DEVELOPMENTAL ANALYSIS OF THE ACHAETE-SCUTE SYSTEM OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The interpretation of the wild-type function of a gene depends on our knowledge of the phenotype caused by its absence. We have first defined the genetic extent of the achaete-scute system by studying the phenotype of different terminal and intercalary deficiencies including these genes. When these deficiencies were lethal, we have defined the phenoeffective phase of lethality and studied their phenotype in genetic mosaics (gynandromorphs and mitotic recombination clones). The achaete-scute system affects two functions, one necessary for the differentiation of the embryonic (central?) nervous system and the other necessary for the differentiation of peripheral nervous elements of the chaetes and sensillae of the adult cuticle. The possibility that these functions correspond to differential expression of a single mechanism is discussed.

THE aim of a genetic analysis of a developmental process is twofold. It should first define the elements or genetic signals that control it by isolating the mutants that interfere with it, and second, assign them a function in developmental terms. This approach, however, usually results in a vicious circle, for the assignment of a function to a gene requires the previous knowledge of the steps involved in the process and of the entire informational content of the gene.

The study of the process of chaete formation is a case in point. Among many mutants known in Drosophila to affect the differentiation of chaetes, the alleles of achaete and scute have a visible phenotype consisting of the suppression of particular chaetes in particular positions of the adult cuticular pattern (DUBININ 1929). A genetic analysis soon revealed that these viable mutations represent hypomorphic condition of genes that, when completely deleted, manifest a more complex phenotypic syndrome (DUBININ 1933; AGOL 1932; MULLER and PROKOFIEVA 1935). The genetic and cytological analysis of deficiencies and duplications of the region where these mutants map have defined the extent and organization of the achaete-scute genetic system (MULLER and PROKOFIEVA 1935; GARCÍA-BELLIDO, in preparation).

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In this analysis, new phenotypes including lethality and impaired motility, not obviously related to the chaete phenotype, were discovered. It is the aim of the present paper to trace that complex phenotype back to one (or few) primary gene disfunctions. Allelic forms retaining some wild-type function (hypomorphs) are modifiable in their expression by genetic, developmental and environmental conditions. Thus, an understanding of the nature of a genetic signal requires the consideration of the effects of its total absence. Genetic deficiencies are then ideal objects for this analysis. Deficiencies of the entire achaete-scute system are zygotic lethals. However, their phenotypic study can be carried out in genetic mosaics. Several considerations support the view that the genes achaete and scute are involved in the mechanism of differentiation of nervous system derivatives from cells of ectodermal origin.

MATERIALS AND METHODS

The lethal phases of the different genetic combinations were evaluated as follows. Eggs laid over a 4 hr period were counted immediately after removal of the parents, and those which had not hatched 24–28 hr later were recorded. The deficiencies used were balanced over either *FM6* or *In(1)d1–49*, *y Hw* m^2 g^4. Since these genotypes have the tendency to accumulate extra *Y* chromosomes, we marked the *Y* chromosome of these stocks with $B^S Y$, and only non-B^S females were used as mothers. Pupae and emerged adults were subsequently counted. The differences in counts between stages were used to evaluate the fraction of dead individuals per developmental interval. Dead embryos were studied under the microscope to distinguish an early from a late (complete morphogenesis) lethal phase. Mitotic recombination was carried out using a Philips MG X-ray source operated at 100 kv, 15 mA (2 mm Al filter, 300 r/min) at a total dose of 1000r.

Developmental time at the moment of irradiation was calculated in hours before (BPF) or after (APF) puparium formation. The deficiency chromosomes were marked with f^{36a} a cell marker that express itself in chaetes as well as in the trichomes of the wing surface and notum. Most of the deficiencies used either carried *y* or were deficient for *y*. In order to increase the size of the mitotic recombination spots, the deficiency chromosome was in some experiments in *trans* to $M(1)o^{Sp}$, so that *M(1)o+* clones were produced (MORATA and RIPOLL 1975). In the analysis of twin spots, the chaete marker sn^3 or f^{36a} was used.

Gynandromorphs were generated making use of the spontaneous loss of the *R(1)2*, $In(1)w^{vC}$ chromosome, $R(1)w^{vC}$ for short. $R(1)w^{vC}$ males were crossed to heterozygous deficiency females. Occasionally $R(1)w^{vC}/FM6$ females were crossed to deficiency/duplication males. The emerged adults were scored under the dissecting microscope for *y* f^{36} spots or *y* spots, and their frequency and distribution over the body surface were compared with those of *dl-49*, *y Hw* m^2 g^4 (or *FM6*) male tissue of controls. The adult structures labeled with *y* f^{36a} or *y* were plotted on a map of the presumptive structures in the blastoderm (GARCÍA-BELLIDO and MERRIAM 1969).

RESULTS

I. *The lethal phase*

The different terminal and intercalary deficiencies of the scute region can be phenotypically defined by their lethal phase. Table 1 and Figure 1 show the data from such an analysis. In these experiments, females carrying the tested chromosome, balanced with *FM6* or *dl-49* were crossed to wild-type males, *X/Y*. As controls *y* $f^{36a}/FM6$ females were crossed to *X/Y* males. From the number of segregating genotypes and the number of eggs laid, we can infer how many

TABLE 1

Lethality phase of different deficiencies and deficiency-duplications

Maternal genotype	Dp *via* male	Eggs	Eggs/ Genotype	Mortality Embryo	larva	pupa	Adults Total	sc	Adult genotype max. viab.
$y\ f^{36}/FM6$		241	60	4	23	8	206	–	59
Df(1)svr/FM6		787	196	159	211	27	390	0	172
Df(1)svr/FM6	$Dp(1;4)sc^{H}$	123	15	19	28	4	72	0	19
Df(1)260–1/FM6		1614	403	352	261	52	949	0	338
Df(1)260–1/FM6	$Dp(1;4)sc^{H}$	1050	130	150	230	60	610	0	160
$Df(1)sc^{H}/FM6$		521	130	187	63	15	256	0	111
$Df(1)sc^{H}/FM6$	$Dp(1;Y)sc^{S1}$	324	81	76	13	3	232	0	87
$Df(1)sc^{19}/FM6$		923	230	233	115	20	555	0	230
$Df(1)sc^{19}/FM6$	$Dp(1;Y)sc^{S1}$	952	237	252	73	13	614	0	234
$Df(1)sc^{19}/FM6$	$Dp(1;Y)sc^{8}$	249	62	70	9	4	166	0	67
$Df(1)sc^{8}/FM6$		214	53	28	55	3	128	0	47
$Df(1)sc^{8}/FM6$	$Dp(1;Y)1J1+$	314	78	44	60	4	206	4	74
$In(1)y^{3PL}sc^{9R}/dl\text{-}49$		576	144	102	141	7	326	0	114
$In(1)sc^{8L}sc^{9R}/dl\text{-}49$		214	53	37	62	3	112	0	44
$In(1)sc^{4L}sc^{9R}/dl\text{-}49$		639	160	121	165	3	350	0	122
$In(1)y^{3PL}sc^{S1R}/dl\text{-}49$		298	74	1	136	11	150	0	57
$In(1)sc^{8L}sc^{4R}/dl\text{-}49$		154	38	0	43	6	105	18	34
$In(1)y^{3PL}sc^{8R}/dl\text{-}49$		356	89	6	69	12	269	50	92
$sc^{10-1}/FM6$		1130	282	40	230	43	817	141	248

correspond to each genotype. This number should correspond to the number of adults of the genotype of maximal viability, assuming that there was no mortality due to uncontrolled causes in the experiment. The most viable genotypes are those corresponding to heterozygous females. *FM6* males show a considerable mortality in the early larval period. In the experiments, the deficiency chromosome is as a rule y^{-} so that hemizygous larvae of this genotype can be distinguished from those of *FM6* males, which have y^{2} coloration of the mouth parts.

