DEVELOPMENTAL ANALYSIS OF THE WING DISC IN THE MUTANT ENGRAILED OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

The engrailed (*en*) mutation leads to the transformation of the posterior structures of the dorsal mesothoracic disc into those characteristic of the anterior region of the same disc. Similar posterior-anterior duplications have been detected in dorsal as well as ventral structures of all the thoracic segments. ——Genetic combinations of *en* with other pattern mutants have shown their synergistic effect on the posterior wing pattern.——A clonal analysis of the *en* wing disc shows that *en* affects its development in a characteristic way. The genetic change, by induced mitotic recombination, of *en*+ into *en* cells is followed by the corresponding transformation, except when it takes place some cell divisions prior to differentiation.——The *en* posterior wing disc cells show positive affinities with normal anterior wing disc cells in aggregates.——The mode of action of the *en*+ locus controlling wing disc development is discussed.

THE epidermal cells of insects differentiate into distinct cuticular structures whose spatial distribution characterizes the cuticular pattern of different body regions. In Drosophila, pattern mutants lead to the abnormal distribution of otherwise normally differentiated epidermal cells. A current approach to understanding how patterns, and morphogenesis in general, are genetically controlled is by the comparative analysis of the development of normal and mutant body regions.

As with other genes, those genes controlling pattern organization are expected to have their primary expression within the cell itself. Thus, our first concern is an analysis of how these genes, or their mutant alleles, manifest themselves in single cells. STERN (1938, 1954, see also 1969), making use of mitotic recombination, initiated the analysis of pattern mutants in clones of recombinant homozygous cells, surrounded by proliferating heterozygous cell populations. One such pattern mutant is *engrailed*. The mutant engrailed has a broad pleiotropic morphogenetic syndrome (ECKER 1929). BRASTED (1941) first discovered the appearance of a secondary sexcomb in the posterior part of the male basitarsus, and TOKUNAGA (1961) studied its development by clonal analysis. The existence of a similar duplication in the structures of the posterior wing margin, pointed out to the senior author by E. B. LEWIS, has led to the present work. We studied the expression of this mutant in the wing disc by comparing the behavior of normal and mutant cells in clones initiated at different developmental stages, in aggre-

gates of normal and mutant cells, and in genetic combinations of engrailed with other morphogenetic mutants.

MATERIALS AND METHODS

Mitotic recombination was induced with X-rays (PHILIPS MG 151 Be, 300r/min, 100Kv, 15 mA and 2 mm Al filter) at a total dosis of 1000r. The age of the larvae at the moment of irradiation was calculated from the time lapsing from irradiation until pupariation (PF). This method permits us to time accurately the last stages of larval development (GARCIA-BELLIDO and MERRIAM 1971a).

Clonal analysis was carried out in two ways: First, a "cell lineage" analysis of the engrailed wing disc development was performed in *y / Df(1)sc*^{8} ; *en* ; *Dp(1;3)sc*^{14} *y+ jv/mwh* individuals. Following mitotic recombination in the left arm of chromosome III, twin clones *y*; *mwh* (*y*: *yellow* 1–0.0; *mwh*: *multiple wing hairs* 3–0.0) and *jv* (*javelin* 3–10.2) appear over the surface of the *en* adult wing (GARCIA-BELLIDO and MERRIAM 1971a). *yellow* and *javelin* are bristle markers and *multiple wing hairs* is a trichome marker.

Second, recombination was induced in heterozygous *en/+* individuals with the aim of detecting homozygous *en/en clones* ("morphogenetic mosaicism"). To this end, engrailed (*en*: 2–62.0, to the right of 45 E1–2, BRIDGES *et al.* 1936) was genetically coupled in *cis* configuration with *straw* (*stw*^{3} ; 2-55.1, 41-BC LINDSLEY and GRELL 1968), a cell-marker mutant which produces yellowish transparent bristles, although it does not mark trichomes. According to data from X-ray-induced mitotic recombination in the abdominal histoblasts, recombination between the centromere and *stw* is as frequent as recombination between *stw* and the tip of the right arm of chromosome II (GARCIA-BELLIDO 1972a). Since the cytogenetic distance between *stw* and *en* is small, recombination proximal to *stw* should be several times more frequent than between *stw* and *en*. In fact, *stw+ en* clones, i.e. transformed but genetically unmarked structures, were never found (see below).

The experiments of cell dissociation and aggregation of the imaginal wing discs were performed by the method already described (GARCIA-BELLIDO 1968a). In order to recognize the origin of the cells in the metamorphosed reaggregates, normal wing disc cells carried the cell marker mutants *y*; *sn*^{3} and *mwh*, against which the unmarked *en* cells are easily identifiable (see LINDSLEY and GRELL, 1968, for details of the mutants used).

RESULTS

The mutant phenotype: The morphogenetic effects of engrailed are multiple (ECKERT 1929, BRASTED 1941). However, this pleiotropic manifestation seems to conform to a rather simple scheme, namely the transformation of the posterior regions of the thoracic segments into the corresponding anterior ones. BRASTED (1941) and TOKUNAGA (1961) described the appearance of a secondary sexcomb area as a mirror image duplication of the anterior one in the posterior part of the male basitarsus. MUKHERJEE (1965), studying *en* in combination with other mutants, detected its manifestation in regions larger than the sexcomb area, including secondary transverse rows. Similar posterior–anterior duplications can be found in other ventral appendages. We have observed duplications in the transverse rows in the female prothoracic legs and an enlargement of the transverse row area in the metathoracic legs. No duplications could be detected in the mesothoracic leg, possibly due to the lack of characteristic patterns in it.

In the dorsal mesothoracic structures, engrailed is characterized by having a cleft between the half scutella and by a spatulate shape and an abnormal vena-

tion pattern in the wing. A detailed analysis of the wing surface reveals the presence of a triple row of bristles in the posterior wing margin instead of the normal double row of long hairs (Figures 1 and 2). In this duplicated triple row, the dorsal and ventral elements keep the anatomic position of the anterior margin. The ventral row sometimes shows a parallel row of extra bristles with a similar disposition to that of the dorsal row. The alula is generally reduced in size and its long, socketless structures are substituted by long bristles similar to those found in the anterior costal region (Figure 1c). A comparative analysis of the wing venation leads to the scheme presented in Figure 2. The normal veins are assymmetric in a dorsoventral section of the wing surface, being flat on one surface and swollen on the other. If we analyze these assymmetries in the different

