SELECTION AND THE EFFECT OF TEMPERATURE ON SCUTELLAR BRISTLE NUMBER IN *DROSOPHILA*¹

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SELECTION of a genotype which gives rise to similar phenotypes in different environments is one evolutionary strategy which may result in high adaptedness of a population to the environments in which it lives. Presumably, the stability of certain traits is the outcome of a long history of natural selection tending towards an adaptive phenotype. Several investigators have attempted to select strains of *Drosophila* for phenotypic stability of variable traits (FALCONER 1957; THODAY 1959, 1960; RENDEL and SHELDON 1960; PROUT 1962; SCHARLOO 1964). Interestingly, the stability of a trait with respect to small (microenvironmental) and to more drastic (macroenvironmental) variations generally seemed to be independent. When selection reduced phenotypic variance in a given environment, responsiveness to macroenvironmental variables was not affected. Likewise, selection for decreased responsiveness to major environmental differences did not increase microenvironmental buffering (WADDINGTON 1960; KINDRED 1965). RENDEL and SHELDON (1960) obtained different results. They succeeded in reducing the phenotypic variance of scutellar bristle number around a mean of two bristles in scute *Drosophila*. This reduction in variance at 25°C was accompanied by lowered sensitivity of bristle number to temperature changes.

The experiments reported in the present article involved selection for altered temperature responsiveness of scutellar bristle number and were designed to study the relation between microenvironmental and macroenvironmental buffering. These experiments are in some ways similar to those of KINDRED (1965) although they were done independently.

MATERIALS AND METHODS

Flies from an unselected *sc w^{bl}* stock used by KINDRED (1965) and by RENDEL and SHELDON (1960) provided the basic material for this investigation. In order to assure a heterogeneous background, *sc w^{bl}* females were crossed in separate groups to wild-type males from two Florida and two Syracuse strains. Flies homozygous for *sc w^{bl}* were re-extracted from the F₂ of each cross, and crosses among these flies provided the foundation stock for the selection. Flies from this stock raised at 18°C had a distinctly higher mean scutellar bristle number than replicate samples raised at 28° (Figure 1).

Four lines were derived from the foundation population: two lines (H1 and H2) were selected for increased difference in mean scutellar bristle number between 18° and 28°C, and two lines (L1 and L2) were selected in the opposite direction. For each selection line, family selection was practiced in accordance with the cyclical mating scheme used by RENDEL and SHELDON

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(1960). Cultures were designated A, B, C and D. Each generation, 20 subcultures were made involving five pair matings each between A females $\times$ D males, B females $\times$ A males, C females $\times$ B males, and D females $\times$ C males, resulting respectively in subcultures A_{1-5} , B_{1-5} , C_{1-5} and D_{1-5} . Each generation, eggs were collected at 25° within 24- to 30-hour intervals and were placed at test temperatures of 18° and 28°, and, for propagation, at 25°. The family that showed the greatest (or the least) difference in means between 18° and 28° among the five pair-matings from each subline provided the parents for the next generation. Usually, 30 females and 30 males were scored from each pair-mating at each temperature, totalling about 1200 flies scored at each temperature for each line per generation. Parents for the next generation were chosen from samples raised at 25°. An attempt was made to keep the mean bristle number constant at 25° by mating females having two bristles and males with one or two bristles at 25° each generation. Parents with specific bristle patterns were chosen in order to evaluate any influence that position of scutellar bristles might have on the outcome of the selection. Whenever possible, parents having anterior bristles only were used in H1 and L1, whereas flies with anterior and posterior bristles on opposing sites were mated in H2 and L2. Lines H1 and L1 were run simultaneously, and H2 and L2 likewise. Due to incubator failures, data for generations 21 for L1 and H1, and generations 14 and 15 for L2 and H2 are not available.

RESULTS

Selection response: Figure 1 illustrates that selection has produced populations that differ strikingly in their responsiveness to the 18° and 28°C temperature range, as measured by the selection criterion. For both sets of lines, the greatest response was in the direction of lowered phenotypic responsiveness to temperature. By generation 10, flies from the L2 line had the same mean scutellar bristle number at 28° as at 18°. This lack of responsiveness was also achieved in L1 but at a later generation. It was especially interesting to observe families exhibiting a higher mean bristle number at 28° than in replicate samples raised at 18°. When such families were observed, parents for the next generation were chosen from those families showing the greatest negative response. This apparent reversal of usual temperature effects has been consistently maintained in L1 for nine generations (except for generation 28) for both sexes, and for 11 consecutive

FIGURE 1.—Difference between mean scutellar bristle number of replicate samples raised at 18° and 28°C in successive generations.

generations for L1 males. Line L2 showed this effect for 14 out of 16 generations scored (after generation 10) for females; males did not show this effect regularly. Selection for increased reversal of the temperature effect has not been particularly successful thus far, although data for L1 males suggest a consistent trend in this direction during later generations.

