Genet. Res., Camb. (1962), **3**, *pp.* 110–138
With 7 text-figures
Printed in Great Britain

The effects of inbreeding and artificial selection on reproductive fitness

By B. D. H. LATTER* and ALAN ROBERTSON†
Institute of Animal Genetics, Edinburgh

(*Received* 12 *September* 1961)

INTRODUCTION

The response of an equilibrium population of a cross-fertilized species to artificial selection for extreme expression of a chosen quantitative character is a phenomenon which is as yet only very imperfectly understood. The change in the mean of the character under selection can be predicted on the basis of a well-developed theoretical framework, from an analysis of the equilibrium phenotypic variation displayed, and the experimental work which has been devoted specifically to checking predictions of this sort has shown reasonable agreement between expectation and observation over the early generations (Kyle & Chapman, 1953; Clayton *et al.*, 1957*a*). The theory is, however, incapable of giving useful predictions of the long-term response in the mean, and the factors which are important in defining the ultimate level reached under selection are not yet well understood.

Existing theory also deals with the response to be expected in characters other than that directly selected, but is limited to those whose additive genetic association with the primary character is such as to be usefully described by a linear regression equation. Critical tests involving characters showing relatively high genetic correlations have shown the predictions of short-term correlated response to be adequate (Reeve & Robertson, 1953; Falconer, 1954), though more recent work has underlined the necessity of breaking observed genetic covariances down into additive and non-additive components, if accurate predictions are to be obtained (Robertson, 1957).

As a result of the disturbance of the initial genetic equilibrium, it is to be expected theoretically that the reproductive ability of the individuals comprising the selected population will be adversely affected, and changes of this sort have been a general feature of artificial-selection programmes in a number of laboratory and domestic species. However, we have no way of predicting the rate of decline in reproductive fitness for a given character under selection, nor can it be clear what part the changes in fitness will play in determining the ultimate limits to selection. There is no clear understanding of the relative importance of changes in gene frequency due to the effects of selection and those due to the effects of inbreeding in promoting changes in fitness under selection, nor is the importance of linkage in this process at all well established.

The first step towards an understanding of the phenomenon must be observa-

* Present address: Division of Plant Industry, C.S.I.R.O., Canberra, Australia.
† Member of the Agricultural Research Council Staff.

tional rather than analytical, and the real need is for accurate measurements of the associated changes in fitness with selection, for a number of different quantitative characters in a controlled experimental programme. An attempt has therefore been made, using *Drosophila* as the experimental animal, to measure the changes in reproductive fitness consequent upon selection for three separate quantitative characters, using the technique developed by Knight & Robertson (1957). Suitable control populations have been maintained to enable the effects of inbreeding on fitness to be measured independently of the effects of selection. In addition, the direct effect of rapid inbreeding on fitness has been studied in some detail to throw further light on the nature of the equilibrium genetic variance in reproductive fitness itself. Observations of this sort can be expected to provide a useful basis for further research of a more analytical kind.

EXPERIMENTAL MATERIAL AND TECHNIQUES

The population which has been used in this investigation is a wild-type laboratory cage population of *Drosophila melanogaster*, described previously by Clayton *et al.* (1957*a*). At the start of the work, the stock had been maintained under a constant regime for some eight years (approximately 160 generations) at an average size of roughly 5,000 individuals. From all practical points of view it can therefore be taken to have been in genetic equilibrium.

Three different types of competition test have been used in this work; a 'male × female' test which involves all three major components of fitness, a 'female' test which brings in only egg-laying ability and larval survival, and a 'male' test designed to measure male mating ability alone. In all the fitness testing, the standard laboratory medium was used, cultures were kept at 25° C., and a laboratory bottle population of *Cy/Pm*, maintained throughout in large numbers, was used as the tester stock. The detailed procedure were as follows:

(*a*) *Male × female test.* Inseminated females of the inbred or selected line to be tested, and inseminated *Cy/Pm* females, were allowed to lay for 5 days in one-pound jam jars. One hundred females in all per jar were used, the relative numbers of the two types being varied according to the anticipated fitness of the inbred or selected line. From the emerging adults, balanced sets were collected of twenty-five males and twenty-five virgin females of each of the two types, preferably from a single emergence. Where a complete set could not be made up from the flies obtained from a single emergence from one jam jar, care was taken that the wild-type and *Cy/Pm* flies of both sexes were balanced for age and parental culture.

The twenty-five pairs of males and the twenty-five pairs of females were stored separately in vials for a period of 3 days in the constant-temperature room, and were then placed in a fresh half-pint culture bottle to mate and lay eggs for 5 days. The numbers of wild-type and *Cy/Pm* flies emerging from the competition bottle were recorded over a 4-day period. The ratio has been used as a competitive index, expressing the competitive ability of individuals of the inbred or selected line relative to the tester stock, and will be denoted by C.I. (♂ × ♀).

(*b*) *Female test.* Balanced sets were collected and matured for 3 days as in the previous test. The wild-type and *Cy/Pm* females were then mated separately to their own males, the males removed after 8 hours, and the inseminated females of the two sorts placed in a fresh culture bottle to lay for 5 days. The numbers of wild-type and *Cy/Pm* flies emerging were then recorded as before, and the resulting ratio used as an expression of the relative fecundity and larval survival of the wild-type line, denoted by C.I. (♀).

(*c*) *Male test.* A balanced set of twenty-five pairs of males was collected from each jam jar, matured for 3 days, and the wild-type and *Cy/Pm* males then allowed to compete for mates among a group of fifty virgin *Cy/Pm* females for 8 hours. The females were then placed in individual vials to lay, and the resulting progeny examined to determine for each female which of the two types of male was responsible for the insemination. Double matings were not infrequent, but could of course only be detected when a wild-type male and a *Cy/Pm* male contributed to a single progeny group. It is easily shown that, if triple matings do not occur in the period allowed, an unbiased estimate of relative male mating ability is obtained by giving a score of $\frac{1}{2}$ to each type of male for each detectable double mating, and a score of 1 to the appropriate type of male in all other cases. The ratio of the number of inseminations due to wild-type males to the number due to mutant males has then been used as an index of the relative mating ability of males of the wild-type line, denoted by C.I. (♂).

Variance between replicates

As pointed out by Knight & Robertson (1957), replicate determinations of competitive ability show considerable variation, and a number of competition bottles must be set up for each line to be examined. The statistical treatment of the replicate observations has been as follows: individual results have been expressed as proportions (p) of the form (wild-type)/(wild-type + *Cy/Pm*) and the mean proportion ($\bar{p}$) taken over all replicates. A competitive index has then been calculated as C.I. $= \bar{p}/(1-\bar{p})$ with standard error

$$\text{S.E. (C.I.)} = \frac{s_p}{\bar{n}(1-\bar{p})^2},$$

where n is the number of replicates and s_p^2 the variance of the proportions p.

Our experience has been that for values of $\bar{p}$ in the range 0·2 to 0·8, there is no apparent relationship between the value of $\bar{p}$ and the variance between replicates,

Table 1. *Variance of replicate measurements of competitive ability*

Competition test	s_p^2	d.f.
Male × female	701×10^{-5}	426
Female	874×10^{-5}	152
Male	297×10^{-5}	224

and pooled estimates of s_p^2 for each of the three types of competition test (Table 1) have been used in the calculation of standard errors.

Primary tests

Before extensive use was made of the competition techniques, it was thought advisable to carry out preliminary tests to check the following points:

(i) Are measurements of competitive ability repeatable in tests made at different times?

(ii) Is competitive ability (relative to the tester stock) independent of the particular tester used?

(iii) Do the competitive indices given by the three different tests show the expected relationship C.I. (♂ × ♀) = C.I. (♂) × C.I. (♀)?

It was to be expected that the repeatability of measurements made at different times would be very similar to that of replicate contemporaneous measurements, since the design of the tests ensured that competing wild-type and *Cy/Pm* individuals shared a common micro-environment. On the other hand, Lewontin (1955) has shown larval survival to be dependent upon the composition of the competing population, and it was conceivable that the results obtained by the use of the *Cy/Pm* tester might not be the same as those given by tests using a different marked stock. It was therefore important to determine how large such effects might be, relative to the differences in fitness which the technique are capable of detecting.

Four lines available in the laboratory were therefore chosen, and two replications set up of each of five competition tests: the male × female test, the female test and the male test using *Cy/Pm* as tester; and the female test and male test using a white-eyed stock (WK) as tester. It was not possible to conduct male × female tests with the WK stock, since the white-eye is recessive to wild-type, and cross-matings could not therefore be detected. The tests were repeated at 7-day intervals over a period of 4 weeks.

For each replicate of the female and male × female tests, the proportion of emerging flies which were wild-type (the progeny of cross-matings being neglected in the male × female tests) has been calculated; for the male tests, the proportion of inseminations due to wild-type males in each replicate has been recorded. Analyses of variance of these proportions are presented in Table 2.

The four lines used were the Kaduna cage population, with which all subsequent work has been concerned, and three lines derived from it by slow inbreeding over many generations. The least fit of the partially inbred lines had a C.I. (♂ × ♀) which was approximately 50% that of the cage population, and over this range there is no indication that the relative competitive ability of the lines was in any way dependent on the tester used, nor is there any evidence that repeatability in time was any less than that of contemporaneous measurements. The significant second-order interaction shown by the female tests seems most likely to be a chance effect, in view of the non-significant first-order interactions.

The WK stock was chosen as a check on *Cy/Pm* in these preliminary tests because of its close genetic relationship to the Kaduna population: it was in fact derived by repeated backcrossing to Kaduna of a cross between Kaduna and

a laboratory white-eye stock. Competition with WK should therefore come very close to simulating that provided by the equilibrium array of genotypes within the Kaduna population itself.

