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The expression of 'Hairless' in *Drosophila* and the role of two closely linked modifiers of opposite effect

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1. INTRODUCTION

The chaetae, epidermal sense organs, of *Drosophila* are affected by many mutant genes. These have been studied extensively, and a complete understanding of their action would increase our knowledge of the genetic control of development very considerably. Yet it is still difficult to provide any single scheme which clearly explains the expression of any one of these mutants, let alone to integrate all of the described effects into a single, coherent picture of the development of the chaetae.

The development of chaetae involves two distinct sets of events. First must come '*pattern formation*', the events which decide where bristles will develop. Then a series of alterations to an originally single epidermal cell which constitute '*chaetogenesis*' leads to the production of chaetae at these previously determined sites.

The present paper describes work on a mutant, *Hairless* and two of its major modifiers, *Suppressor of Hairless* and *Enhancer of Hairless*. *Hairless*, itself, is a mutant with dominant effect on macrochaetae. Sometimes a particular chaeta is absent without any trace on a *Hairless* fly. At other times a vestige may remain. The morphologically distinguishable chaetae are consistently more or less liable to one or other of these effects, despite a wide range of levels of expression which have been observed. The *Enhancer* and *Suppressor* mutants are also dominant, and, although unlinked to *Hairless*, are very closely linked to one another. An attempt to interpret all of the observed effects on all of the chaetae studied has met with some success, but complex assumptions have to be made about development in order to do so. In particular, it seems useful to invoke genetic control mechanisms both to explain the mutant effects and to allocate a role to the modifiers.

2. THE DEVELOPMENT OF CHAETAE AND CHARACTERISTICS OF THE HAIRLESS MUTANT

The changes which take place in the development of insect chaetae are known from light microscopic studies (see Stern, 1954). The externally visible adult chaeta consists of two parts, a 'hairlike' trichon and a basal ring generally called a 'socket'.

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The form of these two structures, which are chitinous, is determined by the action and interaction of the two cells, the trichogen and tormogen, which can be identified in the presumptive epidermis 5 to 10 hours after pupation (in *Drosophila*). The two cells arise by division from a single cell, itself a division product of a '*bristle initial cell*'. The second product of the earlier division (at least in some of the insects) yields the connexions of the chaeta to the central nervous system.

The *Hairless* mutant is a dominant mutant which shows recessive lethality and, in the heterozygote, leads to a variety of effects on eyes, wings, body colour, size and, most strikingly, on chaetae. It is located at 69·5 on chromosome III of *Drosophila melanogaster* (see Bridges & Brehme, 1944). The H^1 allele was used in this work. When *Hairless* effects a chaeta it often removes the trichogen leaving a vestigial structure at the site of the chaeta. The vestige has been called a 'socket' on account of its resemblance to the basal ring of a normal chaeta. On closer inspection, however, most 'sockets' can be seen to be double structures. The two parts were shown by Lees & Waddington (1942) to be products of two cells, presumably the aberrant tormogen and trichogen.

Based upon this observation and the abnormal relative orientation of these cells, Lees & Waddington supposed the primary abnormality of the mutant to be in the mechanism determining the orientation of cell division.

After an extensive study, I am led to reject this hypothesis. The rejection in no way alters the validity of Lees & Waddington's conclusions about the normal relationship between the trichogen and tormogen which was based on many mutants and to which the particular mode of action of any one mutant is not essential.

The initial basis for questioning the hypothesis is a more detailed study of mutant effects. A vestige is not always a double structure. If it is single it is very small, compared with the largest double structure. These are extremes in a continuous size range. The larger the vestige, the more disparate the size of its two parts. The larger the size, the further from hemispherical symmetry is the vestige. The largest vestiges consist of two crescent-shaped structures, with concave edges facing and touching at the tips. These have bilateral symmetry and polarity which define an axis which is nearly constant at a given site and more or less related to the normal orientation of the chaetae on the adult fly. (The trichon of a given chaeta runs obliquely and in a fixed direction on a normal fly.)

The multipel levels of effect can be extended. Sometimes well-developed chaetae are, nevertheless, deformed. At the other extreme it is very common for no vestige to exist at an affected site. Such a large range of effects does not seem likely if only orientation of cell division is involved. Indeed there is evidence that a measure of orientation is retained despite effect. It *looks* more as if the mutant halts development at various stages and what has been achieved up to then is retained. It will, indeed, prove to be at least a part of the interpretation of *Hairless* expression that different mutant effects represent different stages in normal development.

Now it is a well-known fact that *Hairless*, like many other mutants, does not affect all chaetae to the same extent. (Plunkett, 1926). The small microchaetae, for instance, are little affected. And, given a particular stock, the frequencies of effect on

different macrochaetae will not all be the same. It has been often assumed that such differences necessarily reflect the process of pattern formation (Goldschmidt, 1937, Sturtevant, 1961) but there has been and still is no simple way of relating pattern formation to them. However, these differences, together with the multiple effects of *Hairless* on chaetae, led the author to suppose that the mutant might be suitable material to resolve this problem. But it now seems possible that the differences, in this mutant at least, may fairly be related to the spatial disposition of the chaetae with respect to the ring-gland, an endocrine organ and, hence, only to an already established pattern.

