CELL AFFINITIES IN ANTENNAL HOMOEOTIC MUTANTS OF *DROSOPHILA MELANOGASTER**

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DISSOCIATED embryonic cells show, in culture, cell affinities and morphogenetic movements which are tissue characteristic. While cells which derive from the same organ and tissue are able to reconstitute the original cell arrangements in mosaic fashion, cells from different organs sort out and regroup separately. This situation holds for vertebrate cells as well as for cells of many other lower organisms (see TRINKAUS 1966 for discussion).

In Drosophila the adult cuticle consists of several cell structures specifically arranged into segment characteristic patterns. The adult cuticular structures are formed from cells of the imaginal discs of the larvae which undergo differentiation during metamorphosis. A variety of mutants in *Drosophila melanogaster* change the color or form of single cuticular structures (cell mutants). With the aid of cell marker mutants HADORN, ANDERS and URSPRUNG (1959) have shown that dissociated cells of the same imaginal disc are able to reaggregate and reconstruct the adult patterns of this disc integrated in mosaic fashion. However, dissociated cells deriving from different imaginal discs (NÖTHIGER 1964; GARCIA-BELLIDO 1966b) or from different regions of the same disc (GARCIA-BELLIDO 1966a) sort out or segregate in aggregates. Furthermore, cells from homologous imaginal discs (different leg discs) reconstruct the common patterns in a typical mosaic integration; whereas they segregate when they correspond to different patterns (GARCIA-BELLIDO 1966a). These results are indicative of a highly specific cell determination correlated with highly selective cell affinities in the dissociated cells of the imaginal discs.

When cultured *in vivo*, aggregated cells may change their prospective differentiation into that typical for other imaginal discs. The underlying phenomenon is called transdetermination and is assumed to be based upon a non-mutational change in the activity of sets of genes (HADORN 1966). When studied in cell aggregates, the transdetermined cells show new cell affinities which correspond to their new cell determination (GARCIA-BELLIDO 1966b). The hypothesis was advanced then that the same specific genes are responsible for both the selective affinity and the correlative differentiation of the imaginal disc cells.

There are mutants in Drosophila which specifically change the arrangement of the differentiated structures in the adult cuticle. Such mutants can be con-

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sidered as pattern mutants. Among the pattern mutants the homoeotic mutants transform one segment or organ of the body into another. The mutants aristapedia (ss^a) and antennapedia (*Antp*) determine the specific transformation of certain antennal segments into leg-like segments. Such mutants allow us to test further the above hypothesis. The aim of the present work is to analyze the behavior of dissociated cells of the mutant antennal discs in cell aggregates. Under these conditions the question arises whether the isolated mutant cells will show the characteristic cell affinity of antennal cells (the segment in which they arose) or the affinity characteristic for the homoeotic differentiation.

MATERIALS AND METHODS

The homoeotic mutants employed correspond to two different loci: ss^a(3–58.5) and *Antp* (3–47.0 ±). At the ss^a locus two different mutants were studied: ss^a (BALKASHINA 1929) and ss^{a40a} (BUZZATI-TRAVERSO 1940), both recessives. HEXTER, LEZNER and BUNN (1967) find that these mutants are separable by crossing over and are, therefore, pseudoallelic. At the *Antp* locus only the allele $Antp^{50}$ (found by PITTERNICK, see LINDSLEY and GRELL 1968) was studied. The latter mutant is dominant and lethal when homozygous. A cytological analysis of $Antp^{50}$ showed no major chromosomal rearrangements (HANNAH-ALAVA, personal communication).

Dissociation, aggregation and culture of imaginal disc cells was performed according to the following method. Dissociation of imaginal disc cells was carried out in a 0.5% trypsin solution in phosphate-buffered (pH 7) Drosophila Ringer's solution. The dissociation is achieved by means of a microhomogenizer which disperses the imaginal disc cells in a siliconized centrifuge tube. The cell suspension obtained in this way is then centrifuged for one minute at 3,000 × *g* and the recovered clump of cells is washed in Ringer's and implanted into the abdomen of adult host flies. After two days the implants are recovered and transferred into larval hosts, where they undergo metamorphosis and differentiate into adult cuticular structures. Further details of this method are given elsewhere (GARCIA-BELLIDO 1966a; 1967).

In order to elucidate the origin of the adult cells in the aggregates, genetic cell markers were used. For this purpose donor larvae carried body color mutants: either *e* (ebony, 3–70.7) or *y* (yellow 1–0.0), and mutants producing bristle and hair abnormalities: either sn^3 (singed 1–21.0) or *mwh* (multiple wing hair, 3-left end; DI PASQUALE 1952).

