Genetic Analysis of Cell Heredity in Imaginal Discs of *Drosophila melanogaster* (chaetae/trichomes/crossing-over/puparium formation/cuticle)

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ABSTRACT Somatic crossing-over was used in heterozygous *Drosophila melanogaster* to effect clonal homozygosity for three mutants (*h*, *Hw*, *ac*). In these mutants, one type of process, chaetae, replaces another type, trichomes, in specific patterns on the adult fly. Heterozygous individuals were irradiated at different larval or pupal ages with x-rays and the somatic crossing-over was identified in the adult cuticle by genetically coupled mutants serving as cell markers of chaetae and trichomes. Induction of homozygosity more than 8 hr before puparium formation resulted in autonomous differentiation of the mutant pattern in the homozygous patch of tissue for the three mutants tested; homozygosity induced within 8 hr of puparium formation was not followed by expression of the corresponding genotype. 8 hr before puparium formation, each cell type still has to divide twice before metamorphosis and differentiation. Thus, the genetically conditioned decision of a specific cell to differentiate either a chaeta or a trichome is made during the growth of the wing imaginal disc and is transmitted clonally to descendant cells. Once this decision has been made, subsequent changes in the genotype of the cell or of its daughters are not material to the fate of the cell. We introduce the term "perdurance" to designate the persistence of a cellular developmental fate for several cell generations after the loss of the genetic basis for that cellular development.

The adult integument of insects is formed by the differentiation of a continuous sheet of cells into a chitinous surface including distinct cellular elements such as trichomes, chaetae, and sense organs. These elements differ in appearance and in distribution over the regions of the body, giving rise to characteristic cuticular patterns. Little is known about the mechanism determining the localized differentiation of such cuticular elements from the histologically homogeneous cell population found in the imaginal discs (1).

One approach to this problem has been to follow the cell lineage of regions of the adult cuticle by use of somatic crossing-over to make homozygous in a few cells mutant markers that are heterozygous in the remaining cells (2). Mutants of *Drosophila* are known that alter the shape, color, or number of the differentiated cellular elements (termed "cell-marker mutants") and can be used to record in the adult cuticle the clonal descent of cells propagated during the larval stages. Other mutants are known that modify the organization of the cells into the regional patterns (termed "pattern mutants") and can be made homozygous in patches to analyze the effect of mixing mutant and normal patterns of the same regions.

We use here the technique of somatic crossing-over to make clones homozygous for pattern mutants that insert chaetae

in regions normally void of them, coupled with cell-marker mutants, to analyze the time of the determinative event leading to chaeta differentiation. Somatic recombination is induced in larvae of various ages in order to determine if there is a time in development of the imaginal discs beyond which the mutant pattern of the marked cells in the adult cannot be distinguished from the normal pattern of the unmarked cells. Such an inability to express a change in genotype would reflect an irreversible, somatically heritable determination of the cells to form a particular pattern.

EXPERIMENTAL DESIGN

The pattern mutants *Hairy-wing* (*Hw*), *hairy* (*h*), and *achaete* (*ac*) influence the appearance of chaetae in the cuticle derived from the wing imaginal disc. *Hw*/+ or *h*/*h* individuals have extra chaetae on the notum and have chaetae on the wing surface in regions where none usually appear (Fig. 1). Individuals homozygous for both *achaete* and *hairy* (*ac/ac*; *h/h*) resemble wild type (no extra chaetae). The wing surface normally consists only of trichomes; in this sense, the pattern mutants condition the fate of an imaginal-disc cell to differentiate either a chaeta or trichomes.

The parameters of induced somatic recombination in wing disc cells have been described in detail (3), and will only be summarized here. Fig. 2 presents the data of somatic recombination marking trichomes on the wing surface with the mutant *multiple-wing-hair* (*mwh*). The number of marked clones scored in the adult increases exponentially with the age of the larvae (or early pupae) at the time of irradiation, while the size of the clones (i.e., the number of cells marked per clone) decreases exponentially. This reflects the increase in the number of target cells per wing imaginal-disc over this period, with the corresponding reduction in the number of mitoses each cell ultimately undergoes before cell division stops at metamorphosis and differentiation.

