

Selection for and against a canalized phenotype in *Drosophila melanogaster*

By S. S. Y. YOUNG

*C.S.I.R.O., Division of Animal Genetics, Delhi Road, North Ryde, N.S.W.,
Australia*

(Received 22 August 1966)

1. INTRODUCTION

The existence of canalization of development of different traits in *Drosophila* (Waddington, 1953; Rendel, 1959; Smith & Sondhi, 1960; Fraser, 1963) and in mice (Dun & Fraser, 1959) has been well established. The above authors were able to show that once the canalization with respect to a trait was removed the canalized character became more variable and successful selection could be carried out.

It was suggested by Dun and Fraser that the degree of canalization of a trait was dependent on the action and dosage of major genes; thus, with respect to the vibrissae, wild-type mice (+/+) were more canalized than mice heterozygous for the tabby gene (Ta/+), who, in turn, were more canalized than the homozygous Ta/Ta animals. On the other hand, Rendel (1959) produced evidence to show that the degree of canalization of the scutellar bristles in *Drosophila* was determined by the expression of the character; the number of scutellars was canalized at the four-bristle level, in both the wild-type and the scute genotypes, despite the fact that the mean and the variance of scutellars in a scute population were both greatly different from those in wild-type populations. He further proposed that the strength of canalization of scutellar bristles could be measured by the probit distance between the three-bristle and five-bristle thresholds (Rendel, 1962).

Rendel & Sheldon (1960) and Rendel, Sheldon & Finlay (1966) showed that it was possible to develop a new level of canalization of scutellars, by a suitable method of selection. They found that after more than 120 generations of selection for high and low variances around a mean of two bristles, the low variance line had a width of zone for two bristles, measured in probit units, about twice that of the same zone in the high variance line. The low line thus selected was also less sensitive to changes in temperature. Since a new level of canalization can be created by selection, it seems reasonable to expect that the existing 'natural' zone of canalization at four scutellar bristles could also be altered by selection. The present paper reports an attempt to increase and decrease the strength of canalization in two selection lines.

2. MATERIAL AND METHODS

Variation in scutellar bristles in a population may be considered as a result of interaction between the degrees of canalization of development and the genetic variation in potential bristle numbers in that population. It is postulated therefore, that a fly which has other than four bristles may deviate from this value for two reasons; either it has a 'make' (Rendel, 1962) which deviates too far from the mean of the population or it is poorly canalized. This being so, then on the average, flies with four bristles are better canalized than those with bristle number other than four. If this were true then the offspring produced by parents each with four bristles would be expected to be better canalized than offspring produced by matings of flies with three and five bristles, if the degree of canalization were heritable.

The two groups of offspring might differ in bristle counts due to the difference in degrees of canalization between groups, even though their mid-parent values were identical.

In order to test this hypothesis, it was necessary to establish selection lines from a stock of flies with a large number of individuals having three and five bristles so that the matings mentioned earlier would be carried out. A suitable stock, which was a sample taken from a population selected for high scutellars, was kindly supplied by Drs J. M. Rendel and B. L. Sheldon. The population was maintained by matings of scw^{bl}/yw ♀♀ × scw^{bl} ♂♂ and cultured at 25°C. The distribution of the number of scutellars in different genotypes, observed from a sample taken from the original population was as follows:

Genotype	Number of bristles				
	2	3	4	5	6
scw^{bl}/yw (♀♀)	0	0	42	31	11
scw^{bl}/scw^{bl} (♀♀)	2	14	92	0	0
scw^{bl} (♂♂)	2	18	136	0	0
yw (♂♂)	0	0	62	35	11

From this sample scw^{bl}/yw ♀♀ and scw^{bl} ♂♂ were selected at random and mated in five bottles. The offspring of this mating were reared at 25°C. and selected as parents for three selection lines. The selection lines were:

- (1) The canalization line (N) Maintained by matings of scw^{bl}/yw (♀♀) × scw^{bl} (♂♂) all with four scutellars.
- (2) The decanalization line (D) Maintained by matings of scw^{bl}/yw (♀♀) with five scutellars × scw^{bl} (♂♂) with three scutellars.
- (3) The control line (C) Maintained by matings of scw^{bl}/yw (♀♀) × scw^{bl} (♂♂) selected at random.