In the present paper the following cell combinations were studied:
Experimental Series A. Antenna-ID (*e*) with ♂ I+II leg-ID (*y sn mwh*)
Experimental Series B. Antenna-ID (ss^a *e*) with antenna-ID (*y sn mwh*)
Experimental Series C. Antenna-ID (ss^a *e*) with ♂ I+II leg-ID (*y sn mwh*)
Experimental Series D. Antenna-ID (ss^{a40a}) with ♂ I+II leg-ID (*y sn mwh*)
Experimental Series E. Antenna-ID ($Antp^{50}$) with ♂ I+II-ID (*y sn mwh*),
where ID stands for imaginal disc, and I+II for first and second leg pair. All of the dissociated cells came from imaginal discs of mature larvae (100–120 hr after egg laying). Wild type color (instead of *e* Experimental series D and E) is dark enough to contrast in aggregates with *y* (*sn mwh*) cells.

RESULTS

Description of the adult structures in situ: The fine cuticular structures of the normal antenna *in situ* were described by FERRIS (1950) and GEHRING (1966). Those of the normal legs were described by HANNAH-ALAVA (1958) and GARCIA-BELLIDO (1966a). Figure 1 shows the general anatomy and distinctive cuticular structures of the normal antenna and of the normal male first and second thoracic leg.

TABLE 1

Penetrance of regional leg transformation in homoeotic antennae in situ

Genotype	Number of antennae	Regional transformation in percent			
				Claw	
		Femur Tibia	Tarsal segments	Claw	Claw-arista†
ss^{a}	104	. .	100.0 (4.0)*	97.1	1.3
ss^{a40a}	115	. .	100.0 (2.8)*	0.9	15.6
$Antp^{50}$	100	5.0	1.0	. .	. .

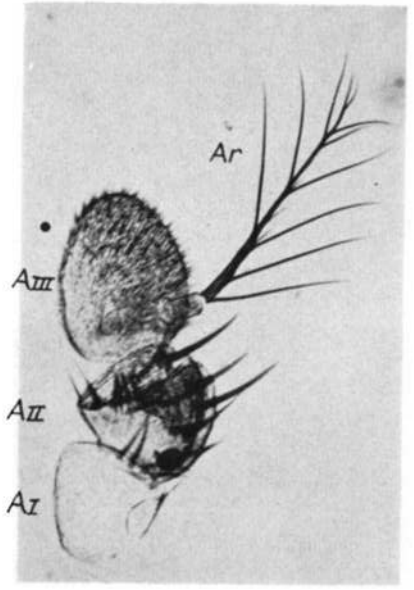
* Mean number of tarsal segments.
† Claw-arista: intermediate structures between a normal arista and a claw.

The homoeotic mutants studied here show leg-like outgrowths in antennal territories. The expressivity of these antennal transformations varies from one mutant to another (Table 1). Typically, in ss^{a} individuals the distal part of the third antennal joint is transformed into four or five tarsal-like segments which end in a claw (Figure 2b). In ss^{a40a} individuals the antennal transformation is medio-distal only; the third antennal joint develops only two or three tarsal segments which terminate in an arista (Figure 2c). The $Antp^{50}$ mutants develop, only rarely, tibia- or femur-like outgrowths in the proximal part of the third and on the second antennal joint, leaving the arista unmodified (Figure 2d). The general morphology and the cuticular structures of these outgrowths are similar to some segments of the thoracic legs. However, they lack any characteristic bristle type or bristle pattern which identifies them as belonging to any specific thoracic leg.

The aim of the present work is to analyze the morphogenetic behavior of the antennal homoeotic cells in cell aggregates with cells from wild-type thoracic legs. The experiments described below were designed to answer two questions: a) Do antennal cells derived from the homoeotic mutant individuals possess the same cell affinity as thoracic leg cells derived from wild-type individuals? b) Will dissociated homoeotic antennal cells in combination with such thoracic leg cells differentiate cell structures of all the leg segments or only of those leg-like structures found *in situ?*

Control experiment: Since antennae and legs show very different cuticular structures (Figure 1) we expect, by analogy with the findings in other heteronomous combinations, that their presumptive cells will segregate, that is, they will sort out and differentiate separately in aggregates.

