would tend to disappear by the elimination of the less favourable alleles.

The suggestion that characters associated with inbreeding depression will be properties of the organism as a whole rather than of one of its parts is more difficult to justify. If a character can be readily altered by selection in either direction, then the processes of development must be alterable so as to produce changes in the selected character, without involving correlated, and almost certainly deleterious, changes in other characters. If such correlations in development exist, then changes in the character artificially selected for will be opposed by natural selection. To take Falconer's example of lactation, increased milk production must be accompanied by a changed nutritional balance in the mouse. Thus characters associated with vigour and inbreeding depression will tend to be those which cannot be altered without involving, through causal connexions in development, changes in many other characters, or whose improvement depends on raising the general metabolic efficiency of the organism.

Most characters of economic importance in agriculture are of this kind. The problems of animal and plant breeding have more in common with attempts to increase fertility or rate of development in *Drosophila* than to increase the bristle number. Inbreeding is likely to be fatal to such attempts. As far as the practical problems of animal breeding are concerned, the present paper can be regarded as an illustration of how not to do it.

8. SUMMARY

Two brother-sister mated lines of *Drosophila subobscura*, the **O** and **NF** lines, were established from wild-caught females. In each generation fast and slow developing flies were selected and mated together. There was no difference in either line between the rates of development of the progeny of fast and slow pairs in the first selected generation.

In the **NF** line heritability of rate of development (i.e. the difference between the mean eclosion times of the progeny divided by the difference between the mean eclosion times of the parents) increased in subsequent generations, and reached 0·89 after five generations of selection. Mating together slow-developing flies from the fast-selected (**NFF**) and slow-selected (**NFS**) sublines gave progeny which developed slightly faster, and were less variable, than **NFF** flies. These results are consistent with the hypothesis that most genetic variance of rate of development is due to genes or chromosome regions with heterotic effects.

In the **O** line no significant difference was observed in the rate of development of the fast and slow selected lines. Infertility developed in all the inbred lines, but its appearance was most rapid in the slow-selected **O** sublines. All four such sublines were lost in the first five generations. This association between slow development and infertility in the **O** line probably explains the absence of any response to selection for rate of development.

The mean number of adult progeny produced per day per pair in the **O** line fell from forty-eight in the F_1 to from one to three in the F_{7-8}. This was followed by a slight rise in productivity in the only surviving **O** subline in the F_{9-15}. A major cause of the decline was the failure of eggs laid by fertilized females to hatch. By outcrossing inbred flies to flies known to be fully fertile, it was found that this failure was mainly due to male infertility, i.e. to some inadequacy of the sperm produced by inbred males. This male infertility was not due to the segregation of a single mutant.

Infertility developed, although less rapidly, in the **NFF** and **NFS** lines. As in the **O** line, the major cause was male infertility.

The fertility of two brother-sister mated lines, **B** and **K**, derived from structurally homozygous laboratory stocks, was also studied. In these lines the males are almost fully fertile, and female infertility accounts for the majority of failures in egg hatching.

In all lines except **O**, there is an indication that some eggs fail to hatch due to zygotic inviability.

The **O** line was still segregating for inversions in two of the four long autosomes in the F_{17}.

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