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THE GENETIC CONTROL OF DEVELOPMENTAL COMPETENCE AND MORPHOGENETIC TISSUE INTERACTIONS IN GENETIC MOSAICS*

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With 5 figures

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I. Introduction

The differentiation of the macrochaetae on the surface of wild type *Drosophila melanogaster* occurs according to a specific pattern of distribution and individual size. In the early pupa the imaginal hypodermis of the head and thorax consists of numerous small cells of equal appearance. Then, at a few characteristic locations, groups of enlarged cells can be distinguished which differentiate into bristle organs. Combining the results of workers on the origin of the bristle organ in several different

* Dedicated to KARL HENKE on the occasion of his sixtieth birthday.

The basic data were collected a considerable time ago but have only now become intelligible. I wish to acknowledge earlier helpful, critical comments by Dr. A. H. STURTEVANT on an unpublished manuscript dealing with these experiments and of Dr. ALOHA HANNAH-ALAVA on the present report. The analysis was completed during tenure of a John Simon Guggenheim Memorial Fellowship while I enjoyed the hospitality of Professor C. W. WADDINGTON at the Institute of Animal Genetics, Edinburgh, and of Professor A. KÜHN at the Max-Planck-Institut für Biologie, Tübingen.

insects, it appears that such an organ consists of four cells which are derived from a single cell by two mitoses. These four cells form, respectively, the bristle proper, its socket, a sensory nerve cell and its sheath (LEES and WADDINGTON 1942; SCHWENK 1947; HENKE 1951; WIGGLESWORTH 1953). Obviously, the characteristic locations in which these differentiations are initiated must have properties which distinguish them from their surroundings and from one another. In other words, a "prepattern" must anticipate and condition the appearance of the observed pattern of bristles.

The bristle pattern is subject to genetic control. Some genotypes decrease the number of places in which macrochaetae are formed, others increase them. Moreover, changes in the position of specific bristles may accompany the changes in their total number. There are at least two methods by means of which different genes may determine different bristle patterns. The genes may either affect differently the paths of development prior to the appearance of the prepattern and thus lead to different prepatterns in different genotypes, or the differential effect of different genes may not set in until after the establishment of one and the same prepattern. In the first alternative different bristle patterns would originate in consequence of different prepatterns; in the second, the bristle pattern would depend on the genetically controlled competence of the hypodermal cells to respond or not to respond to the singularities of the constant prepattern.

The results of previous studies involving male vs. female genotypes as expressed in presence or absence of a sex-comb, and normal vs. achaete genotypes as expressed in presence or absence of certain thorax bristles have been interpreted in terms of constant (nonvarying) prepatterns but genetically controlled varying competence of cells (STERN and HANNAH 1950; STERN 1954a, b). Other data by HANNAH (reported in STERN 1954b) suggest that genes for the formation of extra sex-combs and for the development of legs in place of antennae may act by introducing new prepatterns into the embryonic anlagen of appendages to which tissues with unchanged competence may then respond by differentiations at unusual locations.

The present report continues the analysis of the genetic control of developmental patterns. Specifically it is concerned with the determination of a particular "extra" bristle not found on normal flies of this species. Two different mutant genetic conditions were studied, one which leads to the formation of this bristle, and another which, superimposed on the first, leads to suppression of this specific differentiation. The results show that different genotypes endow the hypodermal cells with different competence. In addition, the data demonstrate morphogenetic interrelations between genetically different tissues in mosaic individuals.

II. Materials and Methods

In certain stocks of *D. melanogaster* in addition to the normally present 11 pairs of macrochaetae a twelfth pair is differentiated on the dorsal mesothorax (Fig. 1). It is located anterior to the posterior postalar bristle at or posterior to the level of the posterior supra-alar bristle in a region normally covered only by microchaetae. This bristle is a member of the normal bristle complement of some other diptera where it is known as the interalar. The specific stocks employed in the following experiments owed their interalars to the presence of Theta (Θ; PATTERSON 1930), a small, cytologically visible duplication of part of the X-chromosome.

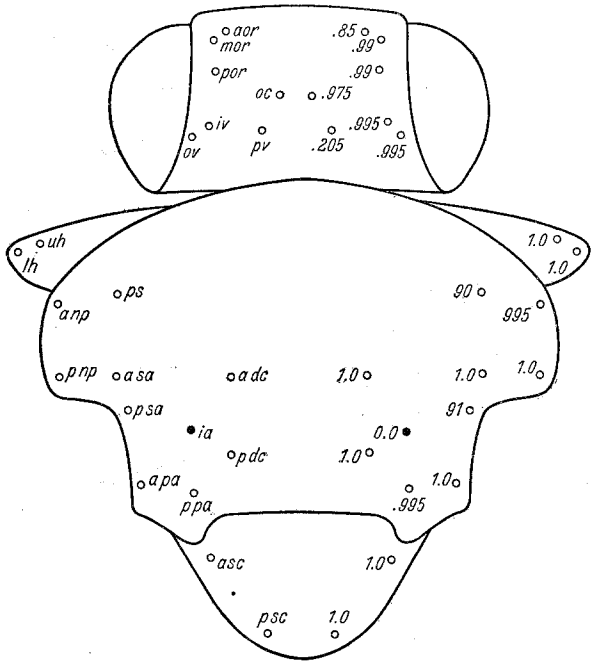

Fig. 1. Dorsal view of head, pro- and mesothorax with the positions of the bristles. *a* anterior; *p* posterior; *m* median; *or* orbital; *oc* ocellar; *iv-ov* inner and outer vertical; *pv* postvertical; *uh-lh* upper and lower humeral; *ps* presutural; *np* notopleural; *sa* supra-alar; *pa* postalar; *dc* dorsocentral; *sc* scutellar; *ia* interalar. Normally located bristles, in *D. melanogaster* are indicated by a circle (○) and the extra (interalar) bristle by a filled-in circle (●). The numbers indicate the fraction of cases in which the bristles were present in M-n/scute[1] females

(Essentially, Theta may be regarded as an X-chromosome which, as a result of irradiation with X-rays, had lost most of its material except a short terminal segment at both its left and right ends. It includes the normal loci for yellow and bobbed.) The Theta fragment is attached, by its right end, to the right end of an X-chromosome. "Homozygous Theta" females ($X \cdot \Theta/X \cdot \Theta$) have two Theta fragments, one attached to each of their X-chromosomes; "heterozygous Theta" females ($X \cdot \Theta/X$) possess one normal X-chromosome and one with the Theta attachment. In Theta males ($X \cdot \Theta/Y$) the single X-chromosome carries the attachment.