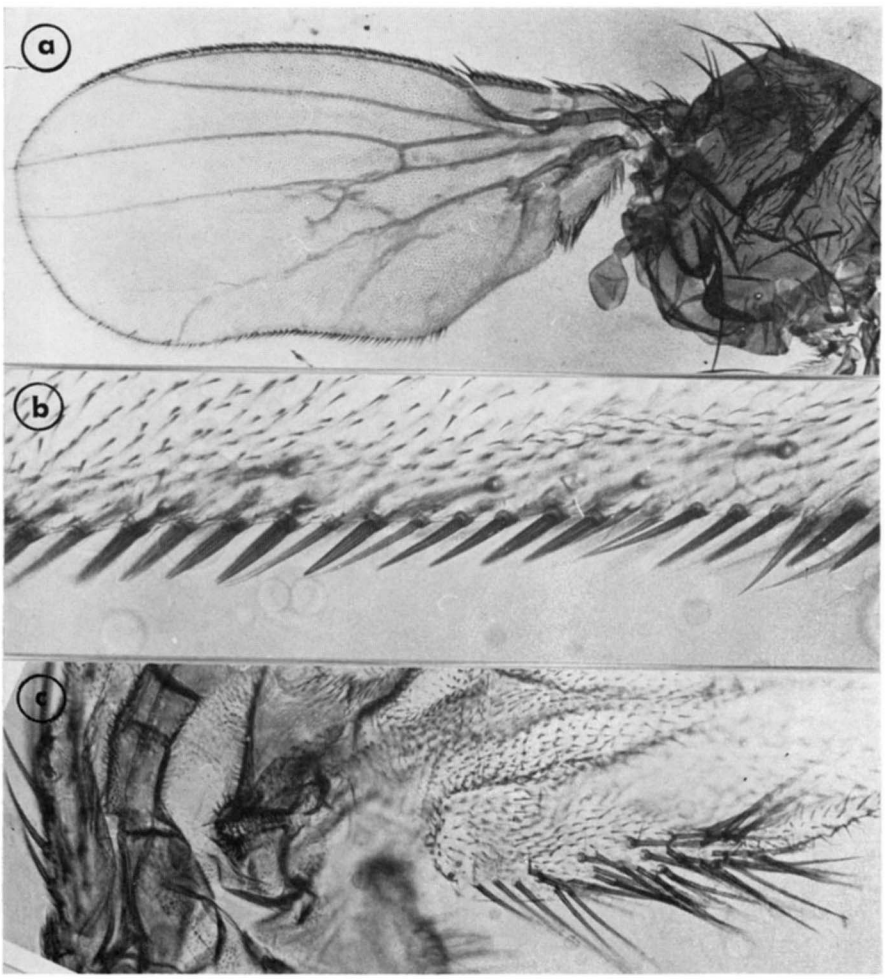


FIGURE 1.—The *en* transformation in the dorsal mesothoracic structures. (a) general aspect. (b) transformed double row in the posterior margin. (c) transformed alula. (See schematic representation in Figure 2.)

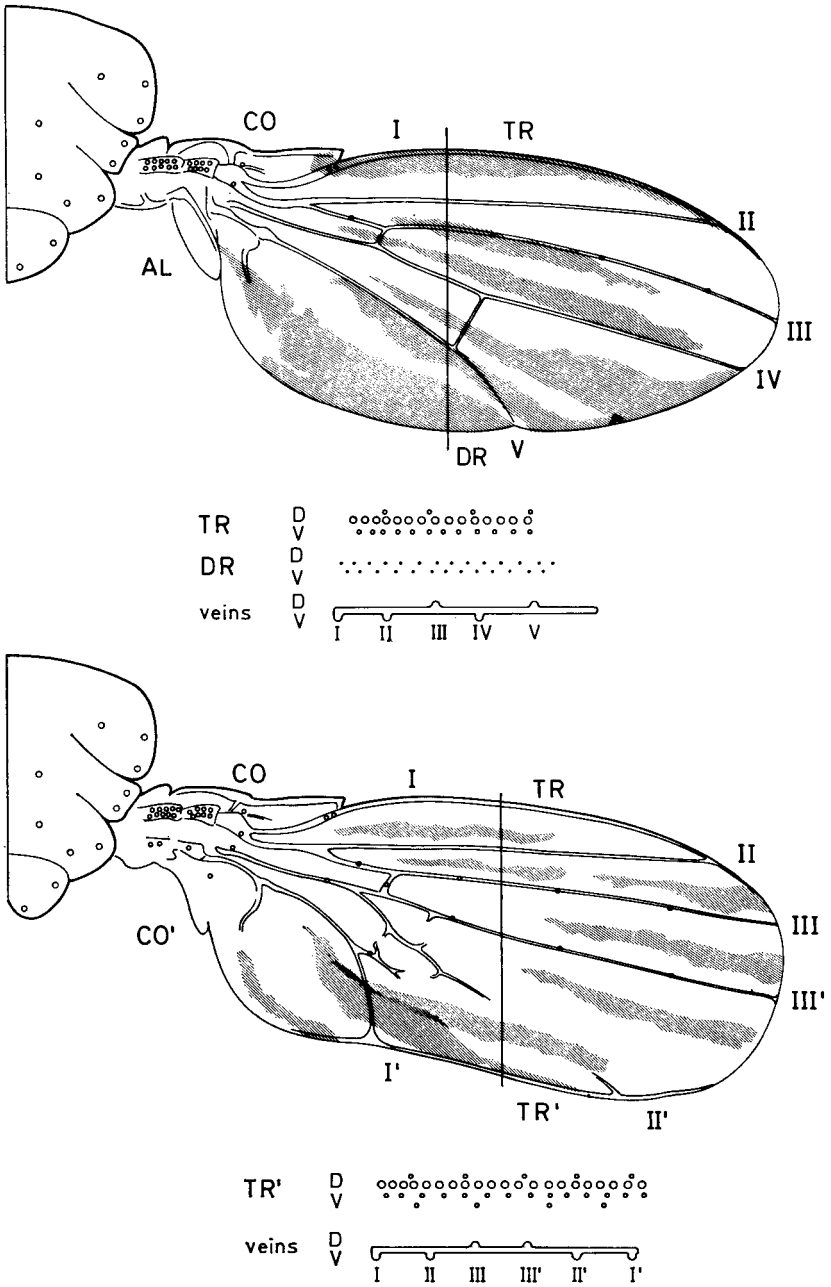


FIGURE 2.—A comparison of the general pattern of the normal (a) and *en* (b) dorsal mesothoracic structures. Co.: costa, TR: triple row, DR: double row, Al: alula, I-V: veins, D: dorsal, V: ventral. Circles in the wing veins: sensillae; in the notum and TR: bristles. Veins: outlines of the shown sections cutting the wing surface. Homoeotically-transformed structures labelled (′). Dashed: clones of *mwh* trichromes in wild type and *en* wings.

veins we would observe that whereas veins I, II and III are similar, veins IV and V are different in normal and in engrailed wings. Veins IV, V and VI of engrailed seem to correspond to III, II, and I respectively. Thus, in the engrailed transformation, veins IV and V of the normal wing would disappear giving rise to veins corresponding to III′, II′ and I′. The same conclusion can be drawn from a comparison of the distribution of the sensilla of the wing surface (Figure 2). The variable venation and sensilla pattern of the posterior part of the wing possibly results from the incomplete transformation of the posterior part of the wing into the anterior one. Apparently this posterior–anterior transformation does not affect the postnotum of the mesothorax which might be expected to appear as a mirror-image duplication of the anterior mesonotum. Perhaps the cleft of the scutellum represents such a transformation in an incipient way (see below).

The expression of the engrailed character in the wing, as in the other appendages, is variable. The transformation of the double row into a triple row varies in the cuticular elements of the posterior wing margin. Since the manifestation of the character is measured in single-cell structures we will refer to variations in the penetrance of the mutant character in cells. The transformation of the hairs of the double row into bristles is an all-or-none response, i.e., we may find normal double-row hairs or bristles of the ventral, dorsal or medial triple row, but intermediate structures rarely appear (Figure 2b). The variation in penetrance of these characters for different regions of the posterior margin of the *en* wing is shown in Figure 6.