The plan of these experiments is to induce homozygosity of a pattern mutant by recombination in wing disc-cells at various points throughout larval and early pupal development and to score the results in the adult. In order to record the fact of chromosomal homozygosity, it is necessary to genetically couple mutants marking chaetae: *yellow* (*y*), *stubarista* (*sta*), *singed*³ (*sn*³), and *javelin* (*ju*), with the pattern mutants. This is accomplished (as shown in Fig. 3) for the mutant *h* in females by use of chromosomes bearing *T(1,3)sc*^{J4}, in which the *y*⁺*ac*⁺ tip of chromosome I has been translocated to the tip of chromosome arm IIIL, which also carries *ju* (and *mwh*⁺ and *h*⁺). Of the remaining two homologues, chromosome I carries *y*, and chromosome arm IIIL carries

Abbreviation: PF, puparium formation.

mwh and *h* (and *ju*⁺). The phenotype of the fly is wild type. Somatic recombination occurs at the 4-strand stage. Thus, a crossover in chromosome arm III L proximal to *h* may lead after segregation to nonidentical daughter cells, giving rise to a "twin spot" on the adult cuticle. One of the spots will be *ju*, the other *mwh* (and homozygous for *h*). The latter spot will be phenotypically *y*, as the genotype for chromosome I is *y/y*⁻. The recombinant event causing homozygosis for *h* can then be scored in the chaetae by the *y* marker, and in the trichomes by the *mwh* marker. In the chaetae, the twin spot is *ju*; in the trichomes the twin spot is not detectable.

MATERIALS AND METHODS

All the mutants and chromosomes used here have been described (4). The specific genotypes of the flies tested in four experiments are: Genotype 1: *Df(1)sc*⁸, *y*⁻*ac/y*; *mwh h/Dp(1;3)sc*^{J4}, *y*⁺*ac*⁺ *ju*. Genotype 2: *Ins(1)y*^{3PL}*sc*^{8R}, *y*⁻*ac*⁻/*sta*, *sn*³; *mwh h/h*. Genotype 3: *Ins(1)y*^{3PL}*sc*^{8R}, *y*⁻*ac*⁻/*Df(1)sc*⁸, *y*⁻*ac*⁻; *mwh h/Dp(1;3)sc*^{J4}, *y*⁺ *ac*⁺. Genotype 4: *y Hw/sta sn*³.

Somatic recombination was induced in third-instar larvae by x-irradiation with 1000 R (50 kV, 20 mA, 1 mm Al filter, at a rate of 330 R/min). Treated larvae were collected as prepupae at intervals of 8 hr after irradiation, set aside in vials, and allowed to complete metamorphosis. The interval elapsing between treatment and puparium formation (PF) accurately defines the age of each larva at the time of irradiation (3, 5). This amount of irradiation causes no significant delay in PF with respect to unirradiated controls. Other pupae were irradiated at 0-4, 4-8, 8-12, and 12-24 hr after PF. Adult flies were classified according to sex and genotype. The dorsal thorax was dissected from each female, boiled 5-10 min in 10% KOH to remove nonchitinous material, washed, and mounted in Euparal for microscopic examination. Somatic spots in the notum and wing cuticle were then scored under the microscope.

RESULTS

Description of the pattern mutants (4)

The distributions of the "extra" chaetae specific for each of the mutants are characteristic, although largely similar to the pattern shown in Fig. 1. They can be distinguished in the scutellum, postnotum, and wing blade, but not in the large background of chaetae normally present in the notum. The number of extra chaetae, given in Table 1, varies with the

FIG. 1. Chaetae distribution characteristic of pattern mutants examined. Normal chaetae (open circles) and extra chaetae (closed circles) on the scutellum (S), post notum (PN), and wing blade in a *Hw/+* female.

FIG. 2. Somatic crossing-over induced at different ages, marking trichomes on the wing with *mwh*. Logarithms of frequencies (open circles) and sizes (closed circles) of spots in samples plotted against time of larval irradiation (minus hours from PF) or pupal irradiation (plus hours from PF).

genetic background. The *h* mutant is completely recessive. Expression of the *Hw* is recessive in the scutellum and dominant in the wing blade, with homozygous females having about twice as many chaetae on the wing blade as do *Hw/+* females (compare rows *j* and *k* in Table 1). The *Hw* mutant is inseparable from a recessive *yellow* mutant.

Homozygous *achaete* flies have a characteristic reduction in the number of chaetae normally present in the notum. In homozygous (*ac*⁻/*ac*⁻; *h/h*) individuals, it suppresses the extra chaetae in the notum (6) and in the wing blade (rows *g* and *i* in Table 1). This *ac* effect on *h* is largely recessive (compare row *b* with rows *d* and *f* in Table 1, see ref. 7).

