VARIATION FOR METRICAL CHARACTERS IN DROSOPHILA POPULATIONS

II. NATURAL SELECTION

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1. INTRODUCTION

POPULATIONS of *Drosophila melanogaster* contain extensive genetic variation for sternopleural chaeta number (Rasmuson, 1955; Robertson, 1966). However, little is known of the mechanisms involved in maintaining this variation and indeed, there is some controversy as to whether chaeta number is subject to natural selection (Robertson, 1955).

In a laboratory population derived from two inbred lines, Barnes (1968a) showed that natural selection had a differential effect on chaeta number. Selection was towards equilibrium values of 23 chaetæ at 25° C. and 21 chaetæ at 18° C. In each environment maximum fitness was associated with phenotypes around the population mean and changes away from this value were accompanied by a marked reduction in fitness. However, the two lines used in this study (Oregon and Samarkand) have widely different origins, and hence the population produced was artificial. Therefore the selective effects which were demonstrated may have little relevance to natural populations.

The present paper is concerned with a preliminary attempt to investigate the relationship between chaeta number and fitness among genotypes extracted from a single wild population. The origin of the population used (Texas) was described in an earlier paper (Barnes and Kearsey, 1970). In order that the fitness of different phenotypes might be assessed accurately, it was considered necessary to examine a wide range of genotypes. For this reason a population derived from the F_2 between high and low selection lines was used in preference to the base population. The phenotypic variances of these populations are approximately 42 and 3 respectively. It will be shown in the discussion that the results obtained are, in fact, relevant to the base population.

2. MATERIALS AND METHODS

The cage population used in this study was derived from an F_2 cross of two lines of *Drosophila melanogaster*, originating from the " Texas " population, which had been selected for high and for low sternopleural chaeta number. The " Texas " population was initiated from a sample of inseminated females captured in October 1965 at Austin, Texas, U.S.A. The details of the method of maintaining the cage populations and the establishment of the selection lines may be found in an earlier paper (Barnes and Kearsey, 1970).

Progeny were raised from eggs sampled from the cage at essentially two different densities. In the initial samples used to estimate the mean and

variance of the population no attempt was made to control egg densities rigorously, except that the laying period was limited to prevent obvious overcrowding. In subsequent experiments, once it became apparent that density dependent factors were operating, densities of 40 per tube or 100 per bottle were used. These will be referred to as the low density samples.

3. RESULTS

The first egg sample was collected one month after the cage was set up, and subsequently at approximately monthly intervals. There was no consistent change in either the mean or the variance over the first six months, but such changes may have been obscured by fluctuations in the density of the progeny raised. However, over this period the average estimates of the mean and variance for females were 21·33 and 35·17 respectively.
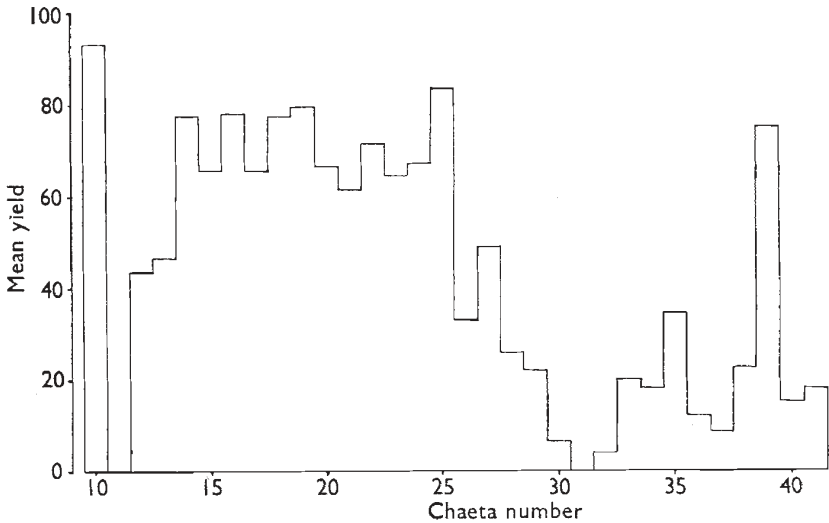

FIG. 1.—Average number of progeny produced by females of different chaeta numbers.

In view of the apparent absence of change in the population an attempt was made to demonstrate whether different phenotypes differed in fitness. Barnes (1968a) has shown that differences in reproductive capacity were responsible for the change in mean hair count in populations derived from two inbred lines. Reproductive capacity of females was used first as a measure of fitness.