Genetic combinations: The posterior–anterior duplication of the wing anlage might be produced by a variety of mechanisms. We tested whether the anterior part determines the organization of the posterior part, either because the cells in the posterior part derive from presumptive anterior ones or because the growing posterior anlagen somehow "copy" the anterior blastema organization, or alternatively, the *en* transformation is an autonomous character of the posterior wing disc cells. The pseudoallelic homoeotic mutants *bithorax* (bx^s), which transforms the anterior structures of the metathorax into those of the mesothorax, and *postbithorax* (*pbx*), which leads to the corresponding transformation of posterior structures (LEWIS 1964), were genetically combined with engrailed. Figure 3 shows schematically the transformations found in the double homozygous *en*; bx^s and *en*; *pbx* flies. In *en*; bx^s flies the posterior part of the metathorax remains unchanged. This indicates that the posterior part of the anlage does not derive from, or copy, the anterior part. This conclusion is reinforced by the analysis of *en; pbx* flies (Figures 3 and 4a). The appearance of an anterior wing in the posterior metathorax indicates that the *en* transformation is an autonomous feature of the posterior region of these segments. In this case the effect of *en* is superimposed to that of *pbx*. The lack of function of pbx^+ in the metathorax leads to a developmental situation which permits us to detect the function of en^+, for when this fails, due to the *en* mutation, anterior wing structures develop in the posterior part of the metathorax. These results indicate that en^+ also controls metathoracic development.

Genetic combinations of engrailed with other pattern mutants serve to test

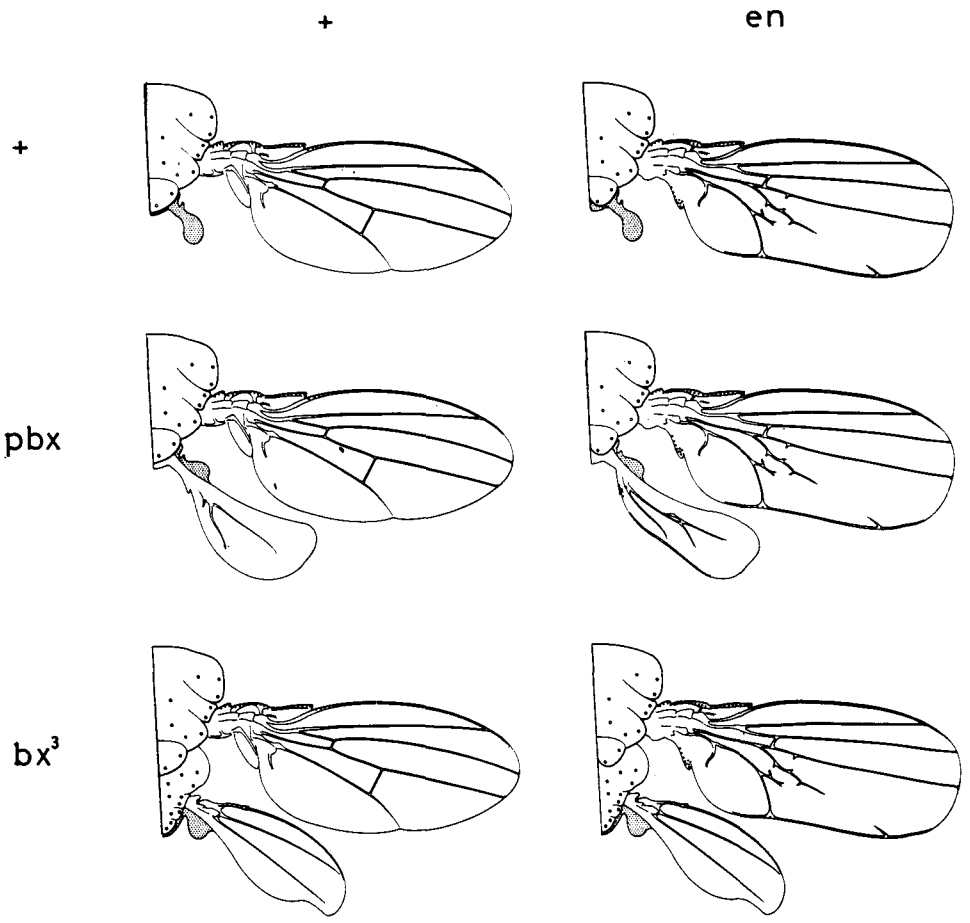


FIGURE 3.—Schematic representation of the dorsal mesothoracic and metathoracic structures in the genetic combinations of *en* with bx^3 and *pbx* mutants.

further whether the posterior region of the dorsal mesothorax of this mutant is developmentally similar to the normal anterior region. The mutant hairy (*h*) leads to an anterior–posterior asymmetrical pattern of extra bristles, on the wing surface. These extra bristles appear preferentially along vein II and are very scarce in veins IV and V (GARCÍA-BELLIDO and MERRIAM, 1971b). In the double mutant *en* ; *h*, numerous extra bristles appear as normal along vein II but also along its symmetrical vein II′ or its region in the posterior part of the wing (Figure 4b).

Clonal analysis: A). *Cell lineage of the engrailed wing disc:* We first studied the growth of the *en* wing disc by the use of clones of cell-marker mutants initiated at different developmental stages. This is the same method used in the clonal analysis of the wild-type wing disc development (GARCÍA-BELLIDO 1968b; GARCÍA-BELLIDO and MERRIAM 1971a; BRYANT 1970). Mitotic recombination was induced in *en* individuals heterozygous for *y* and *mwh* in order to obtain clones of

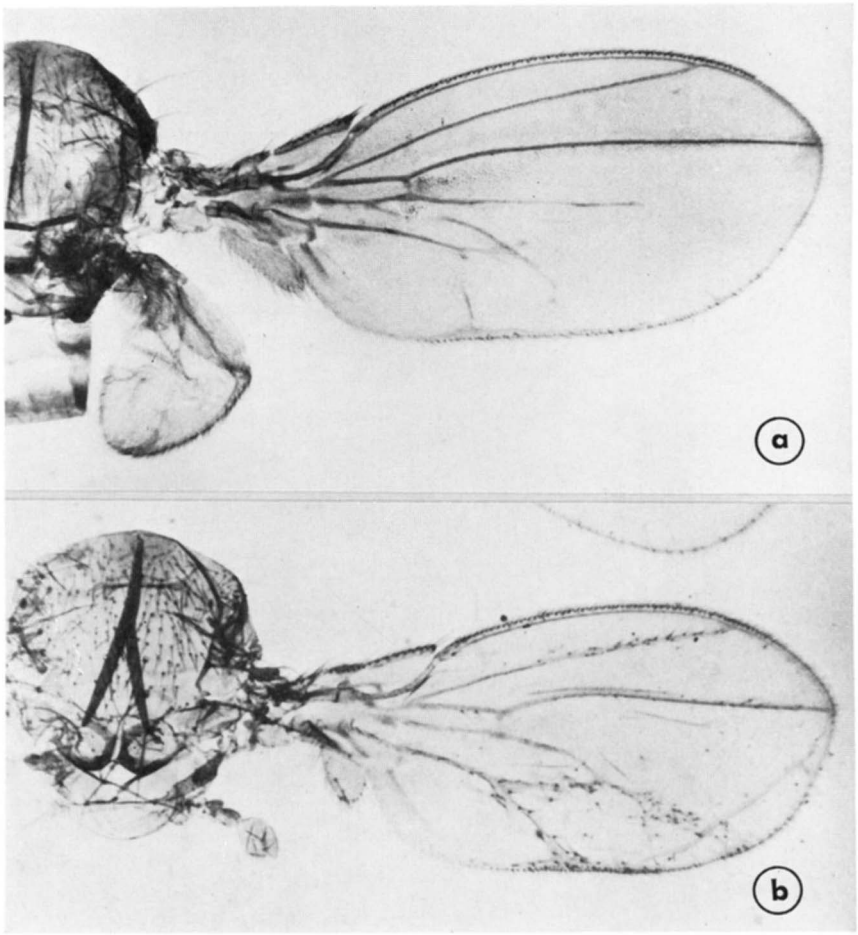


FIGURE 4.—Genetic combinations. a) *en*; *pbx* b) *en*; *h*.