Each line consisted of six cultures, and four pairs of parents were mated in each culture. In every generation the parents were discarded after 7 days of mating.

On the 11th day progeny flies found in each bottle were scored and discarded. Virgins were collected at about 5-hour intervals beginning from the 11th day. As each mating produced four genotypes (scw^{bl}/scw^{bl} ♀♀, scw^{bl}/yw ♀♀, scw^{bl} ♂♂, yw ♂♂), about 100 or more offspring in each genotype and in each line were scored in every generation. Scoring was done by counting the number of scutellar bristles as well as by noting the positions of the bristles in each fly on prepared bristle pattern sheets.

On day 15 a new generation was set up. To minimize inbreeding each bottle was labelled and the selected flies of each sex from each bottle were first kept in separate vials. When sufficient numbers of parents were available, male and female flies selected from two different bottles were used to set up a new culture, according to a cyclical design. The same routine was followed for eighteen generations.

Matings of parents in each line followed the description given earlier, except in generations 3 and 5 where a male with two bristles and a female with six bristles were used in the D line on each occasion, because insufficient numbers of suitable flies were found during the period of collection.

3. RESULTS

(i) *Variations between bottles within each generation*

Variation between bottles within each line and in each genotype was first examined. Analyses were made on data obtained from generations 1 and 10; in each generation for each genotype, within each line, the data were analysed by one-way analysis of variance, and twelve such analyses (3 lines \times 4 genotypes), with unequal sub-class numbers, were performed for each generation. The mean contribution of the variation among bottles to the total variance, calculated by expressing the 'between bottles' variance component as a fraction of the sum of all components, amounted to only about 2% and 3%, for generations 1 and 10 respectively. Variation among bottles was therefore ignored in subsequent analyses.

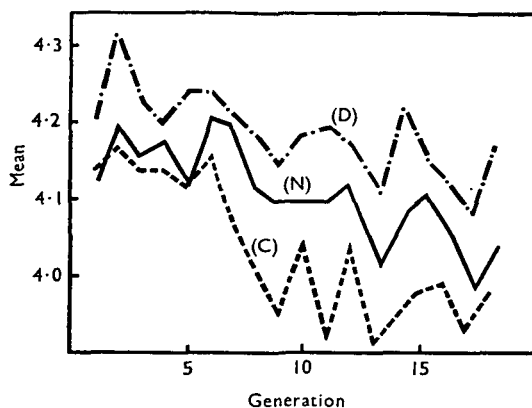
(ii) *Changes in mean*

Overall means of each line in different generations were calculated and plotted in Fig. 1*a*. Each value was calculated as the unweighted mean of the means for the four genotypes (*sc*, *sc.sc*, *yw* and *sc.yw*) in each line, the unweighted mean being preferred because differences in means among genotypes were large and unequal numbers of flies were scored for different genotypes.

There was a general decline in mean bristle numbers with increasing generation number over all the lines. As the selection differentials for bristle number in the N and the D line in each generation were almost always negative, the decline in these lines was expected. In addition, the original population from which the lines were derived had been selected for high bristle number, so that a general regression of the means (Latter, 1963) in all the lines was not surprising. It is interesting to note that the values for the control line showed a greater rate of

decrease than the N and the D lines. It seems probable that the selection for a fixed mid-parent value, practised in the selected lines, although slightly negative, had prevented a more severe regression, due to the exclusion as parents of flies having very low bristle counts. The means of the D line were consistently higher than those of the N line, but the difference between the lines remained more or less unchanged throughout the generations.

