THE FORMATION OF DUMPY VORTICES IN MOSAICS OF *DROSOPHILA MELANOGASTER*¹

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INTERMEDIATE patterns in development have been termed "prepatterns" by STERN (1954a,b). STERN hypothesized that the determinations of some cells depend on the particular environments of their positions in intermediate patterns and that some genes may act to alter these patterns. A genetically based change in the prepatter could thus indirectly affect the final pattern of differentiated cells.

There is good evidence for dependent differentiation in *Drosophila* metamorphosis. Regulative morphogenic fields within imaginal discs have been shown (HADORN 1953; for review in English see URSPRUNG 1963). Earlier evidence that imaginal disc development is not strictly determinative was the fact that cell lineage is not constant, as shown by the overlapping shapes of the mosaic patches themselves (STERN 1940). Induction of ectodermal structures by mesodermal tissue has been shown in other insects (BOCK 1939).

In *Drosophila* it is possible to produce somatic mosaic such that areas of one genotype appear on a background of a contrasting genotype. X-irradiation during development can cause mitotic crossing over so that the genotype of a proliferating cell can be changed from heterozygosity to homozygosity for a group of linked alleles (BECKER 1956a, 1957). Morphogenic mutants linked to marker genes can be used to study the developmental interactions of tissues of different genetic compositions.

Marked mosaics of a number of morphogenic mutants have been studied, including mosaics of some homoeotic mutants: the bithorax series (LEWIS 1963), engrailed (TOKUNAGA 1961), and extra sex comb (C. TOKUNAGA, in preparation). All pattern forming genes studied in this way have proven to be autonomous, each cell differentiating according to its own genotype. These genes apparently effect differential responses to an unchanging prepatter. In the present study, mosaics of a pattern determining mutant dumpy (*dp*), show a different aspect of pattern formation.

Before the mosaics would be meaningful a careful description of the mutant phenotype was necessary.

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Description of the vortex phenotypes: methods and materials. Stocks of eight *dp* alleles were kindly supplied by DR. E. A. CARLSON (see CARLSON 1959). Detailed examination was limited to the thoracic effects in *dp^{v2}* homozygotes and in *dp^{v2}/dp^{lv}* and *dp^{ov}/dp^{lv}* heterozygotes. Dorsal thorax dissections were cleared and mounted in André medium as described by GOTTLIEB (1963) for external examinations, and wax imbedded specimens were dissected for examination of the muscles and other internal relationships.

For the wax embedded dissections a thin layer of melted beeswax and asphalt was poured into a Petri dish and allowed to harden. About 15 etherized flies were turned onto their sides on the wax. The wax was softened over a hot surface, and quickly cooled when the flies had sunk into it to about three quarters of their width. The specimens were thus held firmly in place for dissection under alcohol at 25 $\times$. The wax embedding technique was also used for the examination pupal development. The pupal case was cut open under alcohol leaving the pupa proper in place for examination.

Description of the vortex phenotypes: results. The *dp* vortices, which have been described as "whorls," "pits," "volcano-like prominences," and "vortices," are hypodermal irregularities of the scutum, involving darkened folds and depressions in the cuticle and changes in the distribution and orientation of the surrounding bristles and cell hairs. Several alleles are recessive lethals which show the vortex phenotype only in combination with nonlethal vortex alleles. Expression is irregular in every viable allelic combination, but the combinations with the recessive lethal alleles are consistently more extreme in expression. In the homozygotes *dp^{v2}* and *dp^{ov}* there is typically a single posterior pair of vortices, each centering just lateral to the anterior dorsocentral macrochaeta. Penetrance is incomplete and sometimes unilateral. In the more pronounced phenotype of the heterozygous lethal combinations, the same posterior vortices are larger and

FIGURE 1.—Dorsal view of the thorax of a *dp^{v2}/dp^{lv}* fly showing the large posterior vortices, the smaller anterolateral vortices, and the anteromedial comma vortices. Cell hairs are not depicted. Hatched areas represent the vortex pockets seen through the transparent cuticle. In homozygous *dp^{v2}* flies the posterior vortices are usually present, but the anterolateral and comma vortices seldom occur.

penetrance is complete; a more anterior and lateral pair of vortices, and a medial and anterior pair of "commas" (vortex-like grooves) are usually present.