A sample of eggs was collected from the cage and the female progeny separated into the different chaeta number phenotypes. Females from each class were placed individually in tubes with several males and allowed to lay, a maximum of 25 females in any one class being tested. The mean yield of each phenotype, averaged over both fertile and infertile females, is shown in fig. 1. The number of progeny produced is fairly consistent over much of the phenotypic range and is reduced markedly only for the high phenotypes. In addition, the percentage of females of different chaeta numbers producing viable offspring follows a very similar distribution to that shown in fig. 1.

Thus, given that a female is fertile, the number of progeny produced varies little with chaeta number.

The relative constancy of the mean and variance of the population as estimated from low density samples, in addition to the relatively minor variation in reproductive capacity of females of different phenotypes suggests that selective pressures were not intense. However, samples of adult flies captured from the cage population had means and variances as follows:

	Females	Males
Mean	18·47	17·78
Variance	8·23	9·13
Number scored	908	890

These means and variances are in marked contrast to those quoted earlier for the low density samples. Both the mean and more particularly the variance of individuals raised under the highly competitive conditions of the cage are considerably reduced. These reductions may be due either to a direct effect of the environment or to a selective elimination of extreme phenotypes, particularly those with high chaeta numbers.

On *a priori* considerations, the reduction in mean chaeta number could be a direct effect of food shortage under the intensely competitive cage conditions. However, the effect of this type of stress would be more likely to increase the phenotypic variance.

Two approaches were available to distinguish these alternative hypotheses. If the reduction of variance in the cage was purely environmental, then both cage adults and low density samples should contain the same array of genotypes in the same frequencies.

1. If we progeny test males obtained from both cage and from low density samples by crossing them individually to a common inbred tester line and raising the progeny at low densities, then we would expect the variance of progeny family means to be the same for both. Whereas the regression of offspring on parent will be greater for the cage males than for the low density males. This results from the restriction of the phenotypic range under cage conditions. However, if the reduction in variance is due to the selective elimination of extreme phenotypes we would expect these slopes to be the same.

Males from both environments were crossed to females from the inbred line, Oregon, and female and male progeny were scored. There was no significant difference between the slopes of the regression of son on father or of daughter on father within both cage and low density samples, and therefore these data were pooled. The combined estimates of the regression coefficients are:

Cage males   0·41679 ± 0·06662
Low density males   0·38326 ± 0·05025

These estimates are not significantly different suggesting that the difference in variance is due to selection rather than an environmental restriction of the phenotypic range.

2. We may obtain further information on the nature of the reduction in the variance of cage adults in the following way. If the reduction results solely from an environmental effect, then on randomly mating representatives from the cage and from low density samples we should find that the progeny

variances are the same. In contrast, if the reduction is due to selection then the variance of progeny raised from low density samples should be greater than the variance of cage progeny. However, we should note that this second approach may be less sensitive if stabilising selection for chaeta number is an important process in the cage. We may illustrate this by a simple example. Let us consider a single locus model with equal gene frequencies. In low density samples the genotypes will occur in the following frequencies, $\frac{1}{4}AA$, $\frac{1}{2}Aa$ and $\frac{1}{4}aa$. If these genotypes are randomly mated then there will be no change in their relative frequencies. However, in the cage, intermediate types will be at a selective advantage, i.e. the heterozygote, Aa. If these individuals are randomly mated and the progeny raised at low densities then the following genotypes will be obtained, $\frac{1}{4}AA$, $\frac{1}{2}Aa$ and $\frac{1}{4}aa$. Thus differences in the progeny variance may be more difficult to detect.

The results of such test crosses were as follows:

(a) Variance of progeny from cage adults   32·7422 ($N = 500$)

(b) Variance of progeny from low density adults   38·5620 ($N = 500$)

This confirms the previous findings. The reduction in the variance of cage adults results from a selective elimination of the extreme phenotypes.

