INFLUENCE OF TEMPERATURE ON THE EXPRESSION OF POSITION EFFECTS IN THE SCUTE-8 STOCK OF *DROSOPHILA MELANOGASTER* AND ITS RELATION TO HETEROCHROMATIZATION

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IN *Drosophila melanogaster* translocation of a euchromatic chromosome region to a heterochromatic is frequently accompanied by an effect, known as a position effect, on the activity of genes located near the point of breakage and reunion.

It has been assumed (e.g. by SCHULTZ 1941; PROKOFYEVA-BELGOVSKAYA 1945, 1947) that position effects of genes located in euchromatic regions are due to, or closely correlated with, "heterochromatization" of the bands (as observed in the salivary gland chromosomes) where the affected loci are supposed to be situated.

In the papers cited, PROKOFYEVA-BELGOVSKAYA gives data on the influence of temperature on the condition of the chromosomes in the scute-8 (*sc*⁸) stock. The region studied was the extreme left end of the X chromosome, including bands 1A1-1B1.2 (BRIDGES' map). By virtue of the long scute-8 inversion, which includes the loci of scute (*sc*) and bobbed (*bb*), these bands while still at the left end of the chromosome, are adjacent to the heterochromatic region 20 ABC.

PROKOFYEVA-BELGOVSKAYA's analysis of the salivary gland chromosomes of female larvae heterozygous for the scute-8 inversion, raised at different temperatures, showed that heterochromatization of the region 1A1-1B1.2 in the scute-8 X chromosome was most frequent at 25°C (71 percent of the nuclei showed the effect), while at 30°C and 14°C this region had the normal euchromatic structure in a majority of the nuclei examined (63 percent and 67 percent, respectively). The results indicate a significant effect of temperature on the phenomenon of heterochromatization.

In view of these results, it seemed of interest to study the influence of temperature on the manifestation of position effects associated with the scute-8 inversion.

The influence of temperature on some position effects is already known. Thus, in the position effect associated with In(2L)40D, affecting the eye, HINTON (1949) finds the most extreme manifestation at 17-23°C in a tested range from 15° to 29°C, with increasing normality as the extremes of temperature are approached. In T(1; 4) *w*²⁵⁸⁻¹⁸, a translocation between the X and fourth chromosomes with a position effect at the white (*w*) locus, the eye deviates more from normality at 25°C than 29°C (DEMEREK and SLIZYNSKA 1937), and deviates still more at 18°C than at 25°C (author, unpublished). Again, unpublished data of the author show no difference in phenotypic ex-

pression of a light (*lt*) position effect, associated with $T(1; 2)w^{258-39}$ at 17°, 25°, and 29°C. As a final example, position effects at the *cubitus interruptus* locus are more extreme at lower temperatures (STERN, MACKNIGHT and KODANI 1946), following the same pattern as the recessive mutant *cubitus interruptus* and the white-mottled 258-18 position effect.

In the first two examples given, the affected loci are located in euchromatic regions which have been transferred to heterochromatin, in the last two the affected loci normally lie near the centromere, and have been removed from a heterochromatic to a euchromatic region. Even from these few examples, it is clear that the influence of temperature on position effects is not consistent. If one assumes a direct relationship between position effect and heterochromatization (or the converse "euchromatization" of heterochromatin), it follows, either that the effect of temperature on heterochromatization varies with different chromosome regions, or that the phenotypic expression of some position effects is modified by temperature, not through its influence on heterochromatization, but through an effect on some other stage in the chain of events which link together the gene and its phenotypic effect.

This paper deals specifically with the influence of temperature on two bristle characters of the scute-8 stock—the Hairy-wing (*Hw*) effect and the scute (*sc*) effect.

The Hairy-wing, or extra bristle effect, is presumably associated with the band 1B1.2 of the X chromosome (see DEMEREC and HOOVER 1939), which is included in the region studied by PROKOFYEVA-BELGOVSKAYA, and adjacent to heterochromatin of 20 ABC.

The scute, or missing bristle effect, is probably associated with the band 1B3.4 (see BRIDGES and BREHME 1944), which is not included in the region in question, but has been transferred to the chromocenter, where it too is adjacent to heterochromatin.