In contrast to Theta which leads to the differentiation of the interalar bristle another mutant, the dominant, sex-linked, Minute-n [M(1)n], when present together with Theta tends to suppress interalar differentiation. Apart from its interaction with Theta, Minute-n causes the production of shorter, more slender macrochaetae than the not-Minute wild type. Minute-n is an important tool for the study of gene action in development, since under its influence somatic crossing over between the two X-chromosomes of a female and between its X-chromosomes and a Theta fragment occurs relatively frequently. As shown in Figure 2 somatic segregation in consequence of such crossing over and followed by multiplication of

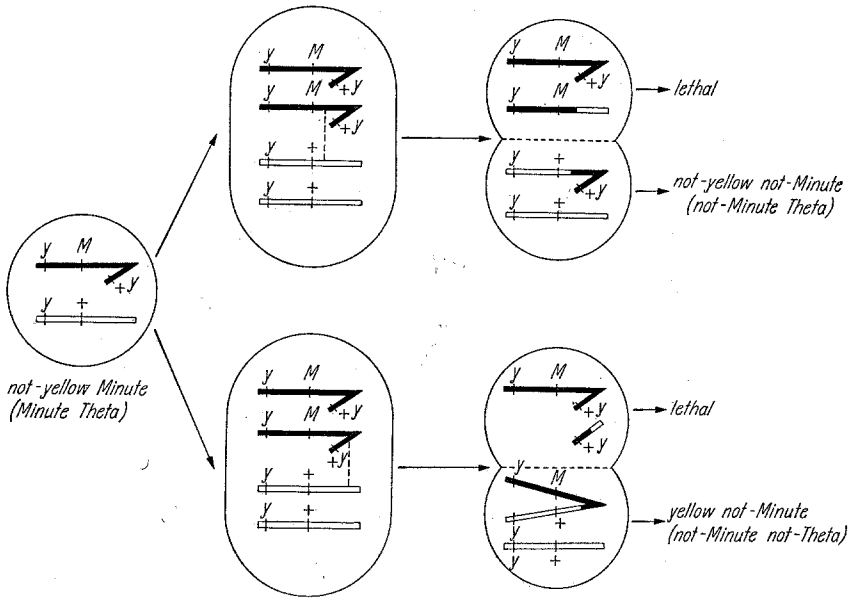

Fig. 2. Two examples of somatic crossing over and segregation in not-yellow, Minute cells of the genotype yM/y +. Left: a cell before crossing over. Upper line, middle: doubling of the chromosomes and crossing over between two of the X chromosome strands in the region to the right of M. Upper line, right: daughter cells resulting from segregation of the four strands. One of the daughter cells being homozygous for M cannot form a viable cell patch. The other will form a not-yellow, not-Minute patch. Lower line, middle and right: crossing over between the proximal regions of the Theta fragment and one of the free X chromosome strands results in a free Theta fragment and a pair of attached X chromosomes. One of the daughter cells, being hemizygous for M, cannot form a viable cell patch. The other will form a yellow, not-Minute patch (its genotype M/+/+ produces a not-Minute phenotype)

the resulting cells leads to the appearance of patches of tissue which possess a genic content different from that of the main part of the individual. In the examples represented in the figure the original genotype of the developing individual (left) was yM · Θ/y +, corresponding to a not-yellow Minute phenotype, the not-yellow but grey body coloration and black bristles being due to the wild type dominant allele of yellow in the Theta fragment. In the upper row (right) one of the two cells formed following somatic crossing over and segregation has lost Minute-n. Since the size of each bristle on a fly mosaic for Minute and not-Minute genotypes is determined by the genotype of the specific bristle-producing cell itself, patches with the segregated constitution y + · Θ/y + will form not-Minute bristles.

In the lower row (right), one of the two cells has lost Theta. This results in the appearance of surface patches with yellow body coloration and brown bristles. (In spite of some interdependence in pigmentation of yellow and nonyellow in mosaics (HANNAH 1953), the relative cell-autonomy of expression of the two genotypes is sufficient to serve as a clear marker of the borders of the relevant thoracic mosaic areas described below). Since somatic crossing over in different regions of the X-chromosomes and between the X-chromosomes and the homologous portions of the Theta fragment leads to a variety of genotypes, it is necessary to determine both the bristle size and the coloration of segregated patches in order to define their genotype comprehensively.

A third marker gene, present in the majority of experiments, is of minor importance for the following developmental analysis, though valuable in the determination of genotypes of segregates. It is the recessive X-chromosomal mutant singed (sn) which causes growth of crooked instead of the normally nearly straight or slightly curved bristles. Whenever somatic segregation results in the production of homo- or hemizygous singed areas on a heterozygous nonsinged background, the shape of the hairs and bristles is a marker for the genotype of the cells which have produced them.

Omitting singed as well as other genes of no importance to the problems of pattern differentiation, all females were of one or the other of two genotypes: $yM \cdot \Theta/y$ and $yM/y \cdot \Theta$. Accordingly their phenotype, apart from segregation patches, was not-yellow, Minute. Two kinds of relevant patches were observed, namely (1) not-yellow not-Minute, and (2) yellow not-Minute. The validity of the developmental analysis depends on the essentially accurate designation of the genetic constitution of the patches. The deductions leading to these designations are more reliable in some experiments than in others. One-half of the 110 mosaics which form the raw data for this paper were obtained among females in which both X-chromosomes had the normal genic sequence. Here the assignment of genotypes to the patches is relatively simple. The other half occurred in females in which one of the X-chromosomes possessed the long inversion associated with a bobbed-deficiency (bb^{Df}). These genotypes pose problems to the interpretation of somatic crossing over and segregation which have not been fully resolved. Nevertheless, it is believed that, on the whole, the designation of the genetic constitution of the segregates in bb^{Df} individuals is correct. This belief is supported by the fact that correspondingly designated genotypes of patches in bb^{Df}-carrying and bb^{Df}-free flies give essentially identical bristle effects as will become apparent. It has seemed desirable, however, to record the data for the two groups of genotypes separately.

For the detailed genetic analysis of the mosaics the reader must be referred to an earlier publication (STERN 1936; particularly pp. 656—664, 669—678, and 698—708 including Figures 8, 9, 12 and 13). According to the interpretations given there, the two phenotypes of patches presumably correspond either exclusively or in the majority to the following genotypes:

1. Phenotype not-Minute Theta (= not-yellow, not-Minute): Genotype $y+ \cdot \Theta/y+$ ♀ (in bb^{Df}-free flies) and $y+ \cdot \Theta$ ♂ (in bb^{Df}-carrying flies).

2. Phenotype not-Minute, not-Theta (= yellow, not-Minute): Genotype triplo X $yM \cdot y+/y+$.

III. The frequency of the interaler bristle in flies containing Theta and the influence of Minute-n

Table 1 summarizes data on the frequency of the interalar bristle obtained under different genetic background conditions and somewhat

variable external influences such as uncontrolled temperature fluctuations. In the right-most column the strength of differentiation is expressed as number of interalar bristles per 100 sites, a site being the area potentially capable of forming an interalar. A study of the data shows that the left and right sides of a fly, although derived from two separate imaginal discs do not behave completely independently in differentiating interalar bristles but may show significant positive correlations. Such correlations do not necessarily indicate developmental interrelations within an individual but are often the expression of genetic or environmental heterogeneity in a population which affects the two sides of an individual in similar ways (PLUNKETT 1926; see also REEVE, RAND, and F. W. ROBERTSON 1955.)

It is seen that in two experiments females homozygous for Theta, and without Minute-n, developed 58 and 100% of interalar bristles respectively. Theta males without Minute-n had somewhat lower frequencies, namely 36 and 78%. The difference between the homozygous Theta females and the hemizygous Theta males is undoubtedly significant since the y·Θ/y·Θ females and y·Θ males developed as segregating offspring in the same series of cultures. This is thus an interesting lack of dosage compensation.

Females heterozygous for Theta, and without Minute-n in two experiments differentiated 26 and 31% of interalars. Females heterozygous for Theta, but also containing Minute-n, show a striking reduction in the frequency of the interalars. In six experimental groups the percentages of interalars varied between less than 1% to 22%, with only one value, based on a small sample, higher than 10%.

