STABILISING SELECTION IN *DROSOPHILA MELANOGASTER*

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1. INTRODUCTION

THE action of selection has been classified by Mather (1953) into three basic types, stabilising, directional and disruptive. Selection is said to be stabilising when an intermediate phenotype is favoured at the expense of both extremes. In contrast, one extreme phenotype is favoured by directional selection, and both extremes simultaneously, at the expense of the average, by disruptive selection. If we consider the frequency distribution for metrical characters which are not apparently directly associated with fitness, we find that the majority of the population are, in Galton's words, mediocre. This suggests that for this broad category of characters the mean expression approximates to the optimum phenotype. However, in populations with high genetic variance, segregation and recombination will continuously produce numerous individuals that depart from the optimum and hence have lower fitness (Mayr, 1963). In these populations, therefore, we would expect stabilising selection to be of principal importance. This, of course, assumes that the environment remains reasonably constant.

Although there is a large body of experimental evidence on the genetic consequences of directional selection, and more recently on disruptive selection, evidence relating to the consequences and indeed the occurrence of stabilising selection is much less. Bumpus (1898) observed that sparrows dying from storm damage departed more from the mean phenotype than did those which survived. Dunn (1942) showed that the total variation in scales of adult samples of reptiles was less than in juvenile samples, and a similar situation was reported by Mertens (1947) in the snake *Natrix natrix*. Hecht (1952) demonstrated selection against extremes of size in the lizard *Aristelliger praesignis*. However, in these cases little is known about the genetic control of the character. Birth weight in man has been shown to have a central optimum value (Karn and Penrose, 1951; Jayant, 1966).

Thoday (1958) has argued that sternopleural chaeta number in *Drosophila melanogaster* is a character of adaptive significance as it is affected by natural selection. This inference was made from quite general evidence. Different populations of a species have characteristic chaeta numbers and within a population the variance of chaeta number can be shown to be much less than the potential genetic variance by appropriate selection experiments (Sismandes, 1942; Mather and Harrison, 1949; Rasmusson, 1955; Thoday, 1958, 1959; Gibson and Thoday, 1963). These facts must imply a history of effective stabilising selection (Mather, 1953).

In the present study an attempt has been made to assess the importance of stabilising selection in determining the distribution of sternopleural chaeta number in two large cage populations of *Drosophila melanogaster* maintained at 18° and 25° C. If stabilising selection is an important process in these

populations, then we would expect maximum fitness to be associated with phenotypes around the respective population mean. Furthermore, changes away from the optimum value would be accompanied by a reduction in fitness.

2. MATERIALS AND CULTURE METHODS

Careful consideration must be given to the method of maintaining large populations of *Drosophila*. At the present time techniques which require the transfer of a population to a fresh food source at frequent intervals present insuperable difficulties in the sampling technique. The nearest approach to a successful solution of the problem was proposed by L'Héritier and Teissier (1933) and L'Héritier (1937). They devised population cages into which fresh food is introduced and from which the worked-out food is removed sufficiently frequently for the size and age distribution of the population to remain approximately stationary.

The population cages used in the present experiments are modifications of the original model. The cage is a perspex box with outside dimensions $32 \times 27 \times 13$ cm. The bottom surface has twenty circular openings $2\frac{1}{2}$ cm. in diameter, into which specimen tubes can be inserted, these are held securely in position by a $\frac{1}{4}$ -inch rubber grommet. The top of the cage is covered by copper gauze, thereby allowing free gaseous exchange with the atmosphere.

The populations were formed from F_1 crosses of two long inbred lines of *Drosophila melanogaster* Oregon, O, and Samarkand, S. When progeny began to emerge, fresh tubes containing approximately 2 cm. of oatmeal medium with fresh yeast were introduced into the cages in a cyclical system, such that any one tube remained in the cage between 17 to 18 days at 25° C. and between 33 to 35 days at 18° C. This rotation of tubes ensures that a representative sample of the genotypes in the tube cultures contribute to the population. The number of adults in the populations rapidly built up to numbers that were compatible with the quantity of food available. In these experiments the numbers fluctuated around a value of 2000.