MATERIAL AND METHODS

Homozygous females of the scute-8 stock were mated to *sc cv v f* males (*sc*=scute, *cv*=crossveinless (wing), *v*=vermillion (eye), *f*=forked (bristles)), and were then kept in half-pint bottles at each of the following temperatures: 14°, 18°, 25° and 30°C. The stocks used were not isogenic. The parents of flies raised at 18°, 25° and 30°C were taken from a single bottle of each of the stock cultures within a few days of each other. Female parents were thus of the same generation and likewise male parents. The flies at 18°, 25° and 30°C were raised on a single batch of medium. It was not possible to raise flies at 14°C simultaneously with those raised at the other temperatures. The 14°C cultures were raised on a different batch of medium, from parents derived from the same stock culture bottles and separated not more than three generations from those used in the other experiments.

In order to reduce sampling variations, flies from three or more bottles were counted at each temperature, thus multiplying the number of parents for each sample.

F₁ females (*sc*⁸/*sc cv v f*) and males (*sc*⁸) were scored separately, with regard

to four characters, viz:—1) extra thoracic bristles (*Hw* effect); 2) presence or absence of anterior and posterior scutellar bristles; 3) presence or absence of anterior and posterior supra-alar bristles; 4) presence or absence of anterior, median and posterior sternopleural bristles. These particular bristles were chosen because they are supposed to be the most frequently affected in the scute-8 stock (BRIDGES and BREHME 1944).

Two hundred females and two hundred males were scored at each temperature for all the above characters, with the following exceptions: At 25°C, only 75 of the 200 females and 75 of the 200 males, chosen at random, were scored for scutellar bristles. At 30°C, only 75 of the 200 females were scored for scutellar bristles; while the total number of males examined was 80, and only 65 of these were scored for scutellar bristles.

RESULTS

The data obtained are presented in table 1 and in the form of histograms in figure 1. The number of flies showing a given abnormality is shown in the histograms as a percentage.

TABLE 1
Numbers and percentages of individuals with extra bristles, or lacking specific bristles, at different temperatures.

TEMPERATURE °C	EXTRA BRISTLES				ANT. SCUTELLAR				POST. SCUTELLAR			
	♀		♂		♀		♂		♀		♂	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
14	41	20.5	174	87	0	0	0	0	1	1.3	1	1.5
18	52	26	178	89	0	0	0	0	0	0	0	0
25	9	4.5	157	78.5	1	1.3	6	8	2	2.7	5	6.7
30	68	34	77	96	51	68	64	98.4	7	9.3	54	84.6

	ANT. SUPRA-ALAR				POST. SUPRA-ALAR			
	♀		♂		♀		♂	
	NO.	%	NO.	%	NO.	%	NO.	%
14	5	2.5	100	50	0	0	8	4
18	0	0	48	24	1	0.5	2	1
25	0	0	12	6	1	0.5	2	1
30	0	0	33	41	0	0	0	0

Owing to the much lower incidence of abnormalities in females than in males, it has proved necessary to deal largely with the data for males, but, as will be seen, the data for females provide corroborative evidence in certain important instances.

FIGURE 1.—Histograms showing percentages of individuals with extra bristles, or lacking specific bristles, at different temperatures.

The results may be summarized as follows:

Extra bristles. Females and males show similar reactions to different temperatures, apart from the lower frequency of individual females with one or more extra bristles. The data for females show a significant temperature effect as

between 14° C and 25° C, and as between 25° C and 30° C $\left(\frac{\text{Diff.}}{\text{S.E.}} = 4.8 \text{ and } 7.3 \right)$

respectively, $P = 10^{-5}$ and 10^{-8} respectively). The males show a possibly significant difference between the proportions of affected individuals at 14°C and

25°C $\left(\frac{\text{Diff.}}{\text{S.E.}} = 2.29, P = 0.02-0.03 \right)$ and a clear cut difference as between 25°C

and 30°C $\left(\frac{\text{Diff.}}{\text{S.E.}} = 3.6, P = 10^{-8} \right)$.

Scutellar bristles. All scutellar bristles appear to follow the same general pattern in relation to temperature in both females and males, with a maximum of missing bristles at 30°C, fewer at 25°C and virtually none at the lower temperatures.

The differences in anterior scutellars between 25°C and 30°C are significant

for both females $\left(\frac{\text{Diff.}}{\text{S.E.}} = 11.1, P = 10^{-9} \right)$ and males $\left(\frac{\text{Diff.}}{\text{S.E.}} = 10.4, P = 10^{-9} \right)$.