As seen in Table 1 and Figure 1 all the terminal deficiencies are hemizygous lethal. With the exception of $Df(1)sc^{8}$ males, they die early in the embryonic period. In males carrying terminal duplications as well it is possible to ascertain the lethal phase of the noncovered region of these deficiencies. *Df(1)svr/FM6* females crossed to *X/Y*; $Dp(1;4)sc^{H}/ci^{D}$ males give in the offspring two kinds of males: *Df(1)svr/Y*; $Dp(1;4)sc^{H}/+$ and *Df(1)svr/Y*, $ci^{D}/+$. Both combinations are lethal, but from the distribution of lethality during the different phases of development it appears that the first genotype, y^{+}, dies in the larval period, whereas the second, y, dies during the embryonic period, as in the previous cross. Similar considerations lead us to conclude that, whereas *Df(1)260–1* is an embryonic lethal (DEMEREC and HOOVER 1936), $Df(1)260\text{–}1/Dp(1;4)sc^{H}$ is a larva-pupal lethal. $Df(1)sc^{H}/Y$ or $Df(1)sc^{H}/Dp(1;Y)sc^{S1}$ males die in the embryonic period. $Df(1)sc^{19}$ covered with $Dp(1;Y)sc^{S1}$ or with $Dp(1;Y)sc^{8}$ die in embryos. $Df(1)sc^{8}/Y$ dies as embryo/larval boundary lethal (POULSON

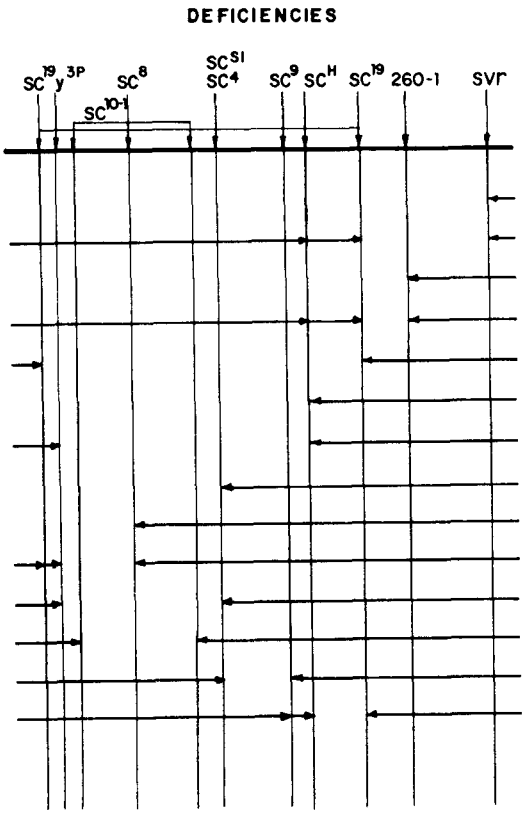

FIGURE 1.—Phenotype of different deficiencies of the achaete-scute region. Vertical arrows: The genetic breakpoints. Horizontal arrows: Extent of the duplications and nondeleted material of deficiencies. E: embryo, L: larvae, P: pupae.

1940). The presence in these males of *Dp(1;Y)1(1)J1*$^{+}$ shifts the lethality to the larval and pupal period, and even adults occasionally emerge. Thus, distal to the breakpoint of *Df(1)svr* at salivary band 1B 9–10, the only factor responsible for early embryonic lethality lies between the breakpoints of *Dp(1;Y)sc*^{S1} and *Dp(1;4)sc*^{H} (Figure 1). This region corresponds to the lethal of scute (*l'sc*).

The intercalary deficiencies within the achaete-scute region fall into two groups: those being deficient for *l'sc* [*In(1)y*^{sPL}*sc*^{9R}, *In(1)sc*^{8L}*sc*^{9R} and *In(1)sc*^{4L}*sc*^{9R}], which die during the embryonic period, and those deficient for both *ac* and *sc* only [*In(1)y*^{sPL}*sc*^{S1R}] and in part *Df(1)sc*$^{10-1}$ or *sc* only [*In(1)sc*^{8L}*sc*^{4R}] or *ac* only [*In(1)y*^{sPL}*sc*^{8R}], which die in advanced larval stages, in larval and pupal stages, or emerge as adults, respectively (Figure 2).

It is interesting to note an additional trait of the phenotype of the viable deficiency males, besides the characteristic achaete or scute chaete phenotype. *In(1)y*^{sPL}*sc*^{8R} males are fully motile and fertile. However, *In(1)sc*^{8L}*sc*^{4R} and the *Df(1)sc*$^{10-1}$ males, besides being strongly achaete and scute, are nonmotile. That is, they may emerge and remain idle on the food, but many of them die