Induction of hairy clones

Flies of Genotype 1 (heterozygous for *h*) do not have extra chaetae. Somatic recombination in chromosome arm III L (schematically represented in Fig. 3) may lead to cells homozygous for the mutants *y*, *mwh*, and *h*, as discussed above in *Experimental Design*. Fig. 4 presents the results of scoring *y* spots among 207 adult flies previously irradiated at various larval and pupal stages. Since the *h* phenotype can only be distinguished in the wing, the frequency of *y* spots in the notum serves as an internal monitor for somatic recombination; the results for the notum shown in Fig. 4 are identical to those obtained in previous experiments without *h* (3). The frequencies of *mwh* spots on the wing surface represents another internal control, which in this experiment was identical to the results shown in Fig. 2. The *y* spots (chaetae) observed on the wing surface, found in flies irradiated more than 8 hr before PF, represent the autonomous differentiation of the *h/h* genotype in those clones. All of the extra chaetae observed on the wing surface were phenotypically *y*, and in some cases (irradiated as young larvae) groups of *y* chaetae were surrounded by *mwh* trichomes. That is consistent with the fact that irradiated flies (Canton-S) not carrying *h* do not show *h*-like phenocopies.

FIG. 3. Schematic representation of the marker arrangements of Genotype 1, heterozygous for *y*, *mwh*, *h*, and *ju*, with a crossover figured in chromosome arm III L (each chromosome arm shown in two of four strands).

TABLE 1. Distribution of extra chaetae in pattern mutants

Row	Genotypes	Number of wings scored	Number of extra chaetae per disc			
			Scutellum and post notum		Wing	
			Mean	±SD	Mean	±SD
a.	<i>y/y;h/+</i>	26	0.0	—	0.0	—
b.	<i>y/y;h/h</i>	40	14.4	3.2	71.4	9.3
c.	<i>y/Dfsc⁸;Dpsc¹⁴/h</i>	40	0.2	0.01	0.15	0.01
d.	<i>y/Dfsc⁸;h/h</i>	40	6.7	1.2	41.1	7.6
e.	<i>y/Dfsc⁸;Dpsc¹⁴ h/h</i>	40	8.7	1.5	38.0	8.4
f.	<i>y⁻ac⁻/sta sn³;h/h</i>	40	6.4	1.8	52.3	8.5
g.	<i>y⁻ac⁻/y⁻ac⁻;h/h</i>	28	1.6	0.4	2.2	0.1
h.	<i>y⁻ac⁻/Dfsc⁸;Dpsc¹⁴/h</i>	26	0.01	0.01	0.02	0.01
i.	<i>y⁻ac⁻/Dfsc⁸;h/h</i>	36	4.8	1.1	8.5	2.3
j.	<i>y Hw/sta sn³</i>	20	0.7	0.05	49.5	4.2
k.	<i>y Hw/y Hw</i>	20	11.0	0.4	110.2	5.4

Flies carrying different doses and combinations of the mutant genes studied here were scored for extra chaetae in the scutellum, post notum, and on the wing blade. Means and standard deviations are given; data from dorsal and ventral surfaces of the wing are pooled.

We conclude that presumptive wing surface cells made homozygous for *h* more than 8 hr before PF are as capable of differentiating into a chaeta (responding to pattern) as are newly marked cells in *h/h* flies. In the first place, comparison of the frequencies and sizes of spots induced here with marking the extra chaetae with *y* in *h/h* flies [*Df(1)sc⁸, y⁻ac⁻/y;Dp-(1;3) sc¹⁴, h/mwh h*] shows them to be the same. In the second place, the (*y*) extra chaetae produced as a consequence of the homozygosis of *h* are predominantly located along the wing veins, as are the extra chaetae found in *h/h* flies.

However, in flies of genotype 1 irradiated within 8 hr of PF, or after PF, no *y* extra chaetae are found in the adult wing, although somatic recombination has occurred. *mwh* clones induced at this time in the wing include 2–4 trichomes per clone, which is not substantially different from the size of *mwh* clones induced 12 hr or earlier before PF. This makes it unlikely that the abrupt change in expression of the *h* phenotype in spots is due to diffusion of some *h⁺* substance into *h/h* cells that is limited by the size of the clone.

Induction of *achaete* clones in *hairy* flies

The reverse of the above experiment is to remove the extra chaetae in spots. This was attempted in flies of Genotype 2,

FIG. 4 (left). Results of scoring *y* chaetae in the wing blade (*W**) and notum (*N*) from somatic crossing-over induced in flies of Genotype 1 (heterozygous for *y* and *h* in coupling). * Indicates marked "extra" chaetae. Logarithms of frequencies of spots in samples plotted against time of larval irradiation (minus hours from PF) or pupal irradiation (plus hours from PF).