For posterior scutellars, the difference between 25°C and 30°C is not significant

for females $\left(\frac{\text{Diff.}}{\text{S.E.}} = 1.74, P = 0.08-0.09 \right)$, but significant for males

$\left(\frac{\text{Diff.}}{\text{S.E.}} = 9.27, P = 10^{-9} \right)$.

Supra-alars. A very small number of females with missing anterior supra-alars was obtained but these were all raised at 14°C, the temperature at which the greatest number of affected males was found. The males show a minimum of affected individuals at 25°C, with greater effects at the temperature extremes. The differences between 14°C and 25°C and between 25°C and 30°C are

highly significant $\left(\frac{\text{Diff.}}{\text{S.E.}} = 9.75 \text{ and } 7.1 \text{ respectively. } P \text{ in both cases} = 10^{-9} \right)$.

Missing posterior post-alars were infrequent and the numbers are too small to justify any conclusion about the influence of temperature.

Sternopleurals. Absence of any of the sternopleurals occurred very rarely (in one male at 18°C and in four females and one male at 14°C) and is not represented in the table and histograms.

DISCUSSION

While it would have been desirable to have used isogenic stocks in these experiments, it seems probable that the conclusions drawn below are valid. As it was pointed out under Materials and Methods, the experiments at 18°, 25° and 30°C were carried out under otherwise identical conditions. The comparisons between flies raised at 25° and 30°C are therefore legitimate. Although this is not true of comparisons between 14° and 25°C, these are put on a sounder basis by the fact that, wherever they show a significant difference there is also a significant difference in the same direction between flies raised at 18° and 25°C.

It is clear from what was said in the introduction that one might expect to find correlation between PROKOFYEVA-BELGOVSKAYA's data on heterochromatization and the phenotypic expression of the extra bristle (Hairy-wing) effect, located in the region 1A1-1B1.2 which she studied.

PROKOFYEVA-BELGOVSKAYA found a maximum of heterochromatization at 25°C. One would assume, therefore, that there should be a maximum abnormality of phenotypic expression at this temperature for genes located in the 1A1-1B1.2 region. The present data show that the extra bristle effect has an opposite relation to temperature, with minimum abnormality at 25°C and maxima at the more extreme temperatures.

The scute locus does not lie within the region studied by PROKOFYEVA-

BELGOVSKAYA and would not necessarily be expected to show a correlation with her data.

The different bristles affected do not all show the same relation to temperature. For all except the scutellar bristles the incidence of abnormalities in the females was negligible, and it might be argued that there is some doubt as to whether all missing bristles in the scute-8 males are attributable to an effect of the scute locus, or whether other bristle-determining loci in the same region may not be affected. Similar results were found by CHILD (1935), however, in a temperature study of the scute-1 stock, a mutant scute stock in which the X chromosome is normal and no position effects are involved; so that it seems justifiable to consider the scute locus responsible for the whole missing bristle complex.

In this case, it is evident that one cannot expect any simple relationship between the phenotypic manifestation of the scute effect and heterochromatization of the scute end of the inversion in the scute-8 stock.

The effect of temperature on the phenotype of flies carrying the Hairy-wing duplication is not known. The only bristles affected by the scute locus which are considered in common in this study and CHILD's (*loc. cit.*) are the scutellars, and the data for the anterior scutellars in scute-1 and scute-8 differ markedly. We are, therefore, not in a position to judge the nature of the temperature effect on these position effects—whether it influences them *qua* position effects, or merely affects one or more steps in the chain of “bristle-forming reactions” initiated by the pertinent loci.

SUMMARY

The effect of temperature on the expression of the Hairy-wing and scute position effects in the scute-8 stock was studied in males and females from the cross $sc^8/sc^8 \times sc \text{ cv } v f$, raised at 14°, 18°, 25° and 30°C.

The Hairy-wing effect occurred with least frequency at 25°C and greater frequency at the more extreme temperatures on either side of 25°C.

The different bristles affected by the scute locus were not consistent in their relationship to temperature, scutellar bristles being most frequently absent at the higher temperatures and anterior supra-alars at the lower.

It is concluded that the expression of the Hairy-wing position effect is not directly related to heterochromatization, and that there can be no simple relationship between the manifestation of the scute effect and heterochromatization.

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