(a)



(b)

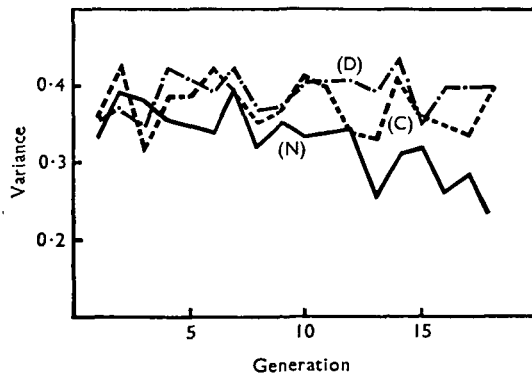


Fig. 1. Means and Variances of scutellar bristles pooled over genotypes in three selection lines.

For all figures (1-4): — = Canalization line (N)
 - · - · - = Decanalization line (D)
 - - - - = Control line (C)

Despite the relatively unsatisfactory performance of the control line, it does reflect some environmental trend common to all lines during the period of selection (Fig. 1). Using data from the C line as corrections for environmental fluctuations, the realized heritability for each of the N and the D lines were 0.05 ± 0.01 and 0.03 ± 0.01 for the N and the D lines respectively. Both values differed significantly from zero but not from each other. The estimates may be biased, however, because of the behaviour of the C line mentioned earlier.

It was pointed out by Rendel (1959) that the score for scutellar bristles was extremely non-linear; the interval between three and five bristles, measured in standard deviation units, was many times greater than those between other numbers. Hence higher heritability values than those estimated here might be obtained if probit transformations were first carried out. However, in eighteen generations of the present material, only a few wild-type flies with three bristles and a few scute flies with five bristles were found. The data therefore were unsuitable for probit transformations, as the width of the four-bristle zone from most

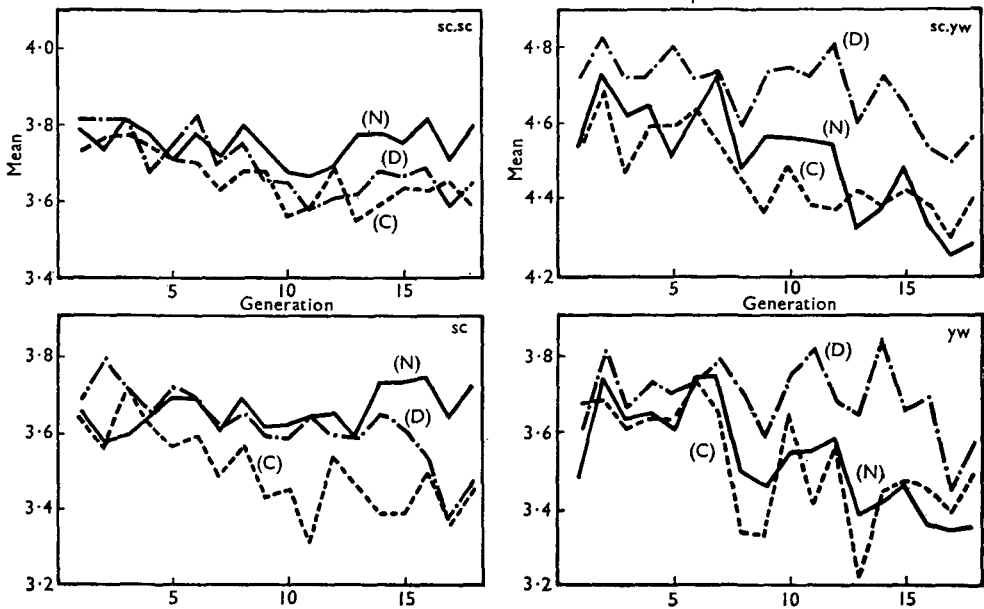


Fig. 2. Means of scutellar bristles for each genotype in three selection lines.

generations could not be estimated. The heritability values calculated here should therefore be considered as minimal estimates.

The mean bristle numbers for each genotype, over the generations, are plotted in Fig. 2. In the early generations, no apparent differences in means were found between lines. But after seven generations of selection, the means of the scute flies in the N line began to show higher values than those for the D line, and the differences increased with selection until the end of the experiment. In wild-type flies the situation was reversed; the D line flies had higher means than those for the N line from the beginning of the experiment, and the differences increased with generations of selection. Analyses of variance were carried out within each genotype and generation. Each analysis partitioned the total sum of squares into the between- and within-lines portions and the usual test of significance was applied (detailed analyses are not presented here). In three genotypes (*sc*, *yw* and *sc.yw*)

mean differences between lines were mostly significant after seven to eight generations and in *sc. sc* females the differences were usually significant after twelve generations of selection.