The inhibition of bristle formation by Minute-n includes other than the interalar bristle. A census was taken of the presence or absence of all bristles on the head, pro- and mesothorax of 100 flies heterozygous for Minute-n without Theta. This provided 200 sites for each bristle. The results are shown in Fig. 1 where 9 out of 20 bristles are listed as having been present in 100% of the cases, 6 bristles in 99 or 99.5%, 3 more bristles in between 90 and 97.5% and 2 bristles in 85 and 20.5%, respectively. (Although the flies were also heterozygous for scute-1, it is unlikely that this gene is responsible for the specific reductions in bristle numbers in these Minute-n flies. Its effect on bristles even when it is present in homo- or hemizygous constitution, follows a completely different pattern than shown here.)

It was seen that the actions of Theta and Minute-n at the interalar site are antagonistic. Theta frequently causes the differentiation of a bristle apparatus while Minute-n reduces the incidence of this developmental event. The following sections contain information which will serve to analyse the actions of Theta and Minute-n.

IV. Genetic mosaics

The production of genetic mosaics by somatic segregation or other means is equivalent to transplantation experiments involving tissues of different genotypes. Somatic segregation results in the appearance of a cell, or of two sister cells, which are different in genic content from the rest of the tissue. Depending on how early or late in development segregation had taken place, subsequent cell divisions will result in the presence of a large or a small group of cells, products of the original segregate. In nearly all cases in which segregation involves the mesothoracic imaginal disc, a coherent patch of segregated tissue is formed.

The outlines of different segregated spots vary greatly even if they have some area in common. Not only are the total areas covered of different sizes, which is a measure of time of origin of the initial segregated cell, but in addition the outlines of different spots frequently overlap if superimposed on a single diagram of a dorsal mesothorax (Fig. 3). This is interpreted as a consequence of a considerable degree of indeterminate cell lineage within a mesothoracic disc (STURTEVANT 1929, NOUJDIN 1936, STERN 1940).

As stated earlier, two main types of genetic mosaics were available: (1) those in which not-Minute, Theta tissue was present in an otherwise Minute, Theta female and (2) those in which not-Minute, not-Theta tissue was present in an otherwise Minute, Theta female. Among the segregation patches only those were of value for the present purpose which included an interalar site. In these mosaic flies the term site was interpreted somewhat more liberally than would appear justified from the location of interalar bristles on nonmosaic Theta flies. In mosaics the place of differentiation of a bristle varied over a greater area than in a nonmosaic. This phenomenon will be discussed later.

The mosaics permit an analysis of the problem stated in the introduction. If the same prepattern is present in all genotypes dealt with and thus the genotypes act by way of differential competence of hypodermal cells, then the mosaics should show presence or absence of an interalar bristle independent of the main body of the fly but corresponding to the genotype of the interalar region. If, however, different prepatterns are produced then no such autonomy of differentiation would be expected.

1. Not-Minute Theta tissue

Seventy-one females were found in which the cells of the segregation area had lost Minute-n but retained Theta, and in which the interalar site was included in the segregate. The genotypes of the segregated areas most likely were (a) female and heterozygous for Theta in the

41 flies from experiments not including bb^{Df} and (b) male and hemizygous for Theta in the 30 flies in which bb^{Df} was present. The interalar bristle had developed in 32% of (a) and in 43% of (b). These frequencies are in acceptable agreement with the mean frequencies of 27% of interalar bristles observed in nonmosaic not-Minute heterozygous Theta females [equivalent to (a)] and of 39% of interalar bristles in nonmosaic not-Minute Theta males [equivalent to (b); cf. Table 1]. Thus, the absence of Minute-n in the cells of the segregation patch restored in full the potency of the heterozygous Theta genotype for differentiation of an interalar bristle, independently of the presence of Minute-n in the cells of the major part of the mosaic individuals.

Table 1. *Frequency of interalar (ia) bristles in flies of different genotypes.* y = yellow; w^e = eosin; sn^3 = singed-3; g = garnet; B = Bar; Mn = Minute-n; bb = bobbed; bb^1 = bobbed lethal; bb^{Df} = bobbed-deficiency; Θ = Theta

Genotype	Number of interalars per individual			Percent ia on total sites
	0	1	2	
$y \cdot \Theta/y \cdot \Theta$	133	96	197	58[1]
$y\ w^e\ sn^3 \cdot \Theta/y\ w^e\ sn^3 \cdot \Theta$	—	—	20	100
$y\ B/y+ \cdot \Theta$	108	58	19	26
$y\ w^e\ sn^3 \cdot \Theta/y\ w^e\ sn^3$	16	11	4	31[4]
$y\ Mn\ bb^{Df}/y+\ + \cdot \Theta$	660	1	2	<1[2]
$y\ w^e\ sn^3 + Mn+ \cdot \Theta/y + + g^2 + bb$	84	14	2	9
$y\ w^e\ sn^3\ Mn/y\ w^e\ sn^3 + \cdot \Theta$	21	1	—	2[5]
$y\ w^e\ sn^3\ Mn\ bb\ /y\ w^e\ sn^3 + + \cdot \Theta$	14	1	—	3
$y\ sn^3\ Mn\ bb/y\ sn^3 + + \cdot \Theta$	10	5	1	22
$y + +\ Mn\ bb^{Df}/y\ w^e\ sn^3 + + \cdot \Theta$	93	7	—	3
$y \cdot \Theta$	339	208	150	36[3]
$y\ w^e\ sn^3 \cdot \Theta$	5	12	33	78

[1, 2, 3] segregated in one progeny group.
[4, 5] segregated in another progeny group.

The fact that only a fraction of the patches heterozygous or hemizygous for Theta, but free from Minute-n forms an interalar bristle is not remarkable since it corresponds to the limited penetrance of these genotypes in whole flies. It became apparent, however, that superimposed on the incomplete penetrance, a relation seems to exist between the size of the area of the patch and presence of the interalar bristle. In order to clarify this aspect the outlines of all not-Minute Theta patches were drawn on copies of PLUNKETT's (1926) diagram of the dorsal and dorsolateral surface of the mesothorax of *Drosophila* which shows the positions and distances from each other (as measured along the surface of the fly) of the various bristles. The area of each spot, in arbitrary units, was then determined by means of a planimeter.

The mean area of the 71 spots was 11.4 units (Table 2) slightly above onehalf of the total area of a half-mesothorax which is 21 units. The spots of the series which involved bb^{Df} were larger than those not involving bb^{Df}. This fact is probably of no special significance in the present consideration, since it is known that the genetic background frequently acts as a modifier for spot size (STERN 1936). It is, however, of great interest, that in both series the mean area of patches which had formed an interalar bristle was smaller than the mean area of patches without such a bristle [7.5 vs. 9.2 in series (a) and 12.9 vs. 17.0 in series (b); Table 2]. Unfortunately, the data though derived from a study of numerous flies of which only a very small fraction satisfied the requirements of this study, are too small to permit a rigorous proof of statistical significance of the observed differences. There are, however, various considerations which suggest strongly that the association between presence of the bristle and small area of the segregates is a

Table 2. *Mean areas, in arbitrary units, of not-Minute Theta segregates*

Genotype	ia present	N	ia absent	N	All segregates
(a) not-bb^{Df}	7.5	13	9.2	28	8.6
(b) bb^{Df} . .	12.9	13	17.0	17	15.3
Total	10.2	26	12.2	45	11.4

Table 3. *Not-Minute Theta segregates. Grouped data for size of area. (a) and (b) refer to the genotypes designated in Table 2*