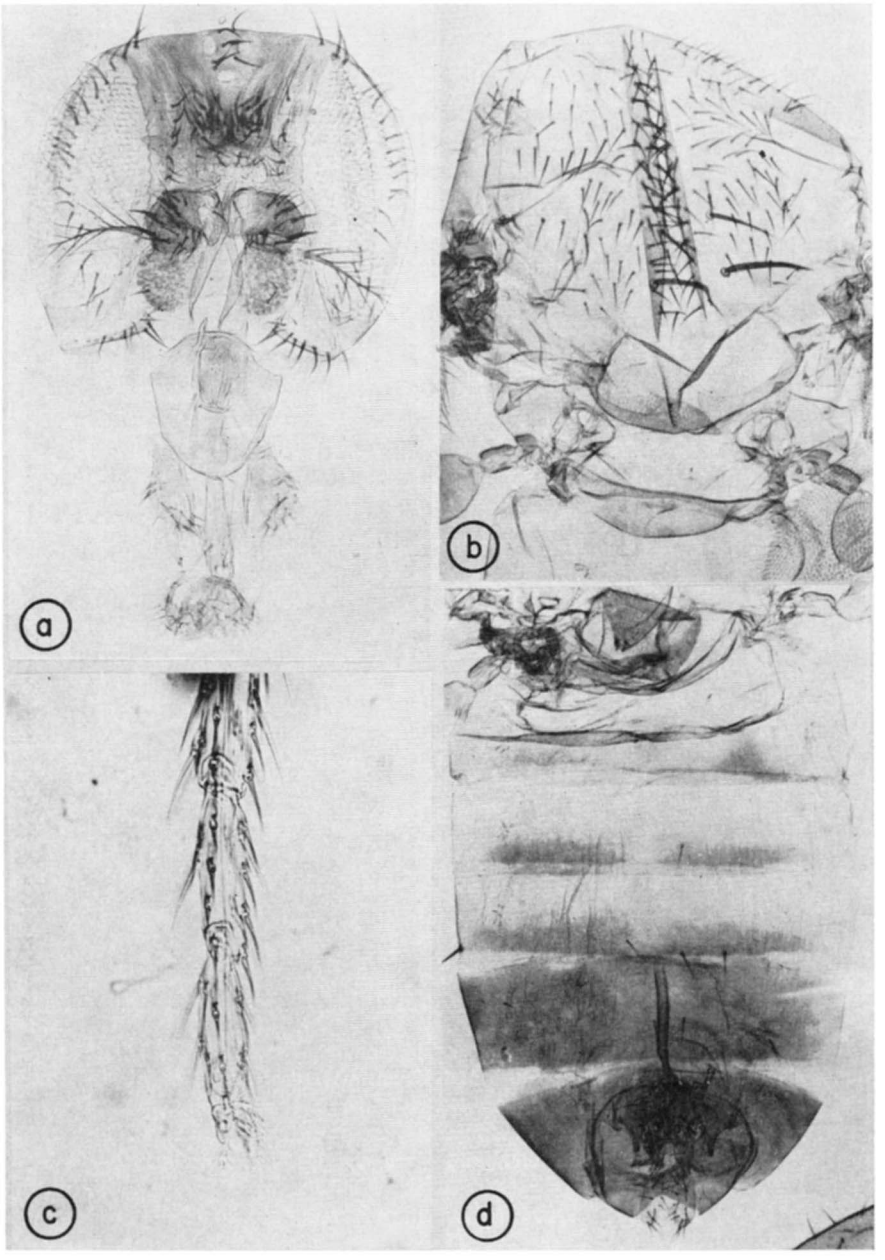

FIGURE 2 (a-d).—Chaete pattern of different achaete-scute deficiencies viable in flies. (a, b and d) $In(1)sc^{8L}sc^{4R}$. (c) $In(1)y^{3PL}sc^{4R}$.

while trying to emerge from the puparial case. This abnormal behavior is also characteristic of genetic combinations that leave deficient either the left scute region (scute$^{\alpha}$, *e.g.*,*Df(1)sc^{4}/Dp(1;Y)sc^{8}* males) or the right one (scute$^{\beta}$, *e.g.*, *Df(1)sc^{19}/Dp(1;f)sc$^{7.2}$ males*) without being deficient for the lethal of scute (see Figure 1; GARCÍA-BELLIDO, in preparation).

II. *Gynandromorphs*

Different lethal deficiencies of the scute system were studied for their viability in male spots in gynanders. To that end, the deficiency chromosome was marked with either *y* or *y^{2}*, or was deficient for *y*, and in some cases also carried the cell marker *f^{36a}* (Table 2). In order to produce control gynandromorphs, the *R(1)w^{vC}* chromosome was carried by the father, so that two kinds of *R(1)w^{vC}* females appeared in the offspring: (1) heterozygous over the deficiency chromosome, or (2) over the balancer chromosome (*FM6*, marked with *B* and *y^{2}*, or *In(1)dl-49*, marked with *y* and *Hw*). The fraction of gynandromorphs exposing the balancer chromosome among the number of heterozygous *R(1)w^{vC}/* balancer females provides an estimate of the frequency of loss of the *R(1)w^{vC}* chromosome per experiment. The comparison of gynanders exposing mutant and balancer chromosomes measured the mortality of gynanders due the deficiency chromosomes. Of the mutant kind, some gynandromorphs always appeared. Based on the extent and location of male tissue that appeared in these gynanders, we plotted on the blastoderm map the possible outlines that the male cells occupied in this early stage (Figure 3). The adult landmarks showing the male phenotype for individual gynanders have been included in single outlines made of the shortest distances between landmarks. The superposition of the largest patches of the different gynanders of a given genotype shows the area of the blastoderm map that can be occupied by male tissue of this genotype without killing the zygote. Male tissue hemizygous for control chromosomes is viable over the entire body surface, and the outlines of its patches cross the dorsal and ventral medial line, thereby affecting all the germ layers of the embryo (see POULSON 1950 and Figure 3). However, the deficiency male tissue can extend over areas of the presumptive epidermis and even cross the dorsal medial line,

TABLE 2

Offspring of Deficiency/Balancer females crossed to R(1) w^{vC}/sc^{8}Y *males*

Maternal genotype	F_1 genotypes *Df/R(1)w^{VC}*	Gyn	F_1 genotypes *Bal/R(1)w^{VC}*	Gyn	Bal/O
Df(1)260-1, y f^{36a}/FM6	310	0	390	>51	200
Df(1)sc^{19}, y f^{36a}/FM6	690	42	1396	>200	250
In(1)y^{3PL}sc^{9R}, y w^{a}/dl-49	138	2	260	42	45
In(1)sc^{8L}sc^{9R}, y^{2}w^{a}/dl-49	71	5	132	32	48
In(1)sc^{4L}sc^{9R}, y w^{a}/dl 49	346	31	743	145	269
In(1)y^{3PL}sc^{4R}, y/dl-49	192	12	302	62	120
In(1)y^{3PL}sc^{S1R}, y/dl-49	324	18	677	120	100

Gyn: gynanders; Bal: balancer, Bal/O:males without paternal Y.