(iii) *Changes in variance*

Changes in overall variance in different lines are shown in Fig. 1*b*, where it can be seen that the variance for the D and the C line showed no consistent changes, and the values fluctuated around 0.35 from generation to generation. The variance for the N line however showed a general decline after seven generations of selection,

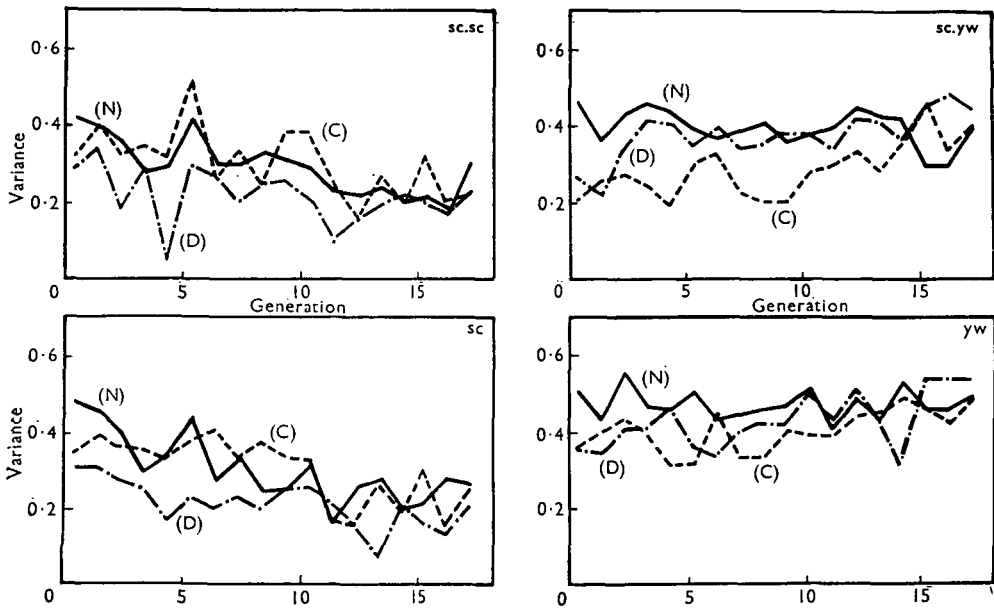


Fig. 3. Variance of scutellar bristles for each genotype in three selection lines.

and towards the end of the experiment the difference in variance between the D and the N line was very marked. Variances for each genotype, within selection lines, over the generations were plotted in Fig. 3, and it was apparent that the variance in the D line was in general higher than that for the N line in all genotypes. Over all lines, the variance for the scute flies increased with selection, while that for the wild-type flies, decreased with selection. The differences in variance between the D and the N line were inconsistent in early generations, but by the ninth generation the variances of the D line were almost always higher in all genotypes.

(iv) *Bristle distribution*

The data were examined in terms of the model proposed by Rendel (1962), mentioned earlier. As pointed out before, the widths of the four-bristle zones, in the present material, often cannot be estimated. Among the few cases where it

could be, the values ranged between 2.4σ to 3.5σ . As these values were obtained from different lines, genotypes and generations, no meaningful comparisons could be made. In addition, estimates were based on one or two flies in the terminal classes, the total number of flies involved for each estimation being always less than 200, and estimates therefore had large standard errors.

One possible, though less satisfactory, way to observe the effect of selection on canalization was to calculate changes in proportions of flies having four scutellars in different lines. The proportions were calculated and are plotted in Fig. 4.

