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Action of natural selection in lines of *Drosophila* selected for a new level of canalization

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Summary. Four lines of *Drosophila melanogaster* previously selected for a stabilized phenotype of two extra dorsocentral bristles were examined for 20 generations of canalizing selection and relaxation of selection. A substantial frequency of flies with either two anterior or two posterior extra bristles was maintained in the relaxed lines. These patterns were the only ones tolerated by natural selection, i.e., the only symmetric ones. It was concluded that anterior and posterior dorsocentral bristles are two independent development structures, and the results are discussed in relation to two proposed genetic systems for bristle determination.

Key words: Canalization – Stabilizing selection – Natural selection – Dorsocentral bristles – Positional pattern

Introduction

Canalization is a concept used to explain the permanence of a great hidden genetic variability in some polygenic characters. Individuals tend, in their development, to express a uniform phenotype of these characters, even when the genotypes of these individuals may be very different; these genetic differences can be easily detected (e.g., Scowcroft 1966; Parsons 1980).

Rendel (1967, 1979) interpreted canalization in terms of thresholds. The level of canalization is the interval between two thresholds in which a genotypic difference do is not reflected in a phenotypic difference.

Scutellar bristles of *Drosophila melanogaster* are a widely studied system. An attractive model to explain their formation is the following: an extra bristle appears when a substance called chaetogen surpasses a determined threshold in a certain position; below this threshold we can only obtain the normal phenotype (Fig. 1). The amount of chaetogen may change between two consecutive thresholds without a change in bristle number (Richelle and Ghysen 1979).

Because symmetry is important in the development of the fly (Maynard-Smith and Sondhi 1960), canalization will only allow an even number of extra bristles: zero extra bristles (normal phenotype), two extra bristles, four extra bristles, etc. This theoretical reasoning has been proven in selection experiments (e.g., Ménsua 1966).

However, few people have noticed the position of these extra bristles. Rendel (1965) and Robertson (1965) initiated an old discussion: Rendel believed that the unity of development and canalization was the total scutellum; Robertson proposed that genetic determination of anterior and posterior bristles was, at least partially, independent. This latter idea has been confirmed by selection experiments on the scutellum (Latter and Scowcroft 1970; Scowcroft 1973) and by data on the developmental genetics of the scutum (e.g., different temporary determination; Poodry 1975).

According the Rendel's theory, the four positional phenotypes in the "two extra bristles" that appear in Fig. 1 are equally symmetrical; according to Robertson, only *aa* and *pp* are symmetrical. We are able to study the possible independence of anterior and posterior positions by relaxing selection in lines with dorsocentral extra bristles, because natural selection will eliminate asymmetrical phenotypes more quickly than symmetrical ones.

Material and methods

We used 4 lines of *D. melanogaster* selected for 25 generations for 2 dorsocentral extra bristles (e.g.). Two lines called ADC

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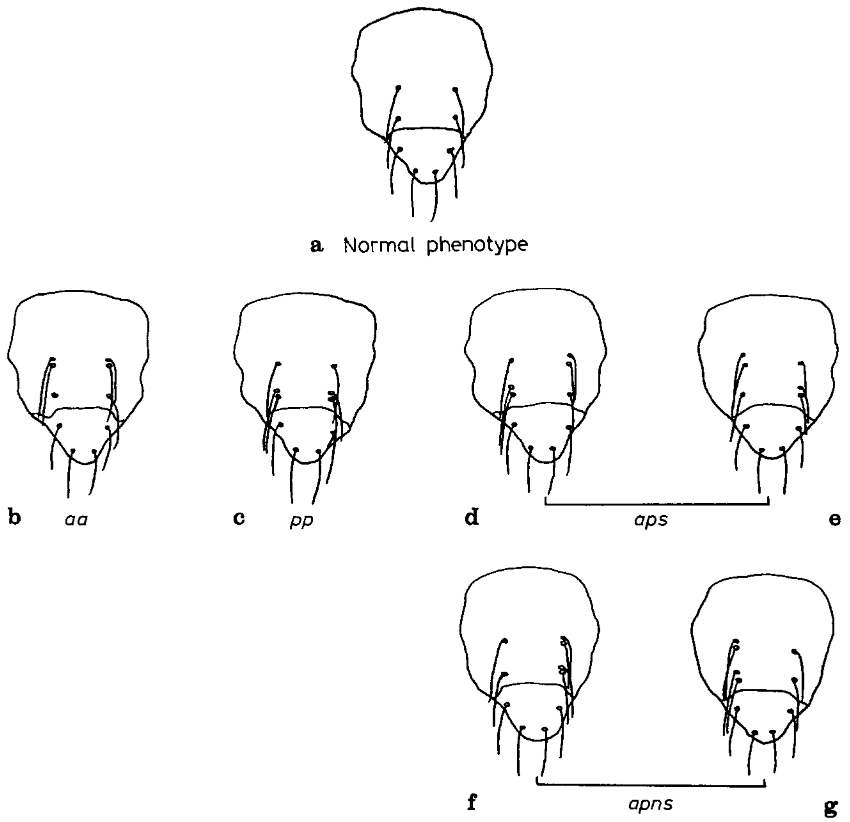