3. EXPERIMENTAL RESULTS

The changes in chaeta number in the two environments were followed over a period of time. In order to obtain a random sample of genotypes from the population, four freshly yeasted tubes were placed in the cage, and a sample of eggs was collected over approximately three hours at 25° C. and six hours at 18° C. These samples were then transferred to fresh culture bottles and incubated. The adults obtained were assayed for sternopleural chaeta number by scoring a random sample of ten females and ten males. The changes in chaeta number with time in the two populations are shown in fig. 1.

The two populations showed a consistent divergence over the course of the experiment. After 600 days the difference in chaeta number of the two populations was highly significant, and both populations seemed to have achieved equilibrium values. At this point we should consider whether the difference between the two populations resulted solely from a direct influence of the temperature environment on the expression of chaeta number of the type that has been reported in the literature by Plunkett (1926), Beardmore

(1960), Gibson, Parsons and Spickett (1961) and Lawrence (1964). This would seem not to be the case for two reasons. First, temperature had no marked effect on the expression of chaeta number in the parental, F_1 and F_2 generations, as can be seen from table 1. Second, in the cases reported in

FIG. 1.—Changes in sternopleural chaeta number in populations maintained at 25° C. and 18° C.

the literature a reduced temperature caused an increase in the expression of the character. However, the divergence of the populations used in this study was to a higher value at 25° C. and a lower value at 18° C. The reverse of the situation we would expect if the divergence was a manifestation only of the direct effect of temperature.

TABLE 1

Effect of temperature on the expression of chaeta number

Generation	25° C.	18° C.
0	22.28	22.46
S	20.07	20.11
F_1	21.36	21.42
F_2	21.13	20.99

In a large random mating population the average level of heterozygosity will remain constant at an equilibrium value. In the absence of selection the value of a metrical character is expected to remain in the region of the F_2 value, within the limits of sampling variance. However, as different equilibrium values were achieved in the two populations we may postulate that natural selection had a differential effect on chaeta number in the two environments. At 25° C. selection was towards a chaeta number of 23.2, and at 18° C. towards a value of 21.4. Selection will, of course, only be effective in bringing about a change in the mean of a metrical character if there are differences in fitness between the various phenotypes. The nature of the selective process responsible for maintaining the difference between the populations may be examined further, therefore, by determining the fitness of the phenotypes within each population. If stabilising selection is of principal importance, then maximum fitness will be associated with the chaeta number classes around the respective equilibrium population values.

A change away from the optimum will be accompanied by a reduction in fitness.

We require, therefore, some measure of the fitness of the phenotypes within each population. Dobzhansky (1955) in a paper "Concepts and Problems of Population Genetics" discussed some of the problems involved in studying changes in fitness of a population. Indeed, he pointed out that "the work in this field is severely handicapped by the lack of reliable methods of comparing the fitness of populations, and, of course, by the lack of clarity in the concept of fitness itself". This confusion must arise in part as the term fitness may be applied to many levels of biological integration.

In experimental investigations the fitness of a particular genotype or population may be examined in terms of the various components of the life-cycle, for example, fecundity, hatchability, rate of development, competitive ability, differential mortality, sexual activity, sterility, etc. However, all these are components of fitness and not exclusive estimates of it.

Attempts to measure fitness have included innate rate of increase (Birch, Dobzhansky, Elliot and Lewontin, 1963) biomass (Carson, 1957) number of males and females hatching (Beardmore, Dobzhansky and Pavlovsky, 1960) number of flies per food unit and equilibrium population size (Ayala, 1965). In this later study it was concluded that both production and population size were satisfactory statistics for characterising a population.

In the present investigation, differences in fitness have been assessed by measuring the total number of progeny per single pair mating. This metric may be criticised on the grounds that we have no idea of the relative importance of the various components of fitness, and cannot separate the influence of the parental genotype from the progeny genotype. However, this is not a serious difficulty in the present context. As fitness must in all cases refer to the ability of an organism to leave offspring, and thereby contribute to the gene pool of subsequent generations, differences in the number of progeny produced must reflect differences in fitness.