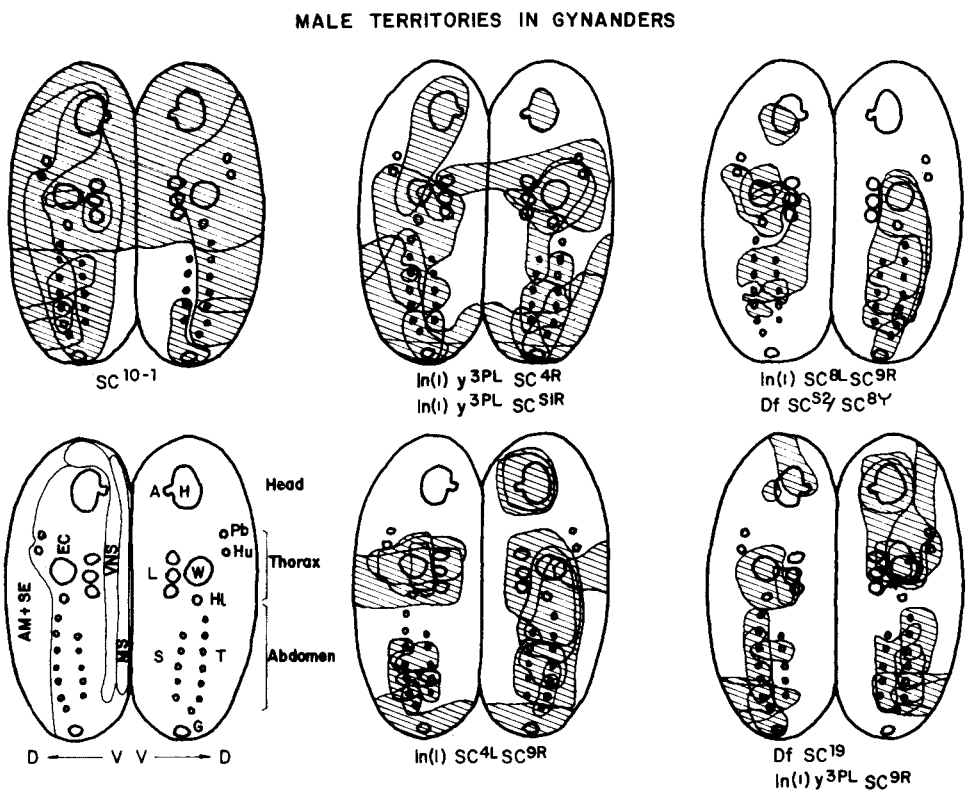

FIGURE 3.—Extent of male territories of different genetic constitutions upon the blastoderm map of gynandromorphs. A: antenna. H: head, L: legs, W: dorsal mesothorax, HL: dorsal metathorax, T: tergites, S: sternites, G: genitalia, AM+SE: amnios and serosa, EC: ectoderm, VNS: ventral nervous system, MS: mesoderm. Lined areas, individual male patches.

but not the ventral one. The only exception is *In(1)y*^{3PL}*sc*^{4R}. We found four gynanders, dead within the puparium, with the entire cuticle of that male genotype. The fact that no gynander was found of the other deficiencies that affected ventral organs of both sides of the body suggests that gynanders with mesodermal or nervous tissue of the deficiency genotype are inviable. In three cases we observed gynanders, once recovered from etherization, in which those legs showing the male phenotype did not shake. This happened in two gynanders of *Df(1)sc*^{19} and in one of *In(1)sc*^{S1L}*sc*^{9R} genotype.

The cuticle of the male patches showed a characteristic *ac* or *sc* phenotype, depending on the nature of the deficiency. Thus, *Df(1)sc*^{19} and *In(1)y*^{3PL}*sc*^{4R} patches are absolutely devoid of chaetes, claws and several types of sensillae, similar to but more extreme in appearance than the phenotype of the viable *Df(1)sc*$^{10-1}$ males. *In(1)sc*^{8L}*sc*^{4R} patches show only some chaetes, those not affected by achaete, a phenotype similar to that of *sc*$^{10-1}$*/Dp(1;Y)sc*^{8}. However, it was surprising to notice that *In(1)sc*^{4L}*sc*^{9R} patches contained macro- and microchaetes. Their phenotype was similar to that of *sc*^{4} or *sc*^{9}, both strong scute

mutants, but with several macrochaetes and sensillae still present. Since *In(1)sc^{4L}sc^{9R}* is deficient for the lethal of scute, this suggests that the function of this gene is not related to chaete or sensilla differentiation. That its function is required either in the central nervous system or in the mesoderm derivates is suggested by the observation that spots of this deficiency, or of other deficiencies that also include the *l'sc* locus, never include the ventral germ layers of the embryo.

The viability in gynanders of *Df(1)sc^{8}* was studied in a cross of *R(1)w^{vC}/dl-49* females to *Df(1)sc^{8}, f^{36a}/Dp(1;Y)sc^{8}/B^{S}Y* males. We found in the F_1 19 *R(1)w^{vC}/Df(1)sc^{8}f^{36a}/Dp(1;Y)sc^{8}* females and 10 gynanders with large male *y^{+} f^{36a}* patches and 18 *R(1)w^{vC}/Df(1)sc^{8}, f^{36a}/B^{S}Y* females, but no gynanders with large areas of male *y f^{36a}* tissue. Only in three cases were small *y f^{36a}* spots found in the abdomen. In these cases, the chaetal pattern was normal. These findings indicate that *Df(1)sc^{8}* tissue is not viable in large territories in gynanders, due not to variegation of bobbed on *XO* tissue (BAKER 1971), but to a viability gene present in the *Dp(1;Y)sc^{8}*. The *y f^{36a}* phenotype of the spots indicates that distal to the *Df(1)sc^{8}* breakpoint there is no gene other than *ac* required for the differentiation of chaetes.

III. *Mitotic recombination spots*

The phenotype of the tested deficiencies on the cuticle can also be studied in mitotic recombination clones. This technique has the advantage over that of gynandromorphs in that lethals which are not viable in large male territories of gynander may be viable in mitotic recombination clones within the imaginal discs (RIPOLL and GARCÍA-BELLIDO 1973). Females heterozygous for *M(1)o^{Sp}* and each of the following deficiencies: *Df(1)260–1, y^{-} f^{36a}* and *Df(1)sc^{19}, y^{-} f^{36a}* were X rayed at 24–48 hr of larval development. The deficiency chromosomes were introduced in the cross by males carrying *Dp(1;Y)y^{2-67g}* (kindly supplied to us by M. M. GREEN) in the first crosses and the *Dp(1;2)sc^{19}* in the second one. Minute^{+} clones initiated at these early stages can extend over large territories of cuticle within derivatives of an imaginal disc (MORATA and RIPOLL 1975).

Special attention was paid to the phenotypes of these clones in the dorsal mesothorax and to a lesser extent to derivatives of other discs. *Df(1)sc^{19}, y^{-} f^{36a}* clones are devoid of chaetes, macrochaetes and microchaetes in the notum (10 cases) (Figure 4a). The trichomes were of normal density and of normal appearance, except for being *y f^{36a}*. Of 12 clones affecting different regions of both wing surfaces, 9 extended over the normal region of sensillae campaniformia in the three anterior longitudinal veins and the anterior crossvein, but were devoid of these sensillae (Figure 4b). The chaete pattern of these clones in the wing margin was striking, for whereas all the chaetes of the costa and of the dorsal triple row were lacking, those of the medial and ventral rows in the triple and double row around the margin were present and *y f^{36a}*, but otherwise normal (Figure 4b).