y mwh-marked cells (see MATERIALS AND METHODS). We paid special attention to the shape of the clones in both normal and mutant wings. We studied 61 clones of more than 75 cells in *en* wings, some of which are shown in Figure 2. Whereas the shapes of the clones are similar in the anterior part of both normal and mutant wings, they differ in the posterior part. In this region of the normal wing, clones meet the margin at various angles, while in *en*, clones show the tendency to run parallel to the wing margin as in the anterior part. Thus, possibly the final shape of the mutant wing results from the manifestation of the mutation *en* in successive divisions of the wing-disc cells. In fact, the morphology of the wing discs of mature larvae already reflects those differences. While the wild-type wing disc is asymmetric along the proximo-distal axis, the *en* wing disc is more or less symmetrical about this axis (Figure 7). It is enlarged specially in the region where the presumptive posterior structures will appear (see Figure 2 in HADORN and BUCK 1962).

TABLE 1

Recombinant clones in heterozygous stw^3 en/+; mwh/+ *wings induced at different stages of development*

Age at irradiation	WD	Anterior [*stw* (*en*)] Medial TR				Posterior [*stw en*] Dorsal + medial TR				Posterior [*stw en*] Ventral TR				Wing surface [*mwh*]	
		Clones		Bristles		Clones		Bristles		Clones		Bristles		Clones	Trichomes
	n	*n*	*n*/WD	*n*	*n*/cl	*n*	*n*/WD	*n*	*n*/cl	*n*	*n*/WD	*n*	*n*/cl	*n*/WD	*n*/cl
0– 8	261	192	(.74)	197	(1.0)	3	(.01)	3	(1.0)	3	(.01)	3	(1.0)	(40.4)	(1.8)
8–16	202	147	(.73)	153	(1.0)	7	(.03)	7	(1.0)	6	(.03)	6	(1.0)	(32.4)	(2.8)
16–24	288	114	(.40)	129	(1.1)	4	(.02)	4	(1.0)	6	(.02)	6	(1.0)	(10.9)	(6.7)
24–32	185	55	(.30)	87	(1.6)	6	(.03)	8	(1.3)	5	(.03)	11	(2.2)	(9.1)	(9.9)
32–40	97	12	(.12)	27	(2.2)	2	(.02)	3	(1.5)	2	(.02)	6	(3.0)	(6.6)	(12.3)
40–48	125	18	(.14)	61	(3.4)	5	(.04)	8	(1.6)	5	(.04)	16	(3.2)	(2.8)	(36.7)
48–56	208	25	(.12)	153	(6.1)	14	(.07)	68	(4.9)	8	(.04)	37	(4.6)	(1.9)	(73.2)
56–64	300	17	(.06)	139	(8.2)	9	(.03)	55	(6.1)	5	(.02)	35	(7.0)	(1.1)	(115.4)
64–72	249	8	(.03)	120	(15.0)	5	(.02)	25	(5.0)	3	(.01)	23	(7.7)	(0.6)	(159.3)
72–80	166	6	(.04)	244	(40.7)	3	(.02)	114	(38.0)	7	(.03)	163	(24.3)	(0.4)	(296.3)
80–88	124	4	(.03)	248	(62.0)	3	(.02)	51	(17.0)	3	(.02)	85	(28.3)	(0.2)	(445.6)

WD: wing discs, cl: clones, TR: triple row. In parenthesis the frequency of clones per wing disc (*n*/WD) and of elements per clone (*n*/cl). Anterior: *stw* (*en*) clones; Posterior: *stw en* clones of transformed structures; wing surface: *mwh* clones. Times of irradiation are given as hours before puparian formation.

Clones reaching the margin of the wing were found to embrace transformed and non-transformed double-row structures. These structures appear transformed or not transformed, with no clear relation with their clonal origin. In fact, the coincidence of being transformed or normal is higher between neighboring structures than between structures of the same clone. Apparently, the penetrance of the *en* transformation in the double-row cells is topologically conditioned.

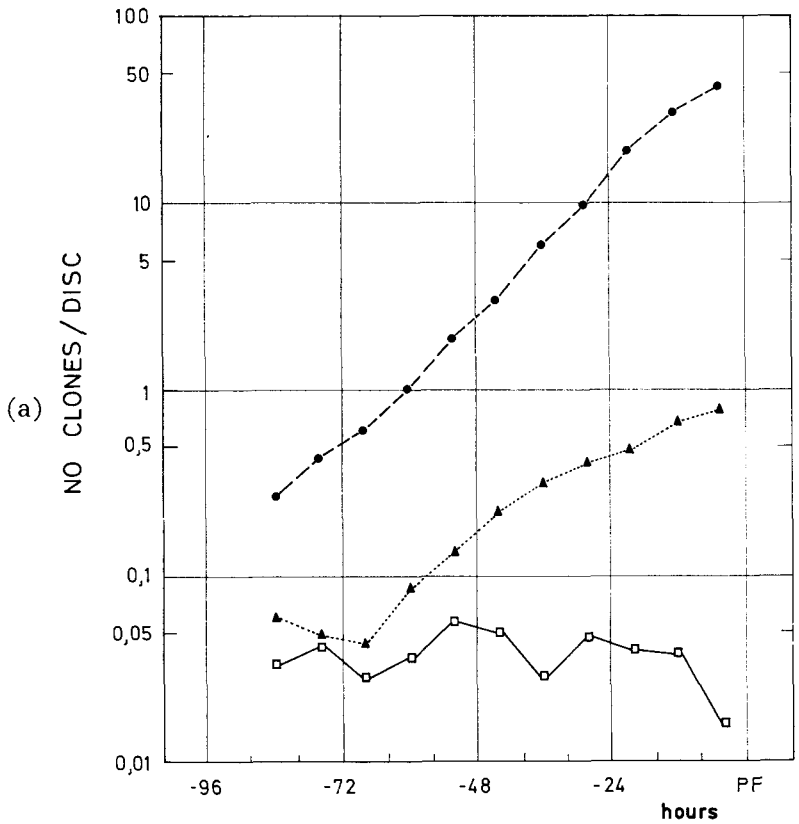
B). *Morphogenetic mosaicism:* The preceding results indicate that the function of en^+ in cells seems to be required from early stages to control a normal wing development. We wanted therefore to analyze the cell autonomy of engrailed and to determine up to what stage in development the function of en^+ is required to prevent the cellular transformation.