The fitnesses of the phenotypes within the two populations were measured by taking a systematic sample, after 176 days at 25° C. and 434 days at 18° C., so that all chaeta number phenotypes were represented. These were then crossed in all possible combinations, *i.e.* at 25° C. individuals in classes 19 to 25 were crossed *inter se*, giving rise to 49 single pair matings, and similarly at 18° C. for individuals in classes 19 to 24. (More extreme phenotypes were observed at both temperatures, but these were too infrequent to be included in the crossing programme.) The single pair matings were set up in the customary 3 × 1 inch culture vials, where the females were allowed to lay for 48 hours at 25° C. and 96 hours at 18° C. The number of progeny emerging over a five-day period at 25° C. and a ten-day period at 18° C. was scored. As the experiment took the form of a diallel cross, the results are shown in fig. 2 as the array mean yield, averaged over female and male arrays.

The crosses from the 25° C. population showed that parents in the 23 chaeta number class produced the most progeny. A change away from this value resulted in a reduction in the number of progeny produced. Indeed, the increase in yield of the 23 class over the 19 and 20 classes was of the order of 20 per cent. In contrast, in the 18° C. population parents with a chaeta number of 21 produced the most progeny, and changes away from this value were accompanied by a reduced yield.

These results are certainly in agreement with the hypothesis that sterno-

pleural chaeta number is subjected to stabilising selection. The optimum phenotypic classes were 23 at 25° C. and 21 at 18° C. We can conclude, therefore, that the changes in chaeta number observed in the populations, and the differences between the two environments, resulted from the action of natural selection.

FIG. 2.—Mean yield of progeny of the chaeta number phenotypes drawn from populations maintained at 25° and 18° C.

The differences in fitness that have been measured are, of course, compounded of differences in fertility, in hatchability and in viability. These differences may be examined further by removing the variation caused by different numbers of eggs being produced in the two environments. If equal numbers of eggs are incubated at 18° and 25° C. then any consistent differences in the number of adults produced must reflect differences in hatchability and viability, which have become manifested under the influence of natural selection.

In this experiment large numbers of eggs of approximately the same age were required. A technique devised by Bakker (1961) and modified by Gale (1964) was used. The flies were obtained from two lines (HI and HII) derived from an Oregon, Samarkand cross which had been subjected to selection for high sternopleural chaeta number at 25° C. At this stage the

mean of the lines was 26.11, which is above the optimum value at both temperatures. About 100 pairs of flies of the line were allowed to lay in a bottle culture containing oatmeal medium well covered with yeast over a period of four days at 25° C. After two days the surface of the medium is completely covered with eggs and larvae and further laying is inhibited. In the next two days females accumulate large numbers of unlaidd eggs. When presented with a suitable oviposition medium the females begin to lay after about half an hour. Laying reaches a maximum during the second and third hours and is normally complete four hours after transfer. The laying surface consisted of a plastic strip which was coated with a starch gel blackened with charcoal, and held in position in the laying tube by the yeasted oatmeal medium. The flies were introduced into the laying tube without anaesthetisation. One hundred eggs were then transferred on to a $\frac{1}{4}$ -inch cube of oatmeal medium. This was then placed in a yeasted tube and incubated. A total of 40 cultures were set up, 20 being allocated at random to the 25° C. environment and 20 to the 18° C. environment. The total number of progeny produced by each culture was then scored.

TABLE 2
Mean number of progeny produced by 100 eggs

	Line	
	HI	HII
25° C.	56.0 (10)	49.2 (9)
18° C.	44.4 (9)	38.5 (10)

The results are shown in table 2 as the mean yield per culture. The figure in brackets indicates the number of cultures contributing to the mean. The missing values were caused by a very rapid drying out of two of the oatmeal cubes, and hence almost complete loss of the eggs.

As we have unequal group sizes the analysis of variance, shown in table 3, was obtained using a multiple regression technique.

TABLE 3
Analysis of variance of yield data

Item	S.S.	d.f.	M.S.	V.R.	P
Temperatures	1175.4459	1	1175.4459	27.9	0.1%
Lines	383.3406	1	383.3406	9.1	1%-0.1%
Temperatures $\times$ Lines	1.6447	1	1.6447	< 1	—
Error	1432.279	34	42.1258	—	—
Total	3067.895	37	—	—	—
Unassigned	75.1848	—	—	—	—

The error sum of squares was obtained in the usual way, by pooling the sums of squares obtained between individual measurements, taken about their own mean, within lines and temperatures. Due to a correlation between the estimates of the mean and the interaction component and between the temperature and line components a proportion of the total sum of squares could not be assigned unambiguously to any of the items.