The actual means of samples raised at 28° and at 18° each generation are shown in Figures 2 and 3. The lowered responsiveness to temperature of L1 is due mainly to a decrease in mean scutellar bristle number at 18° without a corresponding decrease when replicate samples are raised at 28°. In fact, during the early generations, there was an increase in the mean at 28°. In L2, the decreased responsiveness was apparently at first the result of an increase in mean at 28° and a decrease at 18°, but, after generation 10, the change in mean of samples raised at 18° roughly paralleled the trend at 28°. Although erratic, high-line means tended to drift towards higher values at 18° without as much change at 28°, thus accounting for the observed increase in responsiveness.

Means and variances at 25°: Figure 4 shows that means for L2, H1 and H2 at 25° fluctuated erratically. Although high and low lines were apart for a number of generations, they did not tend to diverge from each other in any consistent manner. Line L1 maintained a fairly constant mean at 25° throughout the experiment.

The pooled within-bottle variance at 25° for each generation scored in the present study is shown in Figure 5. The drop in variance seen in L2 males can be attributed to the lowered mean bristle number at 25° after generation 13, since flies pile up in the zero-bristle class, thus decreasing the variance. However, a change in mean is not a sufficient explanation for the decrease in variance observed in L1 females, since the mean value remained fairly constant at 25°.

FIGURE 2.—Mean scutellar bristle number of replicate samples raised at 18° and 28°C in successive generations for females.

FIGURE 3.—Mean scutellar bristle number of replicate samples raised at 18° and 28°C in successive generations for males.

FIGURE 4.—Mean scutellar bristle number of samples raised at 25°C in successive generations.

The measurement of the size of a given bristle class in probits (RENDEL 1959; RENDEL and SHELDON 1960) has been used as an index of the degree to which canalization has been achieved. Figure 6 reveals that the probit distance spanned by the two-bristle class at 25° in L2 and H2 shows no consistent trend towards altered canalization. This finding is consistent with that of KINDRED (1965). However, line L1 shows an increase in canalization at 25° as evidenced by the increase in probit distance of the two-bristle class; no corresponding change was seen in H1.

Bristle positions: In order to ascertain whether the change in responsiveness of mean bristle number to temperature was related to altered temperature effects on specific bristle sites, bristle positions were scored for samples from each line raised at 28° and 18°. This was done for generation 9 in L1 and H1, and for generation 10 in L2 and H2 (Table 1). Since there are four main sites for bristles, more than two bristles will almost invariably mean that at least one anterior and one posterior site will be occupied. In order to avoid possible interference of bristle number with position, flies with one or two bristles were compared. The index of comparison was the frequency of flies with one or two bristles having a bristle at a specific site. The results presented in Table 1 indicate that the selection did produce differences in temperature effects at specific bristle sites. In all lines, the posterior bristles are discriminated against by higher temperature. A con-

FIGURE 5.—Pooled within-bottle variance for samples raised at 25°C in successive generations.

FIGURE 6.—Probit distance spanned by two-bristle class in samples raised at 25°C in successive generations.

spicuous difference between L1 and L2 is the greater anterior bristle frequency in L1 at all temperatures for both females and males. In fact, at 18°, the frequency of posterior bristles is much higher than anterior bristles in L2; the reverse is true for L1. The differences observed may be related to the mating scheme. Parents with two anterior bristles were used in L1 whereas flies with

TABLE 1

Percent flies with one and two bristles having a bristle at site $\begin{matrix} \text{I} & \text{A} & \text{A}' & \text{I}' \\ & \text{P} & \text{P}' & \end{matrix}$