An analysis of *Df(1)sc^{19}, y^{-} f^{36a}* clones in other organs permits the conclusion

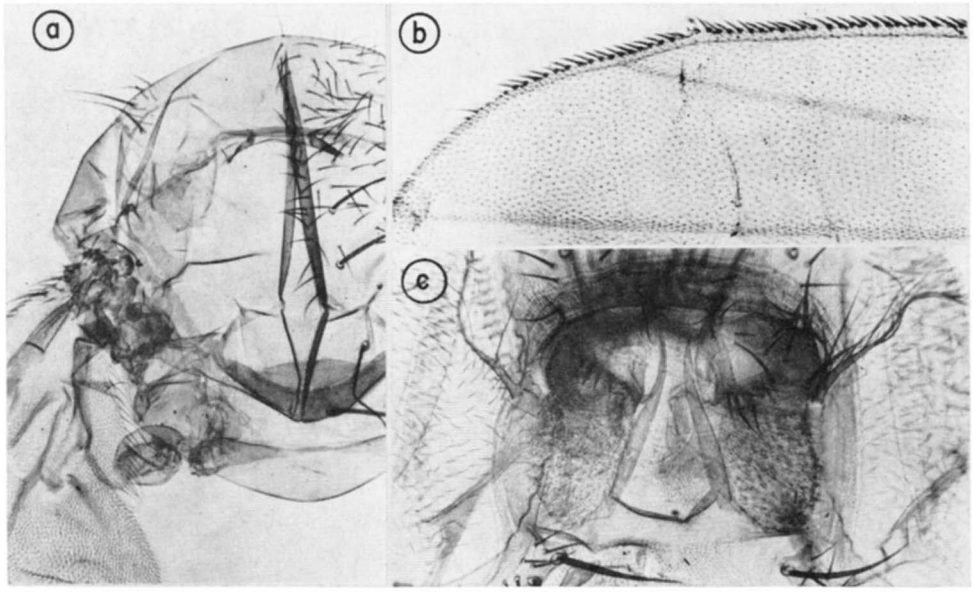

FIGURE 4 (a-c).—*Df(1)y f*^{36a} *M(1)o*+ mitotic recombination clones. In the notum (a), the wing surface (b), and the antenna (c).

that all other chaete types are affected by this deficiency. Sensilla campaniformia and trichodea in the legs and perhaps to some extent the sensilla basiconica in the antenna are affected, for they too are absent in *y* territories. However, structures such as ocelli, eye facets, ommatidial chaetes and aristae in the head differentiate in a normal way. (Figure 4c). In the legs, the bracts normally associated with the chaetes were also absent in the marked territories. In the claw organ of the legs, the empodium and pulvillum were normal, but the ungues were rudimentary. In the tergites, it is difficult to distinguish the absence of *y f*^{36a} chaetes from the nonoccurrence of clones. Therefore, in another experiment, *Df(1)sc*^{19}, *y/sn*^{3} tergites were scored for the presence of *y* chaetes in the proximity of the *sn*^{3} twin spots. No *y* chaetes appeared, but sometimes areas of lower chaete density could be scored next to *sn*^{3} chaetes. A similar finding was reported by WALEN (1961).

Thus, *Df(1)sc*^{19} is viable in imaginal disc cells, but its function is indispensable for the differentiation of chaetes (with the exception of the medial and ventral rows of chaetes of the wing margin) and of sensillae. Since *T(1;2)sc*^{19} males are phenotypically scute, it is conceivable that some *sc* function could remain proximal to its right breakpoint. *Df(1)260–1* is the next more proximal deficiency (1A1–1B6) available. We have seen that *Df(1)260–1/Dp(1;4)sc*^{11} or *Df(1)260–1/Dp(1;2)sc*^{19}*/Dp(1;Y)sc*^{8} flies are lethal, but some of them reach pigmentation stages during metamorphosis. In two cases of each genotype, the pharate adults could be dissected out of the pupa case and studied. The chaetal pattern was that of *sc*^{S2} or *sc*^{19}, respectively; however, the medial and ventral triple and double row chaetes were present. The same phenotype has been found in three *Df(1)260–1, y*$^{-}$ *f*^{36} *M(1)o*+ mitotic recombination clones affecting the

wing margin. Thus, the differentiation of the medial and ventral chaetes of the wing margin escapes the control of the achaete and scute system.

The study of mitotic recombination clones hemizygous for *Df(1)sc*^{8} confirms the data found in gynanders. *Df(1)sc*^{8} *y*$^{-}$; *Dp(1;3)sc*^{J4}*/mwh jv* flies were irradiated as larvae. We found *y mwh jv* spots in normal frequency and size in the notum and abdomen. The clones of notum, however, showed an achaete phenotype.

From these results two main conclusions can be reached: (1) a deficiency as large as *Df(1)260–1* can be viable in mitotic recombination clones, and (2) the only genes responsible for chaete differentiation in the tip of the *X* chromosome, distal to *Df(1)260–1*, are those of the *achaete-scute* system.

IV. *The perdurance of the achaete-scute function*

It has been shown that the removal by mitotic recombination of the wild-type allele of some genes after a given point of development has no effect on the phenotype of the resulting clones (GARCÍA-BELLIDO and MERRIAM 1971c). Thus, the removal of the wild-type allele for hairy (*h*) in *h*/+ flies leads to presence of extra chaetes in the wing surface in clones initiated earlier than 8 hr before pupariation, but not later. Reciprocally, the suppression of the *h* extra chaetes by achaete in *ac*; *h* flies was ineffective in *ac/ac*; *h/h* clones initiated after the same developmental period in *ac*/+; *h/h* flies. It was concluded that the maternal cell genotype was expressed in the cell progeny, irrespective of the actual genotype, probably due to some remnant gene product or process initiated earlier by the wild-type alleles. This phenomenon was named perdurance (GARCÍA-BELLIDO and MERRIAM 1971c).

Here we have analyzed the perdurance of the *ac* and *sc* genes in mitotic recombination clones initiated at different times of development. This analysis is expected to give clues as to the nature of these gene products and their effects on the differentiation of different kinds of chaetes in different positions.

A first analysis was carried out in the tergites of *Df(1)sc*^{19}, *y*$^{-}$*/f*^{36a} females (Figure 5a). The normal parameters of tergite development were analyzed by GARCÍA-BELLIDO and MERRIAM (1971b), GARCÍA-BELLIDO (1973) and GUERRA, POSTLETHWAIT and SCHNEIDERMAN (1973). In flies heterozygous for *Df(1)sc*^{19} *y*$^{-}$*/f*^{36a}, clones initiated before pupariation were only of the *f*^{36a} phenotype (wild type for the scute gene). Only after pupariation did clones of *Df(1)sc*^{19} *y* chaetes appear in frequencies comparable to those of *f*^{36}. However, the average number of chaetes per clone, decreasing from 3.7 to 1.7 in *f*^{36a} clones, was no higher than 1.2 in *y* clones. We interpret these results as indicating that *ac*$^{+}$ and *sc*$^{+}$ products are released only a few cell divisions prior to the actual chaete differentiation, which can then proceed in the absence of these genes. A plot of the location of the *y* chaetes resulting from perdurance upon the tergite pattern is especially relevant (Figure 6). The first *y* chaetes appear in the posterior margin of the tergites and differentiate as macrochaetes. Only later do *y* chaetes occupy the central regions of the tergites. Thus, possibly the marginal chaetes are the first of all the tergital chaetes to be genetically determined.