	Class means							N	Percent $<^1/_2$ max.[1]
	1.5	4.5	7.5	10.5	13.5	16.5	19.5		
(a) ia present .	3	3	3	1	2	—	1	13	73
ia absent . .	2	8	5	3	3	4	3	28	61
(b) ia present .	1	2	1	2	—	2	5	13	38
ia absent .	—	1	1	1	—	5	9	17	12

[1] Determined from individual (not grouped) values.

real one. An inspection of the grouped data for the area of spots (Table 3) shows that in both genotypes (a) and (b) the larger spots are overrepresented among those without an interalar bristle as compared to the larger spots with this bristle and, correspondingly, the smallest spots are underrepresented. This latter point is even more evident when the individual measurements, instead of the grouped data, are considered. In both genotypes the three smallest spots which possess an interalar bristle are smaller than the smallest spot which lacks the bristle [areas in (a): 1.6, 1.6, 2.0 vs. 2.6 and areas in (b): 2.5, 3.8, 4.2 vs. 4.7]. Furthermore, in (a) 73% of the spots with the bristle cover less than onehalf of the maximum possible surface area of their side of the mesothorax

as against only 61% of the spots without the bristle. In (b) this contrast is still greater, being represented by 38 and 12% (Table 3). In one more form the association between presence of the interalar bristle and small area of the spots is apparent from Table 4 where the percentages of spots with areas below that of the mean of their genotype is given separately for spots with and without the bristle: 62 vs. 50% in (a) and 46 vs. 18% in (b). Finally, the situation may be characterized in the following way. The data show that the larger spots with mean areas from 7.5 to 19.5 form interalar bristles in frequencies typical for whole flies of their constitution namely in 28% in group (a) and 38% in group (b), while the smaller spots, with mean areas from 1.5 to 4.5, form interalar bristles more frequently than is typical for whole flies of their constitution, namely in 37% in group (a) and 75% in group (b). A discussion of this phenomenon will be postponed until later.

Table 4. *Percentage of not-Minute Theta segregates with areas below that of the mean of their genotype*

Genotype	ia present	ia absent
(a)	62	50
(b)	46	18
Total	60	42

2. Not-Minute not-Theta tissue

The phenotype of hypodermal cells with not-Minute, not-Theta constitution presents yellow body color and long not-Minute bristles. Genetically, somatic crossing over leads to the segregation into these cells of three X-chromosomes, one of which carries Minute-n, and the other two the normal alleles of Minute-n. While Minute-n, as well as other Minutes, behaves as a recessive in the presence of two normal alleles the bristles produced by the triplo-X, M/+/+ genotype are somewhat shorter than wild-type bristles although larger and thicker than Minute bristles.

We are here primarily concerned with the effect of the mosaic condition of the mesothorax in regard to Minute and Theta. Since, however, the differences in the genotypes of the mosaic areas not only relate to Minute and Theta but also to the 2X—3X condition it is necessary to inquire whether the 3X superfemale genotype may possibly influence formation of bristles in the interalar region. The classical illustration of a superfemale (BRIDGES 1922) shows a specimen with normal bristles on the thorax (exclusive of the scutellum) except for a duplication of the left postalar, but it appeared necessary to inspect a larger number of superfemales to establish the range of variations in bristle number and position. Through the kindness of Dr. CHARLOTTE AUERBACH, 62 superfemales of varying genotypes were provided, thus furnishing 124 interalar sites. On not a single thorax had an interalar bristle been formed and in three cases only was the bristle differentiation somewhat abnormal in the neighborhood of the typical interalar site.

Two of these cases were in a single fly; in both sites the posterior postalar bristles had been "shifted" anterior to a level very slightly behind that of the posterior dorsocentrals. The third fly was distinguished in possessing a bristle clearly lateral to the normal interalar site and pointing at an angle laterally and posterior instead of the typical posterior direction of an interalar bristle. It is concluded that the 3X constitution is not characterized by the production of interalar bristles and does not need to be considered in the interpretation of the interalar situation in the not-Minute not-Theta segregates.

In the experimental groups there were 39 not-Minute not-Theta segregates which covered the site of the interalar bristle. Fourteen of these occurred on females of genotype (a), not carrying the bb^{Df} chromosome, and the remaining 25 on females of genotype (b) carrying bb^{Df}. The genotype of the not-Minute, not-Theta spots is not expected to be substantially different in the two groups and the results in regard to the interalar bristle are essentially alike. While the data are presented separately in the Tables 5—7 the discussion needs to deal with the totals only. In 30 of the 39 segregates no interalar bristle had differentiated. This conforms with expectation on the basis of cell autonomy of differentiation since the segregated tissue patches do not carry Theta. However, in 9 segregates, an interalar bristle had been formed!

Table 5. *Mean areas, in arbitrary units, of not-Minute not-Theta segregates*

Genotype	ia present	N	ia absent	N	All segregates
(a)	7.0	3	10.9	11	9.3
(b)	2.5	6	8.1	19	6.8
Total	4.0	9	8.8	30	7.7

The presence or absence of the interalar bristle is correlated with the size of the segregate on the mosaic thorax (Table 5). The mean area of those segregates which had produced an interalar bristle was only 4.0 units while that of the segregates which had not produced this bristle was 8.8 units. In terms of the total area of a half-mesothorax, as derived from one imaginal disc, the areas of the two types of spots cover 19 and 42%, respectively. The difference would be still larger if adjustment were made for one specific segregate. As seen in Table 6 under "Total" the size distribution of spots without interalar bristle is relatively even but that of spots with such a bristle shows a striking discontinuity. The individual measurements show that eight of the nine spots with an interalar bristle present vary between 0.4 and 6.1 units, while the ninth, covering an area of 15.9 units, lies far outside this range. If this spot were disregarded the average area of spots with an interalar bristle would become 2.5 units or only about 12% of the total possible surface derived from a mesothoracic disc.

This exceptionally large spot which exhibited an interalar bristle may possibly, in spite of its yellow appearance, have contained a Theta chromosome. While a yellow not-Minute Theta spot cannot be formed by a single cross over, such a genotype may be produced by means of double crossing over. This interpretation opens the possibility that one or the other of the supposedly not-Minute not-Theta areas also actually contains Theta but the rarity of multiple crossing over makes this unlikely at least for the overwhelming majority of the segregates.

Table 6. *Not-Minute not-Theta segregates. Grouped data on size of area*

	Class means							N	Percent $<^1/_2$ max.[1]
	1.5	4.5	7.5	10.5	13.5	16.5	19.5		
(a) ia present .	2	—	—	—	—	1	—	3	67
ia absent .	—	3	1	4	3	—	—	11	36
(b) ia present .	4	1	1	—	—	—	—	6	100
ia absent .	4	4	5	2	1	1	2	19	74
Total ia present	6	1	1	—	—	1	—	9	89
ia absent	4	7	6	6	4	1	2	30	67

[1] Determined from individual (not grouped) values.

The relation between size of spot and presence of an interalar bristle is further shown by a number of comparisons. Thus, the grouped data (Table 6) clearly show an overrepresentation of the spots with the bristle present among the smallest classes and a corresponding overrepresentation of the spots without the bristle among some of the larger classes. Furthermore, two of the nine spots with the bristle were smaller than the smallest of the thirty spots without it (0.4 and 0.8 vs. 1.0). Again, eight out of nine or 89% of spots with the bristle had an area below one-half of the maximum possible while only 20 out of 30 or 67% of spots without the bristle covered an area of less than one-half (Table 6). Similarly, 89% of the spots with the bristle were below the mean of the area covered by all spots in contrast to only 55% of the spots without the bristle (Table 7). Finally, the relation may be summarized by the statement that 39% of the smallest spots (with class means of 1.5 and 4.5) had differentiated the interalar bristle as compared to only 10% of the larger spots (with class means of 7.5 to 19.5).

