A Study of the Differentiation of Bracts in *Drosophila melanogaster* Using two Mutations, H^2 and sv^{de}

In *Drosophila melanogaster*, the chitinous parts of the bristle organ consist of a socket and a shaft which develop during metamorphosis from two polytenic cells, the tormogen and the trichogen cell. These arise by differential cell division from a single ectodermal cell within the epithelium of the imaginal disks. Most bristles on the distal leg segments, as well as some on the costa of the wing, are accompanied by a special trichome, called a bract[1,2] (Figure 1). These bracts occupy well defined positions relative to the bristles and represent derivatives of a single ectodermal cell. In genetic mosaics[3–5], a bristle and its accompanying bract may differ in genotype. This indicates that the two structures arise from two different cells which are not necessarily related to each other by immediate cell lineage. In reaggregates of dissociated imaginal disks, isolated bracts without an accompanying bristle were never observed. By definition, this shows that the presumptive bract cell was not yet determined to form a bract prior to dissociation. The hypothesis was advanced that the formation of a bract is induced by the developing bristle[3,4]. When the differentiation of the tormogen cell was preferentially blocked with Mitomycin C[6,7], or with nitrogen mustard[8], no bracts were found near bristles that were devoid of a socket. These data suggest that either a complete bristel organ, or the formation of a socket, is a necessary prerequisite for the induction of a bract. This report provides evidence that the differentiation of a bract requires the presence of a complete bristle organ.

The mutation *Hairless*[2] (H^2: 3–69)[9] is dominant and homozygous lethal. The balanced stock $H^2/T(2;:3)ap^{Xa}$ was used for our investigation. *Shaven-depilate* (sv^{de}: 4–2.0) is recessive and homozygous lethal during the late pupal stage, with a few 'escapers' dying during the 1st or 2nd day after emergence. sv^{de} is kept in a stock balanced with *eyeless-Dominant* (ey^D: 4–2.0). H^2 and sv^{de} were chosen because both of these mutations lead to the formation of bristle organs without or with a grossly reduced shaft. The stocks were raised on standard food (corn, sugar, agar, yeast) at 25 °C. Adult integumental parts were eviscerated for 10 min in hot 5% KOH, washed in distilled water, and mounted in Faure's rubber solution on glass slides for microscopic examination. Photographs were taken with a Zeiss photomicroscope.

H^2 is expressed most distinctly on the head where the occipital, postvertical, and ocellar setae are affected. The bristles of the antennae and vibrissae show the mutant phenotype much less frequently. Sockets without shafts were also found on the thorax, scutellum, abdominal tergites, external genitalia, wings, and legs. No shaft-less

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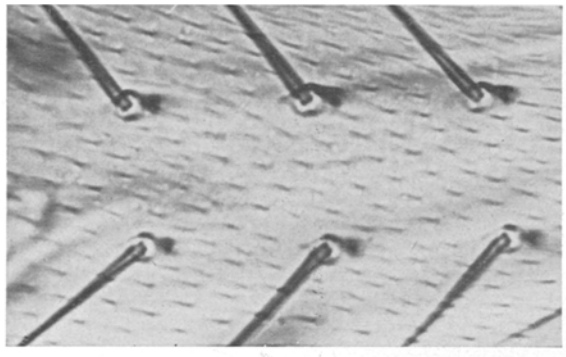