In experiment A, normal antennal (genotype *e*) and leg (*y sn mwh*) imaginal disc cells were dissociated together and subsequently aggregated and cultured. Analysis of the differentiated aggregates shows, contrary to expectation, that normal antennal and normal leg structures do not completely segregate but frequently appear in close association with one another in the same cuticular surface. This association appears as the autonomous yet intermingled differentiation of structures of the antennal joints on the one hand and leg territories on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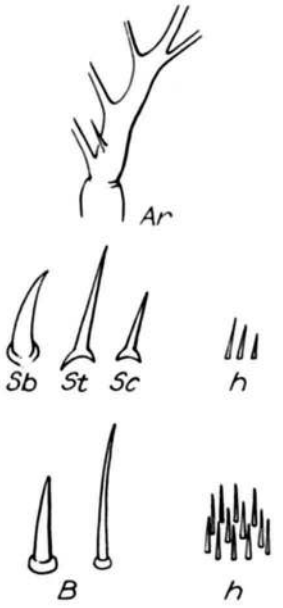
a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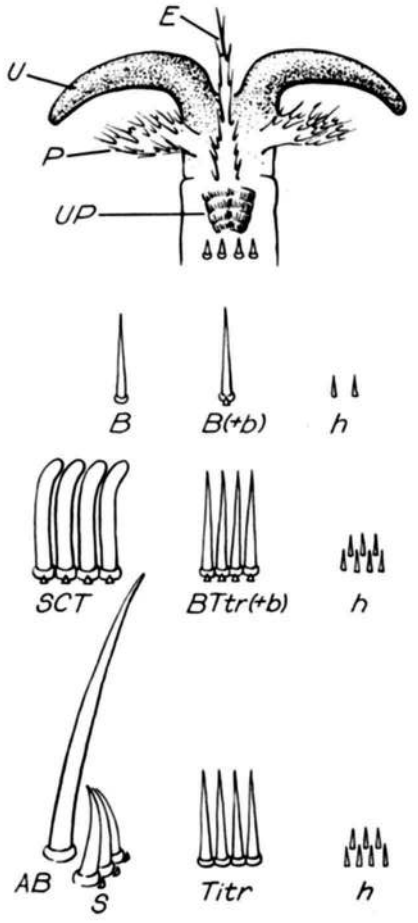
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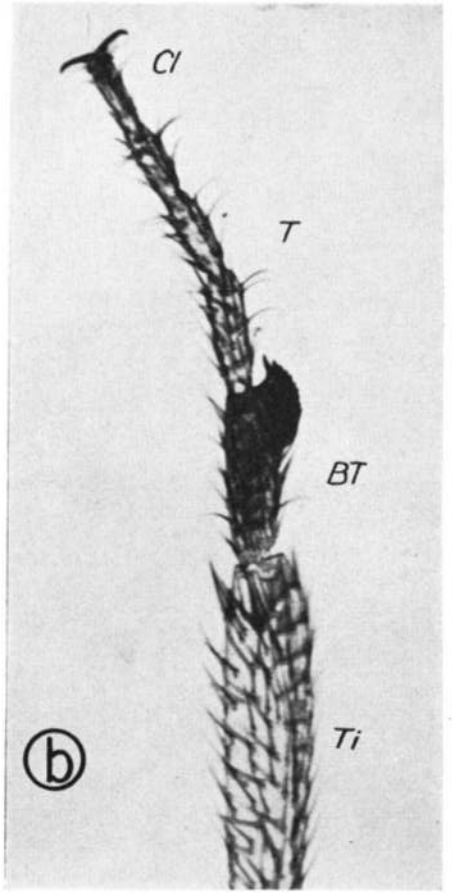
Cl
T
BT
Ti

b

TABLE 2

Cell combinations of homoeotic and normal imaginal discs. Percent of implants having at least one homoeotic leg structure either in monotypic (M) or in chimeric (C) territories.

Experiment	Disc cell combination	Number of implants	Femur	Tibia	Basi-tarsus	Tarsus	cl cl-(cl)†	ar ar-(cl)†	M C
A	AID × LID	59	...	...	...	...	...	12.0	M
	+ +		...	...	...	...	...	13.5	C
B	AID × AID	42	...	...	...	66.6	42.9	10.0	M
	ss^a +		...	...	...	...	19.0*	9.5**	C
C	AID × LID	78	...	...	...	41.0	12.8	5.1	M
	ss^a +		1	5.1	12.8	72.0	27.0	2.5	C
D	AID × LID	103	...	...	...	28.2	4.8	30.0	M
	ss^{a40a} +		...	2.9	8.7	69.0	8.6	19.3	C
E	AID × LID	110	...	1.8	4.5	4.5	0.9	25.4	M
	$Antp^{50}$ +		4.5	17.3	7.3	24.5	3.6	21.0	C

† (cl) = claw structure deriving from the thoracic leg.
* Corresponds here to claw-arista mosaics where the aristal structures derive from the antennal disc.
** Here arista-arista mosaics.

the other (Figures 3, 4 and 5). This type of association will be henceforth called heteronomous association. Most impressive are the cases in which arista and claw structures appear side by side (Figure 3, see also Figure 6). These results are presented schematically in Figure 2a.

Combination of homoeotic and normal antennal disc cells: In experiment B normal (*y sn mwh*) and homoeotic (*e* ss^a) antennal disc cells were combined. This experiment should give us information about the autonomous differentiation of leg-like presumptive cells present in ss^a antennal discs. In such cell aggregates both *e* ss^a and *y sn mwh* cells differentiate structures of all the antennal segments, frequently in mosaic fashion, but *e* ss^a cells differentiate also tarsal structures and claw (Table 2). These leg-like structures correspond in all respects to the homoeotic leg structures found *in situ*, and appear only in *e* territories. Paralleling the results of experiment A there is also heteronomous association of *e* tarsal structures intermingled with *y sn mwh* antennal ones (Figures 6 and 7).