With regard to the expected relationship between the competitive indices of the three types, the results were rather surprising. Data is now available from a total of sixteen lines all of which were derived by selection or mild inbreeding from the Kaduna population: it is clear that a consideration of the C.I. (♂ × ♀) or of the

Table 2. *Analyses of variance of proportions recorded in competition tests using two tester stocks*

		Mean squares ($\times 10^5$)		
Source of variation	d.f.	Male × female	Female	Male
Between lines	3	4,723*	42,288*	1,373†
Between groups of tests	3	235	298	1,005
Between testers	1		133,402*	90
Lines × groups	9	634	451	100
Lines × testers	3		347	375
Groups × testers	3		690	851
Lines × groups × testers	9		1,087†	398
Residual		488‡	358§	416§

* Significant at 0·1% level. † Significant at 5% level.
‡ Based on 16 d.f. § Based on 32 d.f.

composite index C.I. (♂) × C.I. (♀) leads to the same ranking of the lines, bearing in mind that the indices are subject to sampling error, but the differences between lines are considerably magnified when the separate male and female tests are compounded. A possible explanation of this effect lies in the removal of the males after insemination in the female tests, since it is well known that fecundity is greatly stimulated by the presence of the male: to explain the observations on this basis, one must presume the reduction in egg number due to removal of the males to be proportionately less in the lines of high fitness than in those of lowered reproductive ability.

Duration of the effective laying period

One of the main drawbacks of the technique we have used for measuring fitness is that longevity is not considered. Adult males and females of 3 days of age have been used throughout, and females allowed to lay for a period of only 5 days. In addition, flies emerging from the competition bottles have been counted for only 4 days of full emergence, to reduce the labour involved in scoring. It was important therefore to define closely the effective laying period, so that the full limitation of the technique could be appreciated.

Batches of fifty freshly laid WK eggs were placed on the surface of the medium in competition bottles being used for male × female tests with *Cy/Pm* as tester, and the numbers of white-eyed flies emerging during the 4-day counting period

recorded. The eggs were placed in one set of bottles at the time the competing flies were introduced, in a second set after 24 hours, and in a third group after 48 hours. Of the eggs introduced with the competing individuals, 56% came through during the counting period, almost all on the first or second day. Of those introduced at the beginning of the second day, 61% came through to emerge predominantly on the third and fourth days of counting. Of the eggs introduced into the bottles at the beginning of the third day, only 3% came through to emergence during the 4 days of scoring.

It is clear, therefore, that the effective laying period is at most 2 days, and may very likely be as short as 36 hours. However, the early work on egg number in *Drosophila* (Gowen & Johnson, 1946) has shown that relatively high correlations are to be expected between peak daily production and other components of fecundity such as longevity, number of laying days, and lifetime egg production. Bell *et al.* (1955) have in consequence limited their fecundity measurements to the peak period of from 4 to 7 days of age. The restricted laying period in our tests is not therefore likely to be as serious a limitation as might at first be supposed.

EFFECTS OF INBREEDING ON FITNESS

The deterioration in reproductive ability which results from inbreeding in cross-fertilized species is a well-known phenomenon, and has been discussed in some detail by Lerner (1954). A completely general expression for the rate of decline in the mean of any quantitative character in the absence of natural selection has been given by Kempthorne (1957) in terms of the parameters characterizing the genetic variance shown by the trait in the population prior to the commencement of inbreeding. This expression can be applied to fitness itself or any of its components provided the action of natural selection during the inbreeding process can justifiably be neglected. Observations of the rate of decline in fitness under rapid inbreeding therefore throw some light on the nature of the equilibrium genetic variance in fitness in the base population.

The experimental studies which have come closest to measuring the decline in fitness on inbreeding in *Drosophila* have been those in which the mean number of offspring per day of emergence has been recorded for inbred lines under uncrowded conditions (Maynard Smith *et al.*, 1955). It is almost certain that the decline in overall fitness has been underestimated in this work, and it was therefore desirable to obtain measurements with the competition technique at our disposal. Forty full-sib lines were started from the Kaduna cage population, and carried without reserves for ten generations. Competitive ability was measured after 4, 7 and 10 generations of sib-mating, using two replicates per line of the male × female test. The observed decline in the competitive index was so dramatic after four generations that a further fifteen lines were taken off from the base population and full-sib mated for one generation only to provide an intermediate point. The overall generation means are presented in Table 3: from a series of observations of the competitive ability of the array of genotypes in the base population, we know the C.I. (♂ × ♀) to be 3·93 with a standard error of 0·25.

The mean competitive ability of lines surviving to the point of measurement has been calculated on the basis of the proportions of wild-type flies emerging from the competition cultures, and the mean so obtained has then been converted to a competitive index. The corresponding standard errors are based on the observed variance between replicates (Table 1), and on the component of variance between line means at the level of inbreeding concerned. The competitive indices which include those lines lost in the process of inbreeding have been obtained as the product of the proportion of lines surviving and the mean competitive index of the surviving lines. This procedure is of course equivalent to considering all lines lost as having zero fitness, and must therefore lead to an underestimate: an examination of the individual measurements, however, shows that the lines which did not survive were in the main considerably reduced in fitness at the previous point of measurement, and general observation of the sterile cultures made it quite clear that the cause of sterility was in all cases either male or female infertility or poor hatchability of the eggs laid. No culture was lost through the

Table 3. *Mean competitive indices of full-sib lines at progressive levels of inbreeding*

Value of F	Proportion of lines surviving	Competitive index (♂ × ♀)	
		Surviving lines	All lines
0·000	—	3·93 ± 0·25	3·93 ± 0·25
0·250	1·00	1·88 ± 0·22	1·88 ± 0·22
0·594	0·90 ± 0·05	0·89 ± 0·09	0·80 ± 0·09
0·785	0·55 ± 0·08	0·63 ± 0·13	0·35 ± 0·09
0·886	0·42 ± 0·08	0·55 ± 0·12	0·23 ± 0·07

early death of either parent, and all pairs of flies were given the opportunity to lay eggs in at least two vials. The procedure we have adopted for including the extinct lines in the mean competitive index is likely therefore to provide a useful approximate figure.

The relationship between the mean competitive index calculated on this basis and the coefficient of inbreeding is shown in Fig. 1. The curve has been derived as the least-squares linear regression of the logarithm of the mean competitive index (all lines) on F, due weight being given to the points according to their error variances on the logarithmic scale. All the means depart from the curve by one standard error or less, and an analysis of variance of the competitive indices on the logarithmic scale showed the quadratic and cubic mean squares for departure from linear regression to be non-significant.

In considering the implications of this relationship, one can formally make use of the equation given by Kempthorne (1957), expressing the decline in the mean of a quantitative character as a function of the inbreeding coefficient F. However, the decline in competitive index is obviously non-linear in F, and Kempthorne's equation would lead us to conclude that interactions between pairs of loci (in the sense that the loci do not act additively) are of obvious significance in promoting

inbreeding degeneration in reproductive fitness. This conclusion is no surprise in view of the fact that the simplest genetical hypothesis which we can expect to hold is that the effects of different loci on fitness during inbreeding are independent and therefore multiplicative. It is tempting to use log fitness as the quantitative character, and to apply Kempthorne's equation to the relationship between the mean log (C.I.) of the inbred lines and the inbreeding coefficient. The immediate difficulty is that lines which were lost in the process of inbreeding must be given a fitness value zero, if they are to be considered at all, and will therefore have an

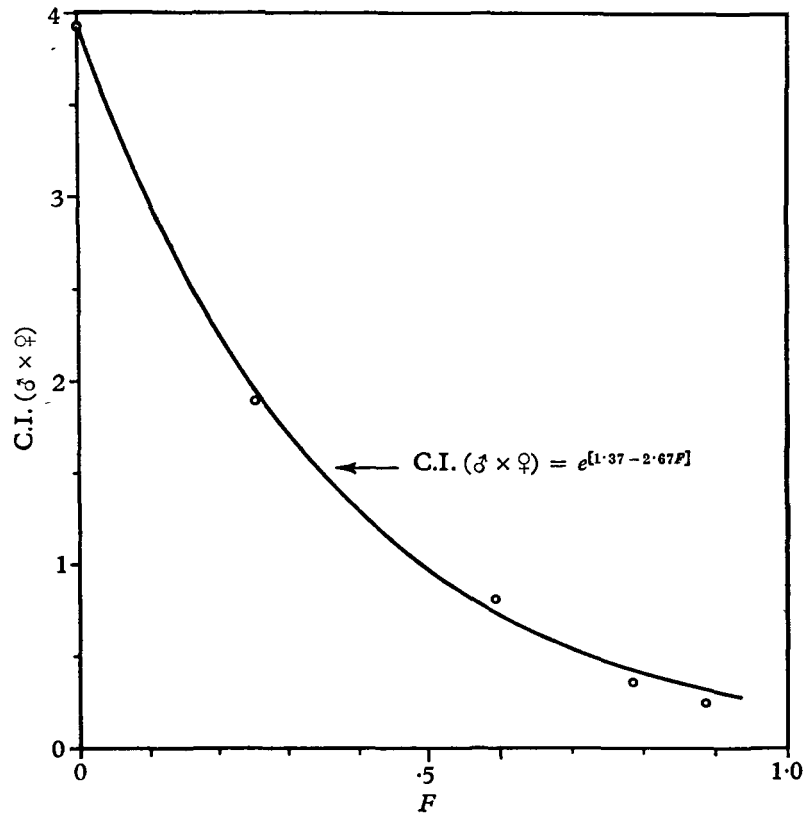

Fig. 1. Decline in competitive index with rapid inbreeding.

infinite value on the log scale: in addition, Kempthorne's derivation cannot be applied to log fitness as a character unless lethal genotypes are non-existent in the population concerned.

However, it can readily be shown that if the effects of different loci on fitness are multiplicative, and the average decline in fitness over the population of possible inbred lines due to single loci is in all cases small, then the logarithm of the mean fitness of the lines will be linearly related to F. The assumption of small overall effects of individual loci on the decline in fitness with inbreeding holds for loci showing a dominance-recessivity relationship, even though one homozygote be lethal, and for overdominant loci provided the heterozygote advantage is small.

https://doi.org/10.1017/S001667230000313X Published online by Cambridge University Press

We must therefore conclude from the present data that the mean reduction in competitive ability on inbreeding is little affected by interactions between loci, being due primarily to the average dominance properties of the alternative genotypes at individual loci. This is not to say that epistatic interactions are necessarily unimportant in the base population, for it is only interaction between loci involving the dominance deviations themselves which are expected to contribute to the decline in mean with inbreeding.