The analysis shows that there was a highly significant difference between the temperatures, and a significant difference between the lines. Thus,

significantly more adults were obtained from the cultures incubated at 25° C. than at 18° C. There was no evidence of a lines $\times$ temperature interaction, and as the covariance matrix showed that the estimates of the temperature and line components were independent of the interaction component no re-estimation of the line and temperature effects was necessary.

TABLE 4
Sternopleural chaeta number averaged over 20 individuals

	Lines			
	HI		HII	
	♀	♂	♀	♂
25° C.	24.55	23.75	23.85	25.35
18° C.	23.20	22.60	22.30	23.15

Thus, of the samples of eggs cultured at the two temperatures those at 18° C. showed a lower hatchability and viability and, therefore, produced significantly less progeny. As we have already established that the optimum chaeta number at 18° C. was significantly less than at 25° C. we would expect this reduction in yield to be accompanied by a differential elimination of individuals with a high hair count. This was investigated by scoring the sternopleural chaeta number of a random sample of 20 males and 20 females taken from the pooled progeny of cultures within lines and temperatures. The results are shown in table 4 as the mean of 20 individuals of each sex.

The analysis of variance is shown in table 5. All items in the analysis were tested against the error mean square with 152 degrees of freedom. There was a highly significant difference between temperatures and a significant sex $\times$ lines interaction.

TABLE 5
Analysis of variance of sternopleural chaeta number

Item	d.f.	M.S.	V.R.	P
Lines	1	0.756	< 1	—
Temperatures	1	97.656	35.811	$\leq 0.1\%$
Lines $\times$ Temps.	1	3.907	1.433	> 20%
Sex	1	2.256	< 1	—
Sex $\times$ lines	1	35.157	12.892	< 0.1%
Sex $\times$ Temps.	1	0.507	< 1	—
Sex $\times$ Lines $\times$ Temps.	1	1.805	< 1	—
Error	152	2.727	—	—
Total	159	—	—	—

The two analyses taken together show that the mean chaeta number of adults in the 18° C. environment was significantly less than in the 25° C. environment. Furthermore, this reduction in chaeta number was associated with a lower yield at 18° C. Thus, from the random sample of eggs cultured in the two environments a significantly lower proportion survived to maturity at 18° C. The reduced numbers correspond to a loss in the high chaeta number classes. Natural selection had, therefore, a differential effect on chaeta number, the higher classes being selected against at 18° C. This is

in agreement with the earlier findings showing that the optimum chaeta number at 18° C. was significantly less than at 25° C.

4. DISCUSSION

The results presented show clearly that sternopleural chaeta number in *Drosophila melanogaster* is subjected to stabilising selection. However, the way in which natural selection operates on a character, and hence the interpretation we place on these results, will depend on the part played by the character in determining differences in fitness. Therefore, before we can consider further the nature of the mechanisms involved in determining the distribution of chaeta number within a population we must look at the functional relationship between chaeta number and fitness. Two quite distinct hypotheses have been proposed in the literature.

Robertson (1955) and Falconer (1960) suggest that chaeta characters in *Drosophila* may be regarded as examples of neutral characters, in that the variation observed for these characters does not lead to variation in fitness. The evidence for this is gained from directional selection experiments. Populations in which experimental selection has been relaxed showed no marked tendency to return to the initial population mean, even though back selection experiments showed that genetic variance still existed (Clayton, Morris and Robertson, 1957). These results suggest that the forces maintaining the variation in the original population are not very strong. Robertson suggests that in these circumstances mutation may be of principal importance in determining the variation observed. If this were the case much of the variation in a population must be determined by alleles at a very low frequency. However, in a selection experiment initiated from four individuals drawn from a wild population the sternopleural chaeta number was reduced to 11 and increased to 45 from a mean of 18 (Kearsey and Barnes, personal communication). In this population, therefore, much of the variation must be determined by alleles at a frequency in excess of 0.125. This indicates that in order to achieve this frequency in the population these alleles must have been subjected to selection.