3. MATERIALS AND METHODS

The large macrochaetae were studied because *Hairless* commonly affects them and because their normal 'pattern' is of remarkable constancy and, hence, of great interest. This constancy itself allows easy identification of mutant effect.

Six macrochaetae on the doral surface of the head (shown in Fig. 1) were chosen because they include the most intensely affected site, the postvertical, and also form a compact region for observation. A seventh chaeta, the median orbital, was not

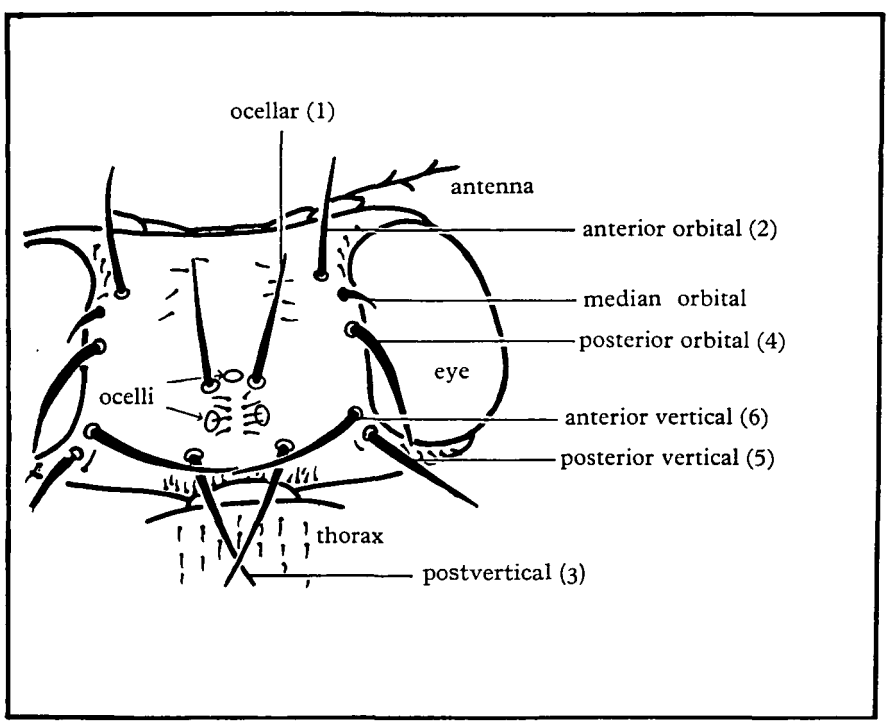

Fig. 1. The positions of the macrochaetae on the dorsal surface of the head of *Drosophila melanogaster*. The normal nomenclature is supplemented by numbers 1–6 at the sites which are affected by *Hairless*. The median orbital is only rarely affected (less than 0·1%). The numbers represent the order of the '*socket-series*', an inherent property of the organization of the pattern of the chaetae which is revealed by the presence of the *Hairless* mutant. Not to scale.

included in the analysis. It is rarely affected by *Hairless*, is intermediate in size between macrochaetae and microchaetae, and might tentatively be thought of as a large microchaeta.

The primary research method was to examine the range of expression of the mutant by selection. This technique has been used for studies on chaetae by Rendel (1959) and Smith & Sondhi (1960). For simplicity, the penetrance and expression of the mutant at a given site was reduced to a three-way classification; unaffected, partially affected and completely affected. These terms are defined below:

Unaffected. The site bears a structure, recognizable as a compound of basal ring and trichon, under 50 × magnification.

Partially affected. The site bears a chitinous structure which shows no sign of a

Table 1. *The criteria used in selecting for modification of the expression of the* Hairless *mutant*

Line		Bristle**					
Name	Symbol	Ocellar (1)	Anterior orbital (2)	Post-vertical (3)	Posterior orbital (4)	Posterior vertical (5)	Anterior vertical (6)
High	(H)	+	+	+	+	+	+
Low	(L)	—	—	—	—	—	—
Socket	(S)	Selected against the presence of any complete effect and for a high frequency of partial effect at all sites					
Anterior	(A)	Not selected	+	Not selected	+	—	—
Posterior	(P)	Not selected	—	Not selected	—	+	+

\+ = selected for high expression at a site, regardless of complete or partial effect.
— = selected for low expression at a site, regardless of complete or partial effect.
** The numbers in parentheses indicate the position of the bristle in the 'socket series'.

trichon. This class includes all structures previously described as 'sockets' or 'vestiges'.

Completely affected. Neither a 'vestige' nor a normal chaeta is present at the site. The effect is identified by a site's being unoccupied.

Five selection lines were bred with respect to the frequencies of these classes. The selection criteria, which are summarized in Table 1, were designed to examine the extent to which partial and complete effect were interrelated at different sites and were also related to the penetrance at particular sites.