Mitotic recombination was used again as a tool for studying homozygous engrailed cells in a normal-growing *en*/+ wing disc. Larvae of the genetic constitution stw^s *en*/+; *mwh*/+ were irradiated at different intervals before puparium formation. Four replicas of this experiment were studied (Table 1). The use of *mwh* serves as an internal control in the analysis of the frequency and size of the induced recombinant clones in the wing surface. The existence of clones of *stw* bristles in the medial triple row indicates that mitotic recombination took place in their presumptive cells and their size and frequency serves as a second internal control. *stw* double-row elements can be detected with difficulty. Thus, mitotic recombination in the presumptive double-row cells were only scored if they showed simultaneously the *en* transformation into triple-row structures. Recombination between *stw* and *en* would give rise to stw^+ *en* transformed structures, but they were never found in the present experiments. We studied the frequency of *stw en* bristle spots in both dorsal and ventral surfaces of the posterior wing margin, in comparison with the internal controls (Table 1, Figure 5a). The *mwh* frequency curve is similar to that found in normal wings (compare with García-Bellido and Merriam 1971a). The frequency of recombinant *stw* bristle spots in the anterior medial triple row runs parallel to that of *mwh* clones, and is similar to that found for a *y* marker in the left arm of chromosome III in normal wings (García-Bellido and Merriam 1971b). As mentioned in this paper the fraction of *stw* phenocopies in the medial triple-row structures is low in larvae irradiated before puparium formation. Thus, recombination proximal to *stw* takes place in the wing disc throughout development. However, the frequency curve of *stw en* spots in the posterior margin runs differently from that in the anterior margin. Whereas the frequency follows more or less that of the control *stw* in the anterior triple row in young irradiated larvae, it decreases relative to controls with irradiation performed later than 40 hr before puparium formation. This drop in frequency has several possible explanations. It could be due to non-autonomy of the *en* character in small spots, or to the absence of mitotic recombination in the presumptive double-row cells. However, the data of Table 1 indicate that the number of *stw* bristles per spot is comparable in the transformed posterior triple row and in the control anterior triple row (Figure 5b). As expected, the control clones contain more bristles than the transformed ones, possibly due to the incomplete penetrance of *en* in the later ones. Interestingly, *en-*

transformed bristles are found even in small clones, suggesting that the penetrance of *en* does not depend on the size of the clone. Since there is no indication of a drop in the frequency of recombination in the posterior wing region in later developmental stages (see GARCÍA-BELLIDO and MERRIAM 1971b) the diminished frequency of spots showing the transformation must be due to other causes (see DISCUSSION).

A further point of the previous analysis deserves attention. The *stw en* spots of irradiated larvae only appear in one of both surfaces, either the ventral, giving ventral triple row structures, or the dorsal, showing structures typical of the medial and dorsal triple row. We never found spots embracing both dorsal and ventral structures of the triple row. This is not surprising since in the normal wing, dorsal and ventral surfaces are clonally separated early in development (GARCÍA-BELLIDO 1968b; GARCÍA-BELLIDO and MERRIAM 1971a; BRYANT 1970). We did not find, either, transformed stw^+ bristles in the opposite surface of a *stw en* triple-row spot. This indicates that the structures on both sides of the wing margin do not arise by reciprocal induction, which is not surprising since the non-transformed cells are genetically en^+ (Figure 6).

Cell affinities: The previous results are consistent with the hypothesis that the



posterior wing cells of engrailed are developmentally identical to the anterior wing cells. This interpretation can be further tested in experiments of cell reaggregation. It has been shown that the affinities of homoeotically transformed cells correspond to those typical of the transformed structures. Thus, aristapedia antennal cells show the same affinity as normal leg cells and segregate from normal antennal cells (GARCÍA-BELLIDO 1968a). In the present work we tested the affinities of cells of the posterior part of the wing disc of engrailed with cells of either the anterior or the posterior part of the normal wing disc, in aggregates.

For that purpose wing discs of mature larvae of *en* were cut along a line separating a stripe of the posterior wing margin, trying not to include the presumptixe notum (Figure 7, see anlage plan of the wing disc by HADORN and BUCK 1962). Although the wing disc of engrailed is more or less symmetrical along the proximo-distal axis, its orientation is facilitated by the asymmetric tracheal trunk and its branches, which have a similar position in both normal and mutant discs. The posterior fragments were then collected and mixed with entire normal wing discs, dissociated, reaggregated and cultured in adult hosts for 2 (series A) and 8 (series B) days, before being implanted in larval hosts for metamorphosis. The origin of the cells in the differentiated implants was deduced by the mutant phenotype of cell-maker mutants *y sn*^{3} and *mwh*, carried by the normal

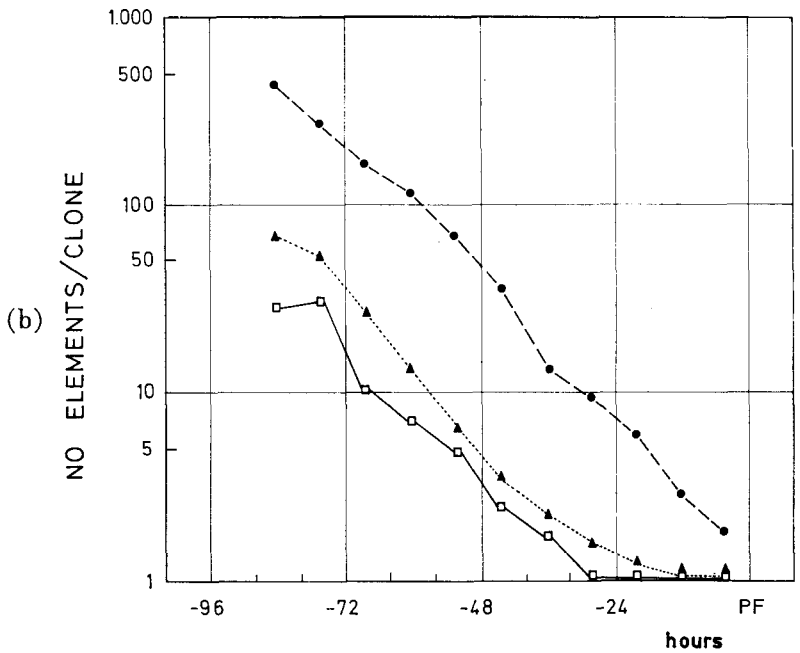


FIGURE 5.—Semilogarithmic representation of the frequency (a) and size (b) of clones initiated at different developmental stages. Age at irradiation in hours before puparium formation (PF). ●, *mwh* clones in the wing surface (control); ▲, *stw* (*en*) clones in the anterior medial triple row (control); □, *stw en* "transformed" clones in the posterior margin (averaged data of both dorsal and ventral clones corresponding to one of the 4 replicas of Table 1).