Table 7. *Percentage of not-Minute not-Theta segregates with areas below that of the mean of their genotype*

Genotype	ia present	ia absent
(a)	67	36
(b)	100	58
Total	89	55

The significance of the relation between size of not-Minute, not-Theta segregate and formation of the interalar bristle can be established

by a χ^2 test. When the data for the totals in Table 6 are grouped into 2×2 tables, in three alternative ways, by combining the numbers on either side of the divisions which separate the first, second or third class from the larger ones, one obtains P values significant below (2 groupings) or very slightly above (1 grouping) the one percent level for the reality of the association between size of area and presence of bristle.

3. The position of the interalar bristle on mosaic thoraxes

In the majority of the not-Minute Theta segregates the position of the interalar bristle was approximately typical for that of nonmosaic

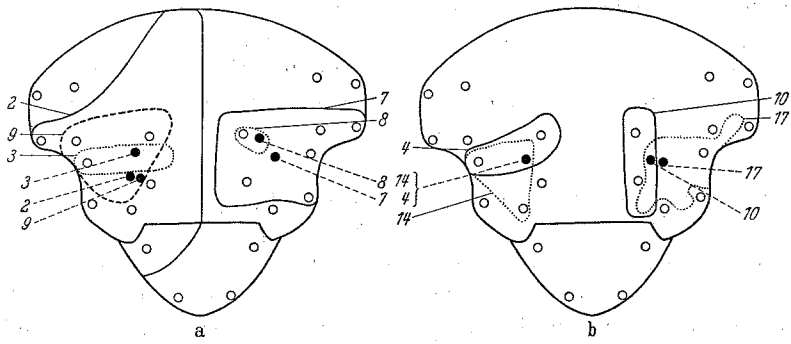

Fig. 3. Outlines of the circumferences of nine not-Minute, not-Theta segregates, and positions of the interalar bristles. The numbers represent code numbers of spots (continuous lines) and the interalar bristle belonging to each (interrupted line)

flies though in four segregates a shift in position toward the midline and simultaneously in an anterior or a posterior direction was noted. This phenomenon was more striking in the not-Minute, not-Theta segregates. An inspection of Fig. 3 shows that the position of the interalar bristle varied considerably in the nine mosaic thoraxes. Only in spots number 2, 7 and 17 was the position typical for that of an interalar bristle on a nonmosaic thorax. In the other six cases it was located nearer to the midline of the thorax or both more dorsally and anteriorly. These shifts in site of differentiation are partly related to the position of the segregated area on the thorax: spots 3, 4, 8 and 10 barely include the typical site of the interalar bristle although they border on it. In the remaining spots (9, 14) with an abnormal position of the bristle, the shift in site of differentiation is related to nearness of the nonsegregated Minute Theta tissue: instead of the typical site which would place the bristle somewhat in the center of the spot, differentiation had occurred close to its border.

It is obvious that in small spots the distance between the site of the bristle and the periphery of the spot is less, on the average, than in

large spots. The facts just reported regarding shifts of differentiation away from the center toward the periphery of spots show that total area as such is not decisive for the development of a bristle on not-Minute, not-Theta tissue. It may be questioned perhaps whether some of the extra bristles, for instance those of segregates 8, 9 and 10, deserve the designation as interalars since they are found considerably removed from the typical interalar site. More specifically, it may be considered whether these bristles could be interpreted as extra dorsocentrals. This, however, is not a likely interpretation. Not only the two normal dorsocentrals but also supernumerary dorsocentrals are arranged along a very narrow anterior-posterior strip which, apart from occasional side-by-side duplications, approaches a straight or only slightly curved line. The position of the extra bristles of the segregates falls outside the narrow range of dorsocentral bristles. On the other hand, some other segregates showed a typical supernumerary dorsocentral bristle as will be seen in the following section.

4. The differentiation of duplicate or supernumerary noninteralar bristles on mosaic thoraxes

Instead of the typical single bristles which are differentiated on the mesothorax, a pair of twin bristles may develop occasionally. No specific data on duplicate bristles have been collected for the Minute Theta, nonmosaic individuals among the material presented but it is certain that such twinning was exceptional. On the other hand, duplication of the bristles was not rare among the 124 half thoraxes of super-females which have been referred to earlier (p. 10). While a bristle was lacking on only one half-mesothorax (it was a posterior supra-alar), the following duplications were recorded: anterior scutellars, 12 times; anterior postalars, 14; anterior supra-alars, 2; presutural, 1 (two well-spaced bristles). In addition, on 23 half-mesothoraxes, three and on one, four well-spaced dorsocentral bristles occurred instead of the usual two.

Omitting, for the present, the dorsocentral bristles, no duplicated bristles were observed within the segregated spots themselves of the Minute Theta flies. However, an apparently significant number of mosaics was encountered in which two instead of one bristle, each with its socket, had developed at the very border of the segregated patches and in such a manner that one of the bristles was inside and the other outside the segregate, thus giving one a not-Minute and the other a Minute phenotype. Among the not-Minute Theta segregates in Minute Theta females "straddling" twin bristles occurred at the following sites: anterior supra-alar (twice), anterior postalar and scutellar (once each). Among the not-Minute, not-Theta segregates Minute Theta

females the types of straddling twin bristles were: anterior notopleurals (twice), anterior supra-alars (3 times), as well as two spots in which 3 and 2, respectively, different bristles were involved. (One case: posterior supra-alar, anterior and posterior postalar; another case: anterior supra-alar and posterior postalar.)

The ten not-Minute not-Theta twin bristles at the border of segregates were probably 3X in constitution. Therefore, the duplications of bristles may possibly have been connected with the tendency of 3X tissues to produce double bristles. Yet, five of the twin bristles in these segregates were found at locations which did not have duplicate bristles in the superfemales studied, namely at the sites of the anterior notopleural and the posterior postalar (twice each), and the posterior supra-alar (once each). Moreover, duplications also occurred in not-Minute Theta segregates which should not have been 3X in genotype. These facts show that the 3X constitution of segregates is not a necessary prerequisite for the phenomenon of straddling twin bristles, although it may possible be a contributing factor.

Somewhat similar to the formation of twin bristles are six cases in which a supernumerary dorsocentral bristle had been formed inside or closely outside a segregate. Five of these are illustrated in Fig. 4, a, b, d—f. In each of the individuals a segregated spot—probably 3X in d—f, and not-3X in a, b—either included the whole dorsocentral region (b) or part of it (a, d—f). In still another individual (c) no supernumerary dorsocentral bristle was found but the site normally occupied by the anterior bristle did not carry the organ, while more anterior in an area included in the tongue of a segregate a dorsocentral bristle had been differentiated. Inspection of Fig. 4, a—f shows that the presence of a not-Minute segregate may lead to the formation of bristles anterior to the anterior dorsocentral bristles, in between the anterior and posterior one, or posterior to the latter. Again, it is clear that the 3X genotype of some of the spots does not appear to be the sole cause of the supernumerary bristles since the cases depicted in figure 4a—c had spots which were not 3X in constitution. Moreover, it is remarkable that the supernumerary dorsocentrals occurred in spots all of which had borders cutting across the dorsocentral line and that the extra bristles developed in the proximity of the border.