In experiments A and B the differentiation of arista and claws in cell aggregates differs somewhat from the corresponding differentiation *in situ*. The aristal differentiation often takes here the form of extended surfaces of long hairs, which sometimes become organized into typical aristal structures (Figures 3 and 6).

FIGURE 1.—Adult structures of normal antenna and leg. a) normal antenna subdivided in segments with their characteristic structures (× 180): Arista (Ar); AIII, third antennal joint with sensillae trichodea (st), companiformia (sc), basiconica (sb); and hairs (h); AII and AI with bristles (B) and hairs (h). b) normal foreleg (× 100) and characteristic segmental structures of the male fore leg and middle leg: Claw (Cl) organ with empodium (E), pulvillum (P), unguitractor plate (UP) and two ungues (U); tarsal (T) bristles (B) with or without bracts (b); basitarsal (BT) sexcomb teeth (SCT) and basitarsal and tibial (Ti) transversal rows (BTtr, Titr) of the foreleg; Apical bristle (AB) with bracteated spurs (S) of the middle leg.

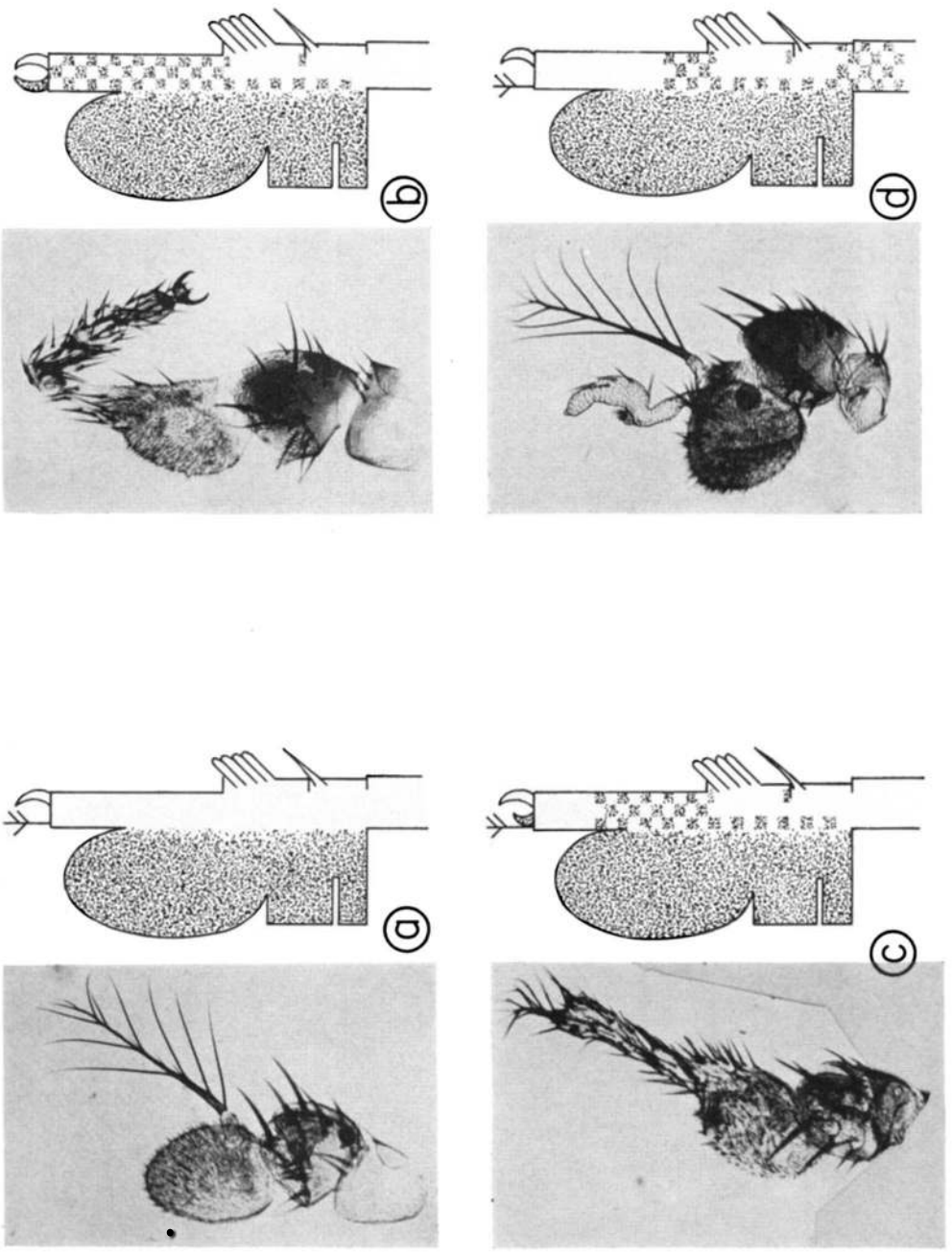

FIGURE 2.—Typical adult structures of the antenna in wild type and in the different homoeotic mutants. a) wild type; b) ss^{a}; c) ss^{a40a}; d) $Antp^{50}$. (× 180). The schematic drawings represent the result of cell combinations between the corresponding antenna and wild type leg imaginal disc cells. The mosaic patches represent the regional formation of chimeric structures. The contiguous black and white structures represent heteronomous association. See text for details.