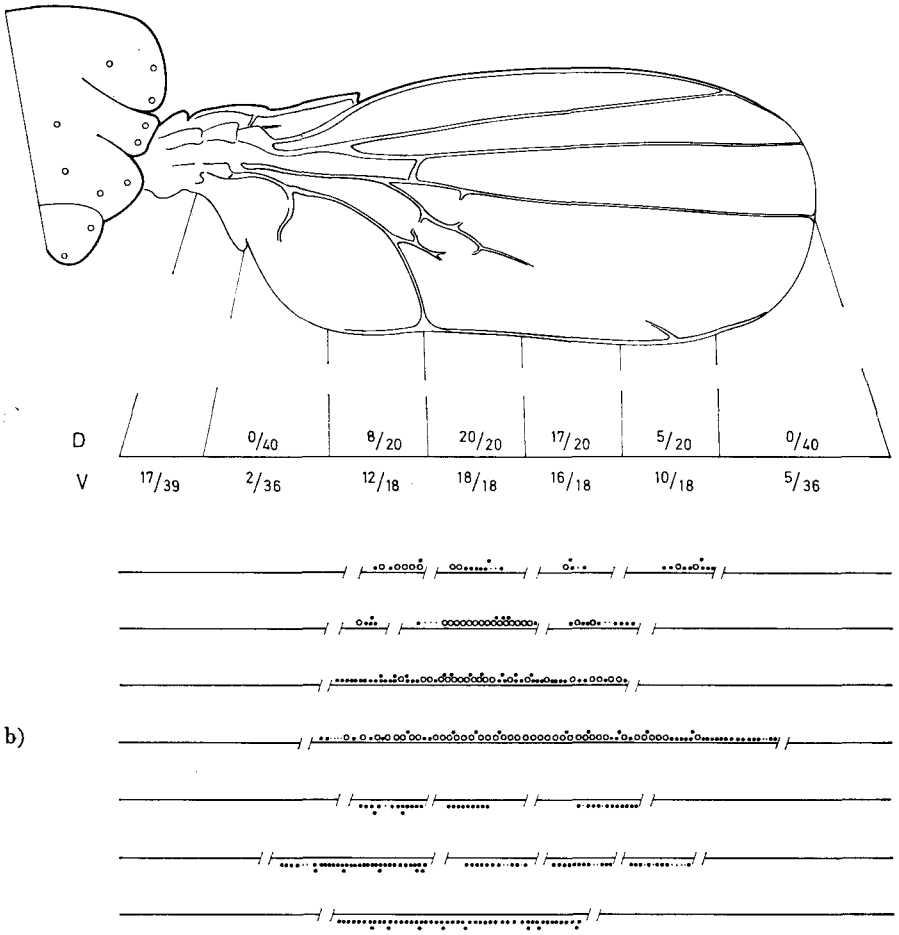


FIGURE 6.—Cell penetrance of the *en* transformation in the posterior margin. a) *en* wing; the figures represent the fraction of transformed structures out of the total number of elements in each region, b) *stw en* clones in a heterozygous background showing the degree of transformation depending on their location in the posterior margin. O: medial triple row bristles; o: dorsal or ventral-row-like bristles; •: non-transformed double row "hairs." Transverse lines separate dorsal (D: above) and ventral clones (V: below).

wing disc cells. It has been shown that normal posterior wing disc cells do not intermingle or differentiate mosaically with normal anterior cells (GARCÍA-BELLIDO 1966). Therefore, the posterior wing disc cells of engrailed were expected both to segregate from normal posterior wing disc cells and to appear in mosaic with anterior ones. We studied 34 metamorphosed implants of the series A. Engrailed cells differentiated, in aggregates, hairs of wing surface and transformed and non-transformed double-row structures. These structures frequently appeared in rows, built up only by *en* elements—monotypic territories—, or in mosaic, i.e., intermingled with normal (*y sn mwh en*$^+$) elements. Interestingly, in these mosaic triple rows the *en* cells differentiated indistinctly transformed or

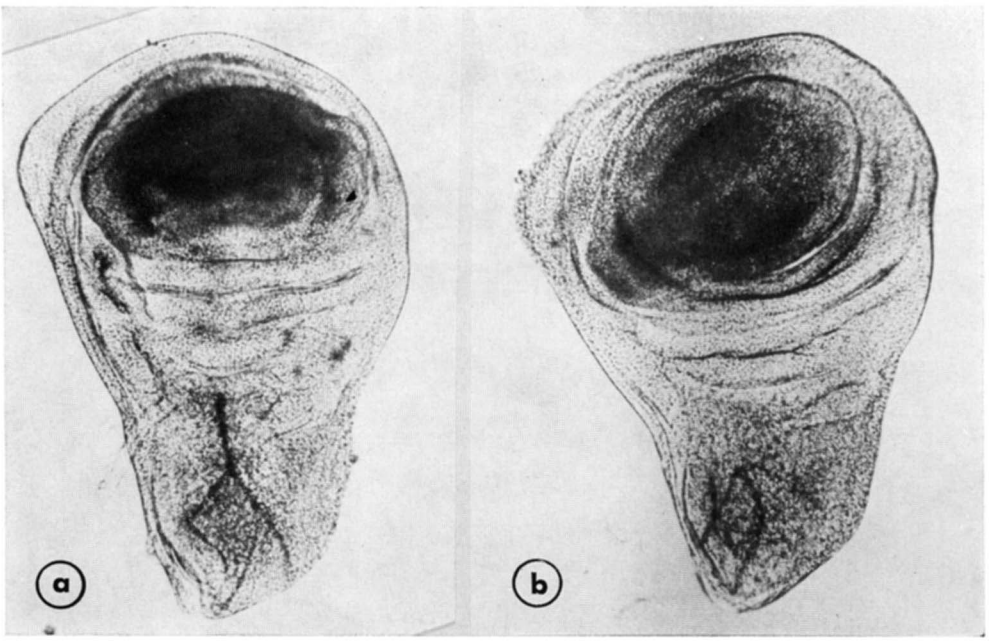


FIGURE 7.—The wing disc of *en*+ (a) and *en* (b) mature larvae. Left wing discs in a dorsal aspect; left: posterior, right: anterior wing regions.

non-transformed structures (Figure 8a). This was not unexpected since recognition properties seem to correspond to topological characteristics rather than to characteristics of the presumptive cell differentiation (see GARCÍA-BELLIDO 1972b). Surprisingly however, *en* double-row structures appeared associated or in mosaic with typical double-row normal structures (Figure 8b). In these mosaics non-transformed as well as transformed cells were found. Both results taken together indicate that the recognition properties of the presumptive transformed triple row cells show incomplete penetrance. The fact that transformed bristles appear associated with long hairs of the normal double row suggests that the penetrance in the transformation into triple-row bristles is not correlated with the penetrance with respect to cell recognition.

In 50 implants of series B we found the same structures as in series A, i.e., transformed triple rows preferentially. In these triple rows the frequency of transformed bristles was higher than in both series A and *in situ*. Surprisingly, *en* bristles differentiating in patterns of the costa (6 cases Figure 8c) and of the mesonotum (5 cases, Figure 8d) were found also. They appeared in monotypic territories as well as in mosaic with normal structures. We interpret their occurrence as due to the autonomous manifestation of *en*, rather than to the inductive influence of the normal cells. Since they did not appear in series A (except for one doubtful case of mosaicism in structures of the notum) they could derive from proliferating cells in the aggregates. In fact, it is known that dissociation and culture conditions may increase the expression of the mutant character