On the scutum of the wild type, the chaetae (bristle organs) are in regularly spaced rows. Both the chaetae and the cell hairs are uniformly oriented, pointing posteriorly. The chaetae of the dumpy vortex areas are irregularly spaced and oriented radially, pointing away from the vortex center. This radial pattern and its gradation into the regularly oriented rows of the normal pattern give the appearance of a whorl or the spiral of a vortex. The vortex area cell hairs are also radially oriented.

In the center of the vortex is a dark depression, or pit. The volcano-like appearance sometimes mentioned is due to a frequent elevation of the cuticle all around the edge of the central depression. In the cleared preparations, these depressions were seen as deep invaginations of the cuticle, somewhat flattened, irregular in expression, sometimes extending a third the diameter of the thorax. Chaetae could be seen inside the vortex "pockets." Although the chaetae of the whorl point away from the center, those observed inside the pockets pointed downward; occasionally one on the bottom of the pocket was seen to point upward, projecting out at the surface. The detailed shapes of the pockets appeared to have been determined to some extent by the downward projecting chaetae; the tips of these chaetae were lodged in small corners and inside ridges. The tips of chaetae in the area around the pockets were frequently bent over where, due to their abnormal orientation, they had come into contact with the pupal cuticle.

The row containing anterior dorsocentral macrochaetae was usually displaced toward the center of the vortex, leaving the more medial chaetae widely spaced. The displacement of chaetae toward and into the vortex pocket was general within the vortex pattern and extended outside the whorl proper into adjoining rows, so that some chaetae which were normally oriented were displaced toward the vortex center. The orientation of the cell hairs followed that of the chaetae, indicating that orientation of both is a property of the tissue. Since the cell hairs are much more numerous than the chaetae, they showed in greater detail the convolutions and extent of the whorls.

The greater part of the posterior vortex whorl occurs in the area bounded by the posterior supraalar, the dorsocentral, and the postalar macrochaetae. Chaeta counts in this area were made of 25 *dp^{vs}* individuals with unilateral penetrance. All individuals except two had exactly eight microchaetae on both sides; one had seven on the vortex side, and one had seven on the nonvortex side. Counts included the chaetae inside the vortex pockets. These were invisible on casual examination, which with the wider spacing of chaetae in the whorl gave a false appearance of a diminished number of chaetae in the vortex pattern.

Adjacent and medial to every vortex pocket was an area of the cuticle devoid of either cell hairs or chaetae. This "bald" area is easily overlooked, since cell hairs are too small to be noticed on casual examination. The bald region cuticle was smooth and normally pigmented. The hairlessness was never seen to extend into the pocket itself, but frequently encompassed part of the darkened, raised edge.

Another phenomenon, observed in about half the vortex specimens, was bristle

organs with doubled chaetae. These usually occurred just anterior to the vortex pocket. In most cases there was a single normal socket.

Cell hair counts in vortex and wild-type half-thoraces were not successful, because of the difficulty in seeing clearly the cell hairs inside the pockets. The spacing of the cell hairs in the whorl is greater than that in the wild type. Since the distribution of the cell hairs appears to follow that of the chaetae, it seems probable that the cell hairs in the vortex phenotype remain unchanged in number.

Out of several thousand *dp* flies, a very few were observed in which the vortex pocket had evaginated rather than invaginated. Figure 2 is a camera lucida drawing of an evaginated posterior vortex pocket in a newly emerged γ ; *dp^{v2}/dp^{lv}* specimen. The commas can also evaginate, giving rise to paired horn-like projections (MOHR 1929).

On one occasion it was noted that the evaginated vortex pocket on a newly emerged *dp^{v2}* fly was transformed into the more usual invaginated form in a few hours. Following this, a number of *dp^{v2}* pupae were observed as described above. In all observed pupae with pigmented chaetae, the pockets were invaginated and fully formed. In the pupae with colorless chaetae, it was not possible to make out the vortex pockets at all, although the chaetae whorls and the displaced and disoriented anterior dorsocentral macrochaetae were clearly visible.