Although a neutral character has no functional relationship with fitness, Falconer (1960) points out a mechanism whereby individuals with different values will differ in fitness in a regular way. If the genetic variance of the character is predominantly additive, then individuals with intermediate values will tend, on average, to be heterozygous at more loci than individuals with extreme values. If in the population heterozygosity is at a selective advantage, for example in an outbreeding species where many of the loci concerned with fitness characters show unidirectional dominance and are in dispersion, this would result in individuals intermediate for the metrical character being superior in fitness. In these circumstances it would be possible to detect a relationship between the metrical character and fitness. However, as Falconer (*loc. cit.*) points out, this would be a relation only at the level of observation, it would not indicate a functional relationship.

In contrast, Thoday (1958), as we have seen earlier, argues that chaeta number is a character of adaptive significance as it is affected by natural selection. Different populations have characteristic chaeta numbers, and within a population the variance of chaeta number can be shown to be much less than the potential genetic variance. These facts imply a history of effective stabilising selection (Mather, 1953). This type of selection is in

agreement with the genetical architecture that has been established for abdominal chaeta number (Breese and Mather, 1957). The results of directional selection experiments of Mather and Harrison (1949) and Barnes (1966) indicated an intimate relationship between changes in chaeta number and changes in fitness. Furthermore, if we look at the type of response to directional selection observed in lines originating from wild populations we see that almost invariably there is an asymmetrical response, more progress being made in the direction for high chaeta number (Clayton, Morris and Robertson, 1957; Ramuson, 1955; Kearsley and Barnes, personal communication). One explanation that may be proposed for this is that the increasing alleles are much less frequent in the population than the decreasing alleles. In order that this situation is maintained in a large population the alleles concerned must be subjected to selection.

In the present study it has been shown that maximum fitness is associated with phenotypes around the respective population mean, and changes away from the optimum value were accompanied by a reduction in fitness. In addition, a directional change in chaeta number was observed particularly in the 25° C. environment. This change cannot be the result of drift as in these experiments the population size was of the order of 2000 individuals. In a large random mating population the importance of linkage in bringing about a directional change of a metrical character as a result of a correlated response to selection for an independent system will be minimised due to the many opportunities for recombination. In the absence of selection the average level of heterozygosity will remain constant at an equilibrium value, and the mean value of chaeta number is expected to remain in the region of the F_2 value. However, as different equilibrium values were achieved in the populations we must postulate that natural selection had a differential effect on chaeta number in the two environments. At 25° C. selection was towards a value of 23.2 whereas at 18° C. towards a value of 21.4. This difference between the environments could not be achieved if chaeta number was a neutral character. Indeed, the evidence suggests that chaeta number and fitness are functionally related, variation in chaeta number being accompanied by variation in fitness. In these populations stabilising selection seems to be an important process in determining the distribution of chaeta number.

5. SUMMARY

1. An attempt has been made to assess the importance of stabilising selection in determining the distribution of sternopleural chaeta number in two large populations of *Drosophila melanogaster* maintained at 18° and 25° C.

2. It has been established that natural selection has a differential effect on chaeta number in the two environments. Selection was towards a value of 23.2 at 25° C. and to a value of 21.4 at 18° C.

3. Maximum fitness is associated with phenotypes around the respective population means, and changes away from these values are accompanied by a reduction in fitness.