Temperature	Line	Generation	Mean $\pm$ SE	Site					
				A	A'	P	P'	I	I'
<i>Females</i>									
18°C	...	F*	2.97 $\pm$ 0.02	48	50	38	37	6	4
	L1	9	2.43 $\pm$ 0.04	60	61	32	30	2	2
	L2	10	2.31 $\pm$ 0.04	22	18	57	61	3	4
	H1	9	2.87 $\pm$ 0.04	45	49	42	43	1	1
	H2	10	3.17 $\pm$ 0.03	63	53	30	38	1	1
28°C	...	F*	1.40 $\pm$ 0.04	53	54	18	19	10	9
	L1	9	2.06 $\pm$ 0.03	88	86	6	6	1	1
	L2	10	2.24 $\pm$ 0.04	39	36	38	37	12	13
	H1	9	1.14 $\pm$ 0.04	50	50	15	22	5	3
	H2	10	1.43 $\pm$ 0.04	54	46	16	22	4	4
<i>Males</i>									
18°C	...	F*	2.02 $\pm$ 0.02	31	33	49	49	4	4
	L1	9	1.14 $\pm$ 0.04	47	43	28	30	0.3	0.9
	L2	10	1.03 $\pm$ 0.03	18	13	53	49	2	4
	H1	9	1.89 $\pm$ 0.04	32	33	48	50	2	2
	H2	10	2.46 $\pm$ 0.04	35	31	54	56	1	3
28°C	...	F*	0.56 $\pm$ 0.01	32	35	21	22	10	12
	L1	9	1.14 $\pm$ 0.04	64	61	8	8	0.8	2
	L2	10	0.98 $\pm$ 0.04	29	34	32	25	9	7
	H1	9	0.38 $\pm$ 0.03	42	41	15	14	4	4
	H2	10	0.39 $\pm$ 0.03	42	31	13	25	5	3

* F refers to random samples of about 2900 flies from the foundation stock.

anterior and posterior bristles on opposing sites were mated in L2. However, the H1 line was mated as in L1, and H2 mated as in L2, and yet, the difference found in the low lines was not detected here. Also noteworthy is the relatively high frequency of off-site bristles in L2 and in random samples of flies from the foundation stock at 28°.

Bristle positions were also scored for generation 14 in L1 and H1 and generation 16 in L2 and H2 at 18°, 25°, and 28°. The data are given in Table 2. The relations between L1 and L2 noted above were also observed in these tests. H1 tended towards greater anterior bristle frequency than H2 at 25° and at 28°, but differences in anterior bristle frequency among L1, H1 and H2 were not apparent at 18°.

DISCUSSION

A lack of correspondence between altered sensitivity of a trait to microenvironmental conditions and responsiveness to major environmental changes has

TABLE 2
Percent flies with one and two bristles having a bristle at site

Temperature	Line	Generation	Mean $\pm$ SE	Site					
				A	A'	P	P'	I	I'
<i>Females</i>									
18°C	L1	14	2.37 $\pm$ 0.04	55	55	28	27	6	4
	L2	16	1.46 $\pm$ 0.04	16	17	56	53	3	5
	H1	14	3.04 $\pm$ 0.04	60	47	32	43	5	5
	H2	16	3.23 $\pm$ 0.03	54	59	40	31	5	3
25°C	F*		2.14 $\pm$ 0.03	55	57	24	23	12	10
	L1	14	2.13 $\pm$ 0.03	77	78	12	13	4	3
	L2	16	1.95 $\pm$ 0.04	32	28	40	40	14	14
	H1	14	1.88 $\pm$ 0.04	61	60	18	22	5	3
28°C	H2	16	1.63 $\pm$ 0.04	55	51	20	25	5	6
	L1	14	2.10 $\pm$ 0.03	84	83	10	7	2	4
	L2	16	1.57 $\pm$ 0.04	29	28	36	30	15	17
	H1	14	1.34 $\pm$ 0.04	60	61	14	10	5	4
	H2	16	1.10 $\pm$ 0.04	46	52	15	15	7	8
<i>Males</i>									
18°C	L1	14	1.20 $\pm$ 0.04	35	37	36	30	5	5
	L2	16	0.54 $\pm$ 0.03	12	7	43	49	6	4
	H1	14	2.11 $\pm$ 0.04	35	37	47	39	6	8
	H2	16	2.57 $\pm$ 0.04	33	34	54	53	3	2
25°C	F*		1.33 $\pm$ 0.03	38	37	29	29	10	10
	L1	14	1.36 $\pm$ 0.04	61	63	16	11	5	6
	L2	16	0.72 $\pm$ 0.04	24	28	32	26	10	14
	H1	14	1.03 $\pm$ 0.04	48	52	15	18	4	5
28°C	H2	16	0.72 $\pm$ 0.03	35	36	25	24	3	5
	L1	14	1.16 $\pm$ 0.04	64	65	9	9	2	2
	L2	16	0.55 $\pm$ 0.04	21	30	30	24	10	15
	H1	14	0.56 $\pm$ 0.03	42	43	15	12	6	4
	H2	16	0.28 $\pm$ 0.02	33	34	13	20	6	5

* F refers to random samples of about 1200 flies from the foundation stock.