A four-pair rotational mating breeding system was used and the selection was for one fly in twenty, unless inviability or infertility prevented the use of the best culture. The dominant *Hairless* was maintained in a balanced lethal system with *LVM*, a third chromosome inversion.

Each of the selection lines responded positively, as can be seen from Fig. 2, which

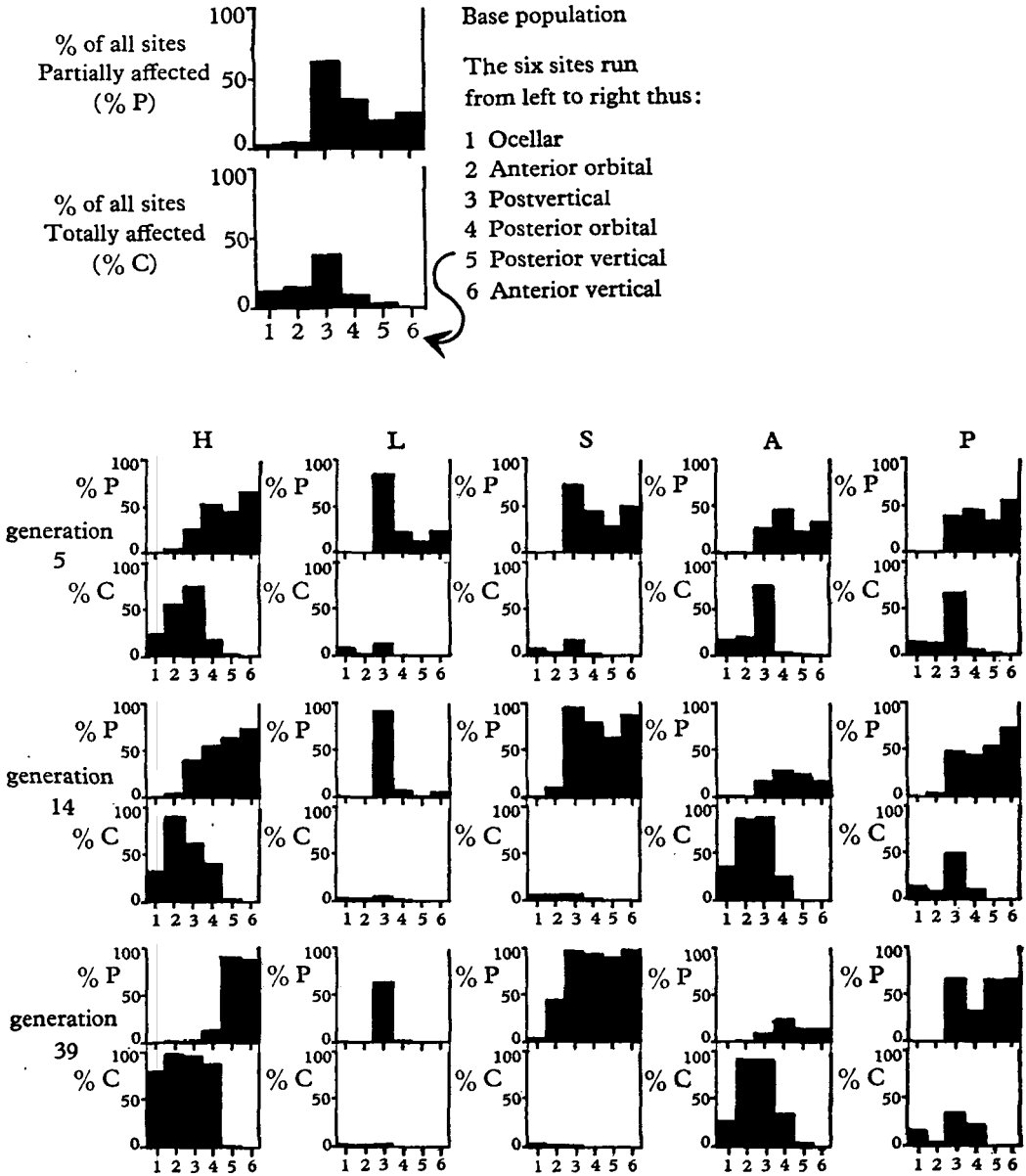

Fig. 2. The frequency of partial and complete effect of *Hairless* in five selection lines. The upper distribution represents partial effect, the lower, complete effect. The six sites studied are arranged in order of the value of 'E' (percentage of complete effect at affected sites), decreasing from left to right. This order is the same one in each population (see Table 2)—the 'socket series'. The figure shows distribution of effect in the base population (200 flies of each sex, i.e. each percentage is based on 800 sites) and generations 5, 14 and 39 (80 flies of each sex, i.e. 320 sites). The selection pressures applied are described in Table 1.

shows the levels in mutant expression at three sample generations during the thirty-nine for which the lines were maintained. In the H line no overall response at the postvertical site was possible; otherwise only the posterior orbital site in the A and P lines showed no overall appropriate response. This singular fact will be discussed later.

