GENETIC CONTROL OF AN ANTEROPOSTERIOR GRADIENT AND ITS BEARING ON STRUCTURAL ORIENTATION IN *DROSOPHILA*¹

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ONE of the fundamental problems in developmental biology is: How do structures become oriented in a specific manner? This problem need further elucidation in terms of genetic mechanisms by which the constancy of structural orientation is realized during development. There are, perhaps, two essential prerequisites for attempting to uncover the mechanisms underlying the orientation of structures. First and foremost, is the availability of a simple developmental system, and second, is the possibility of applying precise genetic methods for the developmental analysis of such a system. In this respect bristles of *Drosophila* provide an ideal material. In *Drosophila* the trichogen and tormogen cells (LEES and WADDINGTON 1942) arising out of the division of a single hypodermal cell give rise to a bristle and its socket, respectively. The bristles are innervated (STERN 1938) by peripheral nerve cells which are bipolar and are situated below the hypodermis. Both the large and small bristles, macrochaetae and microchaetae, respectively, arise by similar processes. Since the microchaetae show variation in number, position and orientation and since they are extremely resistant to environmental fluctuations during development, they have served as a material for the present investigations.

In the present experiments, orientation of microchaetae on the abdominal sternites (Figure 1) was studied. The bristles on the sternites are disposed discretely, showing no fixed number, no fixed position and no fixed orientation. An attempt was made to deduce the nature of developmental processes involved in bristle orientation, by selecting for a specific orientation of microchaetae and by observing the range of adult variation. The results of these experiments show that genes are effective in modifying the orientation of sternal bristles and also reveal, to a certain extent, the underlying mechanisms by which this is realized.

MATERIALS AND METHODS

The Swedish wild-type (a mass-cultured strain, devoid of gross chromosomal abnormalities) of *Drosophila melanogaster* was employed. Fertilized females were allowed to lay eggs in fresh culture bottles. The cultures were raised in half-pint milk bottles on a cornmeal-molasses-agar medium with living yeast suspension added. To eliminate inequalities of nutrition due to over-

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crowding in cultures, each female was allowed to lay eggs for a day in a culture bottle and was discarded thereafter. The first ten males and ten females emerging in each of the ten cultures set up initially constituted the foundation population of the present experiments. The orientation of bristles on each sternite of both males and females in the foundation population was recorded. Starting from this population, selection for bristles, oriented anteroposteriorly parallel to the longitudinal axis, was practised only on the fourth abdominal sternite. There is nearly a continuous variation for the direction of orientation of sternal bristles in *Drosophila*. The orientation of bristles was recorded using a micrometer eyepiece. A vertical line was adjusted to pass through the gap between the hind legs and the middle of the anal plates; if the bristles deviated slightly to the right or left from the mid-ventral line drawn in this manner, they were not regarded directed anteroposteriorly parallel to the longitudinal axis. In each generation, individuals with the most favorable phenotype were selected as parents of the next generation. The selected population consisted in each generation of the offspring of ten pairs, mating being rotational. The procedures of rearing flies in cultures and recording the orientation of bristles were the same as in the foundation population. The orientation of bristles on each sternite was recorded after every third generation of selection. The whole experiment was performed at $25^{\circ} \pm 1^{\circ}\text{C}$.

RESULTS

Table 1 shows the percentage of bristles oriented anteroposteriorly on each sternite in the individuals of the foundation population. The orientation of bristles on the sternites examined reveals a particular order, i.e., the frequency of bristles oriented anteroposteriorly is maximal on the anterior-most sternite and gradually diminishes linearly on the remaining sternites, the posterior-most sternite showing the minimal frequency of such bristles. These results suggest the presence of some kind of an anteroposterior gradient, which is responsible for the observed frequencies of the oriented bristles on the sternites.

TABLE 1

Anteroposteriorly oriented bristles on the sternites of males and of females in the foundation population and in the selected population after three generations of selection. Selection for the anteroposterior orientation of bristles was practised only on the fourth sternite