WADDINGTON (1941) reported, without elaboration, that the *dp* vortices were

FIGURE 2.—Upper left: Dissection of a *dp^{v2}/dp^{lv}* individual showing vortex pockets. The posterior vortex pocket is associated with the anterior muscle mass of the lateral oblique dorsal muscle; the anterolateral vortex pocket is associated with the most lateral of the three muscle masses of the tergosternal muscle. Upper right: Deeper dissection of another *dp^{v2}/dp^{lv}* individual, showing a complex comma vortex pocket. Two separate invaginations meet to form a V. Several balls of cuticlelike material appear to have budded off. Lower: Right half of a γ ; *dp^{v2}/dp^{lv}* thorax with an evaginated posterior vortex pocket. The small anterolateral vortex is present; there is no penetrance of the comma effect. Note the bald areas associated with both vortices, and the double and triple chaetae associated with the anterolateral vortex.

associated with the insertions of the indirect flight muscles. In more than 100 thoracic dissections, the comma invaginations and the vortex pockets in all cases corresponded with insertions of specific flight muscles, confirming this report.

There are four pairs of indirect flight muscles in *Drosophila* each divided into a distinct number of "muscle masses." The longitudinal paired *median dorsal muscle*, made up of six muscle masses, is bounded laterally by three vertical muscles, the anterior *tergosternal muscle*, the middle *tergal remoter of the coxa*, and the posterior *lateral oblique dorsal muscle*, composed of three, two and two muscle masses respectively (WILLIAMS and WILLIAMS 1943; MILLER 1950; ZALOKAR 1947). Internally the comma clefts were seen to be vortex pocket-like invaginations, associated with the anterior insertions of the median dorsal muscles. The posterior vortex pockets were invariably associated with the insertions of the anterior muscle mass of the lateral oblique dorsal muscle, and occasionally with part of the posterior muscle mass of the tergal remoter of the coxa. The dp^{v2}/dp^{lv} anterolateral vortex was always associated with the insertion of the most lateral muscle mass of the tergosternal muscle (Figure 2). The muscle insertions were always on the lateral side of the comma pockets and the medial side of the posterior and anterolateral pockets.

In several dissections, small spherules of pigmented cuticle-like material were imbedded in the muscle just below the tip of the vortex pocket (Figure 2, upper right). No evaginated pockets were dissected; however, when the specimen drawn in Figure 2 (lower) was partially cleared, it could be seen that the flight muscle did not extend into the evaginated pocket.

Mosaics of dumpy: methods and materials. In the scute 19 translocation ($T(1;2)sc^{19}$), a fragment of the first chromosome, containing the wild-type alleles of yellow, achaete, and Hairy Wing, is inserted in the left arm of the second chromosome about one crossover unit from dp^+ . The second chromosome insertion is viable as a homozygous or heterozygous duplication when the sc^{19} first chromosome is replaced with a structurally normal first chromosome.

The test individuals were homozygous or hemizygous for yellow (γ) in a structurally normal first chromosome, and heterozygous for the sc^{19} (γ^+ , dp^+) second chromosome and a structurally normal second chromosome carrying one of the dumpy vortex recessive alleles. These individuals were phenotypically wild type, except for the few mosaic specimens and for a small number of individuals with extra dorsocentral chaetae, attributable to the duplication of the achaete locus. Somatic crossing over in the left arm of the second chromosome produced mosaic patches genetically homozygous for γ and dp , phenotypically yellow because of the loss of the covering duplication.

Although γ has been used as a mosaic marker in many previous studies, its continued use requires some justification in view of evidence that it is not completely autonomous. Apparently γ^+ cells produce a pigment or pigment precursor which can diffuse into adjacent γ cells, masking the mutant phenotype (HANNAH 1954; URSPRUNG and HADORN 1962). HANNAH found evidence of this type of nonautonomy in the cuticle of the heavily pigmented abdominal black bands, but found autonomy for yellow in the rest of the abdomen. URSPRUNG and HADORN found extensive nonautonomy of yellow in the cell hairs of the wing, and a smaller amount in the chaetae of the wing margin. Where there was nonautonomy in the wing margin chaetae, the genotypically γ chaetae were usually intermediate in pigmentation. They reported that there was no evidence of nonautonomy in the chaetae of the thorax. Since, in the present study, only the thoracic chaetae were used for the identification of mosaic areas, γ was felt to be a satisfactory marker.

Test larvae (γ ; $sc^{19}(\gamma^+, dp^+)/dp$) between the ages of 24 and 96 hours were irradiated in bottles with 250 kv X rays for 20 min at 100r per min as measured inside the bottles. The larvae

pupated and eclosed in the same bottles; the adults were killed and preserved in alcohol.