It is natural to inquire what can be deduced from the results as to the dominance properties of the alternative genotypes at individual loci. Since both overdominant loci and loci carrying deleterious recessive alleles will lead to a linear decline of log fitness with F, there is no direct way of discriminating between the effects of the two types of loci: however, one of our lines had dropped by only 50% in fitness after ten generations of full-sib mating, and Knight and Robertson (1957) have recorded a line of 65% fitness after twelve generations of full-sibbing from the same population. If the theoretical level of inbreeding had in fact been reached

Table 4. *Components of variance between full-sib line means of the proportions of wild-type flies*

Value of F	Component	Standard error
0·250	481×10^{-5}	304×10^{-5}
0·594	2032×10^{-5}	554×10^{-5}
0·785	5086×10^{-5}	1603×10^{-5}
0·886	4096×10^{-5}	1483×10^{-5}

in these two lines, it is clear that if overdominant loci are at all common, the mean selective advantage of heterozygotes over their superior alternative homozygotes must be of the order of only 1 to 2%. This conclusion in no way argues against the possible importance of overdominant loci in the sphere of animal and plant improvement; for the increase in performance above the population mean of quantitative characters closely bound up with reproductive fitness, by the synthesis of a hybrid incorporating all superior heterozygotes, can be expected to be roughly equal to the decline in mean due to complete homozygosis at such loci. Dominant-recessive loci do not have the same potentiality for contributing to hybrid performance, as has been pointed out by Crow (1948).

We have so far discussed only the change in the mean competitive index of the inbred lines. At all stages of inbreeding there were found to be significant differences in fitness among surviving lines, and the components of variance between lines are set out in Table 4. The standard errors given have been calculated on the basis of normal distribution theory.

The only theoretical work which has been done on this aspect of the effects of inbreeding on characters showing predominantly non-additive genetic variation is that of Robertson (1952), who has discussed the variance between lines to be expected from the action of independent recessive genes at low frequency. The components which we have observed show the variability between surviving lines

to have increased markedly over the early generations, but little more can be deduced from the data because of the large number of lines lost in the process of inbreeding, and the large standard errors associated with the estimates.

EFFECTS OF ARTIFICIAL SELECTION ON FITNESS

Studies of the effects of artificial selection in the Kaduna population have involved three different quantitative characters; abdominal bristle number on the fourth and fifth sternites, sternopleural bristle number, and body size measured by wing length. In all selected lines, ten pairs of parents have been selected each generation and randomly mated in a single culture. In the abdominal bristle number lines and in the wing length lines, an intensity of selection of 20% was imposed throughout, while in the sternopleural bristle number lines the intensity of selection was 40%. Competitive ability was measured in the abdominal bristle line at generations 5, 10, and 20; in the sternopleural bristle lines at generations 15 and 25; and in the wing length lines at generations 5 and 10. Unselected control lines were maintained throughout using ten pairs of randomly chosen parents per generation, and the rate of inbreeding in these lines can be estimated from our knowledge of effective population size (4·8 effective males and 7·1 effective females; Crow, 1954) to be approximately 4·4% per generation.

Competitive ability of control lines

Four control lines were carried for ten generations, and their competitive ability measured after five and ten generations: two of the lines were continued on to the twenty-fifth generation with fitness measurements at generations 15 and 25. The competitive indices are given in Tables 5 and 6, together with their standard errors. All measurements except those referring to the base population are based on eight to ten replicates.

Table 5. *Competitive indices* (♂×♀) *of control lines**

Line	Generation 5	Generation 10	Generation 15	Generation 25
C1	3·17 ± 0·39	3·08 ± 0·44	2·06 ± 0·25	2·21 ± 0·31
C2	3·74 ± 0·46	2·92 ± 0·41	2·66 ± 0·36	2·58 ± 0·38
C3	3·66 ± 0·41	3·26 ± 0·53	—	—
C4	3·40 ± 0·38	3·33 ± 0·55	—	—
Mean	3·50 ± 0·20	3·14 ± 0·24	2·34 ± 0·21	2·39 ± 0·24
F (%)	20·2	36·2	49·1	61·5

* C.I. (♂×♀) of base population is 3·93 ± 0·25.

The values of the inbreeding coefficient given in Table 5 have been calculated on the basis of the expected loss of heterozygosity of 4·4% per generation. However, we have previously seen that the decline in the competitive index (♂×♀) under rapid inbreeding is approximately 2·7% per 1% increase in F (Fig. 1): by comparison, the more slowly inbred control lines have shown a reduction in competitive index (♂×♀) of the order of only 0·7% per expected 1% increase in F. The higher

fitness of the lines inbred more slowly could be due to maintenance of the equilibrium by selection of heterozygotes or to the greater chance of eliminating deleterious recessives (Robertson, 1960).

Table 6. *Competitive indices (♂) of control lines**

Line	Generation 5	Generation 10	Generation 15
C1	$1{\cdot}34 \pm 0{\cdot}11$	$1{\cdot}16 \pm 0{\cdot}06$	$1{\cdot}06 \pm 0{\cdot}07$
C2	$1{\cdot}42 \pm 0{\cdot}11$	$1{\cdot}18 \pm 0{\cdot}08$	$1{\cdot}14 \pm 0{\cdot}08$
Mean	$1{\cdot}38 \pm 0{\cdot}08$	$1{\cdot}17 \pm 0{\cdot}06$	$1{\cdot}10 \pm 0{\cdot}05$

* C.I. (♂) of base population is $1{\cdot}56 \pm 0{\cdot}09$.

Of particular interest are the measurements of relative mating ability of the males in these control lines (Table 6), since this component of fitness has not previously been studied in any detail. The technique which we have used has been sufficiently sensitive to detect a significant decline of some 30% in male mating ability over a period of fifteen generations.

Selection for abdominal bristle number

The behaviour of the Kaduna population under selection for abdominal bristle number has been reported in detail by Clayton *et al.* (1957*a*, *b*, *c*), and the selection procedure and methods of analysis described by these authors have been followed in the present investigation. It was their experience that problems of infertility were more frequently encountered in the low than in the high selected lines, and that there were obvious differences in fertility after twenty generations among some of the lines selected in the same direction.

In the present study, two high lines (AH1, AH2) and two low lines (AL1, AL2) were selected for twenty generations and tested individually for competitive ability. The responses in bristle score (averaged over the two sexes) are shown in Fig. 2. The low line AL2 was lost through infertility at generation 14: no attempt was made to save the line by relaxation of selection.

Some features of the response are in marked contrast to the observations of Clayton *et al.* (1957*a*, *b*), and it seems worth while to comment on the differences between the two sets of results. Over the first five generations, the response shown in Fig. 2 was symmetrical in the two directions of selection, corresponding to realized heritabilities of 41% in AH1, 28% in AH2, 39% in AL1, and 36% in AL2. In the earlier work of Clayton *et al.*, the response to selection of approximately the same intensity (20/100 instead of 10/50) was equivalent to realized heritabilities in the high and low directions of 53% and 37% respectively, averaged over five replicate lines. The agreement is good for the low lines in the two studies, but our high lines have not shown the response to be expected from the earlier work. Bearing in mind the differences between replicates observed by the previous workers, AH1 has shown something close to the anticipated response, but AH2 has clearly behaved in a descrepant manner. The reason for this is apparent

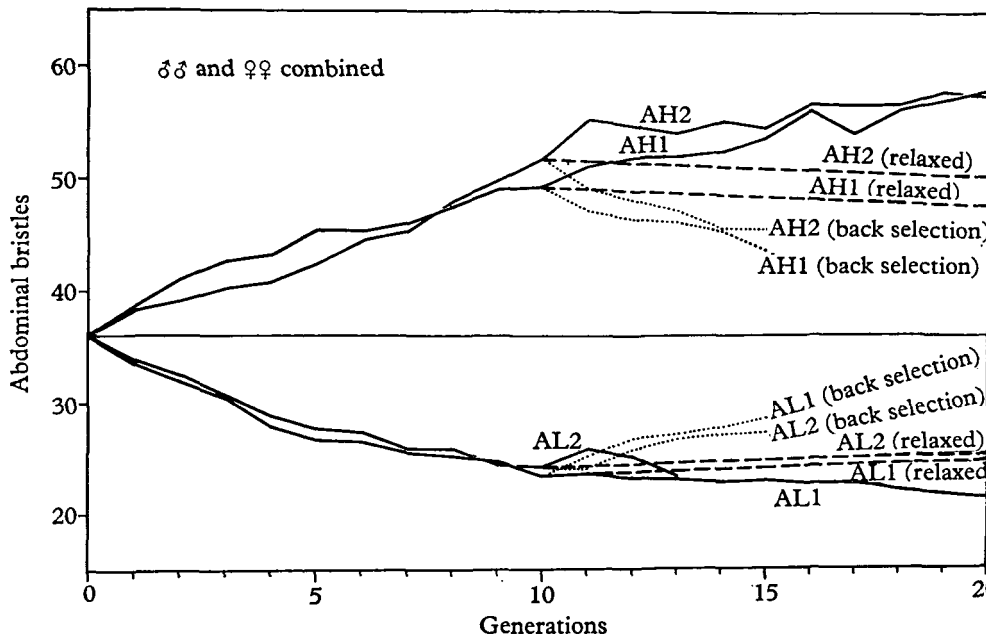

Fig. 2. Response to selection for abdominal bristle number.