For the main purpose of this paper one important fact needs to be extracted from the data in Fig. 2. This will be dealt with in the next section, and summarized in Table 2.

4. THE SOCKET SERIES

The selection lines can be treated simply as five very different populations bearing the *Hairless* mutant. They have an interesting and unexpected property in common. *In each of the populations, indeed in every generation fully analysed (every fourth or fifth), one constant factor interrelates the six chaeta sites.* Table 2 contains values of a measure of the ratio of complete and partial effect at affected sites. This measure of what might be called 'expressivity', is defined as follows for a given site in a given population (say, site X):

$$E = \frac{(\text{Frequency of complete effect at X}) \times 100}{\text{Summed frequency of both types of effect at X}}$$

(E is the percentage of complete effect at affected sites. It is a mistake to suppose that this is a measure of intensity of mutant effect at the site, for as we shall see later, it is the condition of the site and not necessarily the intensity of mutant expression which determines which kind of effect appears.)

It is possible to rank the six sites in a given population in order of the values of E, as has been done in Table 2. What is surprising and important is that in all five populations, and in each generation, the ranking order is identical. It is:

1. Ocellar
2. Anterior orbital
3. Postvertical
4. Posterior orbital
5. Posterior vertical
6. Anterior vertical

This unique series is one out of 720 possible orders, and also applies in many other genetically and environmentally varied populations, including *Hairless* alleles of different mutational origins, different genetic backgrounds extracted from geographically dispersed regions, and cultures grown at 19°C., 25°C. and 29°C. (Nash, unpublished). This unique order will be referred to as the '*socket-series*'.

5. THE EFFECTS OF MODIFIERS *Su-H* AND *E-H* IN THE SELECTED BACKGROUNDS

Suppressor of Hairless (*Su-H*), a mutant described in Bridges & Brehme (1944) and *Enhancer of Hairless* (*E-H*), a new mutant, are closely-linked dominant modifiers of *Hairless*. They are located between 50·0 and 50·5 on chromosome II (see Table 3). In the absence of *H* neither affects chaetae appreciably.

Table 2. *'Expressivity' (E) values at six macrochaetal sites in three sample generations of the selection lines*

Generation	Site	Line H	L	S	A	P
5	1	100	100	100	100	100
	2	93	100	80	100	100
	3	74	15	13	75	62
	4	23	1	3	9	10
	5	3	0	0	6	2
	6	0	0	0	2	0
14	1	99	100	100	100	100
	2	96	100	38	100	78
	3	60	4	5	85	58
	4	42	0	2	47	19
	5	2	0	0	0	0
	6	0	0	0	0	0
39	1	100	100	63	100	100
	2	99	100	6	99	100
	3	97	1	3	91	34
	4	86	0	0	42	6
	5	2	—	0	22	0
	6	0	—	0	0	0

The values shown are calculated thus:

$$\text{Expressivity} = \frac{(\text{Frequency of complete effect}) \times 100}{\text{Summed frequency of both types of effect}}$$

The values are calculated from the effects observed on 160 flies in each generation of each line. 320 examples of each site were inspected, but the number on which the calculation was made depended upon the penetrance. An unfilled value indicates that penetrance was zero.

The naming of sites is as follows:

1. Ocellar
2. Anterior orbital
3. Postvertical
4. Posterior orbital
5. Posterior vertical
6. Anterior vertical

The naming of lines is as follows:

H, high; L, low; S, socket; A, anterior; P, posterior

Su-H is homozygous lethal. *E-H* is viable and gives more extreme modification in the homozygote. In modifying *Hairless* they have such radical effects that the 'socket-series' is disturbed. In this property they are essentially different from the modifier effects revealed by selection. They also differ from these in being of far greater strength than any single chromosomal contribution to the selection responses (Nash, unpublished). Furthermore, in some genetic backgrounds, they produce differences in mutant expression quantitatively greater than those produced by selection over thirty-nine generations.

Figure 3 illustrates the effects of these modifiers in the different genetic

backgrounds of the selection lines. The populations used were first generation intercrosses between a stock bearing the modifier together with the *Cy* second chromosome inversion and the various selection lines. The segregation of *Cy* (*Curly wing*) from the modifier provided a control population, also shown in Fig. 3. Although the selected genetic backgrounds are only heterozygous in these flies, they clearly affect the mutant and modifier expression. Table 4 shows what happens to the values of 'E' (the percentage of complete effect) in these populations. The socket-series loses its integrity (*E-H*) or else becomes meaningless (*Su-H*).