We can consider now the effect of high densities on the mean chaeta number. It has been shown that the regressions of offspring on parent for the cage sample and for the low density sample do not differ in slope. The combined estimate is $0·39675 \pm 0·04052$. The intercepts, however, do differ indicating an overall depression of chaeta number when individuals are raised under cage conditions. In other words, a male from the cage will produce progeny with a higher mean hair count, on average, than a male of the same phenotype raised under low density conditions. In order to compare phenotypes in the two situations it is necessary to estimate the magnitude of this depression. Consider the offspring-parent regression equations given below:

Low density $y = 13·76461 + 0·39675x_1$

Cage $y = 14·77022 + 0·39675x_2$

The average environmental depression is estimated as the difference between values of x_1 and x_2 for a given value of y. Thus the depression in the cage is equal to

$$(x_2 - x_1) = (14·77022 - 13·76461)/0·39675 = 2·5346$$

That is, a male from the cage with 16 chaetæ has the same breeding value as a corresponding low density male with 18·5346 chaetæ.

The type of selection acting on chaeta number may be examined further by comparing the distribution of contemporary phenotypes raised under high (cage) and under low density conditions. However, before making this comparison it is necessary to correct the cage phenotypes for the environmental depression. This was achieved by increasing the chaeta number of all cage adults by 2. The corrected distributions are shown in fig. 2 for females only. The mean and variance of these distributions are

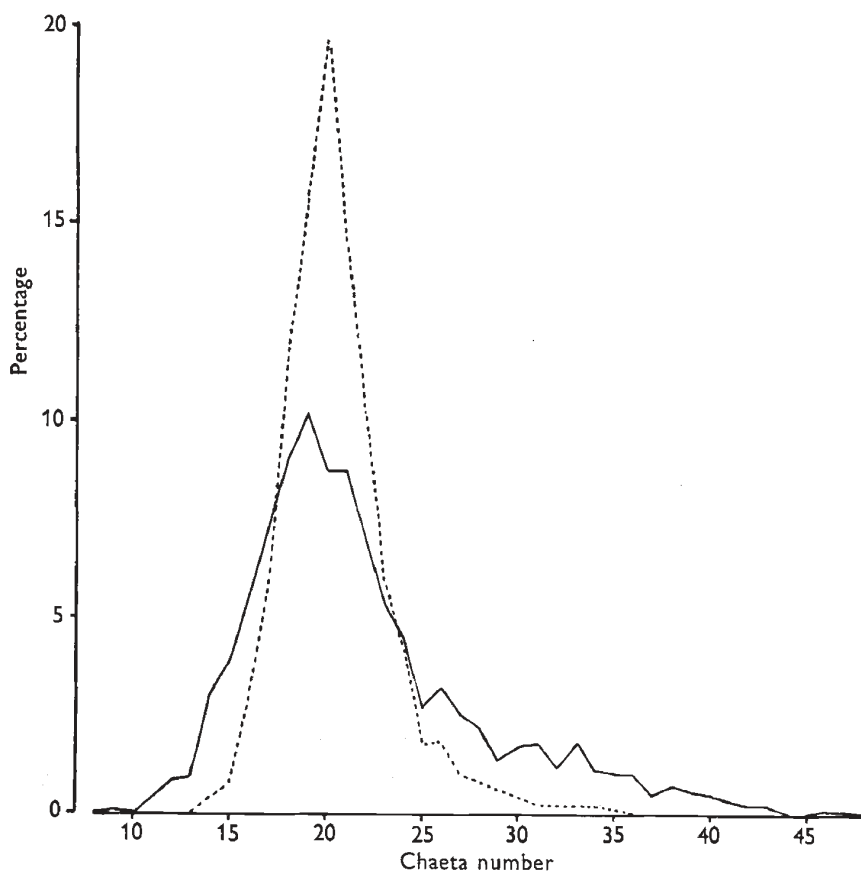|	Mean	Variance
Cage females	20·47	8·23
Low density females	21·91	36·53

Fig. 2.—Frequency distributions of females raised under cage conditions (broken line) and at low densities (solid line).

It is immediately apparent from these distributions that the effect of the high density condition is to remove the extreme phenotypes.

A quantitative assessment of the relative fitness of different phenotypes may be obtained as follows. Let $f(P_{ic})$ and $f(P_{il})$ be the frequencies of the ith phenotype in the cage and in low density conditions respectively. Then, the relative fitness, W_i', of the ith phenotype is obtained as

$$W_i' = \frac{f(P_{ic})}{f(P_{il})}$$

The values of W_i' can be rescaled by dividing all the W_i''s by the largest W_i' so that the maximum relative fitness equals unity. The relative fitnesses, W_i, calculated in this way are given in fig. 3.