For scanning for mosaics, the preserved flies were placed in a wax-bottom dish, covered with alcohol and observed with a dissection microscope at 25 $\times$ and 40 $\times$. A forceps was used to push the legs into the wax, bringing the specimen upright so that the scutum could be examined for yellow chaetae. Seven thousand individuals were screened this way.

Mosaic specimens were mounted whole in André medium in plexiglass depression slides. After clearing, they were reexamined under reflected and transmitted light at 150 $\times$, and camera lucida drawings were made.

Mosaics of dumpy: results. Thirteen mosaics were recovered, a recovery frequency of less than 0.2 percent.

In a recent experiment, X-ray induced first chromosome thoracic mosaics were recovered in frequencies up to 20 percent (F. GOTTLEIB, unpublished). The relatively low recovery frequency of dumpy mosaics appears to have been due

FIGURE 3.—Upper left: Left anterolateral dp^{o1v} mosaic with two evaginated vortex pockets and cell hair whorls. Left side view of thorax. A spherule of cuticle-like material lies beneath the scutoscuteellar suture. Eight yellow chaetae (in outline). Center left: Right anterolateral dp^{lv} mosaic. The three yellow chaetae (in outline) are normally spaced and oriented; a small vortex pocket contains one wild-type (black) chaeta, and three wild-type chaetae outside the pocket are disoriented and bent. Lower left: Large left posterior dp^{v2} mosaic patch (chaetae in outline) includes much of the vortex whorl but does not include the vortex pocket. Cross-hatched area represents the pocket as seen through the cuticle. Right: Three levels of a deep left anterolateral vortex pocket in a dp^{lv} mosaic. The mosaic patch includes the lateral inside wall of the pocket (center) but not the medial inside wall (left). Note the two doubled black chaetae medial to the bald area. Camera lucida.

to a natural difference between the first and second chromosomes in sensitivity to X-ray induced somatic crossing over. This difference has been noted before (C. TOKUNAGA, unpublished). The sc^{19} second chromosome was tested for inversions, and none was detected.

Five dp^{v2} , seven dp^{lv} , and one dp^{olv} thoracic mosaics were recovered, all clearly marked with yellow chaetae. All were associated with chaeta pattern irregularities. Three mosaic individuals had bilateral expression of the vortices, although the yellow chaetae appeared on one side only.

The seven dp^{lv} and the dp^{olv} mosaics show that these alleles are not cell lethal.

A number of the mosaic specimens indicate that dp is nonautonomous in an interesting way: the homozygous recessive areas show the mutant phenotype, but the mutant phenotype extends into the heterozygous wild-type background tissue. Thus in tissue interaction, the recessive tissue seems to be "dominant" over the background tissue. The latter is sometimes strongly involved in the vortex pattern; the whole of the vortex pocket may be composed of cells which carry the wild-type allele, while the recessive mosaic patch is outside the pocket. In the bilateral vortex mosaic specimens, the presence of homozygous tissue in one side of the thorax appears to be capable of inducing vortex formation in the contralateral half of the thorax.

Two of the recovered mosaic specimens had large γ ; dp^{v2} mosaic patches completely encompassing the posterior vortex area. Typical vortices were present entirely within the mosaic area, with no indication of any nonautonomous effect. All other mosaic specimens showed some degree of nonautonomous development. Nine are figured individually (Figures 3 through 6).

In four mosaic specimens (Figures 3, left; 4, top and bottom; 5, top) doubled

FIGURE 4.—Two similar dp^{lv} mosaic specimens with left anterolateral vortex pockets. In each the mosaic patch is limited to two bristle organs inside the pocket, wild-type (black) chaetae are also inside the pockets, and a doubled black chaeta is present anterior to the vortex pocket. The lower figure is a somewhat posterior view; the cell hairs and most of the microchaetae have been omitted. Camera lucida.

FIGURE 5.—Mosaic specimens with bilateral comma invaginations. Dots represent sockets of microchaetae; most microchaetae and cell hairs have been omitted. Upper: dp^{lv} mosaic. Two yellow bristle organs, one with doubled chaetae. Just behind is a bristle organ with doubled black chaeta. Center: dp^{v2} mosaic. Large mosaic patch (chaetae and sockets in outline) covers most of the left thorax, including a small posterior vortex and an atypical dp^{v2} comma. Just anterior to the posterior vortex is a bristle organ with doubled black chaeta. Lower: dp^{v2} mosaic with bilateral posterior vortices. Hatched areas represent vortex pockets seen through the cuticle.