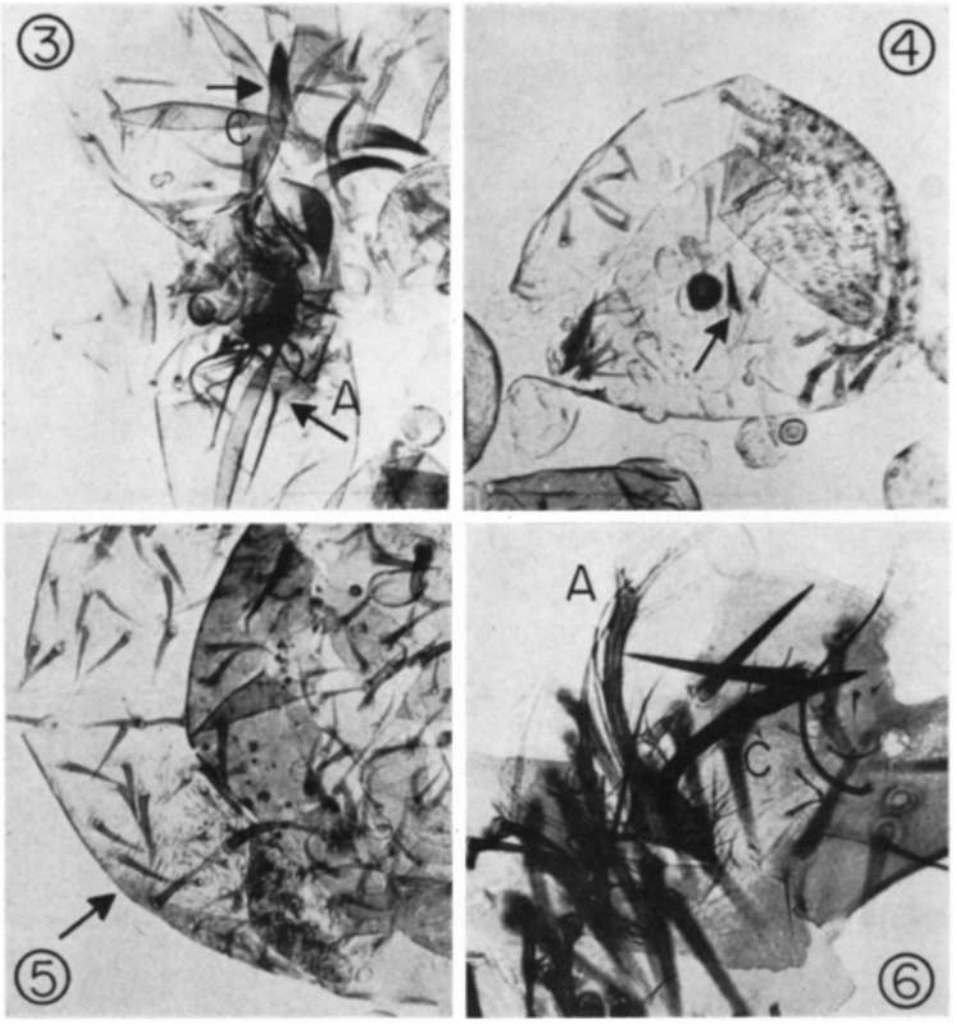

FIGURE 3.—Experimental series. A. Associated differentiation of arista cells (*e* cells) and claw structures (*y sn mwh* cells). Arrow: scattered long hairs of the arista (A). Observe the composite nature of the claws (C). (× 180).

FIGURE 4.—Experimental series A. Associated differentiation of antennal (AIII, *e* cells) and tarsal structures (*y sn mwh*). Arrow: an *e* cell from the antenna differentiating into a bracteated bristle typical for tarsal regions. (× 180).

FIGURES 5.—Experimental series A. Associated differentiation of antennal (AIII, *e* cells) and tarsal (*y sn mwh*) structures. Arrow: *y sn mwh* cells from the leg differentiating AIII typical hairs (× 180).

FIGURE 6.—Experimental series B. Associated differentiation of an arista (A) (*y sn mwh*) and a claw (C) (*e*, deriving from the antennal ss^a disc). Observe the hairs arising from the claw surface (× 400).