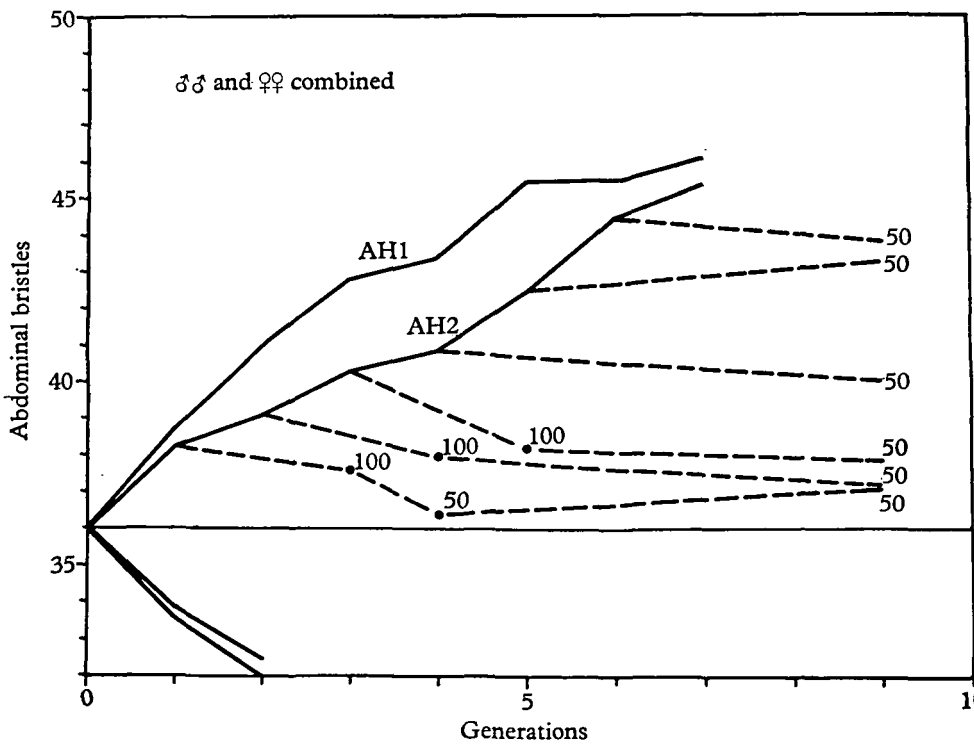

Fig. 3. Relaxation of selection in AH2. (The numbers of observations contributing to each point are indicated in the figure.)

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when we consider the behaviour of the line on relaxation of selection (Fig. 3). All lines were relaxed each generation over the early period of response, and AH2 was the only one of the four which showed a significant regression to the unselected mean after two generations of relaxation. It was therefore subjected to more careful scrutiny. The observations make it quite certain that, for the first three generations of selection, progress in the line was strongly opposed by natural selection.

The long-term response in AH1, AH2 and AL1 was considerably less than that observed by Clayton *et al.*; at generation 20, their five high lines had mean female scores in the range 68–84 (Clayton *et al.*, 1957*b*, Fig. 1), whereas AH1 had reached a score of 62·56 and AH2 a score of 64·40. The five low lines in the earlier work had mean female counts in the range 10–21, and our surviving line AL1 had dropped to only 23·40. The explanation for the difference in response can most plausibly be found in the relative rate of loss of genetic variance due to restricted population size. Twenty pairs of parents were selected per generation by the earlier workers, whereas only ten pairs have been used in the present study.

As regards change in the phenotypic variance within lines with selection, the behaviour of AH1 and AH2 closely paralleled that of the high lines in the earlier study, the coefficients of variation showing a slight decline over the twenty-generation period. The ratio of male to female means remained constant over the period in the two high lines as in the earlier work. The low lines AL1 and AL2, however, showed approximately constant coefficients of variation and ratios of male to female means, in contrast to the odd behaviour of the low lines reported in their work by Clayton *et al.* The lack of corresponding behaviour is no doubt related to the lesser response in the mean observed in the present investigation, and it seems that the apparent threshold postulated by the earlier workers had not been reached in our lines.

Competitive ability in the abdominal bristle lines

The Kaduna base population has a competitive index (♂×♀) of $3{\cdot}93 \pm 0{\cdot}25$ relative to the *Cy/Pm* tester stock. It can be seen from Table 7 that in addition

Table 7. *Competitive indices (♂×♀) of abdominal bristle lines*

Line	Generation 5	Generation 10	Generation 20
AH1	$2{\cdot}63 \pm 0{\cdot}31$	$1{\cdot}32 \pm 0{\cdot}14$	$1{\cdot}36 \pm 0{\cdot}21$
AH2	$2{\cdot}91 \pm 0{\cdot}37$	$3{\cdot}22 \pm 0{\cdot}47$	$1{\cdot}86 \pm 0{\cdot}26$
AL1	$2{\cdot}09 \pm 0{\cdot}24$	$0{\cdot}54 \pm 0{\cdot}06$	$0{\cdot}28 \pm 0{\cdot}06$
AL2	$2{\cdot}49 \pm 0{\cdot}30$	$1{\cdot}63 \pm 0{\cdot}18$	0·00 (sterile)
Mean control	$3{\cdot}50 \pm 0{\cdot}20$	$3{\cdot}14 \pm 0{\cdot}24$	2·37*

* Estimated as mean of controls at generations 15 and 25.

to the decline in competitive index due to the restriction of population size, artificial selection for abdominal bristle number has been responsible for a significant decline in the index of approximately 28% in five generations, 57% in ten

generations, and 76% in twenty generations, averaging over the high and low lines. The most striking feature of the fitness determinations, however, is the contrast in behaviour between the high and low lines: the lines selected for low abdominal bristle number have shown a markedly greater decline in reproductive capacity than those selected for high bristle number, confirming the general observations of Clayton *et al.* (1957*c*). Estimates of male mating ability in the lines show a similar trend.

Table 8. *Competitive indices* (♂) *of abdominal bristle lines*

Line	Generation 5	Generation 10
AH1	1·36 ± 0·11	0·96 ± 0·07
AH2	1·25 ± 0·10	1·16 ± 0·08
AL1	0·98 ± 0·07	0·52 ± 0·04
AL2	1·06 ± 0·08	0·87 ± 0·06
Mean control	1·38 ± 0·08	1·17 ± 0·06

The general pattern of fitness changes in these lines has also been confirmed by measurements of the competitive index (♀). These are presented in Table 9, and the agreement with the other indices makes it quite certain that the technique which we have adopted accurately reflects changes in reproductive capacity in the experimental material.

Table 9. *Competitive indices* (♀) *of abdominal bristle lines*

Line	Generation 5	Generation 10
AH1	3·08 ± 0·43	1·34 ± 0·19
AH2	2·87 ± 0·42	2·10 ± 0·32
AL1	2·20 ± 0·29	0·88 ± 0·12
AL2	2·20 ± 0·27	1·50 ± 0·22
Mean control	3·60 ± 0·37	2·52 ± 0·30

One particularly interesting aspect of the work with these lines has been the demonstration that in the high line AH2, fitness has been affected little more than in the control lines, even after a shift in mean bristle score of some six phenotypic standard deviations over a twenty-generation period. It will be remembered that the response to selection in this line was strongly opposed by natural selection over the first three generations (Fig. 3), and the fact that the competitive ability of the line has not been much reduced by comparison with the controls suggests that the opposing factor had been lost rather than fixed at generation 4. The effect on bristle number of the chromosome segment concerned was obviously considerable, and it is unlikely that the factor should have been lost under selection unless the effects on fitness and on bristle number were in fact separable. The most plausible explanation of the observation is that selection for bristle number had caused an increase in the frequency of the undesirable factor

because of its linkage relationships with some of the loci responsible for the primary response in bristle number. The importance of linkage as a possible mechanism in bringing about correlated response of this sort has been stressed by Wigan & Mather (1942).

The behaviour of selected lines on relaxation of selection provides evidence relevant to the problem of the magnitude of the forces of natural selection maintaining equilibrium gene frequencies at loci concerned with the response to

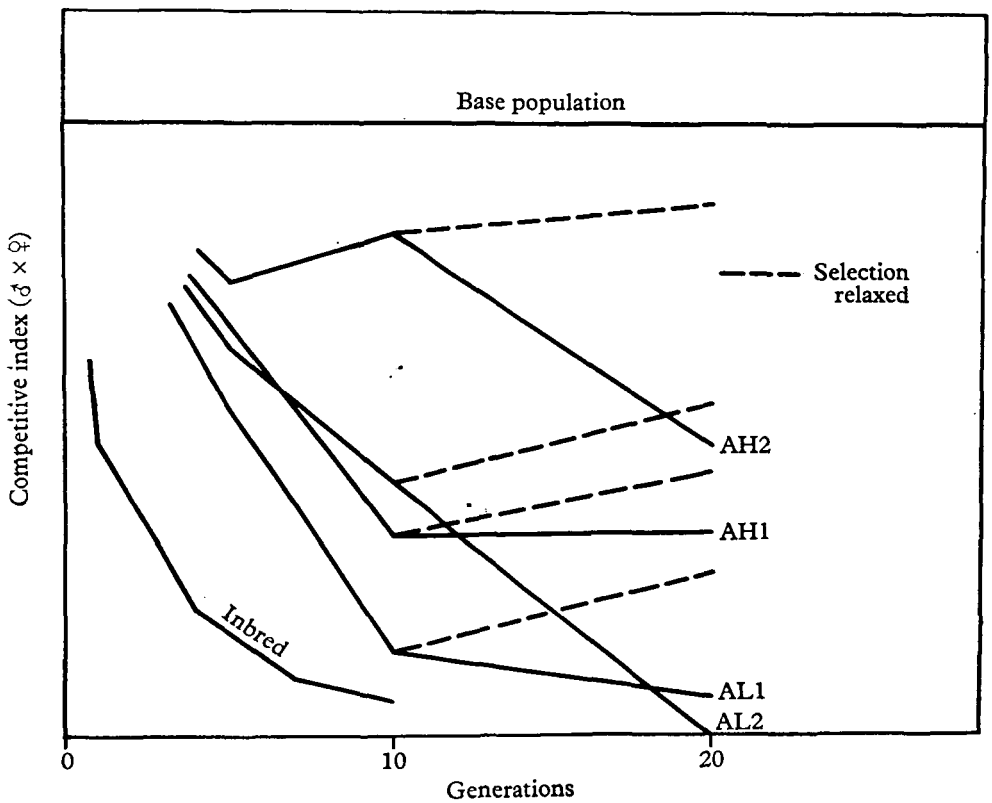

Fig. 4. The competitive ability (♂ × ♀) of the full-sib inbred lines and the abdominal bristle selection lines (A).

selection. In the early generations, it is to be expected that fixation will have been effected at few loci, and cessation of artificial selection followed by random mating under crowded conditions should allow the forces of natural selection gradually to move gene frequencies back to their equilibrium values. The mean of relaxed lines will therefore be expected to move towards the mean of the base population at a rate dependent on the proportion of loci at which fixation has occurred, and upon the magnitude and nature of the forces of natural selection involved at the unfixed loci. The earlier observations of Clayton *et al.* (1957*a*) have suggested that for many loci affecting abdominal bristle score, natural selection plays a minor role in maintaining gene frequencies at their equilibrium values in the base

population, and mutation pressures are therefore likely to be the controlling factors at such loci. After six generations of relaxation under crowded conditions, their high and low lines still retained 65% of the response obtained in five generations of selection, and showed little change after a further thirteen generations.