FIGURE 6.—Mosaic specimen with bilateral vortices. This is a different view of the specimen depicted in Figure 5, lower. Camera lucida.

chaetae were black and almost certainly heterozygous for dp^+ , thus offering the best discrete evidence for dependent differentiation and for nonautonomy at the dumpy locus.

In all three of the bilateral vortex mosaic specimens, the unilateral mosaic patch was involved in one or more well formed "primary" vortices. The "secondary" vortex in each case was smaller and shallower. Two of the secondary

vortices were shallow comma grooves; the third was a well formed posterior vortex (Figure 5).

In the one dp^{olv} mosaic specimen, the anterolateral vortex pocket evaginated rather than invaginated (Figure 3, left top). There was a second evagination in an unusual location, slightly below and posterior to the first, and apparently outside of the yellow mosaic patch. Both evaginations were small and there were no bald areas. Close by, at the prescutal suture, a ball of pigmented cuticle-like material lay just beneath the surface. Similar subsurface spherules were sometimes seen in the same area and other areas of dp^{vz}/dp^{olv} and dp^{vz}/dp^{lv} individuals; they are probably related to the cuticle-like spherules sometimes associated with the vortex pockets (Figure 2).

One of the mosaic specimens is of particular interest (Figure 3, left center): the mosaic patch consists of just three normally oriented yellow chaetae, while a very small pocket and a cell-hair whorl is near by. There is a black (wild-type) chaeta inside the pocket, and three bent, disoriented black chaetae outside the pocket. In this individual the nonautonomy of dumpy is apparently complete.

Several of the mosaic specimens were remounted and drawn again from different angles. The two drawings frequently appeared dissimilar in that chaetae which appeared to be inside the vortex pocket in one view would appear to be outside in another; "pigmented" areas would also seem to shift according to the angle of view. This points out the ambiguity of presenting involved transparent configurations on a two dimensional plane.

Dark pigmentation of the central vortex area has often been cited as a characteristic of the dumpy vortex phenotype. From the way the "pigmented" area changes according to the angle of view, it seems that the darkening is due solely to the folds and convolutions of the semitransparent cuticle, and is not a chemical characteristic of the cuticle.

DISCUSSION

Dumpy vortices and muscle insertions: The association of the vortices with muscle insertions, and the detailed appearance of the vortex pockets, bristle whorls, and bald areas, suggest that the flight muscles have pulled an area of the hypodermis into the interior, stretching and breaking the surrounding tissue. Such an occurrence might be due either to an abnormal contraction of the muscles themselves or to an abnormal weakness of the hypodermis. WADDINGTON (1941) suggested the former hypothesis. He had shown (WADDINGTON 1940) that the dp wing effect was due to an abnormal contraction of the wing epithelial cells, and suggested that the same basic type of activity (i.e., cell contraction) should be responsible for the diverse wing and thorax pleiotropic effects. The evidence of the mosaics indicates, however, that the differential action of the dumpy alleles is in the hypodermis.

WADDINGTON's argument, that the pleiotropic effects of the gene should have a similar basis, is still relevant to the second alternative: that the hypodermis is weakened in some way which allows it to be pulled by the muscle into a vortex configuration. In both the wing and thorax, then, the basic effect of dp might be to cause the epidermis to fail to resist distortion.

There are several indications, however, that the vortex distortion of the thorax antecedes any possible effective contraction of the flight muscles. According to ROBERTSON (1936), these do not form their attachments until 84 hours after puparium formation at 25° C. In the same study ROBERTSON reported that bristle pigmentation on the thorax is complete by 72 hours. By this timetable, hypodermal distortions due to muscle contraction could not begin until some time after the thoracic bristles were fully pigmented (See also BODENSTEIN 1950).

Homozygous γ ; dp^{v2} pupae without any trace of bristle pigmentation were found to have bristle whorls. It could be that the timetable is not the same for γ ; dp^{v2} flies as for normal flies. For instance, the pigmentation of yellow bristles may begin much later than wild-type bristles; or dp itself may alter developmental sequences, inducing a very premature attachment of the muscles at a time when the hypodermis and cuticle are weak. It is known in insects that epidermal tissue can induce the formation of mesodermal structures (Bock 1939); the specificity with which the mesodermal muscles attach to areas of the hypodermis might indicate this type of induction.