It can be seen that flies with 20 chaetæ have unit fitness. If we move away from this phenotype the fitness decreases rapidly and furthermore, phenotypes below 14 and above 35 chaetæ have a fitness of zero. We should note that the optimum at 20 chaetæ is not at the centre of the pheno-
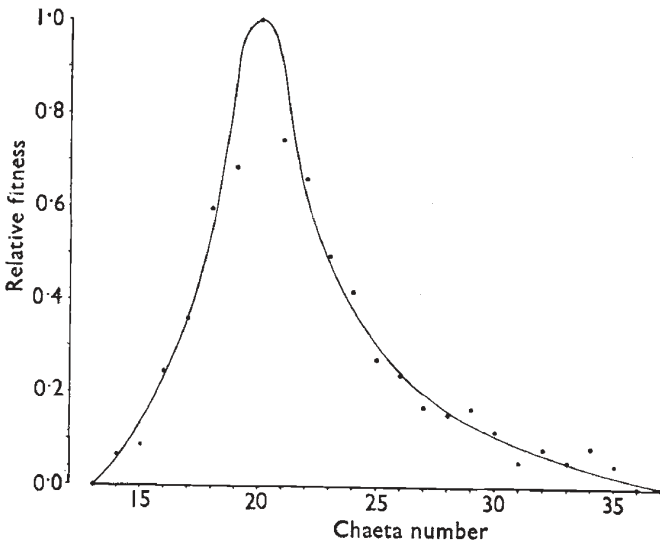
FIG. 3.—Relationship between chaeta number and relative fitness (W_i) under cage conditions.

typic range, and is in fact approximately 7 chaetæ lower than this. In addition, fitness does not decrease symmetrically about the optimum.

Given this type of selection both the survival rate from egg to adult and the mean and variance of chaetæ number should be related to density. This is illustrated by the following experiment. Four samples of 3000 eggs were collected from the cage and the progeny raised at densities of 100, 500, 1000 and 1500 eggs per tube. The results are shown in table 1.

TABLE 1

Effect of density on chaeta number and survival

Density (eggs per tube)	Total number of eggs	Chaetæ		Percentage survival
		Mean	Variance	
100	3000	21·46	36·45	68·50
500	3000	19·83	19·10	33·46
1000	3000	17·98	9·70	13·56
1500	3000	17·77	6·53	5·60

These results show that the intensity of selection is in fact density dependent. A comparison of the mean and variance of chaetæ number estimated from cage adults with the values shown in table 1 suggests that in the cage the egg density per tube is around 1000 and approximately 14 per cent. of the eggs produce adults.

4. DISCUSSION

It has been argued (Robertson, 1955, 1966) that chaeta number is a peripheral character not closely related to fitness. Thus in the absence of strong selective forces the genes involved are likely to be in linkage equilibrium. Furthermore, it has been suggested that mutation may be an important process for maintaining the observed variation in natural populations.

In contrast, Thoday (1958) has argued that the existence of considerable potential genetic variation and the characteristic chaeta numbers of different populations are evidence for the adaptive significance of this character. This implies a history of stabilising selection (Mather, 1953).

The means and variances of chaeta number in several populations are summarised in table 2. The similarity between both the means and the variances of these populations is remarkable in view of their proven genetic potential and their diverse geographic origins. It is difficult to envisage how such a situation arises if all the loci are nearly neutral. On the contrary, it suggests that natural selection is of considerable importance. Indeed the present experiments have demonstrated that under the intensely competitive conditions of a population cage, fitness is related to chaeta number; flies with an intermediate chaeta number having maximum fitness and any departures

TABLE 2

Sternopleural chaetæ in natural populations

Population	Females		Males		Reference
	Mean	Variance	Mean	Variance	
Orëbo	17·12	2·16	16·62	2·67	Rasmuson, 1952
Tunnelgatan	18·56	3·52	18·31	3·95	Rasmuson, 1952
Stäket	16·55	2·56	16·20	2·56	Rasmuson, 1952
Algeria	18·71	2·29	18·44	3·25	Rasmuson, 1952
Kaduna	17·84	(3-4)	17·06	(3-4)	Clayton et al., 1957
Texas	18·40	3·13	17·67	2·94	—
Inhaca	18·02	2·40	17·12	3·26	—

from this optimum resulting in a marked decline in fitness. It must be emphasised that selection acts at a pre-adult stage and hence cannot be for chaeta number as such, but presumably for some pleiotropic effect of the same loci. Furthermore, given this relationship between pre-adult fitness and chaeta number, the variation in reproductive capacity of different females shown in fig. 1 is largely irrelevant.