In the present study, the four selected lines were relaxed under crowded conditions at generations 3, 5, and 10, and the proportion of the response retained after a period of about ten generations of relaxation is given for each line in Table 10. The effects of relaxation in AH2 over the early generations of selection have previously been discussed.

The effects of relaxation at generation 5 are in reasonable agreement with the observations of the previous authors, though there is a suggestion that a slightly greater proportion of the response has been retained in our lines. This was to be

Table 10. *Relaxation of selection in abdominal bristle lines*

Line	Generations of selection	Response (bristles)	Generations of relaxation	Response lost (bristles)	Response retained (%)
AH1	3	6·8	11	2·5	63
AH2	3	4·3	11	2·5	41
AL1	3	5·5	11	0·0	99
AL2	3	5·4	11	2·6	52
AH1	5	9·5	9	2·7	71
AH2	5	6·5	9	1·4	77
AL1	5	9·2	9	2·5	73
AL2	5	8·3	9	2·8	66
AH1	10	13·4	10	1·7	87
AH2	10	15·9	10	1·5	90
AL1	10	12·5	10	1·3	90
AL2	10	11·7	10	0·9	93

expected in view of the fact that the rate of inbreeding was twice that of the earlier lines, and no doubt some fixation had occurred by the fifth generation of selection. Comparison of the results of relaxation at generations 5 and 10 suggests that, by generation 10 at least, genetic fixation had become an important factor in limiting the extent to which the mean could change under natural selection.

Further relevant information has been provided by measurements of competitive ability after ten generations of relaxation of the four selected lines and two control lines relaxed at generation 10 (Table 11). Over the ten generations of relaxation, the control line C2 showed little change, but C1 declined in competitive ability by approximately 25% (non-significant). All selected lines increased in mean fitness, but AL1 was the only one to show a marked and significant change. The competitive index increased by 100% over the period, and in view of the fact that the mean bristle score of this line changed at the same time by only 1·3 bristles, it seems worth while to consider possible explanations of the changes in this line.

The four abdominal bristle lines were tested at the tenth generation of selection for the presence of lethal factors on the second and third chromosomes, using the

standard balanced stock *Cy/Pm*; *Me/H*. Only the third chromosome of AL1 gave evidence of a high frequency of lethal chromosomes (6/19), and cross tests involving five of the six extracted lethal chromosomes showed the lethal factors involved to be identical. Of a further sample of thirty-one third chromosomes taken from AL1, thirteen were found to carry the same lethal factor. The frequency of the lethal gene or chromosome segment in the selected line can therefore be estimated to have been $0{\cdot}38 \pm 0{\cdot}07$. Selection for extreme abdominal bristle number evidently favoured the lethal factor in the heterozygous state, increasing its frequency to somewhere near the theoretical maximum of 33% in ten generations. Similar occurrences were common in the selected lines of Clayton *et al.* (1957*b*).

Assuming the lethal to have been at a frequency of 33% in AL1 at generation 10, we should expect its frequency to have been reduced by natural selection during ten generations of relaxation to approximately 8%. We have to reconcile the observations that the effect of the factor in the heterozygote has been sufficiently great in the direction of selection for the frequency to have been increased

Table 11. *Changes in fitness under relaxation of selection*

	Competitive indices (♂ × ♀)	
Line	Generation 10	Relaxed 10 generations
AH1	$1{\cdot}32 \pm 0{\cdot}14$	$1{\cdot}69 \pm 0{\cdot}19$
AH2	$3{\cdot}22 \pm 0{\cdot}47$	$3{\cdot}40 \pm 0{\cdot}51$
AL1	$0{\cdot}54 \pm 0{\cdot}06$	$1{\cdot}05 \pm 0{\cdot}11$
AL2	$1{\cdot}63 \pm 0{\cdot}18$	$2{\cdot}14 \pm 0{\cdot}26$
C1	$3{\cdot}08 \pm 0{\cdot}44$	$2{\cdot}33 \pm 0{\cdot}29$
C2	$2{\cdot}92 \pm 0{\cdot}41$	$2{\cdot}79 \pm 0{\cdot}38$

under selection to 33%, and that during the partial elimination of the factor under relaxation, the mean score of the line returned by only 1·3 bristles. If we denote the average difference in bristle score of the non-lethal homozygote and the heterozygote by α, it is possible to calculate from tables of the normal distribution the theoretical equilibrium frequency of the lethal factor under selection of constant intensity (20%), as a function of α/σ, where σ is the phenotypic standard deviation in the character concerned. The relationship is presented graphically in Fig. 4 for the case in which the non-lethal homozygote and the heterozygote do not differ in average reproductive fitness.

The phenotypic standard deviation in the base population was of the order of 3·5 bristles, and by generation 10 had fallen in AL1 to roughly 2·5. It is quite likely in the light of previous work (Clayton *et al.*, 1957*b*; Robertson & Reeve, 1952) that the value of α has been progressively magnified under selection, and it is impossible to estimate its original value in the base population. By generation 10, however, we can infer from Fig. 4 that the value of α must have been something in excess of 2 bristles. If the heterozygote were less fit than the non-lethal homozygote, the minimum value of α would be somewhat greater, and the rate of

elimination of the lethal greater than was supposed in arriving at the estimated frequency of 8% after ten generations of relaxation.

On the basis of the previous assumptions, the change in mean bristle score due to the lethal gene or chromosome segment alone would be expected to be approximately $\frac{1}{2}\alpha$ after ten generations of relaxation, and we must conclude that the change of 1·3 bristles on relaxation of AL1 has been predominantly due to the lethal factor we are considering. This discussion has presupposed that the bristle effects and fitness effects of the chromosome segment have remained associated throughout the period of relaxation.

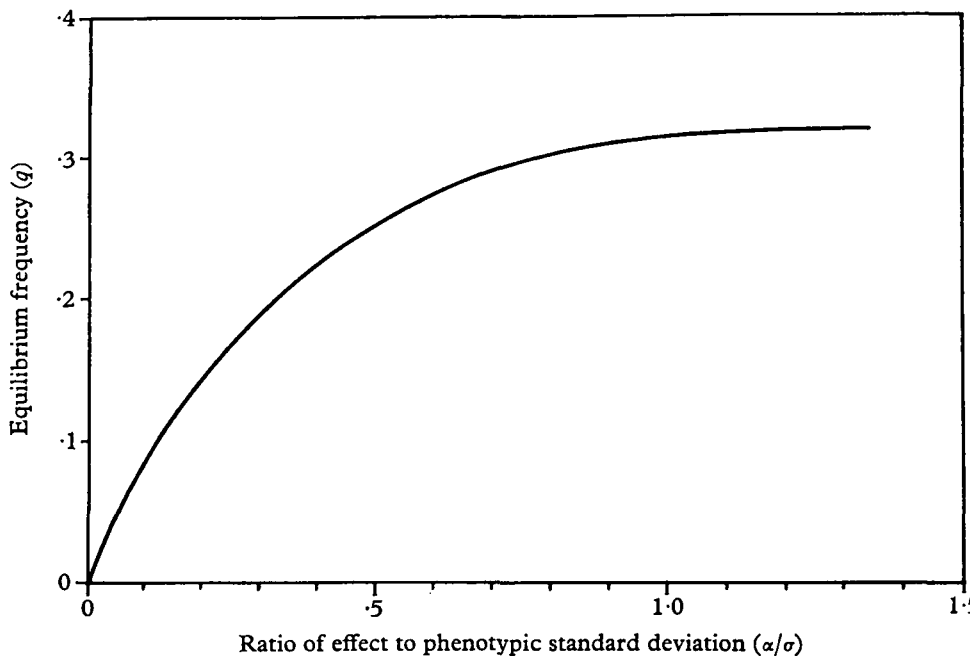

Fig. 5. Equilibrium frequency of a lethal factor favoured by artificial selection in the heterozygous state.

As regards the effect of the lethal on the competitive index, it must be remembered that the competition technique involves one generation of relaxation, and the expected reduction in fitness due to the lethality alone is at most 6%. If the heterozygote and non-lethal homozygote are of the same average reproductive fitness, elimination of the lethal factor can therefore at most account for a 6% increase in the competitive index. A considerable proportion of the 100% rise in fitness on relaxation of selection seems therefore to have been due to unfixed loci which have contributed little to the primary response to selection in bristle number. The most likely explanation is that linkage has played an important part in bringing about the sharp decline in fitness with selection in this line.

Selection for sternopleural bristle number

Starting once again from the Kaduna base population, selection for both high and low sternopleural bristle number at an intensity of 40% (10/25 in each sex) has been carried out with two replicate lines in each direction of selection. The details of the pattern of response are to be discussed elsewhere, and it is only necessary for our purpose briefly to describe the main features.