FIG. 5 (right). Headings and symbols as in Fig. 4. Results of scoring *y* chaetae induced in Genotype 2 females (homozygous for *h*; heterozygous for *y* and *ac*).

which are homozygous for *h* and heterozygous for *ac*. Recombination in chromosome arm I yields twin cells of genotypes *y⁻ac⁻/y⁻ac⁻* and *sta sn³/sta sn³*. The results of scoring *y* spots in the notum and wing blade in 119 flies are shown in Fig. 5. In the notum the *y⁻ac⁻* cell appears as a *y* chaeta; in the wing autonomous expression of the homozygous *ac⁻* cell should lead to suppression of the extra chaetae characteristic of *h* and the spot should not appear. In this experiment *sta sn³* cells are used as an internal control; they appear marking clones chaetae in the notum and in the wing surface (results are not shown) with the usual frequencies expected for each age of induction. The results of this experiment are complementary to those in the previous experiment (Fig. 4): *y* chaetae are found in the notum, but not on the wing surface, in flies irradiated up to 8 hr before PF. That is, the homozygous *ac⁻* clones generated by the somatic recombination show the expected phenotype, since the loss of the *ac⁺* has prevented expression of *hairy*. However, in flies irradiated earlier than 8 hr before PF or as pupae, *y* spots do occur among the extra chaetae on the wing blade, as well as on the notum. The *hairy* genotype is expressing itself even in the absence of the *ac⁺* gene. From this result, it may be inferred that the *ac⁺* gene has functioned at the chromosomal level before 8 hr prior to PF.

As the function of *h* also appears to occur at the same critical period, it was of interest to determine whether simultaneous induction of homozygosis of the two mutants would disclose an overlap in the critical period. Flies of Genotype 3 are heterozygous for both *ac⁻* and *h*. Somatic recombination in chromosome arm III proximal to *h* should generate euploid cells homozygous simultaneously for both *h* and *ac⁻* (and *y*). The frequency of somatic recombination was normal (*y* chaetae in the notum and *mwh* spots in the wing). However, no extra *y* chaetae in the wing blade occurred in flies irradiated at any age (results from 125 flies are shown in Fig. 6).

Induction of *Hw⁺* and *Hw/Hw* clones

Flies of Genotype 4 have about 50 extra chaetae in the wing blade and about 130 chaetae in the notum—slightly over the normal complement of 100 chaetae in the notum. Somatic

recombination in chromosome arm I leads to *y Hw* and *sta sn³* twin cells. If such an event occurs in a presumptive wing cell, where *Hw* is dominant, the *y Hw* cell will appear as a *y* spot among the extra chaetae; the *sta sn³* spot, marking cells which are homozygous *Hw⁺*, would therefore not be detectable in the wing. If the recombination occurs in a presumptive scutellar cell, where *Hw* is recessive, autonomous expression of the homozygous *y Hw* cell will yield extra chaetae, all *y*; the *sta sn³* spot will not be present. The notum serves as the internal control, as in previous experiments.

Flies irradiated prior to 8 hr before PF yield the results expected with autonomous behavior of the mutant: *sta sn³* spots on the notum (not included in the figure) but not on the wing blade, and *y* spots on both notum and wing (Fig. 7). In addition, *y* extra chaetae are found in these flies in the scutellum and postnotum (not included in the figure). There was no indication that homozygous *Hw* tissue in the scutellum acts to induce extra chaetae in the surrounding *Hw/+* tissue, as has been reported for *Hw* spots in the notum (8). In the wing, induction of cells homozygous for *y* and *Hw* may increase the number of extra chaetae to give a distribution characteristic of the higher number (100) found in homozygous *Hw* flies. The relative frequency of *y* chaetae induced in the wing and notum changes about 8 hr before PF, and in flies irradiated as younger larvae, this frequency reflects the observed proportion of chaetae in the wing and notum of *Hw/Hw* adults.

In flies irradiated after 8 hr before PF, *sta sn³* clones appear among the extra chaetae in the wing. The absolute frequency is the same as that of the *y* spots, reflecting their simultaneous generation. Thus, in the *sta sn³* cells, the removal of the dominant *Hw* gene after 8 hr before PF did not prevent its phenotypic expression. The effect is similar to the lack of suppression of *h* by *ac* seen in Fig. 5.