It is significant that four mosaics were observed in which an extra bristle had formed in not-Minute segregates on Minute flies in which no Theta was present in the original constitution (Fig. 4, g—k). The extra bristles included a dorsocentral site and three sites which more or less approached that of an interalar bristle. These mosaics were characterized again by the fact that the place of differentiation was close to the border of the segregate. Here then in not-Minute, presumably

diploid cells, on Minute-diploid females, bristles had been differentiated which would not have formed on a nonmosaic fly of either genotype.

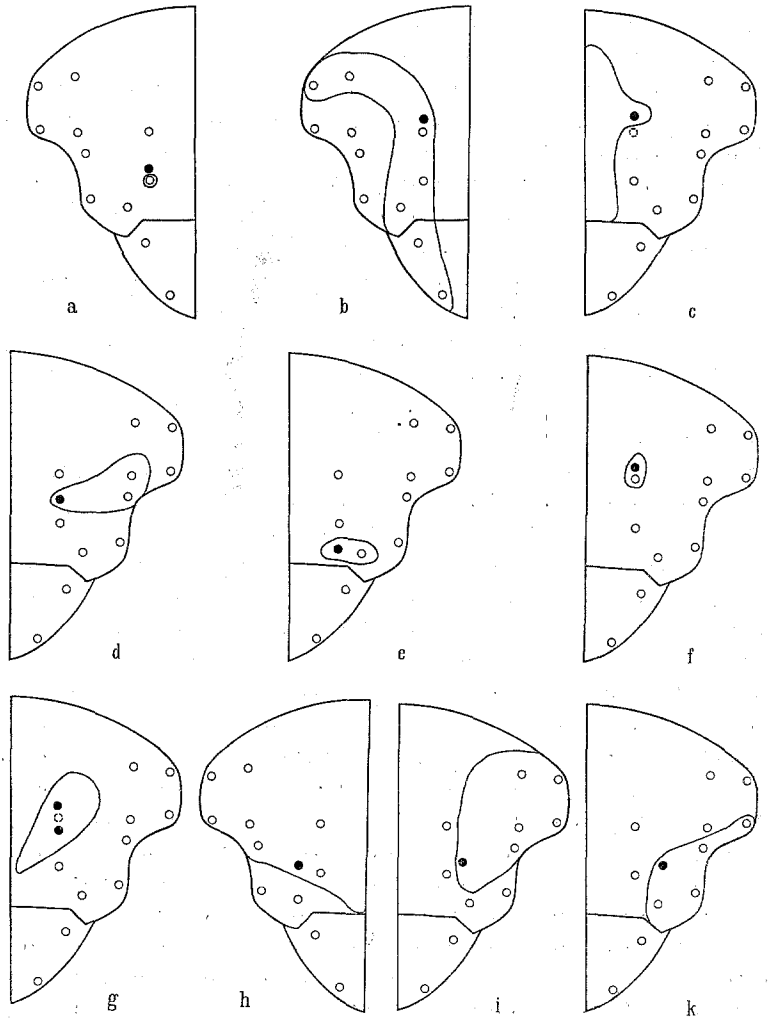

Fig. 4. Outlines of segregates involving supernumerary bristles or bristles at unusual sites. a—c: not-Minute Theta segregates on Minute Theta flies. d—f: not-Minute not-Theta segregates on Minute Theta flies. g—k: not-Minute segregates on Minute not-Theta flies. Presence of normally located bristles indicated by a circle (○), of lacking bristles by an interrupted circle (◌), of extra bristles by a filled-in circle (●).

The mosaics presented in this section are alike in showing the differentiation of supernumerary bristles in not-Minute tissues adjacent or near to a bristle in the nonsegregated Minute tissue. In the case of the twin bristles it is difficult or not possible to designate one of the pair

as the normally located and the other as the supernumerary bristle. The dorsocentral region, on the other hand, may involve development of two bristles in an area where only one would be formed normally, but at two seites neither of which corresponds to the typical position. Lastly, differentiation, in mosaic individuals on not-Theta flies, of bristles in the region of the interalar signifies the origin of bristles which are not characteristic for the genotype of any part of the individual.

V. Discussion

The following facts require further analysis:

1. Flies containing the Theta genotype produce a bristle at a site normally not occupied by such an organ. In Minute Theta flies differentiation of this "interalar" bristle is inhibited.

2. Not-Minute Theta segregates in Minute Theta flies are able to differentiate an interalar bristle in at least the same frequency as whole individuals of this genotype. It is very likely that this bristle is formed in greater than normal frequency if the segregates are small.

3. Duplicate or supernumerary bristles may be formed in the neighborhood of the border between the different tissues of genetic mosaics. Occasionally a bristle in the region of the interalar may be formed on mosaic flies which have no Theta genotype in any of their cells.

4. Not-Minute, not-Theta segregates on Minute Theta flies are able to differentiate an interalar bristle even though in whole individuals this genotype would not lead to such differentiation. There is a clear, positive association between formation of the bristle and small size of the segregate.

1. The action of Minute-n in inhibiting the formation of the interalar bristle and the interactions between Minute and not-Minute tissues

Minute-n reduces the growth of all bristles so that they remain thin and short. It could, therefore, be presumed that the general action of Minute-n might suffice to explain its influence on the interalar bristle. However, the data on the effect of Minute-n on the differentiation of other than the interalar bristles (p. 6) show that a specific pattern of bristle inhibition is involved. If the action of Minute-n were that of relative growth inhibition alone, then it would be expected that it would also frequently eliminate the development of other bristles, perhaps those of a similar size as the interalar, or perhaps the smallest bristles. Actually, with few exceptions no striking reduction of head or thoracic bristles was found on the 200 Minute-n, nonmosaic half thoraxes, and the few bristles affected were of various sizes (Fig. 1). Among those always or nearly always present were such short bristles as the median

and posterior orbitals, the posterior notopleural and the anterior supra-alar, as well as such long ones as the posterior dorsocentral and the posterior scutellar. Among those more frequently missing were the large posterior supra-alar, and the small postvertical and anterior orbital. The lack of relation between size of bristles and their inhibition under the influence of Minute-n suggests that this genotype, apart from its general restriction on growth of developing bristles, acts specifically in inhibiting differentiation of certain bristles. In not-Theta flies the most striking inhibition is seen in the postverticals which appear in only about 20%. In Theta flies when Minute-n is present, differentiation of the interalar bristle is even more frequently suppressed.

In flies of not-Minute but Theta constitution the presence of an interalar bristle is evidence for the existence of a prepattern in the developing hypodermis which endows the interalar site, in contrast to its surroundings, with a developmental singularity prerequisite for bristle formation. If the inhibiting action of Minute-n on the interalar bristle were due to the obliteration of this prepattern singularity, then genetic mosaics in which most of the fly is Minute Theta, except for not-Minute Theta tissue involving the interalar site, should also usually lack the preinteralar singularity. They should, therefore, usually be unable to differentiate an interalar bristle. The fact, however, that the not-Minute Theta segregates at the interalar site do not show a reduction in frequency of the interalar bristle, demonstrates that Minute-n Theta flies posses the same prepattern for an interalar bristle as not-Minute Theta flies and that, therefore, Minute-n acts in reducing the compentence of Theta cells to respond to the unchanged prepattern.