The mean bristle score of the base population was of the order of 17·5 (all bristles on both sides of the animal having been counted), and the phenotypic standard deviation approximately 1·9 bristles. The response to selection was markedly asymmetrical, the high lines changing in mean by a little over five phenotypic standard deviations, and the low lines by less than three standard deviations by generation 15. Over the following ten generations of selection, the high lines showed little change and the low lines responded by perhaps one bristle. The changes in the competitive index over the same periods are summarized in Table 12.

Table 12. *Competitive indices of sternopleural bristle lines*

	Competitive index (♂ × ♀)		C.I. (♂)
Line	Generation 15	Generation 25	Generation 15
SH1	2·53 ± 0·33	2·00 ± 0·24	1·20 ± 0·08
SH2	2·52 ± 0·33	2·21 ± 0·27	1·18 ± 0·09
SL1	1·32 ± 0·15	1·17 ± 0·13	0·93 ± 0·06
SL2	1·50 ± 0·17	1·02 ± 0·11	1·00 ± 0·07
Mean control	2·34 ± 0·21	2·39 ± 0·24	1·10 ± 0·05

By generation 15, the high lines SH1, SH2 had shown a 35% drop in competitive index from the cage level of 3·93 ± 0·25. The unselected controls showed a similar change over the same period, so that the decline in fitness in the high selected lines can be wholly attributed to the effects of restricted population size. A slight further reduction in fitness occurred over the following ten generations of selection, the high lines at generation 25 being 12% less fit than the controls (non-significant). In view of the fact that these lines had been selected to a clearly defined plateau, this result is of great significance for our understanding of the forces of natural selection operative in the base population at the loci concerned with the response to selection.

By contrast, the low lines SL1, SL2 were reduced in competitive ability to 50% of the control level by generation 25; the decline in fitness in both low lines has been closely related to the response in bristle score, having occurred predominantly over the first fifteen generations of selection.

The change in bristle score on relaxation of selection under crowded conditions has also been observed at various stages in the selection programme. The high lines have shown a return to the unselected mean of approximately 14% of the response when relaxed at generation 5 for a period of twenty-five generations;

relaxation of the high lines at later stages in the selection programme has given no evidence of a tendency to return to the unselected level. The low lines, on the other hand, when relaxed at generation 5 showed a return to the base population mean of approximately 50% of the response previously obtained, and consistently

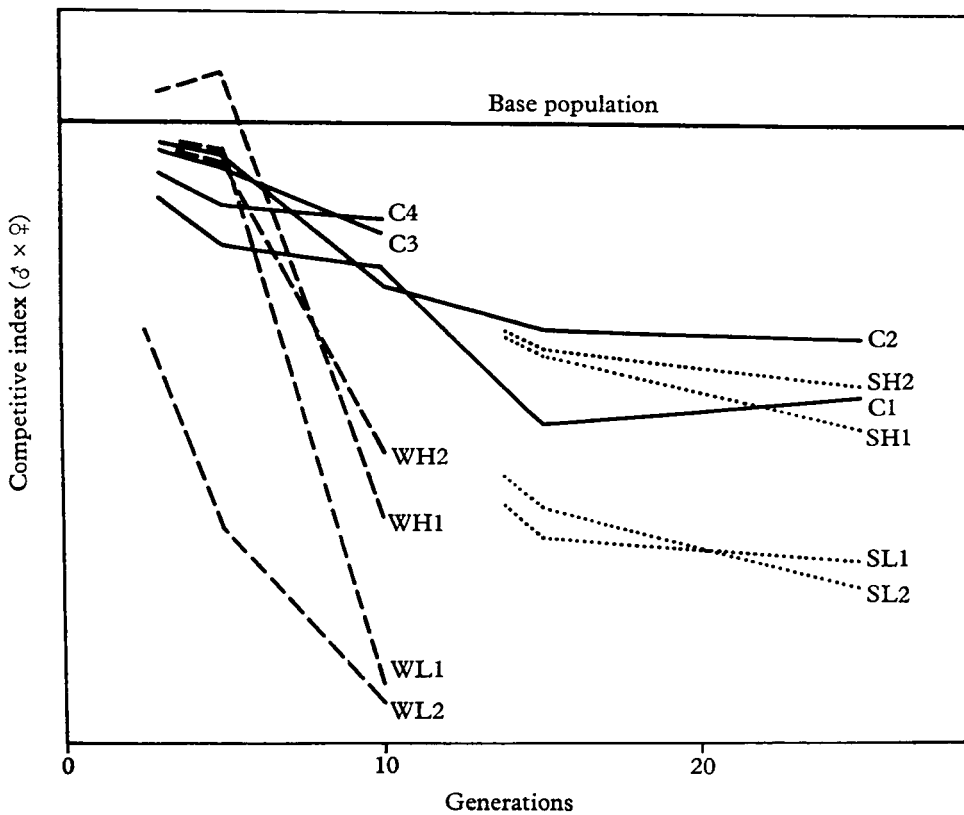

Fig. 6. The competitive ability (♂ × ♀) of the control lines (C) and the wing length (W) and sternopleural bristle (S) selection lines. The C lines at generation 20 have the same inbreeding coefficient as the full-sib inbred lines (Fig. 4) at generation 4.

showed a tendency to move in the same direction when relaxed at generations 10, 20, and 25. These observations tie in well with the changes in competitive index which have been measured, and suggest that the loci responsible for the response in the primary character have also governed the fitness changes in the lines selected for low bristle score.

Selection for wing length

The two bristle characters with which we have so far been concerned show predominantly additive genetic variation in the base population, and no change in mean on inbreeding. According to the views put forward by Robertson (1955), they must therefore be placed at one extreme of the spectrum of quantitative

characters running from traits of trivial importance to the organism to those directly related to reproductive fitness. Body size, on the other hand, shows evidence of inbreeding depression, and the mean of crosses between inbred lines always exceeds the mid-parental value (Robertson & Reeve, 1955). The genetic variance in body size in wild stocks, however, is largely additive in character (Robertson, 1957), and the extent of depression in the mean on complete inbreeding is only of the order of 5%. For our present purposes, therefore, body size provides a contrast to the two bristle characters whose behaviour has been described, though perhaps not so sharp a contrast as we might wish for.

Wing length has been used as the measure of body size, the technique of measurement described by Robertson & Reeve (1952) having been employed. Twenty-five flies of each sex from a single emergence were scored each generation from each of two half-pint culture bottles, and the extreme five individuals in each set of twenty-five selected to be parents of the following generation. The ten pairs of selected parents were placed in a fresh culture bottle to lay for 1–2 days, and were then transferred to a second bottle for a similar period; these two cultures provided the flies to be scored for selection in the next generation. The ten female parents were then separated from the males, and placed in a third culture bottle with gravid WK females to lay for 1 day. The mean performance of the following generation was determined by measuring ten to twenty flies of each sex of the emerging white-eyed and wild-type adults, the difference in mean between the two types providing a measure of response which was unaffected by fluctuations from generation to generation in environmental conditions.

Two long-wing (WH1, WH2) and two short-wing lines (WL1, WL2) were selected for ten generations. The lines WH1 and WL1 were contemporaneous, as were WH2 and WL2, the two pairs being separated in time by two generations. The response is illustrated in Fig. 5 in terms of the uncontrolled means: over the first five generations of selection the realized heritability (calculated from the controlled means) averaged 42% in the high lines and 48% in the low lines. The ratio of male to female means remained constant in all lines over the ten-generation period. In the high lines, the coefficient of variation dropped by 20–25%, the decline being the more rapid in WH2 which made the greater progress under selection. The two low lines differed considerably in the change in variability displayed, WL1 declining in coefficient of variation by approximately 15% while WL2 increased somewhat over the period. The increase in variance in WL2 was partly due to the appearance in the later generations of a small number of individuals in both sexes with considerably shorter wings than their fellows.

Measurements of competitive ability were made on the four lines at generations 5 and 10, and the results are summarized in Table 13. The two high lines and WL1 showed no evidence of a decline in fitness over the first five generations of selection, but were quite dramatically affected by generation 10. At this point the long-wing lines were approximately 50% as fit as the control lines, and the short-wing lines only 10% as fit as the controls. Both low lines were also found to be significantly poorer than the controls in male mating ability by generation 10,

the males effectively inseminating on the average only about half as many females under competition as the control males. Clearly the most striking feature of the results, as with both bristle characters studied, is the contrast in fitness between the high and low lines.

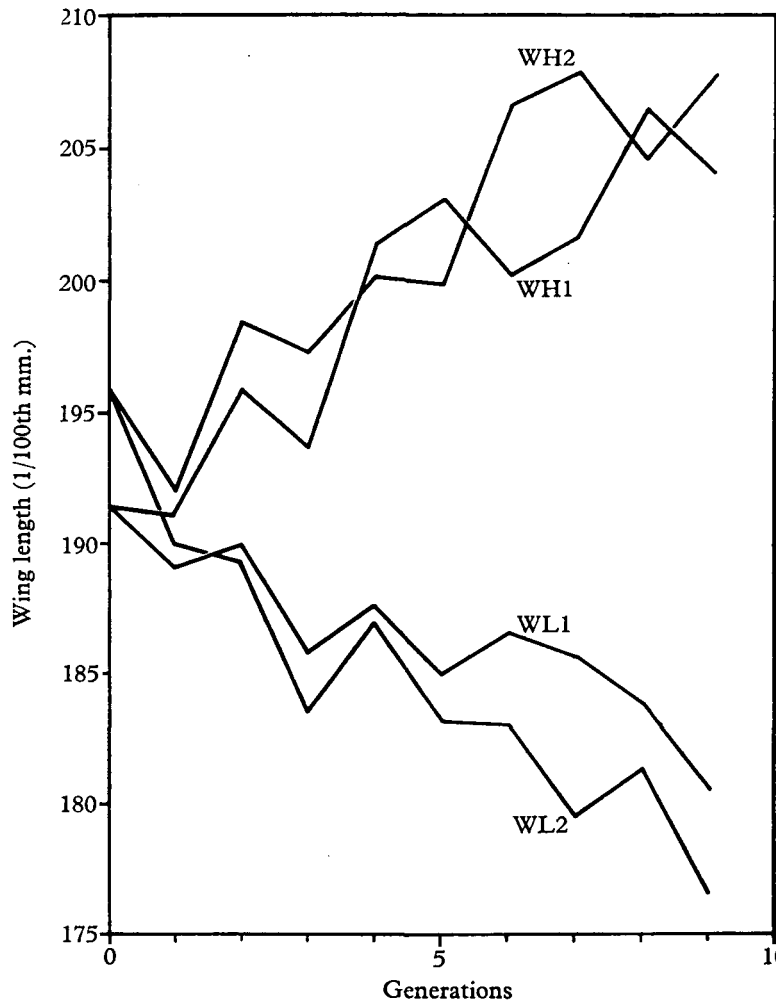

Fig. 7. Response to selection for wing length. (Uncontrolled means.)