Doubled chaetae near the edge of the vortex pocket or bald area are an indication of an effect quite early in pupal development, probably before chaeta growth begins. Doubled chaetae are found regularly in such mutants as split and Dichaete, wherein the effect can be traced back to a reduplication of the first appearing bristle organ cells (LEES and WADDINGTON 1942). LEES and WADDINGTON suggest that, in split at least, the condition is due in part to the improper orientation of the mitotic spindles in early divisions of the bristle forming cells. It seems plausible that a physical distortion of the hypodermis could bring about the same effect. This type of effect has been observed independently by C. TOKUNAGA (work in progress), who has found doubled chaetae, bald areas, and abnormal chaeta orientation to be associated with extensive and abnormal tissue movement in mutant *Drosophila basitarsi*; she finds that chaeta and cell-hair orientation follows the direction of tissue movement. The dumpy mosaics indicate indirect causation of chaeta doubling rather than a direct effect of the gene, since black chaetae are sometimes doubled near the edge of a mosaic vortex pocket.

Since bristle formation begins at 30 hours after puparium formation (ROBERTSON 1936; BODENSTEIN 1950), the indirect action of dp on chaeta doubling occurs at least 54 hours prior to normal attachment of the flight muscles.

Occasionally, the vortex pocket will evaginate rather than invaginate, giving rise to a distinct elevation rather than a pit (Figures 2, bottom; 3, top left). dp^{v2}/dp^{lv} flies sometimes bear horn-like projections in the place of commas (MOHR 1929). Invaginations often occur in the center of volcano-like elevations. Muscle pull cannot account for these effects.

Finally, it should be noted that most of the area of the scutum is taken up by the insertions of muscles. Any regularly occurring hypodermal feature has a good chance of being coincidentally associated with a specific muscle.

Mode of gene action: The total surface area of the dumpy-vortex scutum is greater than that of the wild-type scutum, in contrast with the wing effect of the dumpy-oblique alleles, wherein the surface area is diminished. The opposite nature of these pleiotropic effects led WADDINGTON to look to cellular and muscle

contraction for a resolution. It should be noted, however, that WADDINGTON himself pointed out that the contraction of the *dp* wing epithelial cells is directional; the wing is shortened, but at the same time it is not narrowed and may be slightly widened (WADDINGTON 1940). The underlying similarity of action of the alleles now appears to be in that wing and vortex effects are both due to abnormal epidermal tissue movement.

The polarity changes of the bristles and cell hairs in the mutant phenotype appears to stem from the movement of cells during the formation of the vortex pockets. The degree of polarity change is relative to the size of the invagination; bristle whorls never appear in the absence of vortex pockets in the nonpenetrant individuals.

The edge of the vortex pocket, the pocket itself, and the borders of bald area can each be either homozygous *dp* tissue or heterozygous background tissue; however, the area just anterior and medial to the vortex pocket is homozygous recessive in almost all of the mosaic specimens, secondary vortices excepted.

In mixed tissues the phenotype associated with the recessive genotype prevails, indicating that the action of the homozygous recessive tissue in vortex formation is supracellular and nonautonomous. The homozygous recessive tissue affects the movement of the heterozygous tissue either by failing to produce some supracellular activity which is necessary for normal movement, or by failing to prevent some supracellular activity which disrupts normal movement. In the former hypothesis, the wild-type allele would act directly in tissue movement and the mutant alleles fail to act; in the latter hypothesis, the wild-type allele regulates by intracellular inhibition a supracellular agent, and the mutant alleles fail to inhibit. It is not possible to decide between these hypotheses. Whether the gene acts directly or by inhibition, it would appear to be differentially sensitive to specific developmental environments (e.g., wing and scutum) and these sensitivities can be destroyed or altered independently by mutation. Systems of intracellular, intergenic control by inhibition, sensitive to cellular environments, are known in the operon mechanism of *Escherichia coli* (JACOB and MONOD 1961a,b) and to some extent in maize (McCLINTOCK 1961). FALK (1963) has presented some arguments for the presence of the operon type of genetic control in *Drosophila*.