The paradoxical situation that there is not only no inhibition of the interalar bristles by the Minute surroundings of the not-Minute segregates, but apparently an even higher than normal frequency of the interalar bristle in small spots can be understood in terms of an interaction between the two tissues of the genetic mosaics. Such interaction is obvious in the formation of duplicate or supernumerary bristles in the border zones of Minute and not-Minute components of mosaics, independent of their Theta constitution. All cases of duplicate or supernumerary bristles in mosaics may be interpreted by the assumptions (1) that the prepattern defines not sharp points but larger regions characterized by gradients in strength, (2) that the differentiation of a bristle tends to inhibit the differentiation of other bristles in its surroundings and (3) that under equal circumstances the inhibiting effect of a differentiating large not-Minute bristle is stronger than that of a Minute bristle. Therefore, if a Minute bristle develops in a mosaic and the more competent not-Minute tissue covers nearby parts of the prepatterned region, then the inhibition exerted by the differentiating Minute bristle is insufficient to suppress the formation of a twin or supernumerary bristle in

the segregate. This interpretation accounts for the straddling twin bristles as well as for the supernumerary dorsocentral bristles anterior to, in between, or posterior to the positions of the two usual dorsocentrals and for the increased frequency of interalar penetrance in small segregates. Furthermore, if this interpretation is accepted, it may be suggested that the prepattern feature which in not-Minute Theta flies singles out the interalar region is also present in not-Theta flies which typically do not form an interalar bristle. This suggestion makes intelligible bristle formation in the interalar region of totally not-Theta flies as pictured in figure 4, h—k. Here the presence of Minute tissues in the surroundings of not-Minute segregates reduced the inhibition normally exerted on the interalar region so that it was able to differentiate a bristle organ.

Minute-n thus acts in a similar manner as achaete. The absence of a bristle in the achaete tissue of a mosaic permits the formation by not-achaete tissue of a bristle in an abnormal position (STERN 1954b). The presence of a delicate Minute bristle instead of a strong not-Minute bristle likewise permits the formation of a bristle at an usual place in not-Minute tissue. These observations agree also with those of STURTEVANT (1932) according to whom, "scute, which has as its effect the removal of specific bristles, does not remove these bristles as frequently when they lie [on Minute, not-scute individuals] in small [not-Minute] patches that are scute in constitution as it does in flies that are wholly scute". WIGGLESWORTH (1940, 1948) has provided a similar interpretation by postulating competing interactions for the spacing of sensilla and plaque-forming centers in *Rhodnius*.

Difficulties remain. Inhibition of bristles should be a mutual phenomenon. Why then, when it is assumed that a Minute bristle is not strong enough to inhibit a not-Minute twin, should not a not-Minute bristle inhibit the formation of its Minute twin? One might speculate that twin bristles arise when the Minute partner happens to begin differentiation earlier than the not-Minute duplicate and once having started on its developmental path is not subject to inhibition any more. This speculation implies that contrary to the known differential in speed of development between wholly Minute and not-Minute flies, in mosaic individuals the Minute and not-Minute bristles tend to arise approximately simultaneously making it possible that sometimes the one and at other times the other of these bristles has a slight priority in time of origin. Only further work can clarify these problems.

2. The interactions between Minute Theta and not-Minute, not-Theta tissues

In the preceding section the finding of a bristle in the interalar region of some not-Theta mosaic flies was interpreted as an indication of the

presence of the same interalar prepattern in not-Theta and Theta individuals. It follows from this interpretation that the presence of the interalar bristle in Theta flies is due to the competence of Theta cells to respond to the prepattern, in contrast to the usual lack of such compentence of not-Theta cells.

An independent test of this hypothesis might be provided by mosaics in which a small Theta segregate on not-Theta flies covers the interalar region. Such mosaics, however, cannot be produced by somatic crossing over and segregation which occur typically relatively late in development so that the bulk of the tissue of a mosaic individual is of the original Theta constitution and the smaller segregate is not-Theta. Analogous to the nearly complete autonomy of cells of the normal genotype in differentiating bristles on achaete flies which but for the segregate would have lacked these bristles (STERN 1954a, b), it might be expected that suitable small Theta patches on not-Theta flies would produce the interalar bristle.

The flies studied consist of mosaics in which the main tissues are both Theta and Minute with segregates which are neither Theta nor Minute. If the prepattern of Theta were the same as that of not-Theta then the not-Theta segregates should not produce interalar bristles since they would not be competent for differentiation at the interalar site. If on the other hand, the prepattern caused by the Theta genotype is different from that of not-Theta and the competence of not-Theta cells is adequate to respond to the special Theta prepattern, then the segregated patches should be able to produce interalar bristles. The actual results agree with this last expectation but further considerations show that they are also compatible with the first hypothesis, namely that based on constant prepattern. The clear-cut alternative of either presence or absence of bristles outlined in the present paragraph is based on the assumption that the unknown product of the action of Theta, which according to the hypothesis of a constant prepattern is thought to be responsible for the peculiar competence of Theta tissue, is active only in the cells which produce it. If, however, this unknown substance could diffuse or otherwise spread from Theta into adjacent not-Theta tissue, then the latter would be enabled to have interalar differentiation.

There is still another possibility. Since the not-Theta cells were derived by somatic segregation from ancestral Theta cells, it is conceivable that an early "Theta-reaction" had set up a chain of events which again endowed the derived not-Theta cells with the ability to produce an interalar bristle.

The finding of interalar bristles on not-Theta spots is thus subject to different interpretations, namely (1) the existence of a special prepattern imposed on the segregate by the rest of the Theta fly or by a relevant

region, or (2) the existence of the ordinary prepattern with the competence of the not-Theta tissue provided (a) either by spread of a substance produced by adjacent Theta tissue or (b) by a reaction initiated before segregation of Theta had taken place. The results obtained do not favor unequivocally any one of these alternatives. The fact that spots develop an interalar bristle preferentially when they are small could be interpreted as a consequence of a specific prepattern inherent in the Theta containing surrounding tissue and imposed from there on the small segregate [hypothesis (1)]. Moreover, the frequent "shift" in the position of the extra bristle in segregated spots as compared to the typical site could be explained as the result of a compromise between the prepatterns of the mosaic tissues. Yet the same facts fit the hypothesis (2a) of a constant prepattern and spread of a Theta-dependent substance from the surrounding tissue into the spot, if the reasonable assumptions are made first that there is a relatively large region capable of differentiation of an interalar bristle, and second that diffusion of the Theta substance into this region may cause differentiation at that point where the most favorable combination occurs between the concentration of the diffusing substance and the "strength" of the prepattern. The alternative (2b), that of a continuing Theta reaction set in motion in the cells of the spots before genetic segregation had occurred, might again lead to a correlation between size of spot and appearance of the interalar bristle, since a small spot indicates late segregation and late segregation obviously means presence of Theta until shortly before differentiation. However, this interpretation alone cannot account for the observed "shifts" in position of the bristle which call for the assumption of a process of interaction between the two genotypes of the mosaic.

If the existence of a constant prepattern in Theta and not-Theta individuals were granted, it may be questioned why the assumption of the spread of a Theta-dependent substance into the not-Theta segregates is proposed. Would not the lowered competition of the surrounding Minute tissue be sufficient to account for the formation of the interalar bristle in the not-Minute segregates?

This explanation, however, does not account for the rather high frequency of occurrence of the interalar bristle in not-Theta segregates of Theta flies in contrast to its very great rarity in mosaics which lack Theta throughout all tissues. It seems necessary to assume that Theta is positively involved in the differentiation of the interalars in small not-Theta segregates.