In combinations, *E-H* and *Su-H* in *trans* position produce a phenotype intermediate to those produced by the modifiers and similar to the stock without either modifier. This also applies to the *cis* position, if any of the recombinants shown in

Table 3. *The linkage of* Suppressor (Su-H) *and* Enhancer (E-H) of Hairless *to each other and to the markers black* (b) *and cinnabar* (cn) *on chromosome II*

A sample of recombinants between *black* and *cinnabar* from the cross

$$\frac{b\ E\text{-}H\ cn}{+\ Su\text{-}H\ +}\ \frac{H}{LVM}\ ♀ \times \frac{b\ +\ cn}{b\ +\ cn}\ \frac{H}{LVM}\ ♂$$

were scored for their phenotypic expression of the *Hairless* mutant. The stocks used contained the genetic background from the P selection line. This allowed unequivocal distinctions to be made between the two modifiers and the unmodified expression of the mutant. The results were as follows:

	Enhanced	Suppressed	Unmodified
$+\ cn$	25	103	6
$b\ +$	122	24	3

These data, assuming the published map positions of black (48·5) and cinnabar (57·5), allow the calculation of the linkage between the two modifiers as 0·28 units and their map positions as between 50·0 and 50·5 on the second chromosome. Since phenotypic observation did not indicate which, if either, of the two classes of 'Unmodified' flies were double mutants for the modifiers, it is not possible to give an order for them.

Table 3 are indeed the double mutant. It is, however, possible that these are all wild-types, multiple recombinations accounting for their association with both recombinants of the outside markers.

The terms 'Enhancer' and 'Suppressor' are not sufficient to describe the effects of these mutants. For *E-H* is much more active in enhancing partial effect than complete effect, whilst *Su-H* suppresses partial effect strongly. Indeed, at some sites *Su-H* actually enhances complete effect. This is not simply a matter of more sites being available for complete effect (partial effect being reduced) since the phenomenon is most clearly seen at sites where partial effect is almost unknown (viz., the ocellar site (1)).

The close linkage and opposite effects of these two modifiers suggest that they may be rather unusual mutations. The best-known systems which exhibit similar properties are bacterial control elements. *Su-H* and *E-H* might be considered as formally analogous to the two kinds of mutations at a bacterial regulator locus (Monod &