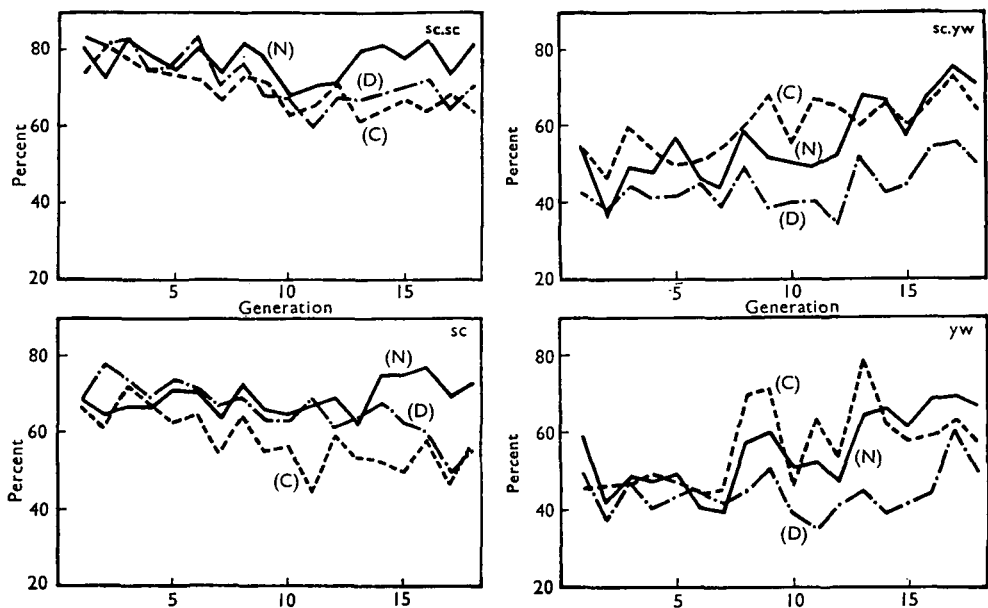


Fig. 4. Number of flies with four bristles, expressed as a percentage of the total number of flies, for each genotype in three selection lines.

Selection increased the proportion in the N line relative to the D line, and differences between lines were appreciable in the later generations. Consistent differences between lines in the wild-type flies were observed from as early as the third generation (*sc.yw*), but in the scute flies such differences occurred only after seven (*sc.sc*) and thirteen generations.

Analysis of variance on the proportion of flies with four bristles was carried out. A three-way (genotypes, lines and generations) fixed model was used, which included the three main effects and all the first-order interaction terms, the second-order interaction being used as the error term. (The analysis is not reported in detail here.) The results showed that all the main effects and the first-order interaction terms were very highly significant ($P < 0.001$).

In wild-type flies a decrease in the proportion of four-bristle flies tends to increase the mean bristle number, while in the scute flies such a decrease would be accompanied by a decrease in mean. Hence, relative to the N line, the mean for the scute

flies in the D line tended to decrease with selection, while the mean for the wild-type flies in the same line tended to increase. The differential response to selection in the two selection lines in bristle number would therefore be attributable to changes in proportion of four-bristle flies in different directions.

Probit values for the three- and the five-bristle zones were also calculated. The widths of these zones in different lines were of interest since they are immediately adjacent to the canalization zone. The estimated values showed no consistent difference between lines or between generations. The means of the estimates for the widths of three- and five-bristle zones are shown in Table 1. There is a faint suggestion of higher means for both the three- and five-bristle zones in the N line but the differences were negligible.

Table 1. *Mean probit distances of the three-bristle zone in scute flies and the five-bristle zone in wild-type flies*

	Scute flies (width of three-bristle zone)		Wild-type flies (width of five-bristle zone)	
	<i>sc</i>	<i>sc.sc</i>	<i>yw</i>	<i>sc.yw</i>
N	1.30	1.32	1.31	1.32
D	1.29	1.28	1.27	1.25
C	1.23	1.32	1.30	1.22

(v) *Changes in bristle patterns*

Selection of parents in the D and N lines with respect to bristle patterns, followed a fixed rule throughout the experiment; flies for the N line were selected for having four bristles on the usual sites, while in the D line the selected wild-type females always had one bristle situated on a site adjacent to one of the anterior sites, in addition to the four bristles grown on normal sites; males in the same line were selected for having three bristles situated in normal sites and usually had two anterior and one posterior bristles.