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6. REFERENCES

- AYALA, F. J. 1965. Relative fitness of populations of *Drosophila serrata* and *Drosophila birchii*. *Genetics*, 51, 527-544.
- BAKKER, K. 1961. An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. *Archives Néerlandaises de Zoologie*, XIV, 2, 200-281.
- BARNES, B. W. 1966. Environment and Selection in *Drosophila melanogaster*. Ph.D. Thesis Birmingham.
- BEARDMORE, J. A. 1960. Developmental stability in constant and fluctuating temperatures. *Heredity*, 14, 411-422.
- BEARDMORE, J. A., DOBZHANSKY, TH., AND PAVLOVSKY, O. A. 1960. An attempt to compare the fitness of polymorphic and monomorphic experimental populations of *Drosophila pseudoobscura*. *Heredity*, 14, 19-33.
- BIRCH, L. C., DOBZHANSKY, TH., ELLIOT, P. O., AND LEWONTIN, R. C. 1963. Relative fitness of geographic races of *Drosophila serrata*. *Evolution*, 17, 72-83.
- BREESE, E. L., AND MATHER, K. 1957. The organisation of polygenic activity within a chromosome in *Drosophila*. I. Hair characters. *Heredity*, 11, 373-395.
- BUMPUS, H. C. 1899. The elimination of the unfit as illustrated by the introduction of the sparrow. *Biol. Lect. Woods Hole*, 1898, 209.
- CARSON, H. L. 1957. Production of biomass as a measure of fitness of experimental populations of *Drosophila*. *Genetics*, 42, 363-364.
- CLAYTON, G. A., MORRIS, J. A., AND ROBERTSON, A. 1957. An experimental check on quantitative genetical theory. I. Short-term responses to selection. *J. Genet.*, 55, 131-151.
- DOBZHANSKY, TH. 1955. A review of some fundamental concepts and problems of population genetics. *Cold Spr. Harb. Symp. Quant. Biol.*, 20, 1-15.
- DUNN, E. R. 1942. Survival value of varietal characters in snakes. *Amer. Nat.*, 76, 104-109.
- FALCONER, D. S. 1960. *Introduction to Quantitative Genetics*. Oliver and Boyd.
- GALE, J. S. 1964. Competition between three lines of *Drosophila melanogaster*. *Heredity*, 19, 681-699.
- GIBSON, J. B., PARSONS, P. A., AND SPICKETT, S. G. 1961. Correlations between chaeta number and fly size in *Drosophila melanogaster*. *Heredity*, 16, 349-354.
- GIBSON, J. B., AND THODAY, J. M. 1963. Effects of disruptive selection. VIII. Imposed quasi-random mating. *Heredity*, 18, 513-524.
- HECHT, M. K. 1952. Natural selection in the lizard genus *Aristelliger*. *Evolution*, 6, 112-124.
- L'HÉRITIER, P. 1937. Étude de variations quantitatives au sein d'une espèce; *Drosophila melanogaster*. *Arch. Zool. Exp. Gén.*, 78, 255-356.
- L'HÉRITIER, P., AND TEISSIER, G. 1933. Étude d'une population de *Drosophiles* en équilibre. *C.R. Acad. Sci.*, 198, 770-772.
- JAYANT, K. 1966. Birth weight and survival; a hospital survey repeat after 15 years. *Ann. Hum. Genet. (Lond.)*, 29, 367.
- KARN, M. N., AND PENROSE, L. S. 1951. Birth weight and gestation time in relation to maternal age, parity and infant survival. *Ann. Eugen. (Lond.)*, 16, 147-164.
- LAWRENCE, M. J. 1964. Environment and selection in *Drosophila melanogaster*. *Heredity*, 19, 105-124.
- MATHER, K. 1953. The genetical structure of populations. *Symp. Soc. Expl Biol.*, 7, 66-95.
- MATHER, K., AND HARRISON, B. J. 1949. The manifold effect of selection. *Heredity*, 3, 1-52, 131-162.
- MAYR, E. 1963. *Animal Species and Evolution*. The Belknap Press of Harvard University Press.
- MERTENS, R. 1947. Studien zur Eidonomie und Taxonomie der Ringelnatter (*Natrix natrix*). *Abhandl. senckenberg. naturforsch. Ges.*, 476, 1-38.
- PLUNKETT, C. R. 1926. The interaction of genetic and environmental factors in development. *J. Exp. Zool.*, 46, 181-245.
- RASMUSON, M. 1955. Selection for bristle number in some unrelated strains of *Drosophila melanogaster*. *Acta Zool. Stockholm*, 36, 1-49.
- ROBERTSON, A. 1955. Selection in animals: synthesis. *Cold Spr. Harb. Symp. Quant. Biol.*, 20, 225-229.
- SISMANIDES, A. 1942. Selection for an almost invariable character in *Drosophila*. *J. Genet.*, 44, 204-215.
- THODAY, J. M. 1958. Homeostasis in a selection experiment. *Heredity*, 12, 401-415.
- THODAY, J. M. 1959. Effects of Disruptive selection. I. Genetic flexibility. *Heredity*, 13, 187-218.