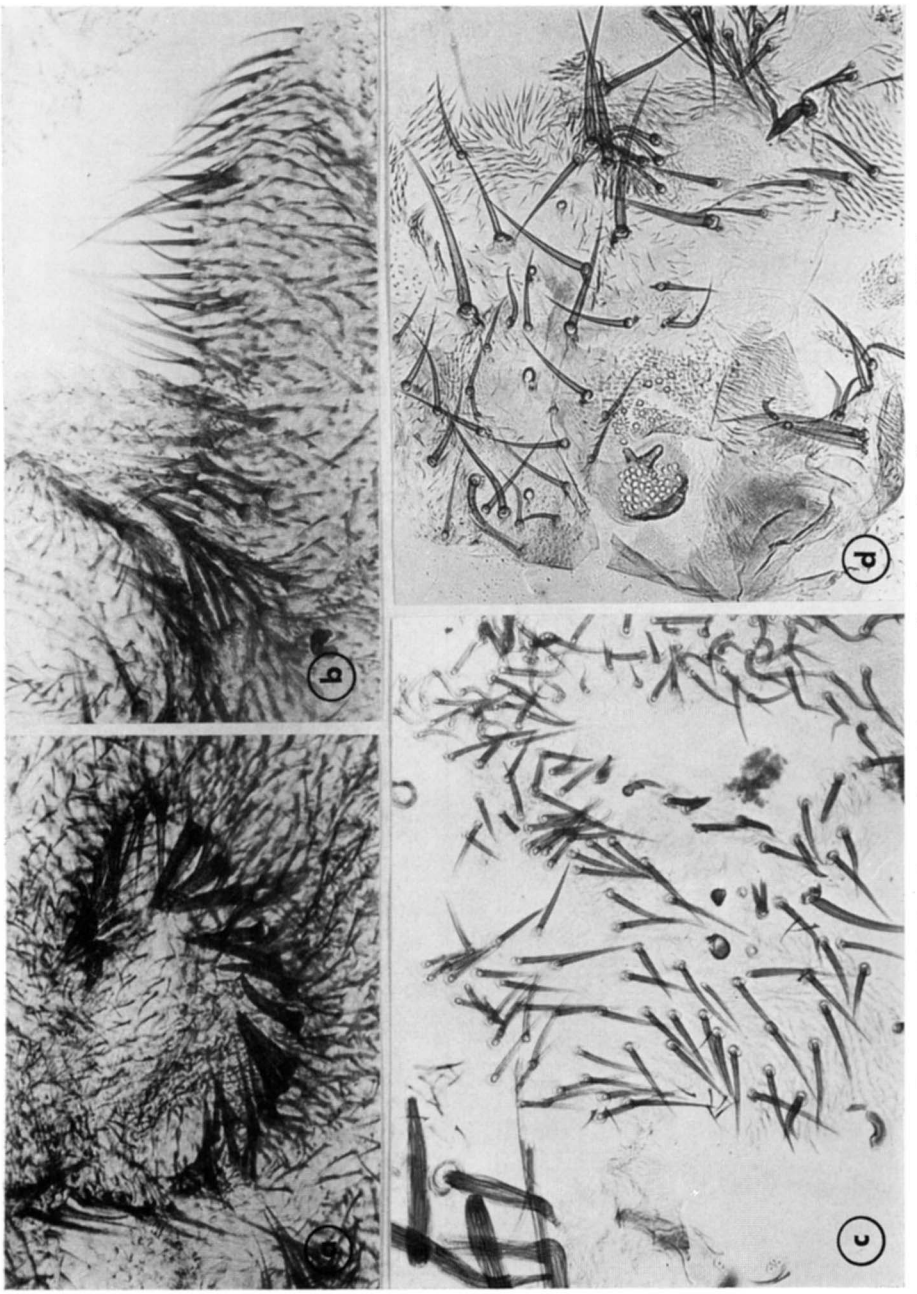


FIGURE 8.—Mosaics of *en* and *en*$^+$ (*y sn*^{3} *muh*) elements in cell aggregates. a) Triple row: straight bristles are *en*; curved bristles are *en*$^+$, b) mosaicism along the double row; straight elements with and without sockets in the upper surface are *en*; in the lower surface are *en*$^+$. Mesonotum (c) and costa (d) structures in mosaic of *en* and *en*$^+$ cells. Observe the bracteated bristles of the proximal costa and the sensilla groups of the basis of vein I in d.

(GARCÍA-BELLIDO 1968a). It is possible that these experimental conditions have uncovered the expected transformation of posterior cells into structures of the costa and notum not manifested *in situ*. However, it should be kept in mind that these new structures can alternatively derive from the old ones by transdetermination (HADORN 1966).

DISCUSSION

The mutant engrailed has a pleiotropic manifestation in all three thoracic segments, which can be interpreted as due to the overall transformation of the posterior structures and pattern organization into those typical of anterior regions. In this sense engrailed represents a homoeotic mutant. In the dorsal mesothoracic disc this transformation shows incomplete expressivity. Thus, in the wing the venation pattern and the posterior margin show intermingled normal and mutant characters. Moreover, the homoeotic transformation shows regional specificities. Thus, the postnotum region does not seem to be affected *in situ*, except for the scutellar cleft. It is interesting to note that regenerating *en* cells derived from the posterior part of the wing disc differentiated typical costal and mesonotal structures. If they do not correspond to transdetermined structures, they would represent the expression of a character which is not manifested *in situ*. Morphogenetic mutants frequently show such variations in the expressivity of the mutant character in specific regions. Thus, whereas bx^s leads to a complete transformation of the anterior metathorax towards both mesonotum and wing (Figure 3), bx^{34e}, a weaker allele, only shows this transformation in the wing (Lewis 1964). Similarly, *en* could represent a weak mutant allele of the *en* locus with a "leaky" manifestation of the normal function in some regions. Alternatively, as in the transformations of antenna into leg in mutants of the spineless locus (see García-Bellido 1968a), more proximal or distal transformations could be controlled by different pseudoalleles, one of which could correspond to the only known engrailed mutant.

The mutant expression in cells: It is difficult to study the manifestation of morphogenetic mutants in single cells because they modify cell arrangements without changing cell differentiation. However, the homoeotic mutants represent interesting exceptions. Postlethwait and Schneiderman (1969, 1971) studied the growth of the Antennapedia antennal discs in clones. They observed that the mutant character manifests itself in an increased growth rate of the presumptive homoeotic leg cells with respect to the non-transformed antennal cells. Moreover, the shape of the homoeotic leg clones is also characteristic of that of the thoracic legs. The differential cell proliferation dynamics start in the mutant antennal disc at the beginning of the third instar. Apparently the *en* wing imaginal disc grows differently from the normal wing disc from early stages of development. Clones of marked cells in the posterior region of *en* wings show characteristic shapes and disposition similar to those of the anterior region and different from the normal posterior region (Figure 2). Since the shape of the clones is indicative of alternating orientations of the mitotic spindle (García-Bellido and Merriam 1971a), this result suggests a continuous manifestation of the mutant genome in cells during development.

However the preceding conclusions can only be applied to cell populations rather than to single cells. We have further analyzed the expressivity of the *en* transformation of the double-row structures of the posterior wing margin in clones. The penetrance of *en* in single cells—the expression of being transformed into triple-row elements or not—varies depending on the position of the cells in

the double row. The cell-lineage experiments have shown that the penetrance of *en* does not seem to result from early cellular decisions passed over to the offspring. In fact, we have found a higher correlation in the transformation between cells related in position than in clonal ancestry. Thus, whereas in other instances, as in position-effect variegation (BAKER 1967), cell differentiation seems to be clonally determined, in the present case the cellular expression of *en* becomes fixed, if ever, late in development. Possibly, the mature wing imaginal disc of *en* consists of a mixed cell population of transformed and non-transformed cells. This conclusion seems to be reinforced by the results of the cell aggregation experiments. Cell recognition of the posterior wing disc cells of *en* is ambiguous, showing positive affinities with normal wing cells of either anterior or posterior regions. Unfortunately, we will not know what the basis for this ambiguity is, until we understand the mechanism of cell recognition in normal cells (for discussion see GARCÍA-BELLIDO 1972b).