In WL2, the egg-laying ability of selected females in the line was noticeably reduced in the later generations. At generation 10, thirty-five WL2 females were mated to vigorous males of the WK stock in individual vials and allowed to lay over a period of 10 days. Of the thirty-five females, twenty gave fewer than ten offspring, sixteen of the cultures failing altogether: the remaining fifteen females gave rise to vigorous cultures. Of twenty-seven WL2 males mated to individual WK females, twenty-one produced vigorous cultures, so that male fertility was apparently not greatly reduced in the line.

The discontinuous distribution of progeny number among the tested WL2 females suggests that at least one female sterile gene was segregating in the line: if a single gene only were involved, its frequency by generation 10 must have been increased under selection to something of the order of 70%, and such a gene would be expected to reduce the C.I. (♂×♀) by approximately 30%.

Table 13. *Competitive indices of lines selected for wing length*

	Competitive index (♂×♀)		C.I. (♂)
Line	Generation 5	Generation 10	Generation 10
WH1	4·23 ± 0·49	1·43 ± 0·16	1·26 ± 0·11
WH2	3·71 ± 0·41	1·87 ± 0·23	0·91 ± 0·08
WL1	3·76 ± 0·43	0·39 ± 0·05	0·63 ± 0·06
WL2	1·37 ± 0·15	0·28 ± 0·05	0·62 ± 0·06
Mean control	3·50 ± 0·20	3·14 ± 0·24	1·17 ± 0·06

The low fitness of the lines selected for short wings by comparison with those selected for long wings is not an unexpected result. The work of Robertson & Reeve (F. W. Robertson, 1955) on body size in a number of different stocks has previously suggested that viability is more affected in the small lines than in the large, though the present results show the difference to be very much more marked when all components of fitness are taken into account.

DISCUSSION

Decline in fitness with inbreeding

Our observations of the effect of continued full-sib mating on the competitive index have led us to the conclusion that the observed depression in fitness can be accounted for primarily by the average dominance properties of the alternative genotypes at individual loci, the effects of different loci being to a large extent independent and therefore multiplicative in combination. We therefore find little need to invoke interactions between loci as an explanation of fitness reduction over a range of F-values from zero to almost 90%.

This is in some conflict with previous observations of the effect of inbreeding in the same species. Tantawy (1957), for instance, plotting percentage emergence in the Crianlarich stock against inbreeding coefficient up to values of 75%, found a greater rate of decline at higher values of inbreeding, and wing length and thorax length tended to show the same pattern of behaviour. Tantawy & Reeve (1956), in a similar study on the Nettlebed stock, found that survival from egg to adult was much less affected than in the Crianlarich stock up to an F-value of 75% but thereafter showed a sharp decline. In this stock wing length showed a linear decline over a range of F from zero to 85%, although less intense systems of inbreeding gave a different picture.

In *D. subobscura*, Maynard Smith *et al.* (1955), studying the effect of full-sib mating on the mean number of adult progeny produced per day per pair under uncrowded conditions, found results rather similar to ours, their measure of fitness declining by 50% after two generations. The observations made by Robertson & Reeve (1955) of the effects on body size and egg production of chromosome substitutions between pairs of different lines are also directly relevant to this problem. They demonstrated widespread interaction between the effects of substituting non-homologous chromosomes on wing and thorax length and also on egg production. The effects of substitution of non-homologous chromosomes were found to be most marked in otherwise homozygous backgrounds and were much less important when the background genotype was already partially heterozygous. This is in good agreement with the inbreeding results of Tantawy and of Tantawy & Reeve mentioned above, in that the effect of inbreeding on fitness would be expected to increase at higher levels of inbreeding.

Similar observations on other species are not abundant, but those available all suggest that characters related to reproductive fitness are affected to a considerable degree by inbreeding. Duzgunes, working with poultry, found that reproductive fitness as measured by the number of chicks per hen which survived to 9 months of age declined by approximately 50% following inbreeding to an F-value of 25%. Dickerson *et al.* (1954) have reported on the decline of litter size and litter weight in pigs under mild inbreeding up to an inbreeding coefficient of 50% and conclude that the data for each character suggest 'no systematic departure from the linear decline'.

In maize the considerable body of data on inbreeding depression in yield under selfing provides little evidence for a departure from linearity of the decline of yield when plotted against inbreeding coefficient. However, Sentz *et al.* (1954) have studied the relationship between heterozygosis and yield in maize in crosses and back-crosses involving two inbred lines from each of two populations and have found good evidence of a non-linear relationship. They observed a marked decline in yield as the level of heterozygosity dropped from 100% to 75%, something of a plateau between 75% and 25%, and a further marked reduction in yield as the heterozygosity dropped from 25% to zero.

The divergences between the different sets of results is probably to be related to the nature of the populations under study. In our case the base population has been maintained over more than 100 generations with a population size of about 5,000 individuals, whereas the stocks used by Tantawy & Reeve were maintained in bottles under optimum conditions with perhaps 100 pairs of breeding individuals each generation. It seems likely that our cage population would carry a greater load of deleterious recessives than would the smaller bottle populations and that under inbreeding these major genes would dominate the situation. Similarly, the inbred lines used in the chromosome substitution study by Robertson & Reeve were derived from a number of different wild stocks and most deleterious genes of major effect may well have been eliminated during the long-continued process of inbreeding.

The behaviour of the control populations when compared to full-sib lines of equivalent inbreeding coefficient does, in fact, illustrate the effect of population size on the nature of the genetic variation present in the population. The maintenance of fitness in the control lines is, however, not necessarily an argument for overdominance. The effect could be due just as well to the elimination of deleterious recessives during inbreeding, which is much more likely in a large population than in a small one. The discrepancy between our results on the effect of inbreeding and those of other workers is then perhaps not so surprising as it seemed at first sight.

Decline in fitness with selection

The effect of continued selection for a quantitative character on reproductive ability may be the result of two types of change in gene frequency: directional changes due to the selection pressure imposed, and random changes due to the restriction of the breeding population in each generation. These two processes are not necessarily independent of one another in that the gene frequency at a locus close to one affecting the quantitative character may well show a directional change in gene frequency in consequence of a random departure from linkage equilibrium brought about by selection in a small population.

The effect of random changes in gene frequency on fitness will be dependent on the effective number of breeding individuals in the population. This will in turn depend on the variation in number of progeny contributed by each parent to the succeeding set of selected breeding individuals. In a *control* line maintained by the random mating of ten pairs of randomly chosen individuals in each generation the effective number of parents is likely to be approximately 4·8 males and 7·1 females (Crow, 1954), leading to an expected rate of loss of heterozygosity of 4·4% per generation. In a *selected* line, however, the rate of inbreeding will be greater than this, in that the variation between families in the character under selection will lead to a variation in the chance of selection of family members as parents of the next generation (Robertson, 1961). Under our conditions the rate of inbreeding would be increased over that in a random control of the same size by about 20% in the abdominal bristle and wing length lines and by about 10% in the sternopleural lines. Differences between selected and control populations will therefore be to some extent accounted for by this difference in rate of inbreeding.

Our results show quite clearly that random changes in gene frequency have played an important part in the decline in fitness on selection. In three of the selected lines, AH2, SH1, and SH2, the observed fitness changes can be accounted for entirely as a consequence of the inbreeding. In some of the other lines, notably AL1 carrying a lethal, and WL2 carrying a female sterile, the decline in effective population size may well have been much greater than in the other lines. For instance, in WL2 the female sterile would cause a high proportion of the selected females to contribute no offspring at all to the succeeding generation.

The importance of linkage as a factor in correlated response to artificial selection has been stressed particularly by Mather in many publications since 1942. Fertility changes on selection were observed in a number of his experiments and linkage was

proposed as the only causal agency involved (Wigan & Mather, 1942; Mather & Harrison, 1949). In our work linkage has been suggested as the most likely explanation of two observations, namely, the slow response to selection in the early generations of line AH2 and the marked decline in fitness of AL1. To what extent linkage has contributed to the decline in fitness in other lines we have no way of telling; however, all the evidence from this and previous investigations points to the fact that fitness has not been affected to the same extent in the lines selected in opposite directions, the low lines in all three characters having been reduced in fitness more than the high lines. Linkage alone cannot account for this, and there can be little doubt that some loci have effects both on the particular character under selection and on reproductive fitness.

The interpretation of these results is made somewhat uncertain by the known segregation of inversions in the base population. These had been declining in frequency up to the time that these present experiments were performed and have now vanished from the population. Their relevance to these present results was unfortunately not realized at the time and some lines were discarded before they could be examined cytologically. We have, however, since examined the A, S and W series of lines. The lines SH1 and SH2 were both segregating for the third chromosome inversion K, but all other lines were homozygous for the standard order. From other information on the state of the base Kaduna population, we believe that this does not constitute a major source of error in interpretation.

From the observed decline in reproductive fitness with artificial selection we would like to make some inferences regarding the magnitude of the forces of natural selection operating on the genetic variation we find in the base population because of linkage and of the enhanced rate of inbreeding under artificial selection. The data do not enable us to estimate the intensity of natural selection for the genes affecting a given metric character with certainty, but it is none the less instructive to make comparisons between the results of selection for different quantitative characters.