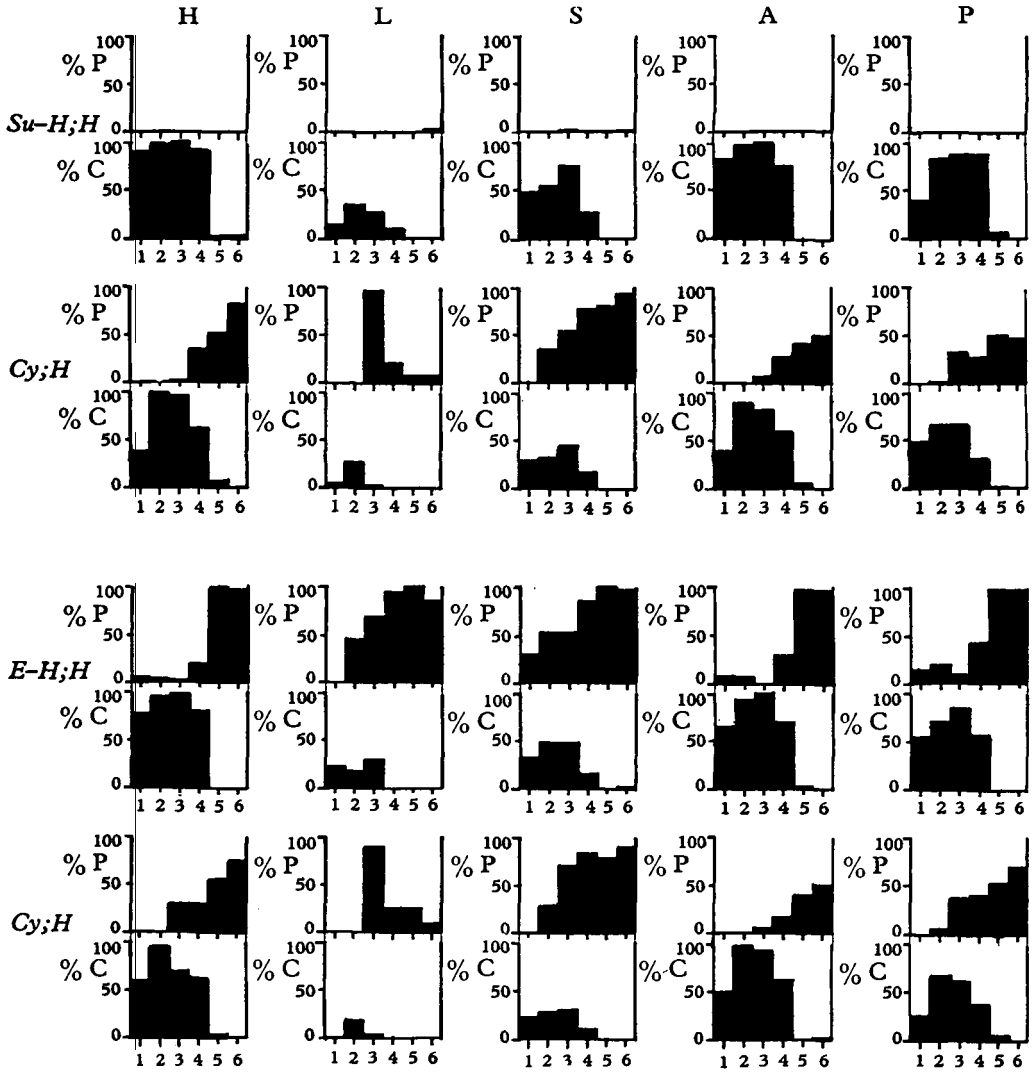

Fig. 3. The distribution of *Hairless* mutant effect in the presence of *Suppressor of Hairless* (*Su-H*) and *Enhancer of Hairless* (*E-H*). Distributions of effect in populations derived from crosses: selection line female × *Cy*/*modifier* (*not-Hairless*) male. The axes are the same as are used in Fig. 2 and the samples used the same as in Table 4.

Jacob, 1961). Their homology is, of course, by no means certain. But it will be argued later that the analogy might at least be extended to calling *Su-H* the 'constitutive' and *E-H* the 'super-repressed' mutant.

6. THE RELATIONSHIP OF PARTIAL AND COMPLETE EFFECT

A somewhat arbitrary division of *Hairless* effects into partial and complete effect, has resulted in two interesting observations: the various sites are related to each other in a rigorous manner with respect to this division—hence the 'socket-series'—

Table 4. *'Expressivity' (E) values at six macrochaetal sites in the presence of* Su-H *and* E-H, *the modifiers of* Hairless, *with genetic backgrounds derived partly from the selection lines*

Chromosome II segregant	Site	Source of genetic background				
		H	L	S	A	P
Su-H	1	100	100	100	100	100
	2	100	100	100	100	100
	3	100	100	97	100	100
	4	100	100	100	100	100
	5	100	—	—	—	100
	6	100	0	0	—	—
Cy	1	100	100	100	100	100
	2	100	100	48	100	96
	3	98	26	45	93	68
	4	64	0	18	69	54
	5	12	0	0	11	5
	6	0	0	0	0	0
E-H	1	94	100	52	90	79
	2	95	28	47	93	78
	3	98	30	47	100	89
	4	80	0	15	70	57
	5	0	0	0	2	0
	6	0	0	2	0	0
Cy	1	100	100	100	100	100
	2	100	100	50	100	93
	3	78	5	30	95	62
	4	68	0	10	78	48
	5	4	0	0	0	9
	6	0	0	0	5	0

The method of calculating 'Expressivity' and a key to the sites and the sources of genetic background is shown in the caption to Table 2.

The flies used were derived from the cross:

Selection line female × *Cy/modifier* (not-*Hairless*) male

The progeny are of four genotypes. Only *Hairless* segregants from the *H/LVM* balanced lethal system used in the selection lines were scored. *Su-H*:*H* flies sometimes have normal chaetae, but can be identified by the pleiotropic effects of H. The values are calculated from ten flies of each sex and genotype, generally taken from two separate cultures.

and the modifiers, whilst operating differently and strongly on partial effect both tend to enhance complete effect, but rather weakly.

One further set of data can be presented which fractionates the two kinds of effect and helps in the interpretation of *Hairless* expression.

Plunkett (1926) described a technique which can be used to demonstrate a limited time period of sensitivity if a mutant is subject to environmental variation. *Hairless*

expression can be varied by differences in the temperature at which the mutant fly develops. Plunkett's '*temperature effective period*' (TEP) is obtained by exchanging developing flies between two temperatures, and is defined as the period before which the expression is typical of the second temperature and after which it is typical of the first temperature. For '*Hairless*' it is possible to find the TEP both for complete and