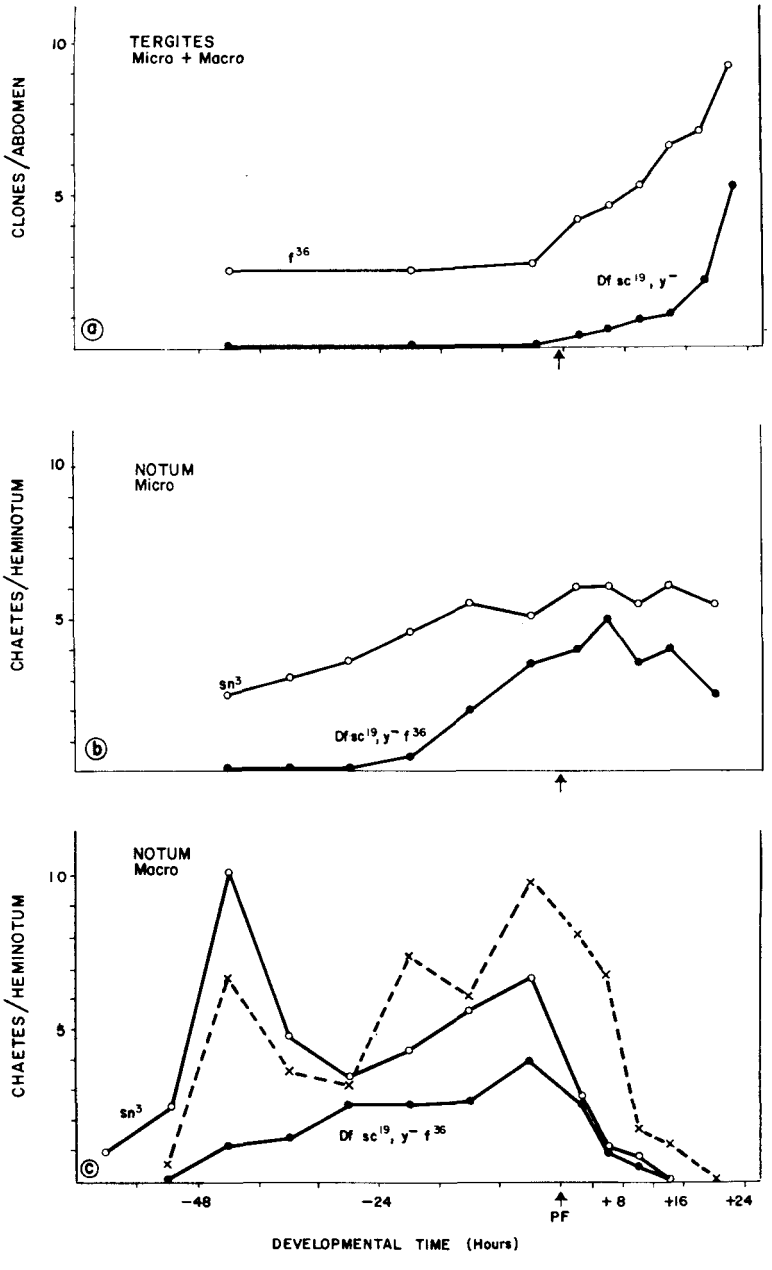

FIGURE 5 (a-c).—Frequencies of clones of chaetes *Df(1)sc*^{19} and its twins in different tissues. (a-c) clones of chaetes originated following X-ray irradiation at different development stages. In (c) X — X: absence of macrochaetes.

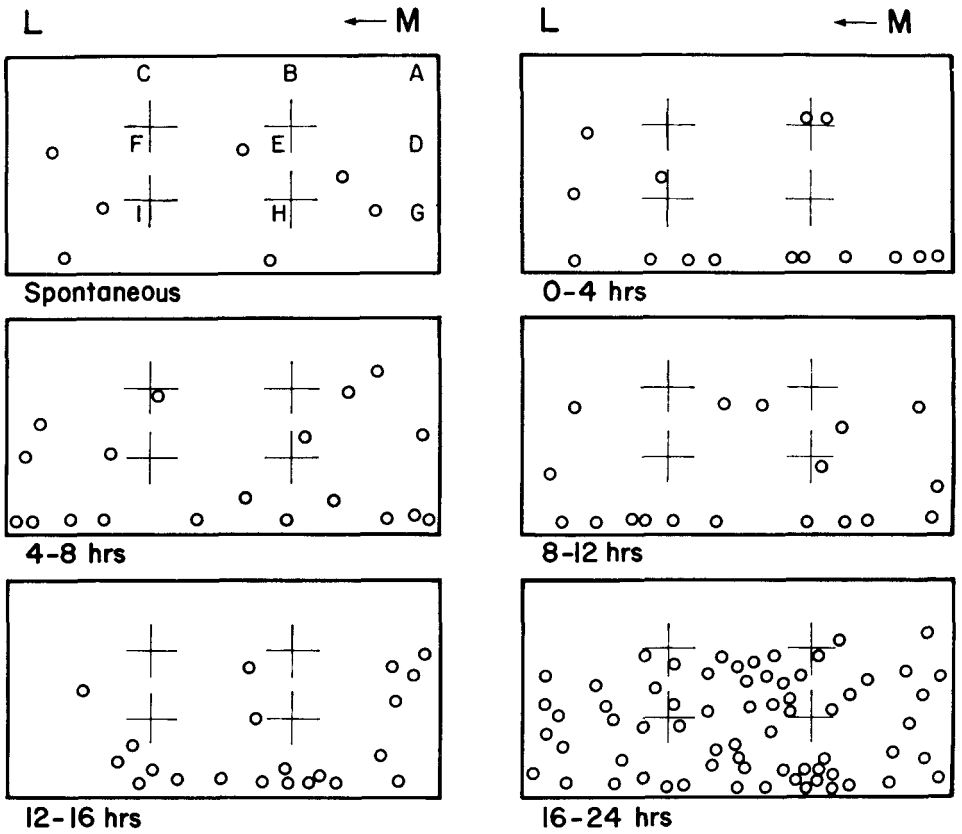

FIGURE 6.—Distribution in tergites of *Df(1)sc*^{19}, *y*$^{-}$ chaetes. Clones appeared following mitotic recombination in *Df(1)sc*^{19}, *y*$^{-}$ *f*^{36a}/*sn*^{3} flies, at different stages (hr) after puparium formation, M: medial, L: lateral border of the tergite. Top: anterior, bottom: posterior. Circles indicate location of individual chaetes summed up from many patches.