Bristle patterns of four-bristle flies were examined for 'normal' and 'abnormal' patterns. The abnormal patterns referred to flies with four bristles but having one or two usual sites unoccupied by bristles. In the present material, all flies with abnormal patterns were found to have additional bristles grown on anterior sites, while leaving one or both posterior sites empty of bristles.

Throughout the eighteen generations no abnormal patterns were found in the wild-type flies, but the proportions of flies having abnormal patterns were large in scute flies. In the D line the proportion increased with selection, while in the N line the proportion decreased and towards later generations the differences between lines were large. For example, in generation 18, the proportion in *sc* males for the D line was 28%, while those in the C and the N lines were only 12% and 9% respectively. Similar differences were also found in *sc.sc* females. The proportions for each line in each generation are shown in Table 2.

Table 2. *Proportions of flies with abnormal patterns among all flies with four scutellar bristles in different lines*

Generation	<i>sc.sc</i> females			<i>sc</i> males		
	N	D	C	N	D	C
1	29.8	23.2	24.2	17.7	9.3	10.3
2	18.0	34.7	24.7	25.6	17.9	19.4
3	35.4	27.9	37.5	12.8	17.5	13.3
4	28.0	38.1	21.7	14.8	23.5	17.4
5	24.3	21.6	22.8	15.2	17.0	11.7
6	27.4	31.1	31.2	22.8	19.8	12.2
7	31.2	35.4	30.8	13.6	10.8	13.0
8	31.0	38.0	36.8	10.5	16.1	13.6
9	33.3	31.8	20.5	5.6	17.1	14.4
10	37.8	36.9	22.5	11.7	18.8	11.5
11	30.6	44.0	28.7	13.6	21.7	14.3
12	26.4	44.7	30.6	10.0	27.7	7.1
13	27.9	38.9	37.2	13.5	21.6	6.2
14	23.4	42.5	28.6	12.9	25.9	7.0
15	20.6	39.7	27.6	11.5	27.5	8.0
16	17.1	38.7	27.0	12.3	21.0	12.6
17	25.0	49.6	39.7	13.6	20.4	10.3
18	24.5	40.6	22.8	8.5	27.7	12.0

Analysis of the results using the three-way analysis of variance of the fixed model (genotypes, lines and generations) mentioned earlier showed very highly significant differences ($P < 0.001$) between lines and between genotypes, while all the other terms were non-significant.

4. DISCUSSION

The selection methods used in the N and the D lines had resulted in two populations differing in many characteristics with respect to scutellar bristles. Relative to the N line, the D line showed a greater variation, a lower proportion of four-bristle flies and a higher proportion of four-bristle flies with abnormal patterns. The lower proportion of flies having four bristles in the D line, in turn had resulted in a relatively higher mean bristle number in the wild-type flies and in a lower mean in the scute flies.

The results may be explained by a relative decrease in the developmental stability of the phenotypes in the D line so that there was, in this line, an increase in the number of scute flies having abnormal patterns. The relatively lower proportion of four-bristle flies in the D line may be taken as an indication of a narrower canalization zone as compared with the N line. We may, therefore, conclude that the N line at the end of the experiment was relatively more canalized than the D line.

An alternative hypothesis, however, may also be used to explain the same results. In this hypothesis it is assumed that there are two classes of genes which jointly affect the potential bristle number in flies; the general modifiers which affect flies

