An interpretation of some bristle pattern modifications caused by directional selection in *Drosophila melanogaster*

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Summary. The variations of the dorsocentral and scutellar bristle patterns founded in two bidirectionaly selected lines are discussed in terms of the Richelle and Ghysen model. The phenotype obtained through selection for bristle suppression can be accounted for by a decrease in chaetogen production. Extra bristles can be accounted for by an alteration of the response of the cells to positional information.

Key words. Drosophila; bristles; phenotype; directional selection; chaetogen.

The occurrence of sensory bristles arranged in defined patterns is a prominent feature of the external morphology of insects. In *Drosophila,* sensory bristles are found at specific locations, arranged in rows, or evenly spaced over defined regions of the cuticule. The latter pattern can be accounted for by a model developed by Wiggelsworth[1]. To explain the formation of bristles at defined sites, two hypotheses have been proposed: a) that the sites correspond to singularities in the distribution of some inducing substance defining a 'prepattern'[2]; and b) that each site corresponds to a particular set of coordinates along gradients of concentration of some diffusible substance defining 'positional information'[3].

The model proposed by Richelle and Ghysen[4] incorporates various characteristics of the models previously developed by Wiggelsworth[1], Stern[2] and Lawrence[3], and is able to generate the different types of bristle (macrochaetae) and hair (microchaetae) patterns that can be found on a fly, including the row pattern. The determination of bristle position occurs in two steps. First, large numbers of imaginal-cells synthesize a freely diffusible inducer, the chaetogen. Second, the cells in which the concentration of this chaetogen reaches a threshold are induced to differentiate into a bristle apparatus. Induced cells synthesize an inhibitor to prevent neighboring cells from also being induced. The synthesis of chaetogen is supposed to be a probabilistic response to positional cues.

Although the application of quantitative genetics to development problems is limited, the possible qualitative changes and some general patterns of phenotypic response can be shown by such an approach[5-8]. Controlled bristle pattern modifications can be produced by artificial selection, and the isolation of individual components of a polygenic system can contribute to finding the specific ways in which the development of the selected individuals has been altered to produce the observed phenotypic responses[9-11].

This paper is concerned with the mechanisms responsible for the appearance of new bristle patterns through directional selection. Wild *Drosophila melanogaster* flies have four dorsocentral and four scutellar bristles located at defined positions. We have established two lines derived from the same population through long-term bidirectional selection: one selected for suppression of dorsocentral and scutellar bristles (S line), and the other selected for extra bristles in the same areas (E line). Variations of the dorsocentral and scutellar patterns are discussed in terms of the Richelle and Ghysen model, which provide a conceptual relation between the action of selected polygenic background and the phenotypes of the selected lines.

Bristle suppression. The S line has reached the plateau and shows a mean of 7.41 ± 0.07 suppressions in this area. The selected phenotype is caused by a recessive allele from a locus in the third chromosome called *bristle-suppression* (*brs,* 104 ± 1.55 cM). The *brs* allele by itself determines bristle suppression, although its expressivity greatly depends on a polygenic system of modifiers with effect only through interaction with *brs*[12].

Pattern alterations that show the S line can be interpreted, in terms of the Richelle and Ghysen model, as a consequence of the chaetogen synthesis reduction:

a) The S line was selected only for suppression of dorsocentral and scutellar bristles, but shows suppression of bristles and hairs from the entire body (fig. 1), to be expected if the selection acted on loci that control chaetogen synthesis[13].

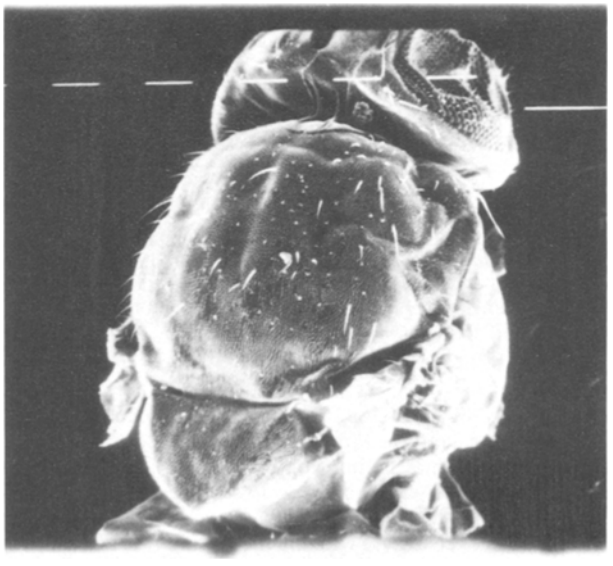

Figure 1. Scanning micrograph of a line S fly showing an extreme phenotype: all bristles and most notal hairs are suppressed.