Fig. 1a–g. Some phenotypes of dorsocentral extra bristles in *Drosophila melanogaster*. Rendel (1965) considers **b–e** phenotypes to be equally symmetric

Table 1. Regression coefficients of mean number of extra bristles on generation number over G.14–G.20

Line	R1			R2		
	a	b	$t_{(b=0)}$	a	b	$t_{(b=0)}$
ADC-4	1.0229	0.0189	0.4985 N.S.	1.0171	0.0004	0.0142 N.S.
ADC-7	0.8357	−0.0361	0.9754 N.S.	0.5843	0.0668	6.2874[a]
AP-2	1.1193	−0.0618	1.3535 N.S.	0.9380	−0.0175	1.1032 N.S.
AP-3	1.4114	−0.0675	1.8424 N.S.	0.9729	0.0189	0.5127 N.S.

[a] $P < 0.01$

were selected for *aa* phenotypes (Fig. 1). They were adequately stabilized: more than 90% of class 2 individuals were *aa*, and they showed a very low proportion of higher classes than the selected one. Two lines called AP were selected for phenotypes *aps* and showed more variety of positional phenotypes in class 2 than ADC, although *aa* were frequently obtained. Ten pairs of virgin flies were taken as parents from each generation (García-Vázquez and Rubio 1982).

Three replicates of each line were kept from generation G.25–G.45. The same type of selection was continued in the control line. Artificial selection was discontinued in the other two, R1 and R2, and ten pairs of parents were taken at random from each generation, without attention to their phenotypes. Bristle phenotypes of 60 males and 60 females in each replicate were saved for 20 generations. To analyse the action of natural selection, we studied the following: (a) the variation in the average of extra bristles in these 20 generations; (b) the individual distribution in e.b. numberical classes at the end of the experiment, comparing it with G.25 (the size of class 2 was measured by probits, following Rendel 1979); and (c) the positional phenotypes of class 2, the main concern of this study.

These phenotypes are shown in Fig. 1. Another phenotype is *ii*, not classifiable because it has one or to e.b. in an intermediate, between anterior and posterior, position.

The experiment was carried out at room temperature. The flies were grown in 200 cc glass bottles and were reared with sugar-yeast-agar medium.

Results and discussion

On relaxation of artificial selection the selected lines tend to return towards the normal phenotype (Fig. 2): the decrease in the average number of bristles in both replicates is clear in ADC and AP. But the supernumerary phenotypes are not removed by natural selection: the replicates became practically stabilized with an e.b. average close to unity (Table 1). Note that the only significant slope (R2 from ADC-7) is positive (the average in-