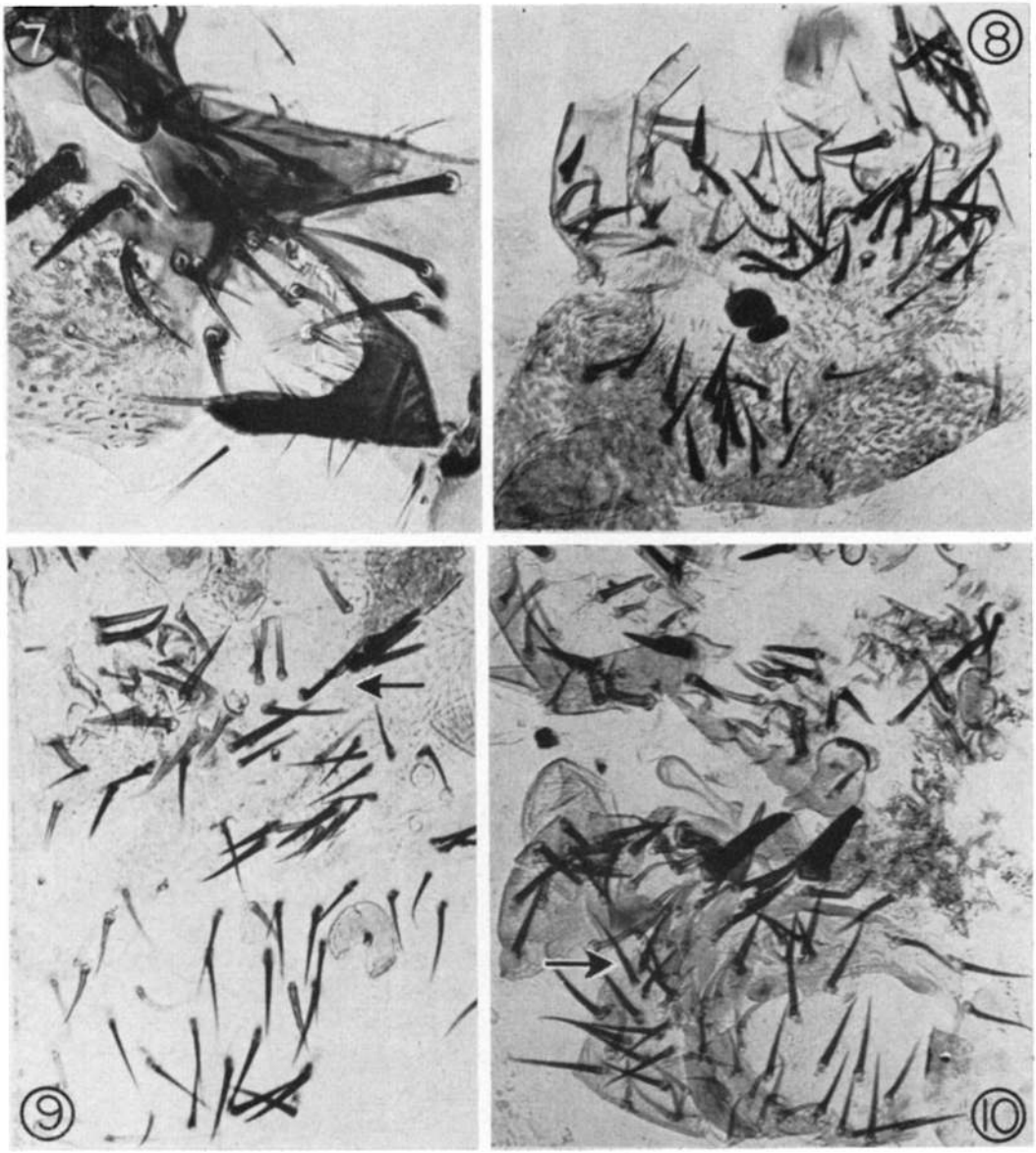

FIGURE 7.—Experimental series B. Monotypic differentiation of an ss^a claw. Observe its fringed and hairy structure. (× 400).

FIGURE 8.—Experimental series C. Chimeric differentiation of ss^a (*e*) and wild-typ (*y sn mwh*) leg bristles. Observe both *e* and *y sn* bristles in mosaic and within typical AIII hair surfaces. (× 180).

FIGURE 9.—Experimental series D. Chimeric formation of ss^{a40a} with normal leg tarsal bristles. Arrow: ss^{a40a} bristles do not differentiate into sexcomb teeth although they appear in proximity to thoracic leg (*y sn*) sex combs. (× 180).

FIGURE 10.—Experimental series D. Chimeric differentiation of $Antp^{50}$ and normal tibial bristles. Arrow indicates $Antp^{50}$ bristles not arranged into transversal rows. (× 180).