b) Bristles that did develop were reduced in size (table 1). This dual effect on the determination itself and on the activity of the differentiated bristle-producing cells is what should happen if the selected genotype reduces the production of chaetogen, and therefore increases the time required before the threshold concentration of chaetogen is reached[13].

c) Another quite frequent pattern alteration is the determination of a bristle located between two normal sites that fail to develop their bristles, at a place where no bristle is normally found (fig. 2). This behavior can be attributed to the diffusible properties of the chaetogen[13]. Since the chaetogen production is reduced by the selected genotype, more time is needed to reach the threshold. Because the chaetogen continually diffuses during that time, the two peaks can be merged and a single maximum can appear halfway between the two presumptive sites.

Extra Bristles. The extra-bristles phenotype, selected in the E line, is determined by a polygenic system on the three major chromosomes. Two segments were located in the third chromosome as polygenic loci of effect large enough to be identified[12]. The E line has also reached the plateau and shows a mean of 7.70 ± 0.12 extra dorsocentral and scutellar bristles in females and 6.06 ± 0.13 in males. The extra bristle pattern is similar to that caused by *polychaetoid (pyd)*[14]; extra dorsocentral bristles are formed anteriorly to normal ones, and are always placed in the same hair row. Extra scutellar bristles are formed anteriorly and posteriorly to normal ASC bristles (fig. 3). This pattern cannot be accounted for by a chaetogen over-production itself

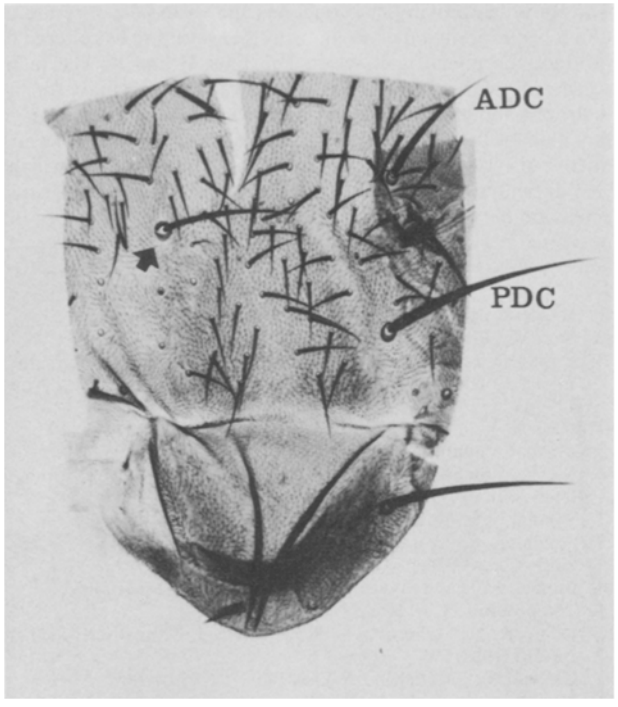

Figure 2. Thorax from a S line fly showing a dorsocentral bristle located between the two normal sites.

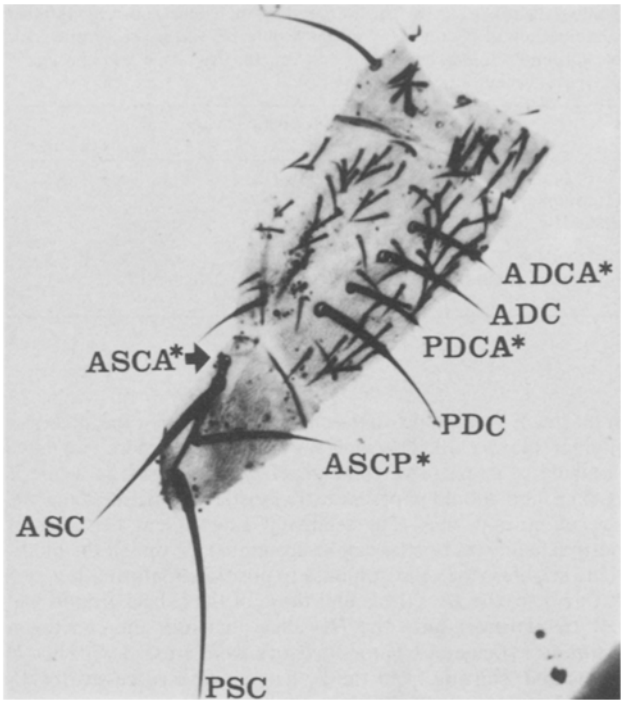

Figure 3. Left half of the thorax from a line E fly showing the extra bristles pattern. Abbreviations as in table 1. *Extra bristles.

or by a reduction of the inhibitor synthesis, because then extra bristles would be formed at any position around the normal bristle, generating an extra bristle pattern like that of *Hairy-wing (Hw)* [14], as has been demonstrated by Ghysen and Richelle[13].