It was pointed out earlier that abdominal bristle number and body size in *D. melanogaster* represent distinct classes of metric characters: in particular, body size shows evidence of inbreeding depression and heterosis whereas abdominal bristle number approximates more closely to the ideal additive genetic character. How do these two characters compare in their decline in reproductive fitness under artificial selection? Perhaps the most useful statistic to summarize this relationship is the ratio $-\log_e \bar{w}/g^2$, where $\bar{w}$ represents the mean fitness of the selected lines relative to that of the controls, following a shift in the mean of the character under selection by g additive genetic standard deviations. Haldane (1954) has defined the intensity of natural selection for a metric character as the natural logarithm of the ratio of the fitness of the mean phenotype to that of the whole population, and if we accept this as appropriate the merit of the above ratio lies in the fact that it is simply related to Haldane's intensity of natural selection on the basis of at least two simple models of the action of natural selection on metric characters. This has been discussed in more detail by Latter (1960).

In Table 14 we give the observed values of this ratio for abdominal bristle number and wing length, estimated after both five and ten generations of artificial selection of intensity 20% in opposite directions. This is based on the average decline in fitness of the four selected lines. In abdominal bristles, for instance, the decline in fitness relative to the controls is by about 30% at the fifth generation of selection when the response to selection had been of the order of four additive genetic standard deviations. If the variation in abdominal bristles is held in the population by natural selection for the heterozygote, then this figure corresponds to heterozygote superiority at the loci affecting this character of the order of 0·5%.

The behaviour of the lines selected for abdominal bristle number is in accord with the simple theory which suggests that the decline in fitness is proportional to the square of the advance under artificial selection. The results of selection for body size do not appear to conform to such a simple pattern, though the enhanced rate of inbreeding in the later stages of selection may partly account for this. However, comparing the average decline in fitness shown by the two characters

Table 14. *Decline in fitness as a function of advance under selection*

	Value of $(-\log_e \bar{w})/g^2$	
Metric character	Generation 5	Generation 10
Abdominal bristles	$0{\cdot}022 \pm 0{\cdot}005$*	$0{\cdot}021 \pm 0{\cdot}002$
Body size	$0{\cdot}011 \pm 0{\cdot}003$	$0{\cdot}033 \pm 0{\cdot}002$

* Standard errors due to variation between replicate determinations of competitive ability.

under selection we must conclude that from the present limited data there is no great contrast in their behaviour. This is perhaps not unreasonable if one is prepared to accept that the degree of genetic complexity displayed by a quantitative character is the result of natural selection on the sub-set of loci contributing to its genetic variance. We are accustomed to thinking of characters which show no inbreeding depression as 'additive characters'; but in fact the available estimates indicate that the genetic variance of abdominal bristle number may be approximately 15% non-additive in nature (Clayton *et al.*, 1957*a*), while that of body size is roughly 12% non-additive (F. W. Robertson, 1957). The type of non-additive genetic variation is admittedly different in the two characters, directional dominance being the rule for body size, but we have really no grounds for considering abdominal bristle number to represent a simpler genetical system than does body size. The implications of this point of view have been further explored by Latter (1961).

SUMMARY

The competitive-index method of measurement of overall fitness in *Drosophila* has been used to measure the effect of inbreeding and of artificial selection for metric characters in a large population of *Drosophila melanogaster*. The technique

itself was examined in detail with particular reference to its repeatability and to the effect on it of the modification of various environmental variables.

With continued full-sib mating the decline in the competitive index was very rapid (it was reduced to a half by a single generation of full-sib mating) and there were no indications that interactions between deleterious genes at different loci were important in determining the rate of decline of fitness as inbreeding increased. Other unselected lines with ten pairs of parents in each generation were carried to serve as a control for the lines under artificial selection. At the same theoretical degree of inbreeding the control lines had a much higher average fitness than the lines produced by continued full-sib mating.

From the base population lines were selected in both directions for abdominal bristles, sternopleural bristles and for wing length, there being two replicates in all cases. Four control lines were kept with the same number of parents as the selected lines. In all cases the selected lines declined in fitness below the value for the base population. However, in three of the lines the fitness was not significantly below the value for the control lines. The effect of artificial selection on fitness was asymmetrical, the decline being greater with down selection for all characters.

The relevance of these results to various theoretical models is discussed. If the variation in these characters is actively maintained in the base population by the selection of heterozygotes then the results are consistent with an average selection disadvantage of homozygotes relative to heterozygotes of about 0·5%.

REFERENCES

Bell, A. E., Moore, C. H. & Warren, D. C. (1955). The evaluation of new methods for the improvement of quantitative characteristics. *Cold Spr. Harb. Symp. quant. Biol.* **20**, 197–212.

Clayton, G. A., Morris, J. A. & Robertson, A. (1957*a*). An experimental check on quantitative genetical theory. I. Short-term responses to selection. *J. Genet.* **55**, 131–151.

Clayton, G. A. & Robertson, A. (1957*b*). An experimental check on quantitative genetical theory. II. The long-term effects of selection. *J. Genet.* **55**, 152–170.

Clayton, G. A., Knight, G. R., Morris, J. A. & Robertson, A. (1957*c*). An experimental check on quantitative genetical theory. III. Correlated responses. *J. Genet.* **55**, 171–180.

Crow, J. F. (1948). Alternative hypotheses of hybrid vigour. *Genetics*, **33**, 477–487.

Crow, J. F. (1954). Breeding structure of populations. II. Effective population number. *Statistics and Mathematics in Biology*. Iowa State College Press.

Dickerson, G. E., Blum, C. T., Chapman, A. B., Kottman, R. M., Krider, J. L., Warwick, E. J. & Whatley, J. A., Jr. (1954). Evaluation of selection in developing inbred lines of swine. *Univ. Mo. Res. Bull.* 551.

Falconer, D. S. (1954). Validity of the theory of genetic correlation. *J. Hered.* **45**, 42–44.

Gowen, J. W. & Johnson, L. E. (1946). On the mechanism of heterosis. I. Metabolic capacity of different races of *Drosophila melanogaster* for egg production. *Amer. Nat.* **80**, 149–179.

Haldane, J. B. S. (1954). The measurement of natural selection. *Proc. IXth Int. Congr. Genet.* **1**, 480–487.

Hull, F. H. (1952). Recurrent selection and overdominance. In *Heterosis* (J. W. Gowen, ed.). Iowa State College Press.

Kempthorne, O. (1957). *An Introduction to Genetic Statistics*. New York: Wiley and Sons.

Knight, G. R. & Robertson, A. (1957). Fitness as a measurable character in *Drosophila*. *Genetics*, **42**, 524–530.

KYLE, W. H. & CHAPMAN, A. B. (1953). Experimental check of the effectiveness of selection for a quantitative character. *Genetics*, **38**, 421–443.

LATTER, B. D. H. (1960). Natural selection for an intermediate optimum. *Aust. J. biol. Sci.* **13**, 30–35.

LATTER, B. D. H. (1961). Changes in reproductive fitness under artificial selection. *Proc. roy. Soc. Vict. Centenary Symposium* (in press).

LERNER, I. M. (1954). *Genetic Homeostasis*. Edinburgh: Oliver and Boyd.

LERNER, I. M. (1958). *The Genetic Basis of Selection*. New York: Wiley and Sons.

LEWONTIN, R. C. (1955). The effects of population density and composition on viability in *Drosophila melanogaster*. *Evolution*, **9**, 27–41.

MATHER, K. & HARRISON, B. J. (1949). The manifold effect of selection. *Heredity*, **3**, 1–52, 131–162.

MAYNARD SMITH, J., CLARKE, J. M. & HOLLINGSWORTH, M. J. (1955). The expression of hybrid vigour in *Drosophila subobscura*. *Proc. roy. Soc.* B, **144**, 159–171.

REEVE, E. C. R. & ROBERTSON, F. W. (1953). Studies in quantitative inheritance. II. Analysis of a strain of *Drosophila melanogaster* selected for long wings. *J. Genet.* **51**, 276–316.

ROBERTSON, A. (1952). The effect of inbreeding on the variation due to recessive genes. *Genetics*, **37**, 189–207.

ROBERTSON, A. (1960). A theory of limits in artificial selection. *Proc. roy. Soc.* B, **153**, 234–249.

ROBERTSON, A. (1961). Inbreeding in artificial selection programmes. *Genet. Res.* **2**, 189–194.

ROBERTSON, F. W. (1955). Selection response and the properties of genetic variation. *Cold Spr. Harb. Symp. quant. Biol.* **20**, 166–177.

ROBERTSON, F. W. (1957). Studies in quantitative inheritance. XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. *J. Genet.* **55**, 428–443.

ROBERTSON, F. W. & REEVE, E. C. R. (1952). Studies in quantitative inheritance. I. The effects of selection on wing and thorax length in *Drosophila melanogaster*. *J. Genet.* **50**, 414–448.

ROBERTSON, F. W. & REEVE, E. C. R. (1955). Studies in quantitative inheritance. VIII. Further analysis of heterosis in crosses between inbred lines of *Drosophila melanogaster*. *Z. indukt. Abstamm.- u. VererbLehre*, **86**, 439–458.

SENTZ, J. C., ROBINSON, H. F. & COMSTOCK, R. E. (1954). Relation between heterozygosis and performance in maize. *Agron. Hourn.* **46**, 514–520.

TANTAWY, A. O. (1957). Genetic variance of random-inbred lines of *Drosophila melanogaster* in relation to coefficients of inbreeding. *Genetics*, **42**, 121–136.

TANTAWY, A. O. & REEVE, E. C. R. (1956). Studies in quantitative inheritance. IX. The effects of inbreeding at different rates in *Drosophila melanogaster*. *Z. indukt. Abstamm.- u. VererbLehre*, **87**, 648–667.

WIGAN, L. G. & MATHER, K. (1942). Correlated response to the selection of polygenic characters. *Ann. Eugen.* **11**, 354–364.