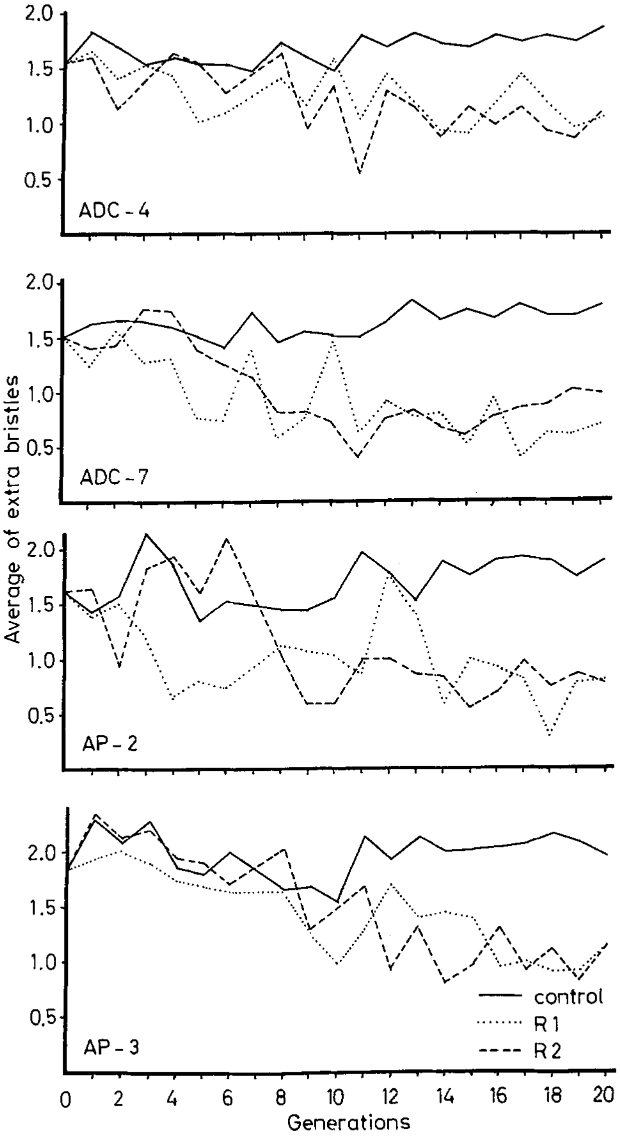

Fig. 2. Average number of extra bristles in each generation

Table 2. Mean value of probits of class 2 for generations G.14–G.20 in which the lines were assumed to be stabilized

Line	Control	R1	R2
ADC-4	5.700	4.783	4.698
ADC-7	5.544	4.238	4.371
AP-2	5.050	4.272	4.280
AP-3	5.510	4.690	4.560

creases). At the end of the experiment, classes 0 and 2 tended to have the highest frequency, but the proportion of classes higher than 2 decreased (Fig. 3). Class 1 remained intermediate.

It is obvious that when selection is interrupted, most parents will be from class 2 and only some from classes

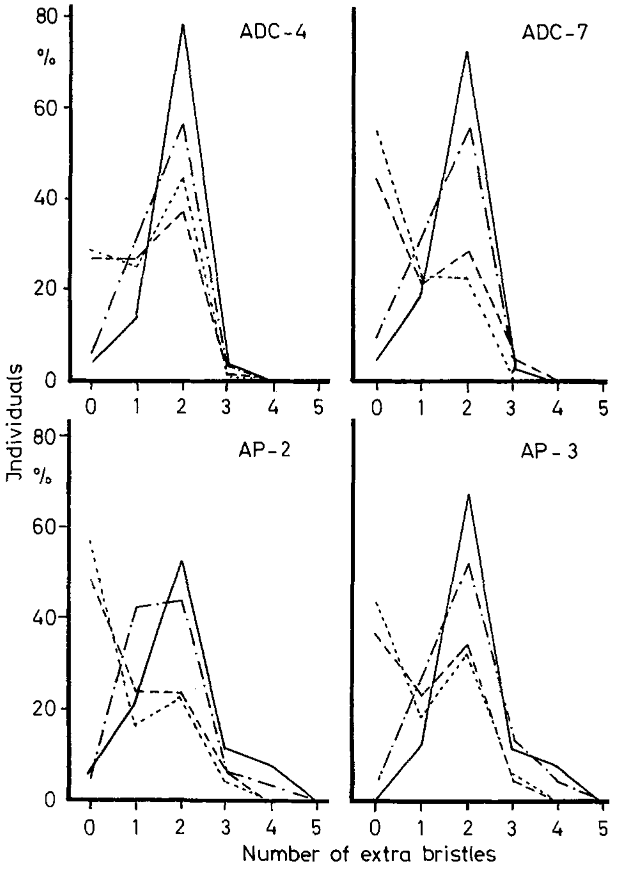

Fig. 3. Distribution of numbers of extra bristles in the first (G.0) and in the last (control, R1 and R2) generations. The last four generations are pooled in each replicate; —·—·— G.0; —— control; ····· R1; ---- R2