The line E pattern can be explained by assuming the existence of an ancestral prepattern whose information has not been eliminated by natural selection and remains at a subthreshold level. This prepattern will be revealed as an atavistic phenotype either by increasing the synthesis of bristle-inducing substance or decreasing the threshold level[6,9,11]. Translating these terms to Richelle and Ghysen's model, the E extra bristle pattern can be explained by assuming that extra bristles are a consequence of the existence of chaetogen synthesizing areas that normally do not reach the threshold level. So, as a result of selection in the E line, there could be a slight increase of the chaetogen synthesis rate that is enough to reveal these peaks without determining a *Hw*-like pattern. There are two features that agree with the prepattern hypothesis:

a) The extra bristles of E flies are smaller than normal ones (table 1), which can be interpreted as a delay in the determination since the threshold concentration in the extra position will be reached later than in the normal one.

b) The line E bristle pattern can be interpreted as an atavistic phenotype since it resembles that of a genus closely related to *Drosophila,* as *Leptocera* is (fam. *Leptoceratidae*)[15], if one accepts, as Garcia-Bellido does, that acrostical bristles of *Leptocera* correspond with the dorsocentral ones of *Drosophila.*

Another hypothesis that can also explain the line E bristle pattern is that selection has modified the response to positional information by epidermal cells, establishing new areas where cells do synthesize chaetogen and, therefore, new regions where extra bristles will eventually be determined.

To test these two hypotheses, we have introduced the *brs* allele on line E, and selected bristle suppression. This line was called *brs* (E). At generation 9, means of bristles were 1.21 ± 0.08 (females) and 2.14 ± 0.09 (males). Around 8% of the individuals have a great number of suppressed bristles and some extra ones. In those individuals, the bristle in a normal position was reduced in size while its neighboring extra bristle was not suppressed (table 2).

Table 1. Comparisons of mean length of dorsocentral and scutellar bristles from Oregon-R and S line (left); and *brs* (E) and E lines (right). The measurements are given in tenths of a millimeter. *Anterior to anterior dorsocentral, ADCA; anterior dorsocentral, ADC; anterior to posterior dorsocentral, PDCA; posterior dorsocentral, PDC; anterior to anterior scutellar, ASCA; anterior scutellar, SCA; posterior to anterior scutellar, PSCA; posterior scutellar, PSC. **Estimated from only two data.

Position*	Oregon-R	S line	t	d.f.	p	E line	*brs* (E) line	t	d.f.	p
ADCA						1.79 ± 0.03	1.51 ± 0.07	3.91	33	< 0.001
ADC	2.30 ± 0.02	1.51 ± 0.15	7.72	20	< 0.001	2.39 ± 0.03	2.14 ± 0.08	3.19	33	< 0.001
PDCA						1.99 ± 0.07				
PDC	3.40 ± 0.03	2.06 ± 0.08	8.71	26	< 0.001	3.19 ± 0.06				
ASCA						2.77 ± 0.04	2.18 ± 0.12	6.18	27	< 0.001
ASC	3.35 ± 0.02	1.96 ± 0.04	14.64	40	< 0.01	3.50 ± 0.04	2.98 ± 0.11	4.52	34	< 0.001
ASCP						2.67 ± 0.13	2.40 ± 0.14	1.33	18	n.s.
PSC	4.08 ± 0.04	2.27**				4.12 ± 0.03				

Table 2. Mean of extra bristle numbers in females bearing different combinations of *Hw* and *Hw*$^+$ alleles with line E and *Hw* stock polygenic backgrounds. Interaction effect between the *Hw* allele and the line E polygenic background.