Fig. 1

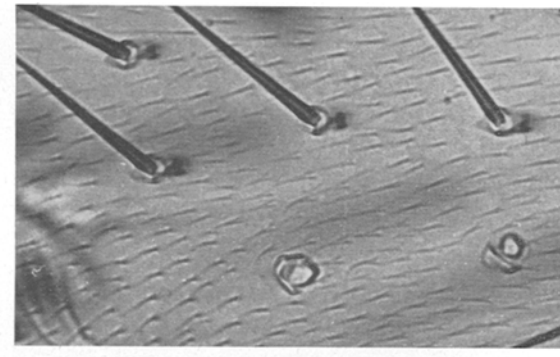

Fig. 2

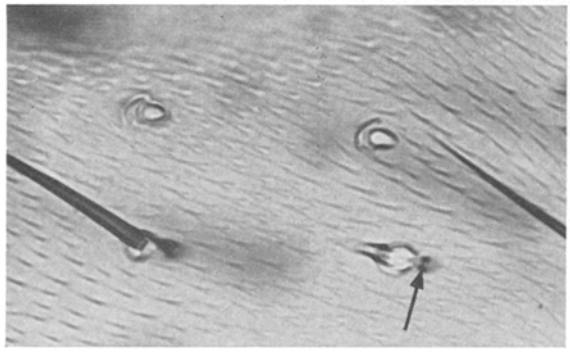

Fig. 3

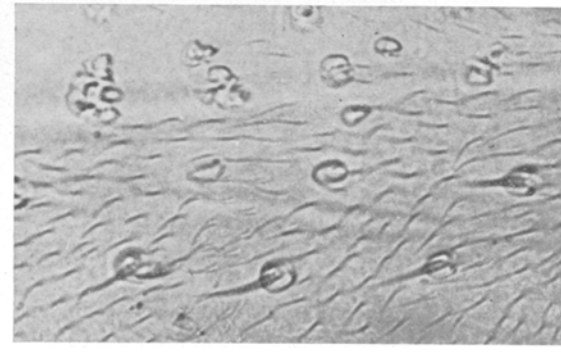

Fig. 4

Fig. 1. *Wildtype:* Distal region of foreleg femur where certain longitudinal rows of bristles are regularly accompanied by bracts.

Fig. 2. *Hairless:* same region as in Figure 1. Note that sockets without a shaft are without a bract.

Fig. 3. *Hairless:* region of the femur showing a very small bristle with a bract of normal size (arrow).

Fig. 4. *Shaven-depilate:* region of the tibia showing the high cellular penetrance of this mutation, and the absence of bracts from bristles without or with a very short shaft. Magnification for all Figures × 780.

sockets appeared on the bracteated costa of the wing. However, some 40% of the bristle organs located on the distal part of the femur differentiate neither a shaft nor a bract in H^2. Bracts were absent whenever a shaft was missing at positions where bracts are always formed in wild-type flies (Figure 2). On the other hand, bract were present near complete bristle organs on mutant forelegs. It is interesting that also a small bristle with a very short shaft may be accompanied by a bract of normal size (Figure 3, arrow).

Compared with H^2, the mutation sv^{de} is characterized by a higher 'cellular penetrance' in the sense that more cells exhibit a mutant phenotype. Sockets without bristles are found on adult integumental derivatives of all imaginal disks. The frequency of this extreme cellular phenotype reaches almost 100% on the thorax leading to the 'shaven' appearance of the adult fly. Where shafts do occur in the sv^{de} mutant, they rarely exhibit normal shape: in most instances they are either bent, twisted, or forked. Up to 97% of the bristle organs of the wing costa or distal leg parts did not differentiate a normal shaft. In all these cases, bracts were also missing, with a very few exceptions ($< 0.1\%$) where sockets that lacked a shaft were nevertheless accompanied by a bract. However, most, if not all, of these rare examples must be ascribed to shafts having been lost during preparation of the flies for microscopic examination[10]. As in H^2, complete bristle organs were accompanied by bracts. On the other hand, bracts were absent near bristle organs that had only developed a very short shaft (Figure 4).