been observed in the selection experiments of several investigators. PROUT (1962, 1963) achieved altered variance in time of development in *Drosophila*, without changing sensitivity to temperature and to other environmental variables. SCHARLOO (1964) decreased the variance in expression of a cubitus interruptus mutant, without changing the sensitivity of the character to temperature. WADDINGTON (1960) was able to decrease responsiveness of the Bar mutant to large temperature differences; however, developmental stability, as measured by asymmetry of eye facet numbers, was not affected. KINDRED (1965) decreased the responsiveness of scutellar bristle number to a temperature change from 19° to 27°, but no sign of increased microenvironmental buffering was detected within a 25° environment. In contrast to the results of the authors cited above, lowered temperature sensitivity of scutellar bristles occurred when RENDEL and SHELDON (1960) decreased variance of scutellar bristle number around a mean of two bristles in scute flies at 25°.

In the present study, decreased responsiveness of scutellar bristle number to a temperature difference of 10° (from 18° to 28°) was rapidly achieved in the low selection lines; a decrease in the within-culture variance at 25° was observed in the L1 line but not in the L2 line. The results suggest that different genes may be responsible for environment-specific buffering and for general resistance to temperature changes. In the L1 line, we have apparently selected those genes conferring stability against microenvironmental conditions and temperature changes. In the L2 line, the genes selected resulted in decreased temperature sensitivity without altering canalization at 25°. Further evidence that different genetic systems may have been selected in L1 and L2 is the finding that these lines differ in the frequency of bristles at different sites.

It is possible to select for increased frequency of anterior scutellar bristles in scute flies at 25° (DRUGER 1966). The result is an increase in the proportion of flies having only two bristles, and hence, an increase in the probit distance spanned by the two-bristle class. In contrast to KINDRED (1965), in the present experiments the parents each generation were raised at 25° and flies with specific bristle patterns were used. Whenever possible, flies having anterior bristles only were mated in L1 and H1, whereas flies with anterior and posterior bristles on opposing sites were used in L2 and H2. The data in Table 2 indicate that selection did indeed result in greater frequency of anterior bristles at 25° in L1 than in the other selection lines. This may have contributed to the apparent increase in canalization of scutellar bristles around a mean of two bristles in L1 at 25°. However, parents with anterior bristles were also used in H1, but no canalization at two bristles was observed at 25°. Apparently, selection in the direction of increased sensitivity of scutellar bristles to temperature was not compatible with the canalization at two bristles that can possibly result from selecting flies with anterior bristles.

Selection for a greater frequency of anterior bristles does not seem to provide a sufficient explanation for RENDEL and SHELDON's (1960) canalization of scutellar bristles about a mean of two. Examination of 1000 two-bristle flies in RENDEL and SHELDON's low and high variance lines revealed no differences in frequen-

cies of bristles at different sites, although both lines had more anterior bristles than the population from which they were derived (DRUGER, unpublished). Also, FINLAY (1965) was able to select for increased frequency of flies with one anterior bristle and one opposite posterior bristle in the low variance line, without decreasing the degree of canalization. Thus, in the experiments of RENDEL and SHELDON (1960), the amount of hypothetical bristle-making "precursor" may have been the character selected, bristle positions being of secondary importance.

An interesting feature of the present experiments was the tendency of families to exhibit a higher mean scutellar-bristle number at 28° than in replicate samples raised at 18°. This phenomenon was also observed by KINDRED (1965), but was not as pronounced as in the present experiments. Also noteworthy in both sets of experiments was the striking asymmetry of the response to selection. Such asymmetry of response commonly occurs in two-way selection experiments, but the explanation for it is obscure.

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SUMMARY

In scute populations, the mean number of scutellar bristles decreases with an increase in temperature. This difference is clear among replicate samples raised at 28°, 25° and 18°C. Using family selection and a cyclical mating scheme, two lines were selected for increased difference in means between 18° and 28°, and two lines were selected in the opposite direction. Pair matings were set up each generation and eggs from each pair were placed at 18°, 28° and 25° for development. Parents for the next generation were chosen from those families showing the greatest (or the least) difference between mean scutellar bristle number at 28° and at 18°.—Selection rapidly produced populations that were strikingly different in their sensitivity to the 18 and 28° range, as measured by the selection criterion. The greatest response was in the direction of lowered phenotypic responsiveness to temperature. Although similar lack of responsiveness was attained in two low selection lines, only one line manifested a decrease in within-bottle variance at 25°. Also, differences between the two lines in frequency of bristles on specific sites suggests that the lack of responsiveness has been achieved by different developmental pathways. In both low lines, families were observed that exhibited a higher mean bristle number at 28° than in replicate samples raised at 18°.

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