		Genetic background	
		E line	Hw stock
		Hw (E)	Hw (+)
Genotype at the Hw locus	Hw/Hw$^+$	6.26 ± 0.23	1.86 ± 0.15
		Hw$^+$ (E)	
	Hw$^+$/Hw$^+$	1.44 ± 0.18	

Interaction effect $(\bar{X}_{Hw(E)} - \bar{X}_{Hw(+)}\bar{X}_{Hw^+(E)}) = 2.96 \pm 0.10$
$z = 9.16 \quad p \ll 0.001$

If the line E genes improve the chaetogen synthesis, the introduction of the *brs* allele (which, as mentioned before, we have assumed to interfere with the chaetogen synthesis) in a line E background should suppress extra bristles before reducing the size of normal ones. The relatively independent behavior of normal and extra bristles can be accounted for only if the modified variable is the cells' response to positional information.

If this is so, the *Hw* effects and those of the E background will not be additives since the *Hw* allele increases the chaetogen synthesis[13]. Females from a *Hw* stock were crossed with line E males and, starting from the F_1 females, five successive backcrosses of *Hw*/*Hw*$^+$ females to line E males were practised in order to obtain females bearing the *Hw*/*Hw*$^+$ or the *Hw*$^+$/*Hw*$^+$ genotype in the same E background. By comparing them with females from the *Hw* original stock (*Hw*/*Hw*$^+$ because of the recessive lethality of *Hw*), an important interaction between the *Hw* allele and the E polygenic background (table 2) was found, which reinforced the hypothesis that selection for extra bristles has modified the cell response to positional information.

The Richelle and Ghysen model has shown to be a very useful tool for interpreting the mechanisms that selection has altered to produce the phenotypes selected in lines E and S. The main conclusion of this work is that there is genetic variability for the interpretation of positional information and, therefore, it can be modified by selection. Confirmation of that hypothesis would involve the generation of somatic mosaicism, but this will be very difficult to perform because of the small effect of the located genes and the importance of their interaction with the rest of the polygenic background.

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Sclerotial and low aflatoxigenic morphological variants from haploid and diploid *Aspergillus parasiticus*[1]

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Summary. Serial transfer of mycelial macerates of a wild type, haploid, aflatoxigenic strain of *Aspergillus parasiticus* in a defined liquid medium resulted in the production of three new morphological classes: a sclerotial form with high aflatoxin production, and two variant forms (*fan* and *fluff*) with lowered sporulation, no sclerotia, and attenuated levels of aflatoxin. A genetically marked diploid containing mutant markers for aflatoxin pathway intermediates yielded the same three morphological classes upon serial transfer of macerated mycelia. When these diploid variants were treated with a haploidization agent, and the phenotypes of the resultant segregants scored, a low frequency of colonies producing aflatoxin pathway intermediates was recovered. These genetic data indicate that the structural genes for the aflatoxin pathway are present but somehow attenuated in the *fan* and *fluff* strains.
Key words. Aflatoxin; *Aspergillus parasiticus;* sclerotia; mycelial maceration; strain degeneration.

Aflatoxins are toxic, carcinogenic secondary metabolites produced via the polyketide pathway by the common molds *Aspergillus flavus* and *A. parasiticus*. Among agricultural isolates of these species there is wide variation in toxigenicity. Some strains produce no detectable aflatoxins, while producers vary several-fold in the amount of toxin synthesized. Moreover, high producers may become low producers after several transfers in the laboratory[3,4]. Experimental induction of lowered or lost aflatoxin-production has been reported after subjecting *A. flavus* to several generations of growth in medium containing barium ions[5]; *A. parasiticus* in a liquid defined medium that suppresses sporulation[6,7]; and both *A. flavus* and *A. parasiticus* to successive transfers on crushed wheat[8]. Experiments on the genetic basis of this variability in aflatoxin-producing ability are difficult to formulate because neither *A. flavus* nor *A. parasiticus* has a known sexual stage and crosses must be established using marked mutants through the parasexual cycle[9,10]. Certain mutants with brightly colored mycelia accumulate anthraquinones which are known precursors of aflatoxin biosynthesis[11]. The fortuitous association of mycelial pigmentation and aflatoxin pathway intermediates allows visual scoring of the aflatoxin pathway in mutants bearing these markers. We have synthesized a parasexual diploid between a norsolorinic acid-accumulating (red) and a versicolorin A-accumulating (yellow) mutant[12] and used this diploid, as well as a wild type, highly aflatoxingenic haploid strain, to extend studies on the serial transfer of mycelial macerates in liquid defined medium

Materials and methods. The wild type haploid strain of *A. parasiticus* was designated SU-1 (NRRL 5862) and its lineage was described by Mayne et al.[4]. The diploid strain (DIP-8) contained contrasting spore color (*wh*-white; *br*-brown), auxotrophic (*lys*-lysine; *ser*-serine) and mycelial color (*nor*-norsolorinic acid; red;