of all genotypes and the specific modifiers which affect only the bristle of one particular genotype. The existence of specific modifiers may be inferred from Rendel's (1959) experiment, where the effect of selection in a scute background was not immediately felt by the wild-type sibs. In the present experiment, no differential selection for general modifiers was practised in the selection lines, but the methods of selection tended to affect specific modifiers in the two lines, in different fashions. In the N line where high scute males and low wild-type females were always selected, the method would promote an increase in the mean of the scute flies, while at the same time tending to decrease the mean of the wild-type flies. In the D line, where a low scute fly was always mated with a high wild-type fly, selection would have had the opposite effect on means. Using Rendel's (1962) model of overlapping normal distributions of scutellar bristle 'make' and fixed thresholds, we may imagine that the scute and the wild-type flies in the original population constituted two normal distributions, with a sizeable portion of each distribution, the four-bristle part, overlapping each other. In the D line, selection for specific modifiers was causing the mean make of the scute distribution to move to the negative direction, and moving the mean make of the wild-type distribution to the positive direction. Now since the bristle thresholds were fixed, a decrease in the proportion of four-bristle flies in both genotypes of this line followed. In the N line reversed movements of the means were accompanied by an increase in the proportions of four-bristle flies in both genotypes. It followed that in the D line there was an increase in the proportion of flies having five or more bristles in the wild-type flies, and an increase in the proportion of flies having three or less bristles in the scute genotype. Within each genotype in the D line the population became more variable, and a relatively higher variance of bristle counts was a direct consequence.

In order to distinguish between the two hypotheses we required direct measures of the relative strength of canalization in the two lines, namely the relative width of the four-bristle zones. Such information as mentioned earlier, was not available from data collected during active selection. It was thought that if the widths of the canalization zones were altered during selection, such changes might be reflected by changes in widths of the zone immediately adjacent to the four-bristle zone. Examination of Table 1, however, failed to reveal any real differences between lines in the widths of the three- and of the five-bristle zones.

The relative width of the four-bristle zone was measured at the end of the experiment, but the measurements were not undertaken until after the lines had been relaxed for eight generations. With the kind assistance of Dr Sheldon, two large batches of cultures were set up on two successive occasions, as very large numbers of flies were needed. The first batch consisted of ten cultures per line with ten pairs of parents ($scw^{bl}/yw \times scw^{bl}$) in each culture and the second batch consisted of fifteen cultures per line with six pairs of parents in each culture. The results were disappointing, for although about 30,000 flies were scored, the width of the four-bristle zone, in many cases, still could not be estimated. The scores obtained from the two batches of flies were pooled and the widths of the four-bristle zones were calculated and summarized in Table 3.

Table 3. *Widths of the four-bristle canalization zone in probit units in different lines*

Genotype	Lines	Total number of observations	Widths of zone	Standard error
<i>sc.sc</i> females	N	1588	(4.45)	0.30
	D	1782	(3.84)	0.28
	C	1640	(3.96)	0.29
<i>sc.yw</i> females	N	2844	4.08	0.21
	D	3023	(4.16)	0.29
	C	3062	3.69	0.17
<i>sc</i> males	N	2938	4.27	0.29
	D	3169	(3.62)	0.28
	C	2822	(3.67)	0.27
<i>yw</i> males	N	2704	3.99	0.21
	D	2834	4.07	0.27
	C	2436	3.42	0.18

Note: Values in parentheses refer to hypothetical minimal values. For calculation of such values see text.

The values appearing in Table 3 in parentheses were hypothetical minimal values, as in these cases a hypothetical fly was assumed to exist in each terminal class; thus when a population consisted only of flies having bristle numbers greater than or equal to four, a three-bristle fly was assumed; similarly in another population where the counts were always four or less, a five-bristle fly was assumed to exist. This was done to facilitate probit transformation, as a minimum value can be estimated by these means.

There is no evidence of any real or consistent differences between lines in the widths of the four-bristle zone. Such lack of difference could be due to regression of zone widths following relaxation of selection. However, the scarcity of flies with three bristles in the wild-type populations and of five-bristle flies in the scute genotypes, both during active selection and after eighteen generations of selection, does indicate that the strength of canalization in different lines has not been substantially altered by selection.