Ster- nite	No. of indi- vid- uals	Foundation population			No. of indi- vid- uals	Selected population				P
		Mean no. of bristles ($\pm$ SE)	Mean no. of oriented bristles ($\pm$ SE)	% of oriented bristles		Mean no. of bristles ($\pm$ SE)	Mean no. of oriented bristles ($\pm$ SE)	% of oriented bristles	% differ- ence	
<i>a. Males</i>										
2	83	9.73 $\pm$ 0.15	2.93 $\pm$ 0.11	30	82	9.58 $\pm$ 0.15	4.03 $\pm$ 0.13	42	12	<0.001*
3	83	15.74 $\pm$ 0.18	2.00 $\pm$ 0.09	12	82	14.91 $\pm$ 0.22	2.56 $\pm$ 0.09	17	5	<0.001*
4	83	16.13 $\pm$ 0.17	1.15 $\pm$ 0.08	7	82	15.26 $\pm$ 0.18	1.34 $\pm$ 0.07	8	1	>0.05†
5	83	17.24 $\pm$ 0.22	0.33 $\pm$ 0.05	1	82	15.64 $\pm$ 0.16	0.43 $\pm$ 0.06	2	1	>0.40†
<i>b. Females</i>										
2	83	11.03 $\pm$ 0.17	2.33 $\pm$ 0.07	21	70	11.38 $\pm$ 0.20	3.47 $\pm$ 0.17	30	9	<0.001*
3	83	19.20 $\pm$ 0.20	1.45 $\pm$ 0.07	7	70	18.14 $\pm$ 0.24	1.91 $\pm$ 0.03	10	3	<0.001*
4	83	19.66 $\pm$ 0.20	0.75 $\pm$ 0.01	3	70	18.85 $\pm$ 0.26	1.21 $\pm$ 0.20	6	3	<0.01*
5	83	20.40 $\pm$ 0.21	0.28 $\pm$ 0.10	1	70	19.47 $\pm$ 0.23	0.64 $\pm$ 0.07	3	2	>0.10†
6	83	19.19 $\pm$ 0.18	0.03 $\pm$ 0.07	0.1	70	18.64 $\pm$ 0.27	0.27 $\pm$ 0.05	1	0.90	>0.40†

* Significant.

† Not significant.

If the anteroposterior orientation of bristles in the individuals of the foundation population is due to the presence of an axial gradient, then the frequency of such bristles should be maximal on the anterior-most sternite, even when selection for bristle orientation is practised on any other sternite. The results of selection for the orientation of bristles on a posterior sternite after three generations of selection show that, although selection for the orientation of bristles was practised on the fourth sternite, the sternites show a linearly diminishing response from anterior to posterior (Table 1, Figure 1). Also, selection was effective in increasing the frequencies of the anteroposteriorly oriented bristles on all the sternites compared with those in the foundation population. Selection for bristle orientation is being continued on the present population, in the hope that prolonged selection may reveal some of the correlated responses which may provide a better understanding of the processes involved in structural orientation.

FIGURE 1.—Orientation of sternal bristles in the individuals of the Swedish population of *Drosophila melanogaster* after three generations of selection. Selection for the bristles, oriented anteroposteriorly along the mid-ventral line, was practised only on the fourth sternite. The sternites, 2–5 in **a** (male) and 2–6 in **b** (female), show maximal response for the orientation of bristles on the second sternite and a linearly diminishing response on the remaining sternites. a.p., anal plate.

DISCUSSION

The results of the present experiments clearly show a linearly decreasing response to selection from the anterior to the posterior sternites, despite the fact that selection for bristle orientation was practised only on the fourth sternite. A hypothesis is proposed to explain the orientation of bristles in terms of genes which are responsible for producing an anteroposterior gradient of some kind and of a certain value of this gradient necessary for bristles to become oriented anteroposteriorly parallel to the long axis. Since the value of the required gradient changes in a linear order, the response to selection is maximal on the anterior-most sternite and gradually diminishes linearly on the other sternites. The importance of these results lies in their demonstrating the genetic control of an axial gradient and its role in structural orientation. The nature of the gradient remains unknown.

If we accept the validity of this hypothesis, an attempt could also be made to show that by similar mechanisms the orientation of bristles in insects could have become diversified during evolution. There is some experimental evidence to suggest that changes in the orientation of large bristles, which show constancy of orientation from individual to individual, could have been produced by changes in the concentration of an anteroposterior gradient (SONDHI 1964, and in preparation).

Continued selection for a reduced number of structures on the ocelliless mutant, variable for the number of head bristles and ocelli, in *Drosophila subobscura* has shown that, only after elimination of the central structures on the head, the marginal bristles undergo rotation at their original sites. Of particular interest are the vertical setae I, directed toward the median line in the individuals of the foundation population of the ocelliless mutant and also in the wild-type individuals, which undergo rotation at the original sites to resemble a homologous pair of bristles in *Aulacigaster leucopeza*, a dipteran of the family Aulacigasteridae closely related to *Drosophila*.

There are reasons to believe that changes in the orientation of bristles in the selected population of the ocelliless mutant are due to alterations in the concentration of an axial gradient. This is so because elimination of central structures on the head is associated with an increase in the number of marginal bristles (SONDHI, 1962b, 1963), despite the fact that selection was practised for a reduced number of structures. This suggests that, as a consequence of elimination of the three ocelli and the associated bristles, the distribution of ocelli-bristle forming substance(s) is altered and corresponding changes in the concentration of an axial gradient are enforced.

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SUMMARY

Selection for bristles oriented anteroposteriorly parallel to the long axis on the fourth abdominal sternite of the Swedish wild-type strain of *Drosophila melanogaster* has shown a linearly diminishing response from anterior to posterior sternites. To account for these results, a hypothesis is proposed according to which genes are responsible for producing an axial gradient of some kind and a certain value of this gradient is required by the bristle forming cells to become oriented anteroposteriorly along the long axis. In the light of this hypothesis, some of the mechanisms responsible for the diversification of bristle orientation in insects are discussed.

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