A similar analysis was carried out in the chaetes of the notum. In this case *Df(1)sc*^{19} cells were doubly labeled with *y* and *f*^{36a} and the twin control cells with *sn*^{3}. Figure 5b and 5c show the frequency of *y f*^{36a} and *sn*^{3} chaetes of the notum following X irradiation of *Df(1)sc*^{19}, *y*$^{-}$ *f*^{36}/*sn*^{3} individuals at different developmental intervals. Normal clones may embrace chaetes and trichomes earlier than 48 hr before pupariation (BPF), but contain a single chaete when initiated later (GARCÍA-BELLIDO and MERRIAM 1971a). The *sn*^{3} (control) macrochaetes do not appear later than 16 hr after pupariation (APF). At that time, the trichogen and tormogen cells can be distinguished histologically from the surrounding epidermal cells, which will differentiate the trichomes (LEES and WADDINGTON 1942). The different temporal response to X rays measured by the frequency of marked chaetes probably reflects different sensitivities of the mother cells of the trichogen, the only element of the chaete organ scorable with the cell markers used. This element appears after two differential divisions from a mother cell. The first one gives rise to nervous system elements, the second one

to both the trichogen and the tormogen (STERN 1938; PETERS 1965). Therefore, the last differential division must have taken place earlier than 16 hr APF and the two differential divisions should occur between 48 hr BPF and 12 hr APF. The differential divisions of the mother cells of the microchaetes probably take place later than that of the macrochaetes, for the trichogen of microchaetes can be marked following mitotic recombination until 21 hr APF (see GARCÍA-BELLIDO and MERRIAM 1971a).

Figure 5b and 5c show the frequencies of sn^3 chaetes in the notum of $Df(1)sc^{19}$ $y^- f^{36a}/sn^3$ flies. For both macrochaetes and microchaetes, the curve of frequencies shows two peaks. As discussed previously (GARCÍA-BELLIDO and MERRIAM 1971a,c), these sensitivity peaks probably correspond to the G_2 phase of the two differential cell divisions. The fact that the pool of all the macrochaetes on one side and the microchaetes on the other still shows clear peaks suggests that the differential divisions are more or less synchronized. The individual macrochaetes show even more pronounced peaks. This inference is confirmed by a Poisson analysis of marked chaetes in the same notum: the frequency of multiple events is much higher than expected from the individual frequencies. It is interesting that both peaks occur earlier in the macrochaetes than in the microchaetes. However, both dorsocentrals and posterior supra-alar macrochaetes affected by achaete behave like the rest of macrochaetes. $Df(1)sc^{19}$, y f^{36a} chaetes start appearing during the first peak, although in low frequencies. They show, however, a clear second peak at the same developmental time as sn^3 controls. Thus, chaetes are capable of differentiating even on the absence of the sc^+ allele, when its removal takes place during or possibly before the first differential divisions of the chaete organ occurs. Small temporal differences in the appearance of y f^{36a} individual macrochaetes exist, but with the data at hand it is not possible to characterize these elements in terms of their differential perdurance.

Depending on the time of irradiation, X rays lead to occasional removal of chaetes. Since both tormogen and trichogen are lacking, it is presumed that X rays affect chaete formation. The frequency with which a given chaete is lacking varies with the chaete position (Table 3). In Figure 5c is shown the temporal distribution of chaete removal for all the macrochaetes. Again this curve follows that of sensitivity to mitotic recombination. This finding suggests that the developmental system of macrochaete formation is fixed prior to meta-

TABLE 3

Number of chaetes affected following X-ray irradiation 48 to 0 hr before pupariation of Df(1)sc^{19}, y f^{36a}/sn^3 *flies*

Phenotypes	SC		PS	NP		SA		PA		DC	
	A	P		A	P	A	P	A	P	A	P
$Df(1)sc^{19}$, $y f^{36a}$	28	36	33	34	19	28	10	39	28	37	29
sn^3	50	49	90	68	45	67	48	76	79	69	52
Absence	76	80	117	54	53	47	251	36	90	106	54

A: Anterior, P: Posterior; SC: Scutellar; PS: Presutural, NP: Notopleural; SA: supra-alar; PA: Postalar; DC: Dorsocentral.

morphosis, and no other epidermal cells can be shifted to substitute for them (but see POODRY, HALL and SUZUKI 1973; POODRY 1975).

The seriation of the different macrochaetes based on their sensitivity to mitotic recombination (sn^3 or $y\,f^{36}$) or to chaete removal due to X rays, are not correlated (Table 3). Again these differential sensitivities seem not to follow the same seriation of chaete suppression caused by the scute allelomorphs.

DISCUSSION

The combined genetic, cytological and developmental analysis of the achaete-scute system was used to try to define the extent of these genes and to infer the nature of their information.

The study of the phenoeffective phase of terminal deficiencies suggests that the only genetic factor responsible for early embryonic lethality in this region is the scute gene, and more specifically the deficiency for the lethal of scute. DEMEREC and HOOVER (1936) had shown that *Df(1)260–2* has a late embryonic-early larval phenoeffective phase and *Df(1)260–1* an early embryonic one. POULSON (1940) confirmed the phenoeffective phase of a genetic equivalent of *Df(1)260–2*, namely $Df(1)sc^8$. We have extended this analysis to terminal deficiencies up to *Df(1)svr* and intercalary ones using left-right inversion recombinants and combination of terminal deficiencies and duplications. Thus, whereas *Df(1)svr* is an embryonic lethal, $Df(1)svr/Dp(1;4)sc^H$ flies have a late embryo-early larval phenoeffective phase. $Df(1)260–1/Dp(1;4)sc^H$ zygotes die as larvae and pupae. Thus, the only factors responsible for the early embryonic lethality of the *Df(1)svr* are within the scute locus. In fact, $Df(1)sc^{19}$ is an early embryonic lethal, and within it also are combinations lacking the "lethal of scute" function, such as $In(1)sc^{4L}sc^{9R}$ (Figure 1).

Several arguments support the idea that all the scute functions are included in $Df(1)sc^{19}$. $Df(1)260–1/Dp(1;2)sc^{19}/Dp(1;Y)sc^8$ individuals dissected out of the puparium have only the typical $T(1;2)sc^{19}$ phenotype, namely a slight suppression of the scutellar chaetes. Thus, between the right breakpoints of $Df(1)sc^{19}$ and *Df(1)260–1* there is apparently no function involved in chaete differentiation. In fact, four lethals corresponding to two complementation groups in that region completely complement $Df(1)sc^{19}$. Within $Df(1)sc^{19}$ there is no viability gene to the left of the achaete-scute system, because $Df(1)sc^{19}/Df(1)sc^8$ flies are yellow and achaete. Thus, $Df(1)sc^{19}$ lacks only the gene yellow and the functions corresponding to the achaete-scute system.

Since the $Df(1)sc^{19}$ is lethal, its phenotypic analysis was carried out in genetic mosaics. EPHRUSSI (1934), STERN (1935), and DEMEREC and HOOVER (1936) studied the viability of *Df(1)260–1* in mitotic recombination clones. Since cells of this genotype were scored only in chaetes, their absence was interpreted as meaning cell lethality of this genotype. Only WALEN (1961) realized that the appearance of territories devoid of chaetes in the tergites could represent the suppression of chaete differentiation in otherwise viable cells. An analysis of the cuticular phenotype of $Df(1)sc^{19}$ in M^+ mitotic recombination clones in all

disc derivatives confirmed this interpretation. This deficiency causes the total absence of chaetes and sensillae (at least the sensillae campaniformia and tricodea) but leaves trichome differentiation normal. This is also the phenotype of *Df(1)260–1* mitotic recombination clones. Thus, there is no gene function essential for the viability and morphogenesis of epidermal cells in this genetic region, a stretch of DNA including about 14 salivary chromosome bands. The only functions affected by these deficiencies related to cuticle differentiation are those of chaete and sensillae differentiation included in $Df(1)sc^{19}$.