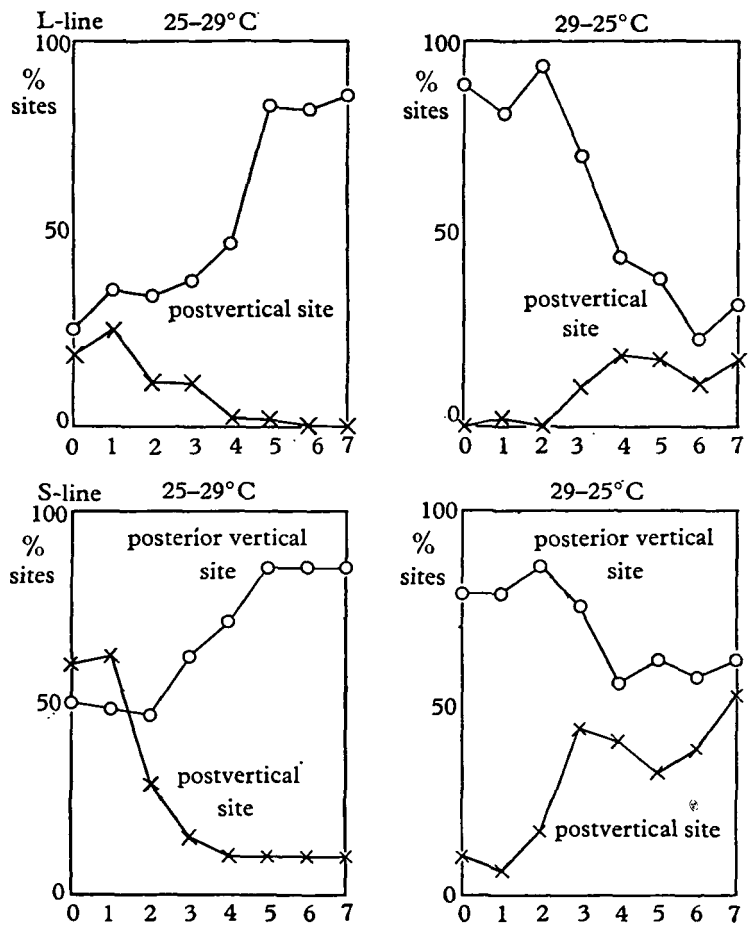

Fig. 4. Temperature effective periods for complete and partial effect as shown by temperature exchange experiments. Each point is based upon at least twenty flies, (ten of each sex) grown in cultures originally containing fifty newly hatched larvae. ––×––×–– = complete effect; ––○––○–– = partial effect. Abscissae show day of transfer. Day '0' is the day of hatching (at 25°C.).

for partial effect. For various reasons, only particularly favourable sites could be expected to show independent TEPs for two kinds of effect.

Figure 4 shows two such examples. In both, the effects are separable in time. The TEP of the partial effect phase follows about a day after the TEP for complete effect. This seems to confirm the view, suggested above, that complete effect is indeed an early phase of mutant effect whereas partial effect arises later.

These TEPs correspond well with other observations on chaetae. Partial effect TEP coincides with the pupal phase when the tormogen and trichogen are enlarging (Lees & Waddington, 1942) and complete effect TEP corresponds with the TEP of *scute* mutants (Child, 1935), which produce only total effect and which may well be operative at the time of induction of chaetae. (Falk, 1963).

7. THE RELATIONSHIP BETWEEN VARIOUS CHAETAE

It is extremely probable, considering the recessive lethality of *Hairless* and the expression of the mutant in triploids ($+/+ < +/+/H < +/H < +/H/H$ [H/H = H/H/H, lethal]) shown by Gowen (1933), that its effects are caused by a deficiency of function of the mutant allele.

If this is assumed, then we must suppose that a site commonly subject to complete effect is encountering deficiency early in its own developmental sequence. Conversely, a site subject to partial effect encounters deficiency late in its development.

Making another simple but justifiable assumption, that there is a single period of deficiency throughout the fly, and that at all other times the productive capacity of the $+^H$ allele present in the heterozygotes is sufficient to support normal development, a prediction can be made:

In wild-type flies sites with high 'E' values (showing much complete effect), develop late and those with low 'E' values (much partial effect) develop early in the total development of the fly.

The best dissection yet obtained to ascertain the correctness of this prediction showed that indeed five (the only ones observable) of the six sites were staggered in the degree to which the trichon was developed. They ranged from the ocellar which showed a trichon shorter than the diameter of its initial cell to the anterior vertical where the trichon was fully developed. This *Oregon wild-type* pupa was raised at 25°C. and dissected 37·5 hours after puparium formation.

The asynchrony and the actual timing of particular sites fit the prediction. It is therefore proposed that the basis of the 'socket-series' is the asynchrony of chaetal development and that the programme of development of chaetae is rigidly fixed in the development of the fly.

The implications of the asynchrony (which contradicts an assertion of Robertson (1936) that all chaetae develop contemporaneously) may be great. It clearly reflects prepatterning of the epidermis in a sense analogous to spatial prepatterning (Stern, 1954). Indeed, it may precede prepatterning and be the source of spatial pattern, as is also suggested by the work of Spickett (1963). Pattern formation would in this case become an example of the 'multiplicative' model provided by Smith (1960) rather than the simultaneous concentration model considered by Stern (1954) and Smith (1960).

8. DISCUSSION

Considering, firstly, the selection effects, it is easy to see how increasing or decreasing duration of deficiency as well as actually raising or lowering the concentration of the normal substance ($+^H$ *substance*) could bring about the response of the

H- and L- lines. The A-, P- and S- lines would require shifts in the timing of deficiency; the A-line to an earlier stage, so as to increase complete effect at the anterior orbital site and the P- or S-lines to later stages, increasing partial effect in both cases.

It is interesting that, as noted above, the posterior orbital failed to respond either in the A- or P-line. It is temporally associated with the vertical sites, yet was selected in conjunction with the nearby anterior orbital site (see Table 1). There was no lack of genetic variability capable of influencing expression at that site, but it was of a kind not available to the particular selection pressures used.

Although these remarks allow us to 'explain' what happened in selection, two major questions remain:

(1) What is $+^H$ substance? and
(2) What regulates the variations of concentration of $+^H$ substance which are required to explain the selection responses?

It is in answer to the second question that a possible role will be assigned to the modifiers.