0 and 1 in the first generations. The increase of the normal class at the expense of the other classes produced a quick decrease of the e.b. average. This is expected because the symmetrical phenotypes can only exist in classes 0 and 2. The symmetrical normal and class 2 phenotypes have a reproductive advantage, so an equilibrium between these phenotypes may be obtained. Nevertheless, class 2 segregates asymmetrical descendants of class 1, even with the artificial selection in which all the parentals belong to class 2. A balance from G.14 is established between these three classes, in such a way that the e.b. average does not vary significantly.

Class 2 shows a canalization level allowed by natural selection, along with the normal phenotype, which is demonstrated by its constant permanence. The probits of this class (Table 2) show this clearly, because they are higher than 4 σ in all cases, even without artificial selection. These probits are higher for the scutellum than reported by other authors (MacBean et al. 1972). The lines have proven to be stable under natural selection, possibly due to a balance between the two symmetrical classes.

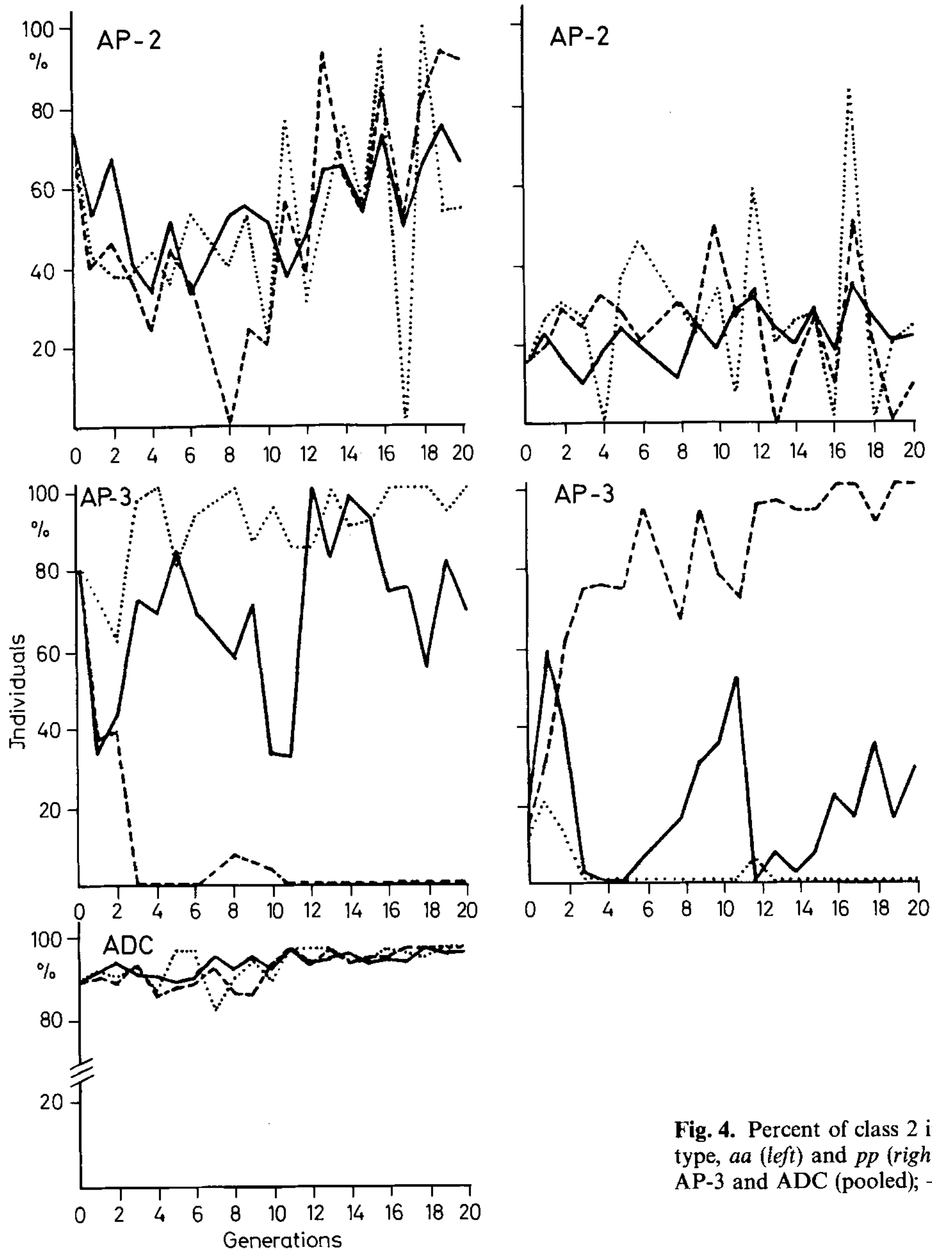

Fig. 4. Percent of class 2 individuals of each positional phenotype, *aa* (*left*) and *pp* (*right*) in each generation for lines AP-2, AP-3 and ADC (pooled); —— control; ····· R1; ---- R2

We can now ask which positional phenotypes remain in class 2. The answer is given in Fig. 4: *aa* and *pp*. Results from the two lines ADC are pooled because there were no significant differences between them. Except in some AP-2 generations, it can be observed that *aa* and *pp* individuals together make up more than 90% of class 2, in most cases 100%. Thus, *aa* and *pp* are then the only symmetrical phenotypes, and this confirms the hypothesis of the independence between the anterior and posterior positions proposed by Robertson (1965). His theory is confirmed here for the scutum.

The appearance of an extra bristle is determined by at least two different genetic systems (Fraser 1967, 1970; Rendel 1976, 1979). We propose, according to the model of Richelle and Ghysen (1979), that one system increases the production of chaetogen for all the positions where the bristles appear. However, the canalizing system regulates the control of each position, letting chaetogen accumulate in them. An extra bristle can probably be induced by reducing the inhibition produced in the normal phenotype around each bristle that obstructs the formation of another in its vicinity.

In the ADC replicates, we suggest that the canalizing system has been modified by our pattern of selection. The strong canalization for the normal phenotype is broken in anterior positions, or at least is relaxed to allow the appearance of extra bristles in these positions. If we had only modified the system that regulates chaetogen, extra bristles should also appear in posterior positions.