Chaetes and sensillae correspond to the group of "Kleinorgane" of HENKE (1953). They derive from epidermal cells through differential divisions which give rise to nervous (peripheral) and cuticular cell lineages (STERN 1938; PETERS 1965; LAWRENCE 1966). Both cell lineages pass through a second differential division. In the nerve cell lineage, this gives rise to a bipolar neuron that contacts the central nervous system, and a neurilemma cell. An analogous division in the epidermal cell lineage gives rise to a tormogen and a trichogen, the visible cuticular elements of the chaete organ. Homologous steps seem to occur in the formation of some sensillae (PETERS 1965).

We have seen that *Df(1)260–1* and $Df(1)sc^{19}$ spots in the wing do differentiate the marginal elements (medial and ventral triple row, dorsal and ventral double row), which are chaetes. In collaboration with DR. I. DEAK, we have studied the innervation of chaetaes in histological sections of wild-type flies. They were stained for acetylcholinesterase, a rather specific nerve cell enzyme. Whereas all the chaetes of the body, the sensilla campaniformia of the wing veins, and the dorsal row of chaetes of the triple row were innervated, those elements not affected by $Df(1)sc^{19}$ did not show a positive stain reaction. This finding suggests that the achaete and scute functions are primarily related to peripheral nervous system differentiation.

CLEVER (1960) found in pupae of Galleria that when the peripheral nerves of the chaetes were poisoned by methylene blue, the tormogen and trichogen elements of the chaete failed to complete differentiation. Thus, the absence of chaetal elements in $Df(1)sc^{19}$ cells could result from the failure of the nerve to grow and, as a consequence, to the subsequent degeneration of the trichogen and tormogen cells. However, LEES and WADDINGTON (1942) did not find in *sc* flies any sign of tormogen and trichogen from the earliest stage at which these cells become recognizable. It is then possible that the scute gene products act at even earlier stages. The study of perdurance of the *sc* gene reinforces that hypothesis. $Df(1)sc^{19}$ cells can differentiate chaetes if these cells result from mitotic recombination induced 40–48 hr (macrochaetes) or 16–24 hr (microchaetes) before pupariation. Some indirect evidence (see above and GARCÍA-BELLIDO and MERIAM 1971a) suggests that at that time the mother epidermal cell initiates the differential divisions giving rise to the nervous and epidermal pathways. This is certainly much earlier than the first histological signs of a trichogen and a tormogen cell (16 hr after pupariation) (LEES and WADDINGTON 1942).

Since $Df(1)sc^{19}$ is an embryonic lethal, a question immediately arises: are genes responsible for chaete differentiation also required for embryonic develop-

ment? No histological analysis of *Df(1)sc*^{19} has been carried out as yet. However, EDE (1956 a, b) describe two mutants, *X-10* and *X-20*, now lost, which mapped in the tip of the first chromosome "near scute" and which were early embryonic lethals. Since there are no early embryonic lethals in the tip of the first chromosome other than the lethal of scute, it is tempting to consider them related to it. The hemizygous embryos exhibited abnormalities in gastrulation, with failures in the segregation of nervous tissue and hypodermis out of the ectodermal cell layer of the blastoderm. This phenotype is consistent with our findings in gynandromorphs. The only viable gynanders with male *Df(1)sc*^{19} tissue are those in which this tissue does not extend over the ventral middle line of the embryo, a region from which the central nervous system derives, according to the anlage plan of the blastoderm worked out by POULSON (1950). SONNENBLICK (1950) showed further, that the nervous tissue originates from differential divisions of ectodermal cells of the blastoderm layer. Thus, it is reasonable to assume that the normal function of the achaete and scute genes is the differentiation of nervous elements or the segregation of the epidermal and nervous pathways in both the central nervous system and the imaginal discs.

It is important to recall now, that the genetic elements responsible for the viability of early embryos are included in the region between the breakpoints of *sc*^{4} and *sc*^{9} (the lethal of scute region). Deficiencies at both sides of it result in lack of chaetes and impaired motility of the emerged adults, but are viable or like *In(1)y*$^{+PL}$ *sc*^{4R} (deficient for achaete and part of the scute functions), lethal in larvae or pupae (Figure 1). An analysis of gynanders with *In(1)sc*^{4L} *sc*^{9R} male tissue shows the same results as that of *Df(1)sc*^{19}. Thus, it is the information lacking in this deficiency that is required for the differentiation of the vital elements of the central nervous system tissue. It is interesting to note that the male tissue in those gynanders shows only the *sc*^{4} or *sc*^{9} phenotype, *i.e.*, it can differentiate chaetes. We must therefore conclude that the genetic information of the lethal scute gene is at least qualitatively different from that of other scute functions. Although it is also related to nerve tissue differentiation, it is conceivable that it is related to motor rather than to sensory (peripheral) elements. The latter could be the ones affected by the viable scute mutations.

Other mutants are known in Drosophila whose phenotype affects both nervous system development and chaete differentiation. Thus, shibire, where several temperature-sensitive alleles are known, has temperature-sensitive phases leading to lethality in early embryogenesis, to the suppression of chaetes or to the appearance of extra chaetes around the time of puparium formation, and to a catatonic syndrome in the emerged adults (POODRY, HALL and SUZUKI 1973). Alleles of Notch that cause an abnormal nervous tissue development during early embryogenesis (POULSON 1940; FOSTER 1973) are also viable in mitotic recombination lones, but they cannot support chaete differentiation (GARCÍA-BELLIDO, unpublished). How these and other genes are interrelated in the control of the process of chaete formation is not known, but previous considerations suggest that the developmental pathways of the nervous system and those of chaete formation are affected by the same mutants.

It is in this context that the problem of site specificity of the different achaete and scute mutants should be now reconsidered. Their genetic behavior together with the comparison of their particular phenotypes and that of the complete deficiency suggests that all of them correspond to hypomorphic mutations of the same wild-type functions. We are entertaining the interpretation that the achaete-scute system contains a sequence of reiterative signals of combined effects, all of which are required for the normal differentiation of all chaetes (GARCÍA-BELLIDO, in preparation). Thus, site specificity of particular mutants could reflect differential thresholds of requirements depending on the position. It is known that these mutants behave autonomously in mitotic recombination clones (see STERN 1954). It is also known that there is no determined cell lineage for chaetes in the notum (STURTEVANT 1929; STERN 1940). Thus, site specificity of the different mutants must depend on the microtopographical conditions where they differentiate. However, attempts to find a site seriation which correlates with response to temperature (CHILD 1935), sensitivity to X rays or to perdurance have so far failed.

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