Wild-type claws appear as cuticular processes lacking any structures homologous to the bristle socket. Furthermore, claws are split and fringed and frequently show branches (Figures 3 and 6). The hairy nature of the claws is even more extreme in ss^a tissue; in aggregates as well as *in situ* we find intermediary structures between aristal hairs and claws (Figures 6 and 7). On genetic as well as developmental grounds the arista is considered a hair derivative (HADORN 1965; PEYER and HADORN 1966). As for the claws the normal ungular structures do not have sockets and ANDERS (1955) has shown scale processes all along their surface. These arguments, taken together with the findings on cell aggregates, strongly support the idea that arista and claw each develop by gathering and compacting of hair processes of several epidermal cells.

Experiments A and B show further the extent to which dissociated cells differentiate eutypically: i.e., according to the determination of the tissue from which they derive. In 89 out of 101 implants wild-type antennal cells (*e* or *y sn mwh*) did not differentiate into leg structures. In the remaining 12 implants (6 of each experiment) some antennal cells differentiated bracteated bristles (Figure 4) which are characteristic of leg structures. The reverse situation is also true: leg (according to the genotype) cells did occasionally differentiate antennal hair structures (Figure 5). Such heterotypic differentiation is never found for large territories or major cell types (sexcomb teeth, and transversal row bristles) and occurs only in the contact zones between the heteronomous territories. However, with the data at hand it is not possible to decide whether this heterotypic differentiation is due to spontaneous transdetermination or to *assimilative induction* involving neighboring heteronomous cells.

Combination of homoeotic antennal and normal leg disc cells: In experiments C, D and E imaginal antennal cells of a given type of homoeotic mutant individuals were mixed with a combination of cells of the first and second leg male imaginal discs of wild-type individuals. In such aggregates the homoeotic antennal cells will have the opportunity of showing preferential cell affinities with characteristic structures of either the first or the second leg (see Figure 1b). In the resulting aggregates antennal structures show heteronomous association with the leg structures as in experiments A and B. However, the homoeotic cells which differentiate into leg-like structures appear normally integrated within *y sn mwh* leg territories in mosaic fashion (Figures 7–10, Table 2). Such mosaics indicate a similar affinity between presumptive leg-like cells from the homoeotic antenna and from the thoracic leg discs. This selective affinity has been shown to be characteristic for homonomous cells and is used as an indication that the cells have identical determination (GARCIA-BELLIDO 1966a; NÖTHIGER 1964).

Homoeotic leg cells in aggregates may appear in either monotypic territories; i.e. territories made up only of leg-like cells or in chimeric territories; i.e., mosaicly integrated with thoracic leg cells. A general feature of the results of experiments C, D and E is that in monotypic territories the homoeotic cells differentiate into the same leg structures and patterns as they do *in situ*. However, whereas the regional specificity of the antennal into leg transformation does not vary, the penetrance of the transformation is much increased by dissociation in com-

parison with the situation *in situ* (compare Tables 1 and 2). This is especially true for ss^{a40a} and $Antp^{50}$ antennal cells which show low penetrance *in situ* for the claw and tarsal transformation. In addition, when antennal cells of the different homoeotic mutant types (ss^{a}, ss^{a40a} and $Antp^{50}$) appear with thoracic leg cells within chimeric territories, the antennal cells differentiate the same leg-like structures and in the same frequency as they do in monotypic territories (Table 2).

Table 2 shows some minor exceptions to this rule. Single homoeotic leg-like structures appear in chimeric territories within leg segments corresponding to more proximal regions than the regions typically transformed *in situ*. For instance, ss^{a} and ss^{a40a} differentiate bristles in basitarsal and tibial territories (Figure 9), and $Antp^{50}$ in femoral ones. However, the homoeotic cells never differentiate into sexcomb teeth, or transversal row bristles of the first leg nor into spurs of the second leg, although they are sometimes found in their close proximity (Figures 9, 10). Thus, the isolated antennal cells of those homoeotic mutants differentiate in aggregates only leg structures and patterns characteristic for the locus and the allele specificity. A scheme of these results is given in Figures 2b, c and d.

DISCUSSION

In experiments involving cell aggregates the single cells have the opportunity of choosing their definitive location among other cells according to inherent cell affinities. In cell aggregates involving either leg or wing (GARCIA-BELLIDO 1966b), or antenna and wing (GEHRING 1966) disc cells, the heteronomous cell types segregate from one another and differentiate into separate cuticular surfaces. The mixture of wild-type antennal and leg cells lead to a different result: heteronomous cells differentiate autonomously yet associated side by side in the same cuticular surface (Figures 3–10). This phenomenon of heteronomous association is comparable to the result of combining cells corresponding to different segments or patterns of the same imaginal disc (GARCIA-BELLIDO 1966a). This phenomenon is even more striking in the case of differentiation of claws and aristal structures in cell aggregates. The prospective cells of both kinds of structures have a strong positive affinity, appearing normally side by side in aggregates (Figures 3 and 6). Moreover, the facts considered on page 495, first support the assumption that both types of adult structures are the result of the gathering and apposition of several independent hair cells. If claw and arista structures are hair derivatives, the positive affinities between the corresponding presumptive cells might be related to the fact that in both kinds of cells common developmental processes are taking place. The same arguments may be extended to explain the cell affinities between antenna and leg imaginal disc cells.