Robertson (1955, 1963) has pointed out that one cannot infer, automatically, a causal relationship between fitness and phenotype for a metrical trait. He illustrates this by showing that both heterozygous advantage and stabilising selection can produce the same relationship between phenotype and fitness. In the model involving heterozygous advantage, Robertson assumes unconditional overdominance for fitness and that fitness increases linearly with increasing heterozygosity. This would certainly result in a stable equilibrium (Kimura, 1956). However, there is little evidence for single locus overdominance for fitness, sickle cell anaemia being the single well-established example.

Heterosis for metrical characters has been shown to result from epistasis and, or, dispersed dominant genes rather than overdominance (Jinks, 1955; Mather, 1955; Jinks and Morley Jones, 1958). Cases where the mechanism of heterosis has been elucidated for fitness characters invariably involve dominant genes which interact in a manner analogous to duplicate genes of classical genetics (Breese and Mather, 1960; Kearsey and Kojima, 1967; Barnes, 1968*b*). If we assume that the genes for metrical traits have the same properties as major genes then it should be possible to fix this superiority in a homozygote. Indeed, Breese and Mather (1960) were able to obtain

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isogenic lines which had the same fitness as the complete heterozygote. This type of system is not capable of maintaining variation, unless the loci involved are prevented from recombining. Furthermore, in laboratory populations derived from the inbred lines Oregon and Samarkand (Barnes, 1968a) and Oregon and 6 C/L (Killick, 1970) the equilibrium chaeta number departs significantly from the F_1 value, and in both cases was outside the parental range. This effect was most marked in Killick's data, where the population moved from the F_1 mean of 28 chaetæ down to a value of 18. These results are inconsistent with a model involving heterozygote advantage. Nevertheless, it may be possible for heterosis to produce an apparent relationship between the primary character and fitness.

TABLE 3

An illustration of the method of calculating $\bar{n}_h$, the average number of heterozygous loci per individual (see text for explanation)

Nos. of increasing alleles (x)	Frequency $\times \frac{1}{64}$ (f)	Genotypes (frequency $\times \frac{1}{64}$)	Total nos. of het. loci $\times \frac{1}{64}$ (n_h)	$\bar{n}_h = \frac{n_h}{f}$
0	1	LLL (1)	0	0·0
1	6	LLX (6)	6	1·0
2	15	LLH (3) LXX (12)	24	1·6
3	20	LXH (12) XXX (8)	36	1·8
4	15	HHL (3) HXX (12)	24	1·6
5	6	HHX (6)	6	1·0
6	1	HHH (1)	0	0·0

Where H = homozygous for increasing gene.
L = homozygous for decreasing gene.
X = heterozygous.

Let us first examine the relationship between the degree of heterozygosity and phenotype. Consider a population in linkage equilibrium with N loci segregating, each locus with two alleles at equal frequency. The frequency of individuals with 0, 1, 2, ... $2N$, increasing genes can be computed from the binomial $(\frac{1}{2} + \frac{1}{2})^{2N}$. For any particular class, for example x increasing genes, the average number of heterozygous loci per individual $(\bar{n}_h)$ may be obtained. This is illustrated in the example in table 3 for $N = 3$.