In answer to the first, we may make a tentative suggestion. *The normal product of the Hairless locus is a hormone produced by the ring gland.* The evidence, which is only circumstantial, is as follows:

A. A circulating compound would fulfil the requirements for synchronous concentration changes throughout the fly.

B. The postvertical site and ocellar site, of all those studied, are closest to the site of the ring gland, an endocrine organ situated above the brain and below the ocelli (see Fig. 1). They also have characteristics which especially differentiate them from the other sites with respect to the effect of *Hairless*. The postvertical site is always strongly effected (never less than 50%). It is posterior to the ocelli and presumably receives haemolymph which, as it passes forward from the heart, has not yet reached the ring gland. This would probably be very poorly supplied with ring-gland secretions. Precisely the converse applies to the ocellar site and this is disproportionately resistant to *Hairless* effects (never more than 80%). The remaining four sites have shown a full range from 0–100% of effect, in various populations.

C. Hormones commonly regulate growth and their deficiency could easily produce the kind of abortive action which the *Hairless* mutant has.

D. Hormonal concentration is commonly subject to negative feed-back control. This last factor could supply the answer to the second question 'What regulates the variations of concentration of $+^H$ substance?'

With respect to this point it should be said that a negative feed-back system might operate as follows: substance a (say, $+^H$ substance) is required at a fixed concentration c_r. A mechanism exists such that if the concentration actually present is c_r then production of a matches its utilization. But if c deviates either downwards or upwards the rate of production is changed (higher or lower as the case may require) so as to return the concentration to c_r. In vertebrates systems are known whereby two hormones produced by different endocrine organs interact to produce precisely this effect.

A lesion in the genetic mechanisms underlying a feed-back controlled system will produce aberrant behaviour of the total system. If only half the productive capacity remains, as may be the case for $+^H$ substance (the mutant being heterozygous) not only will there be lowered initial concentration, but this will not return with the usual rapidity to the normal required level, since the original source of deficiency will also fail to respond appropriately to a signal demanding increased production. In these circumstances, the changes of concentration which occur as time passes will depend on the detailed nature of the components of the system. These are not known in the case of *Hairless*.

One fairly possible time-course would be a transient period of dramatically low concentration preceded and followed by normal or near-normal concentrations. It is suggested that such a time-course is found in the presence of the *Hairless* mutant. If it were, it would serve to explain the fact that only a single and necessarily transient period of mutant effect appears to occur in development.

Furthermore such a system would be susceptible to modification by many subtle influences in development, for example by altering overall development rate. In this connexion it is interesting that flies of the A-line, where early mutant effect was selected, developed more slowly than the H-, L-, S- and a control unselected stock, whereas the P-line developed faster than these other lines (Nash, unpublished). All things being equal, these stocks would, by virtue of the selection procedure, have increased in development rate.

Such considerations led to the original proposal that a feed-back system was at work (Nash, 1963). But the idea that feed-back is involved led to a very simple explanation of the modifiers *Su-H* and *E-H*, whose expression and linkage relationships had not been considered at that time.

As was mentioned above, the modifiers have some of the properties of 'regulator' mutants. It should be possible to find mutants within a hormonal control system with analogous properties to the bacterial control mutants. *Su-H* and *E-H* could be examples of these. If, as has been assumed, *Hairless* is a deficiency mutant at the structural locus for the $+^H$ substance, a suppressor could be a 'constitutive' mutant and an enhancer a 'super-repressed' mutant, at a regulator locus for this structural gene. Furthermore this hypothesis may well be amenable to a direct test. The homozygote *SuH*/*SuH* is lethal. Crosses between flies known to be *SuH*/+ and, as far as is known, containing no other lethal mutants, yield cultures in which appreciable numbers of larvae hatch, but fail to develop, remaining alive but small until siblings have pupated. Such larvae, if they prove to be *SuH*/*SuH* in genotype may provide a direct means for chemical analysis of $+^H$ substance, which should be produced in considerable excess by a constitutive homozygote.

SUMMARY

1. *Hairless* (*H*), a dominant mutant of *Drosophila melanogaster*, affects the chaetae.

2. Various 'intensities' of effect are interpreted as representing different times in

chaetal development at which deficiency of '$+^H$ *substance*' is encountered. 'Temperature effective period' studies confirm this view.

3. The chaetae at different sites are liable to characteristically different 'intensities' of effect. In other words a particular site tends to be affected at a limited time in its development. If it is supposed that a single period of deficiency of $+^H$ substance accounts for effects on all chaetae, then it follows that development of the various chaetae is asynchronous. Some histological observations tend to support this prediction.

The implications of asynchrony with respect to pattern formation are discussed.

4. It is argued that the source of $+^H$ substance may be an endocrine organ, the ring-gland.

5. Two modifiers *Su-H* and *E-H*, whose expression and linkage relations are described in detail for the first time, are, it is suggested, respectively 'constitutive' and 'super-repressed' mutations of a 'regulator' locus controlling negative feed-back on the $+^H$ locus.

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