It may be asked how, if the developmental stability of the flies in the D line had not been appreciably altered, did the higher proportion of abnormal bristle patterns in scute flies come about? One explanation may be found in the method of selection for scute parents used in the D line. In this line the scute parents always had only three bristles and almost always had one of the posterior bristles missing. Selection in the D line among scute genotype therefore might be partly acting on specific modifiers in such a way as to lower the average bristle-making ability at the posterior sites. This is possible, as selection for a different pattern of scutellar bristles, without changing the bristle mean, has been demonstrated by Finlay (1965). If this did, in fact, happen in the scute flies, then when sufficient bristle make was available to form four bristles, competition between sites (Rendel, 1965)

could have led to a higher proportion of flies having more anterior than posterior bristles and, as a result, a higher proportion of four-bristle scute flies having abnormal patterns.

It appears then that results obtained from the experiment can be adequately accounted for by the effects of different selection methods on specific modifiers without recourse to changes in the strength of canalization. Indeed it may be concluded that of the two hypotheses (canalization and specific modifiers) available to explain the results, the evidence favours the one concerning specific modifiers.

SUMMARY

Selection for and against the canalized phenotype in scutellar bristles was attempted in two selection lines and a randomly selected line was used as control. The selection lines were the Decanalization line (D) and the Canalization line (N). The D line was maintained by matings of scute males (*scw^{bl}*) with three scutellars with wild-type females (*scw^{bl}/yw*) with five bristles, in the N line scute males with four bristles were mated with wild-type females also with four bristles, while in the C line males and females of the above genotypes were selected at random. The lines were established from a sample of flies taken from a line selected for high scutellar numbers.

After eighteen generations of selection the C line was characterized by a regression of mean bristle number without appreciable change in variance. Relative to the N line, the D population showed a lower proportion of flies having four scutellars, a higher variance in bristle numbers, and a higher proportion of four-bristle scute flies having abnormal patterns.

Two alternative hypotheses were advanced to account for the results of this experiment. The first postulated a relative change in the widths of the four-bristle canalization zones in the selection lines, while the second suggested a relative change in frequencies of specific modifier genes for scutellars in scute and in wild-type genotypes of the lines. The evidence favours the latter hypothesis.

This work was done during the tenure of a C.S.I.R.O. Overseas Studentship held at the Biology Department, University of Rochester, N.Y. Grateful acknowledgements are made to Professor R. C. Lewontin (now of University of Chicago) for his helpful comments and interest during the course of this investigation and to the Department for the facilities they made available to me. Thanks are also due to Miss E. Smith for computational assistance.

REFERENCES

- DUN, R. B. & FRASER, A. S. (1959). Selection for an invariant character, vibrissa number in the house mouse. *Aust. J. biol. Sci.* **12**, 506–523.
- FINLAY, D. E. (1965). Selection for pattern of an artificially canalised character. *Am. Nat.* **99**, 431–438.
- FRASER, A. S. (1963). Variation of scutellar bristles in *Drosophila*. I. Genetic leakage. *Genetics*, **48**, 497–514.
- LATTER, B. D. H. (1963). Genetic homeostasis and the theory of canalisation. In *Statistical Genetics and Plant Breeding* (W. D. Hanson and H. F. Robinson, eds.). *National Academy of Sciences—National Research Council Pub.* **982**, 455–467.

- RENDEL, J. M. (1959). Canalisation of the scute phenotype of *Drosophila*. *Evolution, Lancaster, Pa.* **13**, 425–439.
- RENDEL, J. M. (1962). The relation between gene and phenotype. *J. Theoret. Biol.* **2**, 296–308.
- RENDEL, J. M. (1965). Bristle pattern in scute stock of *Drosophila melanogaster*. *Am. Nat.* **99**, 25–32.
- RENDEL, J. M. & SHELDON, B. L. (1960). Selection for canalisation of the scute phenotype in *D. melanogaster*. *Aust. J. biol. Sci.* **13**, 36–47.
- RENDEL, J. M., SHELDON, B. L. & FINLAY, D. E. (1966). Selection for canalisation of the scute phenotype II. *Am. Nat.* **100**, 13–32.
- SMITH, J. MAYNARD & SONDEHI, K. C. (1960). The genetics of a pattern. *Genetics*, **45**, 1039–1050.
- WADDINGTON, C. H. (1953). Genetic assimilation of an acquired character. *Evolution, Lancaster, Pa.* **7**, 118–126.