It can be shown that $\bar{n}_h$ may be obtained from the quadratic expression

$$\bar{n}_h = a + k(x-b)^2$$

when $x = 0$ or $x = 2N$
$$\bar{n}_h = 0$$

and further when $x = N$,
$$\bar{n}_h = \frac{N^2}{2N-1}$$

Thus
$$a = \frac{N^2}{2N-1}$$

$$b = N$$

$$k = -\left(\frac{1}{2N-1}\right)$$

and on simplification
$$\bar{n}_h = \frac{x(2N-x)}{2N-1}$$

This, then gives us the distribution of the average number of heterozygous loci over the phenotypic range. If fitness is linearly related to heterozygosity, then the relationship between fitness and phenotype (chaeta number) will follow a similar quadratic distribution. Quite clearly this type of distribution is in marked contrast to that shown in fig. 3, although the optimum phenotype corresponds to the F_1 (Barnes and Kearsey, 1970). Furthermore, the difference between the two distributions will be even more pronounced in the presence of duplicate type interactions. However, the distribution of the average number of heterozygous loci per individual was derived assuming no dominance. If this assumption fails, the general conclusions are still applicable as only the symmetry of the distribution will be affected.
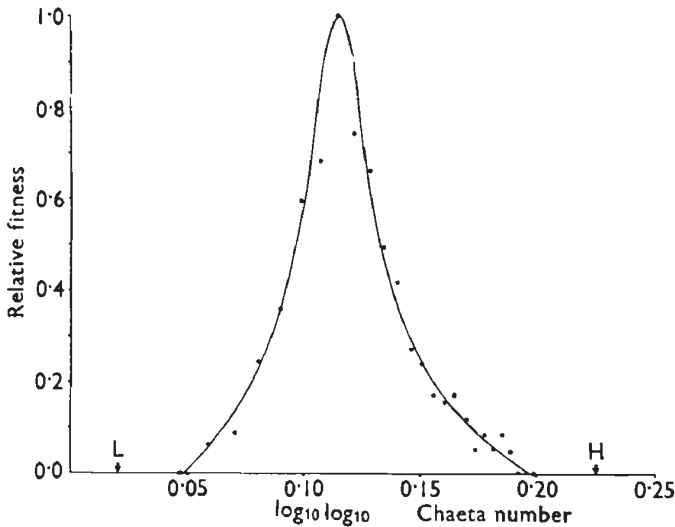

FIG. 4.—Relationship between chaeta number transformed to a loglog scale and relative fitness (W_t). The arrows indicate the positions of the extreme low (L) and high (H) selections.

Heterosis, as we have seen, does not appear to provide a satisfactory explanation. Let us consider now how the results compare with those expected with stabilising selection.

The term stabilising selection has been variously interpreted by different authors. We will use the definition given by Mather (1953). Selection is said to be stabilising when an intermediate phenotype is selected at the expense of both extremes. The relationship between phenotype and fitness estimated in the present experiments, shown in fig. 3, is in agreement with this definition. The observed changes in fitness do not follow a quadratic deviation model (Wright, 1935) which has been used in many theoretical studies, but are in much closer agreement with the linear model (B) suggested by Kearsey and Gale (1968). Here the optimum is fixed at the centre of the phenotypic range, fitness falling off linearly and reaching zero when the phenotype is halfway between the mid-phenotype and the extreme phenotype. However, in the experimental population the decline in fitness is not symmetrical as the optimum is not located at the centre of the observed phenotypic range. This departure from the linear model may be a simple

function of the scale of measurement used. This may be illustrated as follows. The relationship between chaeta number, measured on a $\log_{10}\log_{10}$ scale, and fitness is shown in fig. 4. Here, the optimum lies at the centre of the phenotypic range, approximately, and fitness falls off linearly and symmetrically on either side of the optimum. On this scale of measurement the genes determining chaeta number act in a purely additive manner and, therefore, stabilising selection could lead to stable equilibria if the effects of different loci were unequal. The magnitude of the discrepancy between the loci necessary for stable equilibria is dependent on linkage (Gale and Kearsey, 1968). This discrepancy need be only small if the loci are closely linked. In a genetic analysis of this population (Barnes and Kearsey, 1970) it is suggested that most of the genetic activity occurs on the third chromosome. The assumption of closely linked loci differing only slightly in their effect on the primary character does not seem, therefore, unreasonable. The method of assessing relative fitness used here has been applied also to the Texas population and a population isolated from Inhaca (Mozambique). The results are essentially similar to those described above.

The present results are consistent, therefore, with a mechanism of stabilising selection maintaining variation within the population. The relationship between phenotype and fitness, which is critical to this interpretation, is being investigated further.

5. SUMMARY

1. A method has been developed for assessing the relative fitness of phenotypes, for a metrical trait, within populations of *Drosophila melanogaster*.

2. This method has been applied to a derivative of a wild population to determine the selective forces maintaining genetic variation for sternopleural chaeta number.

3. Selection has been shown to occur at a pre-adult stage and to favour individuals with a chaeta number near the population mean. Fitness declines rapidly with increasing deviations from this optimum, reaching zero within the phenotypic range.

4. The results are discussed in relation to stabilising selection and heterozygous advantage.

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