While the data on mosaics of Theta and not-Theta alone do not lead to a decision between the alternatives outlined above, it might be emphasized that there is independent evidence for a constant

prepattern in different genotypes as well as for diffusion or spread of a substance necessary for development of a bristle (STERN 1954a, b). The constancy of a prepattern is best shown by the fact that in mosaics of achaete and not-achaete a suitably located not-achaete tissue patch nearly always shows its competence by forming a posterior dorsocentral bristle regardless of the size of the spot or the topography of the two tissue types (whole achaete flies under the conditions of the experiment never form this bristle). The diffusion or spread of a gene-dependent substance necessary for bristle development is shown by the complementary mosaics in which an achaete tissue patch covers the region of the posterior dorsocentral bristle. While in the great majority of mosaics no differentiation of the bristle occurs—in conformity with the concept of constant prepattern but lack of competence in achaete tissues—there were exceptions in which a posterior dorsocentral bristle developed in achaete tissue. The presence of this bristle was positively correlated with small size of the achaete segregate, but such nonautonomous differentiation occurred also in some larger segregates. However, in each case, the border between not-achaete and achaete tissue was close to the bristle, either anterior or posterior to it; thus suggesting direct stimulation of the achaete tissue by its not-achaete neighbor, probably by means of spread or diffusion of a gene-dependent substance.

On the basis of these findings on mosaics of achaete and not-achaete as well as of those on Minute Theta and not-Minute Theta, and on the mosaics producing duplicate or supernumerary bristles, it seems very likely that the hypothesis of a constant prepatterning for the interalar bristle and a spreading of Theta-dependent material from Theta into not-Theta tissue is correct. The competence of cells to respond to the interalar prepattern would then have been shown to exist at three different levels. In the wild genotype the competence is below the threshold leading to differentiation. It is increased above it as a result of the Theta genotype, and it is diminished again by Minute-n (Fig. 5).

The differentiations in mosaics show that the prepattern and the realized pattern of bristles do not stand in a fixed relation to each other. The prepattern which is the prerequisite for the formation of an individual bristle defines a peculiarity which extends over a certain region and has its "peak" in a more restricted area. In this it resembles a morphogenetic field and its gradients. The realized pattern is a derived one. It depends on (a) the prepattern, (b) the genetic competence of the tissues and (c) the developmental interactions which are set in motion as soon as either differentiation of a bristle or lack of differentiation has been determined. If a bristle organ once begins to form it exerts a suppressing action on its surroundings while, if it fails to begin, differentiation may occur at unusual places.

In contrast to the result of the analysis given here, Kühn (1955) has interpreted the action of achaete and not-achaete in terms of two different types of fields, equivalent to two different prepatterns: achaete causing a field for dorsocentral bristle formation in the anterior region only, not-achaete causing two fields, one in the anterior and another

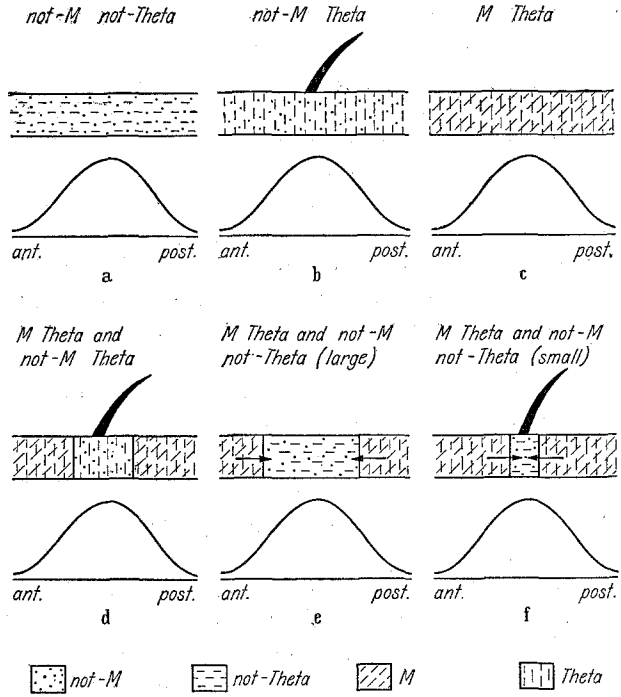

Fig. 5. Interalar competence and prepattern in different genotypes. The upper part of each diagram represents a longitudinal section through the interalar site of the hypodermis, the lower part a graph of the hypothetical "strength" of the interalar prepattern in an anterior-posterior direction. This prepattern is indicated as being the same in all genotypes. a—c: Nonmosaic individuals of three genotypes. Competence for interalar bristle differentiation present in not-Minute Theta only (simplified, cf. text). d—f: Mosaic individuals. Competence in (d) inherent in the genotype of the segregate, in (f) made possible by spread of Theta-dependent substance into the segregate.

in the posterior region. This interpretation is successful over a considerable range of the phenomena reported. It fails, however, to be applicable to the differentiation of a posterior dorsocentral bristle, almost without exception, in patches of normal genotype regardless of the extent of the achaete area. If the achaete genotype really caused the formation of a prepattern different from that of the normal genotype, namely a prepattern which produces a special situation in the region of the anterior dorsocentral bristle only, then a very small segregate of normal cells placed in the posterior region would find no prepatterned

stimulus to which to respond. Therefore no posterior dorsocentral bristle should be produced. The actual finding that this bristle regularly becomes differentiated shows that the posterior region is prepatterned even in prevailingly achaete flies, and that the difference in actual differentiation of achaete versus normal dorsocentral bristle patterns lies in the competence of normal cells to respond and the failure of achaete cells to respond to the prepatterned singularity of the posterior region.

The nature of the prepattern remains unknown. There is evidence that the patterned differentiation of imaginal discs can occur independently of their eversion to form adult surface structures (BIRMINGHAM 1942, STERN 1940), but WADDINGTON (1953) and PANTELOURIS and WADDINGTON (1955) have reported cases of apparent regulation after eversion. Perhaps the prepattern is based upon relatively simple features such as physical stresses in the growing disc. Likewise the evocation of the differentiation of a bristle apparatus may conceivably consist in as unspecific a process as localized stimulation of hypodermal cells to increase in mass, an increase which may automatically entail the sequence of events which truly result in differentiation.

Summary

1. In *Drosophila melanogaster* the genotype Theta causes the development of an interalar bristle-organ which is not formed in the wild type. The genotype Minute-n causes, in Theta flies, a strong reduction in the frequency of the occurrence of the interalar bristle.

2. By means of genetic mosaics, produced by somatic crossing over and segregation, a developmental analysis was applied to the differences in interalar differentiation between the wild-type, the Theta and the Minute-n genotype.

3. Loss of Minute-n in segregated tissues of Minute-n Theta flies leads to differentiation of the interalar bristle. Loss of Minute-n and Theta in segregates of such flies may also lead to differentiation of the bristle, particularly in spots of small area.

4. In the neighborhood of the border between the genetically different tissues of mosaics extra bristles may be formed.

5. The data furnish evidence for interactions between the genetically different tissues of mosaics: inhibition of bristle formation in the surroundings of differentiating bristles, and spread of gene-dependent substances concerned with differentiation.

6. The results can be interpreted in terms of a constant prepattern for the interalar bristle in wild-type, Theta and Minute Theta genotypes, but of different competence of cells with the three genotypes to respond to the prepattern by differentiation of the interalar bristle.

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