The new level of canalization is allowed by natural selection, although the normal phenotype is favoured along with the other symmetrical phenotype (two e.b.). This could be an explanation of the previously described equilibrium.

Both AP lines, which have more positional phenotypic variability, behave in a different way. AP-2 combines both phenotypes, alternating them from generation to generation. On the other hand, AP-3 takes and maintains a symmetrical phenotype from each replicate. This shows that selection for different systems was made in both lines.

It may be assumed that selection in AP-2 altered the chaetogen increase system in such a way that both the anterior and posterior positions remain with higher doses of chaetogen, without eliminating the extra bristles from either position. This dose should be higher than for the ADC lines, because there are extra bristles but not a new level of canalization. The proportion of non-symmetrical phenotypes *ap* and *ii* is higher than appears in the replicates of the other lines (the *aa*+*pp* percentage is not higher than 90% in some generations; see Fig. 4). A new canalization level is not fixed in either position. It is important to note that the signs of canalization in AP-2 control are lower than those appearing in the other lines: class 2 is less frequent, although the percentage of higher class individuals is similar to AP-3 (see Fig. 3). Phenotypes *aa* and *pp* appear constantly in the control, without either phenotype being eliminated and with similar proportions in all positions; both positions are equally uncanalized.

In AP-3, however, selection basically modified the canalizing system for both positions at the same time. The control, which is much more positionally erratic than the AP-2 control (Fig. 4), shows that the canalizing system is being altered. In addition, the pressure to increase the number of bristles is similar in both positions. Because of the alteration in canalisation, this increase is allowed in some positions but not in others, depending on generation. The possibility of a position discanalization is not ruled out because in each generation both types are still being selected, although by a slight selective pressure (only one e.b. in each position). The maximum did not reach the class 4 e.b. without artificial selection, as chaetogen level was not high enough.

Discanalization for anterior positions at random disappeared in R2, and for posterior ones in R1. R1 is similar to ADC replicates, which "choose" and support *aa* phenotypes. R2 behaves in the same way, but "chooses" the equally symmetrical *pp* phenotypes, i.e., the canalizing system that controls the posterior positions is modified.

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