Apparently the penetrance of *en* in single cells is an autonomous character. We have seen that the penetrance of the transformation in clones *en/en* in a heterozygous background is similar to that observed in homozygous flies (Figure 6). Moreover, this penetrance does not vary with the size of clones. The conversion of en^+/en into *en* cells was followed by the corresponding change in phenotype when mitotic recombination was induced throughout most of the larval development. However, in larvae irradiated later than 40 hr before puparium formation there is a decrease in the frequency of transformed *en* clones with respect to controls. As discussed above, this low transformation cannot be due to the non-autonomous expression of *en* in small clones. The possibility that mitotic recombination, precisely in the mother cells of the double-row presumptive elements decreases with progressing development is not confirmed in other experiments where cell-marker mutants other than *stw*, such as *forked* (f^{36a}), have been used (GARCÍA-BELLIDO unpublished). Thus, the low manifestation of *en* in clones initiated late in development must be explained in other terms. A similar situation was found in the genetic change leading to the transformation of bristles into trichomes, or vice versa. In this case the genetic change is no longer manifested in clones initiated later than 8 hr before puparium formation (GARCÍA-BELLIDO and MERRIAM, 1968, 1971b). We concluded then that, although mitoses in the wing disc continue up to 21 hr after puparium formation, the cell determination to become bristle or trichome is established earlier and perdures in daughter cells. In the present case the information leading to the shift of development from posterior into anterior pathways is apparently laid down in the cell, in such a way that the removal of the responsible genetic constitution is ineffective in daughter cells. If this is the correct explanation, we would have to know why this perdurance expresses itself only in some of the clones. This effect is not seen with cell-marker genes—those responsible for the actual cell differentiation —and is possibly characteristic of genes which control general developmental alternatives. Homoeotic genes certainly fall into this category. As in the present case, we have found perdurance of the effects of the normal alleles of *bithorax* in clones initiated later than 40 hr before puparium formation (in preparation).

The mutant pattern: The normal alleles of these homoeotic genes are capable

of preventing the mutant transformation, that is, of functioning throughout most of development. LEWIS (1964) observed transformed bx^s clones in irradiated third instar heterozygous flies and we have seen the same situation in *en* cells. We can try to understand the effects of mutant gene combinations in this context. The superimposed effect of the genetic combination of *en* and *pbx* or *bx* mutants possibly results from the lack of response of the otherwise simultaneously acting, normal genes in cells. Similarly, *h* modifies the posterior bristle pattern of the wing in *en*; *h* flies towards a typical anterior pattern, possibly because the mutant genotype responds in cells to the new "anterior" genetic consituation. TOKUNAGA (1961) interpreted the autonomous expression of *en* cells differentiating sexcomb teeth in the posterior region of the male basitarsus as due to the existence of a common anterior–posterior prepattern. Under this hypothesis it is difficult to understand why the bristle pattern of *h* is different in normal and in *en; h* flies. The same hypothesis applied to interpret the phenotype of *en; pbx* flies would lead to the conclusion that the posterior halter has the same prepattern as the anterior wing. This conclusion weakens the operational value of the prepattern concept. However, prepattern, instead of representing epigenetic blastema organization (WOLPERT 1969), could be understood as indicating patterned genetic pathways in the developing cells. Thus, anterior and posterior wing and halter characteristics would derive from the successive election of patterned developmental pathways in the normal disc cells or their alternative pathways in the homoeotic mutant proliferating cells. In fact, as we have seen in the cases so far analyzed, Antennapedia and *en*, the mutant discs show a different growth pattern than the normal one from early stages of development (see GARCÍA-BELLIDO 1972b for further discussion).

LITERATURE CITED

BAKER, W. K., 1967 A clonal system of differential gene activity in Drosophila. Develop. Biol. **16**: 1–17.

BRASTED, A., 1941 An analysis of the expression of the mutant "engrailed" in *Drosophila melanogaster*. Genetics **26**: 347–373.

BRIDGES, C. B., E. N. SKOOG and J. C. LI, 1936 Genetic and cytological studies of a deficiency (Notopleural) in the second chromosome of *Drosophila melanogaster*. Genetics. **21**: 788–795.

BRYANT, P. J., 1970 Cell lineage relationships in the imaginal wing disc of *Drosophila melanogaster*. Develop. Biol. **22**: 389–411.

ECKER, R., 1929 The recessive mutant engrailed in *Drosophila melanogaster*. Hereditas **12**: 217–222.

GARCIA-BELLIDO, A., 1966 Pattern reconstruction by dissociated imaginal disk cells of *Drosophila melanogaster*. Develop. Biol. **14**: 278–306. ——, 1968a Cell affinities in antennal homoeotic mutants of *Drosophila melanogaster*. Genetics **59**: 487–499. ——, 1968b Cell lineage in the wing disc of *Drosophila melanogaster*. Genetics **60**: 181. Abstr. ——, 1972a Some parameters of mitotic recombination in *Drosophila melanogaster*. Molec. Gen. Genet. **115**: 54–72. ——, 1972b Pattern formation in imaginal discs. In: *Results and problems in cell differentiation*. Edited by H. URSPRUNG and R. NOTHIGER. Springer Vlg, in press.

GARCIA-BELLIDO, A. and J. R. MERRIAM, 1968 Bristles or Hairs: The cell heredity of a genetic decision in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **61**: 1147 (Abstr.) ——, 1971a Parameters of the wing imaginal disc development of *Drosophila melanogaster*. Develop. Biol. **24**: 61–87. ———, 1971b Genetic analysis of cell heredity in imaginal discs of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **68**: 2222–2226.

HADORN, E., 1966 Konstanz, Wechsel und Typus der Determination und Differenzierung in Zellen aus männlichen Genitalanlagen von *Drosophila melanogaster* nach Dauerkultur *in vivo*. Develop. Biol. **13**: 424–509.

HADORN, E. and D. BUCK, 1962 Ueber Entwicklungsleistungen transplantierter Teilstücke von Flügel-Imaginalscheiben von *Drosophila melanogaster*. Rev. Suisse Zool. **60**: 302–310.

LEWIS, E. B., 1964 Genetic control and regulation of developmental pathways. In: *Role of chromosomes in development*. Edited by M. LOCKE. Acad. Press, N. Y.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. No. **267**.

MUKHERJEE, A. S., 1965 The effects of sexcombless on the foreleg of *Drosophila melanogaster*. Genetics **51**: 285–304.

POSTLETHWAIT, J. H. and H. A. SCHNEIDERMAN, 1969 A clonal analysis of determination in Antennapedia a homoeotic mutant of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **64**: 176–193. ——, 1971 Pattern formation and determination in the antenna of the homoeotic mutant Antennapedia of *Drosophila melanogaster*. Develop. Biol. **25**: 606–640.

STERN, C., 1938 The innervation of setae in Drosophila. Genetics **23**: 172–173. Abstr. ——, 1954 Genes and developmental patterns. Proc. 9th Int. Congr. Genet. **1**: 355–369. ——, 1969 Gene expression in genetic mosaics. Genetics **61** Suppl. 1: 199–211.

TOKUNAGA, C., 1961 The differentiation of a secondary sexcomb under the influence of the gene engrailed in *Drosophila melanogaster*. Genetics **46**: 157–176.

WOLPERT, L., 1969 Positional information and the spatial pattern of cellular differentiation. J. Theor. Biol. **25**: 1–47.