The homology of antennae and thoracic legs is supported by observations on homoeotic mutants. Homoeotic antennal legs and and thoracic legs seem to be identical organs on the basis of genetical experiments. Genes which modify the thoracic leg morphology also affect the antennal leg of ss^{a} mutants (*dachs, four-jointed, combgap, thickoid, lozenge-clawless,* WADDINGTON 1939; BRAUN 1940;

VILLEE 1944; ANDERS 1955), and *Antp* mutants (*polycomb*, *extrasexcomb*, STERN 1954; HANNAH-ALAVA 1964), while arista mutants do not modify it (*thread*, *aristaless*, WADDINGTON 1939; BRAUN 1940).

The present results on cell aggregates of homoeotic antennal cells and normal leg cells point to the same conclusion. Presumptive leg-like cells from homoeotic antennal discs have the same affinities as thoracic leg disc cells, since the two kinds of cells integrate in mosaic territories (Experiments C, D and E). Thus, a single mutational change at the *aristapedia* or the *antennapedia* locus appears to change the cell affinity and the subsequent cell differentiation. The degree of this change is dependent upon the allele (ss^a versus ss^{a40a}) as well as upon the locus (ss^a versus *Antp*) considered (Table 2). We assume that the aristapedia mutations allow the normal tarsus-forming genes to function in cells present in the distal part of the third antennal joint, while the antennapedia mutation allows tibial and femoral-forming genes to function in more proximal segments.

The present experiments were carried out with cells of imaginal discs from mature larvae. At this developmental stage the dissociated cells of the homoeotic antennal disc appear to be already determined, since they differentiate in aggregates the same leg-like structures which appear *in situ*. However, VOGT (1946) showed that, for the allele ss^{a-F} whose expressivity may be changed by temperature shocks, the phenocritical point for antennal or leg differentiation lies in the first half of the last larval instar. Thus, the present experiments do not decide whether this cell-specific determination is the indirect result of the location of the cells within an already transformed blastema or is due to the actual expression of the homoeotic mutant in every single cell. Two arguments support the second alternative. ROBERTS (1964) described autonomous leg-like differentiation of ss^a/ss^a cells in mosaic patches within normal antennal ($ss^a/+$) territories, following somatic crossing over in young larval stages. Secondly, dissociated cells of the different homoeotic mutants studied differentiate in aggregates only the allele-specific structures which appear *in situ*. These results suggest that the isolated cells differentiate in aggregates autonomously according to their actual genetical information.

In a previous paper (GARCIA-BELLIDO 1966b) it was shown that in cultures of wild-type cell aggregates a change in determination—transdetermination—leads to a change in cell affinity corresponding to the change in the final differentiation. Likewise, in the present experiments we find that the presence in certain antennal cells of a homoeotic mutation also leads to a change in their cell affinity correlated with the change in their final state of cell differentiation.

The heteronomous association between normal antennal and leg cells (Experiment A), and the mosaic integration between homoeotic antennal and normal leg cells (Experiments C, D and E) might be interpreted on the same basis of positive cell affinities. The affinity between homonomous cells might consist of the presence in such cells of identical gene products characteristic of the identical final differentiation. On the other hand, the affinity between heteronomous cells (like antenna and leg cells, and cells of the different segments of the same imaginal disc) could be due to their having only certain developmental gene products

in common. As a working hypothesis it could be stated that specific genes characteristic of the different developmental states of determination control the production of specific cell recognition substances.

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SUMMARY

In cell aggregates normal antenna and normal leg cells show positive cell affinities, that is both kinds of cells differentiate autonomously but side by side in aggregates. This "heteronomous association" is especially obvious in the case of the differentiation of arista and claw cells together. In addition, both aristae and claws appear to be formed from modified hair cells.—The homoeotic mutations ss^a, ss^{a40a} and $Antp^{50}$ transform specifically and differently the third joint of the adult antenna into leg-like segments. In cell aggregates of ss^a and wild-type antennal imaginal discs, the ss^a cells differentiate only the same allele-specific leg-like structures which appear *in situ*.—In cell aggregates antennal cells of each of the three mutants when combined with wild-type thoracic leg cells are able to differentiate common leg patterns in mosaic fashion. In all cases "mosaic integration" is related to similar final differentiation.—Mosaic integration and heteronomous association being the expression of affinity, the hypothesis of cell affinity as a consequence of the action of developmental genes is discussed.

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