DISCUSSION

Larvae and pupae contain 17 imaginal discs which, on metamorphosis, yield all the external structures of the adult fly except the abdominal sheath. The wing blade, scutellum, notum, and postnotum on one side arise from one such disc. The size of the disc increases by cell division during the period of larval development. The normal time from egg laying to PF is 120 hr; only a very small amount of cell division occurs after PF (9). Thus, a few cells in an undifferentiated disc eventually yield a mature structure containing 50,000 cells exhibiting a wide variety of cell types. The commitment of a given cell to a particular structure must occur at some time during this period of growth. In an earlier paper, we tried to determine the timing of such a commitment by introducing visible cell markers by somatic recombination (3). The induced homozygosity then marks phenotypically all the cells derived from the cell in which the recombination occurred. If the homozygosity for a marker such as *y* occurred very early, before commitment of the cell to a structure, determination at a later time would lead to cells of several types among the descendants of this cell. If the homozygosity occurred after commitment, this would be reflected in a single cell type in the clone.

The final expression of cells as chaetae or trichomes occurs in the final cell division. Trichomes are single-cell derivatives of epidermal cells. Normal chaetae and extra chaetae induced in *h* flies derive from a mother cell by two differential mitoses. Stern (10) found the *h* chaetae to be innervated,

FIG. 6 (left). Headings and symbols as in Fig. 4. Results of scoring *y* chaetae induced in Genotype 3 females (heterozygous for *y*, *h*, and *ac* in coupling).

FIG. 7 (right). Headings and symbols as in Fig. 4. Results of scoring *y* chaetae (open and closed circles) and *sta sn³* chaetae (*x*-*x*) induced in 140 Genotype 4 females (*y Hw/sta sn³*).

as are normal chaetae. The determination of cells to be chaetae or trichomes appears, however, to occur much earlier. If homozygosity for *y* is induced earlier than 40 hr before PF, the *y* clone may contain both trichomes and chaetae. At this time the cell in which recombination occurred gave rise to daughter cells of these two types and was, therefore, not itself committed. Homozygosity within 40 hr of PF yields *y* or *mwh* clones containing either chaetae or trichomes, but not both. The cell has become committed to one of these two possible pathways by this time. Moreover, the resulting clone has a minimum of one chaeta versus 4 trichomes (3).

The experiments reported here were designed to ask when mutants conditioning the appearance of extra chaetae exert their effect, i.e., whether the decision to make extra chaetae (*h* or *Hw*) or to suppress extra chaetae (*ac* or *Hw⁺*) is made at the same time as a cell is apparently committed to chaetae formation. In the present system, homozygosity up to 8 hr before PF permitted phenotypic expression of *h*. Not until 8 hr before PF was the cell line, possibly destined at 40 hr before PF to form chaetae, further defined as to actually differentiate into a chaeta. Similar conclusions were reached from the results with the *ac* and *Hw* mutants.

Such an end point does not reflect merely entrance into the differential cell divisions at metamorphosis. *mwh* clones induced 8 hr before PF contain 2–4 cells, the equivalent of 4–8 cells for the two clones of the twin spot. Trichome-forming cells marked at 8 hr before PF therefore divide once or twice (after homozygosity) before their final differentiation. Similarly, the second of the two differential mitoses giving rise to the chaetae, that in which trichogene and tormogene elements separate, must occur very late in development, since homozygosity for *y* or *sn* in trichogene elements may be induced as late 18 hr after PF. Thus, the decision at 40 hr before PF to become chaetae or trichomes occurs 6–8 cell divisions before the final expression, while the genetically conditioned determination of the extra chaetae formation at 8 hr before PF occurs 1–2 divisions before actual cell differentiation. This could be the explanation of the 1 chaeta–4 trichome ratio mentioned earlier (3).

We introduce the term “perdurance” to describe the persistence of a phenotype after the removal of the conditioning genotype. In the case of *h*, the presence of the gene is required only up to 8 hr before PF; after that time, extra chaetae characteristic of *h* appear whether or not the *h* gene is present. The cells are predetermined. A classical case of predetermination, the maternal effect, is attributed to

metabolic products in the egg cytoplasm (11), to maternal enzymes (12), maternal messenger RNA (13), or "structural matrices" (14) in the oocyte. In the cases treated here, the mechanism is unclear. "Perdurance" does not result from cellular synthesis and storage of all the elements necessary during differentiation, with little or no subsequent cell activity. This may be inferred from the continued expression of *y*, *sn*, and *mwh* on homozygosis: i.e., the *y*⁺, *sn*⁺, and *mwh*⁺ phenotypes do not perdure. The perdurance of *h* or of the *ac* suppression of *h* appears rather to be involved in the actual process of determination. Thus, delimiting the period of perdurance permits identification of the time beyond which the presence of the gene is unnecessary for expression in descendent cells. The critical activity of the gene probably occurs at this time. Such analyses may provide a probe with which to examine parameters of developmental decisions currently inaccessible to study.

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