It has been argued[11] that the formation of bristles without sockets and bracts after treatment with mitomycin C or nitrogen mustard may be due to the 2 drugs inhibiting both the presumptive socket and the bract cell simultaneously and independently. Such a hypothesis is now rendered very unlikely by our observation of a perfect correlation between a normal bristle organ and the formation of an accompanying bract in the two mutants, H^2 and sv^{de}. In the light of the additional evidence now available, this hypothesis would have to make the following two assumptions: 1. that 2 drugs (mitomycin C and nitrogen mustard), and 2 mutations (H^2 and sv^{de}) exert 2 independent effects on 2 different cells (tormogen/trichogen and bract cell); 2. and that the presumptive bract cell exhibits the same sensitivity as the tormogen cell towards mitomycin C and nitrogen mustard, whereas in H^2 and sv^{de} it responds in the same way as the trichogen cell. We think it is much more reasonable to assume that the 2 drugs and the 2 mutations exert only one effect, namely to inhibit the development of either the tormogen or the trichogen cell, respectively, whereas they leave unaffected the competence of the presumptive bract cell to respond to the inductive stimulus of the bristle organ. This latter conclusion is based upon the fact that in mutant flies, both H^2 and sv^{de}, complete bristle organs are always accompanied by bracts, provided they are located in regions where bracts are regularly formed in wild-type flies.

If the additional data obtained with dissociated and reaggregated imaginal disks are taken into consideration, we are forced to accept as a fact that bracts in *Drosophila* are differentiated under the inductive influence emanating from a complete and normal bristle organ. In H^2 even a bristle with a short shaft is capable of induction, whereas in sv^{de} this is not the case. This difference does not present any difficulties for our concept since the two mutations may well affect differently the inductive capacity of rudimentary bristle organs.

Zusammenfassung. Die Mutanten «*Hairless*²» und «*shaven-depilate*» von *Drosophila melanogaster* führen in den Borstenorganen zu einem Ausfall des Schaftes. Bei beiden Mutanten fehlen Borstenschaft und «bract» gleichzeitig in Regionen, wo beim Wildtyp immer «bracts» ausgebildet werden. Die Korrelation zwischen fehlendem Schaft und «bract» wird im Zusammenhang mit früheren Befunden diskutiert. Es wird der Schluss gezogen, dass nur ein vollständiges Borstenorgan die Differenzierung eines «bracts» zu induzieren vermag.

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25. August 1972.

[10] We feel justified to make this statement because we were able to find loose shafts somewhere else in the same preparations. Furthermore, the color and the shape of those shaft-less sockets near which a bract was nevertheless produced were the same as in complete bristle organs.

[11] W. Gehring, in *Results and Problems in Cell Differentiation*, (Eds. H. Ursprung and R. Nöthiger; Springer-Verlag, Berlin 1972), vol. 5, p. 35.

[12] We would like to thank Drs. Ernst Hadorn and David Turner for critically reading the manuscript.

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Coumarins of *Angelica pachycarpa*

Angelica pachycarpa Lge. is one of the Umbelliferae endemic to NW. Spain[1–3] and W. Portugal (Berlanga island)[3,4]. To our knowledge the plant has never been studied for its constituents[5,6], despite the fact that occurrence of coumarins could be assumed. We report in this paper the identification of several of them in the plant.

Materials and methods. Aerial parts of the plants (300 g), exclusive of flowers and fruits, were collected in November and homogenized with methanol (1.5 l) at room temperature. After a 7-day extraction with occasional stirring, the suspension was filtered and the extraction repeated for 2 consecutive 24-h periods. The filtrates were combined, concentrated in vacuo and the residue was taken up in 60% methanol. Pigments and other fat-soluble material were removed by extraction into hexane[7]. The residual 60% methanolic solution was concentrated in vacuo and the methanol-free aqueous residue was continuously extracted overnight with ether.

The ether solution was dried over anhydrous sodium sulphate, concentrated and chromatographed on Whatman 3mm paper with either toluene-acetic acid-water (4:1:5 by volume; upper layer, TAW) or isopropanol-ammonia-water (10:1:1 by volume, IAW) as descending solvents. The chromatograms were observed under shortwave light (254 nm) and bands corresponding to fluorescent compounds were eluted with methanol. Overlapping of substances was showed by rechromatography in different solvents. Consequently, the eluates were sublimed at 130–190° and the sublimates were chromatographed in TAW. Several bands were eluted and