Neither hypothesis provides an adequate explanation for the occurrence of bilateral vortex mosaics. It is conceivable that an excess of morphogenic agent could diffuse over into the other half of the fly and cause the formation of a secondary vortex. This would require of the agent that it affect specific target tissues in specific ways.

Only three bilateral vortex mosaics have been described, two of them in the comma area. In spite of the concrete objections to any hypothesis which holds that the vortices are formed solely by muscle pull, the indirect flight muscles could be involved in some way which is still unclear. Since secondary vortices did not happen to develop any doubled bristles, their time of formation is not indicated. Perhaps they were the result of a chain of events which began early in the hypodermis of the mosaic side, but which eventually involved the muscles of both sides and their hypodermal insertions.

Conclusion: Morphological patterns are formed by the interactions between the process of cellular determination and differentiation and the process of tissue movement. Although descriptive developmental genetic studies have always recognized the importance of the latter process (e.g. WADDINGTON 1940; AUERBACH 1936), theoretical and experimental studies in developmental genetics have more often concentrated on cellular determination as a basis of pattern formation (e.g. HADORN 1953; MAYNARD SMITH 1960; MAYNARD SMITH and SONDHI 1961; SONDHI 1963; STERN 1954a,b, 1956; TURING 1952; URSPRUNG 1963; WIGGLESWORTH 1960).

The action of *dp* is on the tissue movement aspect of pattern formation. With respect to the formation of vortices and whorls, *dp* is not a prepattern mutant since it operates in a pattern forming mechanism quite different from the postulated prepattern-pattern mechanism. A prepattern gene acts by changing "any spatial differentness in development" in a tissue prior to cellular determination (STERN 1954b), and the action of the *dp* vortex alleles occurs after the determination of the bristle organs and hypodermal cells.

The pattern of determination within the imaginal disc may have no definitive topography, but included in the pattern must be a determination for the factors controlling spacing and orientation through tissue movement. There must be some time in imaginal disc formation when cellular proliferation is variable in an unpatterned anlage, but as soon as determinative organization is imposed on this tissue, cell movement can no longer be undirected (STERN 1940; BECKER 1956b, 1957). Perhaps determinative organization cannot be imposed on a tissue until it achieves a critical size (WETMORE 1959), or until sufficient developmental time elapses (URSPRUNG 1963).

In one minor detail *dp* is a prepattern gene in the original sense. Bristle organ determination is not the final cellular determination in chaeta formation; the action of *dp* appears to occur between time of bristle organ determination and the final bristle cell and socket cell determinations. Inasmuch as the action of *dp* sometimes changes the predetermination environments of these cells, thus indirectly affecting their final differentiation, *dp* is a prepattern gene for doubled chaetae. As should be expected, it is nonautonomous in this respect.

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SUMMARY

Certain alleles of the gene *dp* produce vortices, which are changes in the configuration of the dorsal surface of the thorax and in the distribution of thoracic chaetae and hairs. The *dp* alleles appear to affect the chaeta pattern through tissue movement, since they do not change the total number of bristle organs. Cellular changes which occur are bristle organs with doubled chaetae, and small "bald" areas devoid of chaetae and cell hairs.

The vortices are associated with insertions of certain of the indirect flight muscles through deep invaginations of the hypodermis and cuticle. Vortex for-

mation begins prior to normal attachment of the flight muscles: the formation of vortices is indirectly responsible for the development of doubled chaetae, and chaeta differentiation normally occurs about 54 hours before the flight muscles become attached to the hypodermis.

X-ray induced somatic crossing over produced mosaic patches which were homozygous for *dp* and homozygous or hemizygous for yellow on a heterozygous wild-type background. Thirteen mosaics, including eight involving recessive lethal alleles, were recovered from an estimated 7,000 treated individuals. In the mosaic specimens, the dumpy recessives are nonautonomous: homozygous recessive patches bring about the vortex pattern in the adjacent heterozygous or homozygous dominant tissue, and, in three cases, in the heterozygous tissue of the opposite side of the thorax. The doubling of chaetae in the vortices is an indirect effect, since it sometimes occurs in the background tissue.

The wild-type allele of *dp*, in response to specific developmental environments, appears to regulate (either directly or by intracellular inhibition) the production of a supracellular factor, which in turn affects tissue movement. The recessive alleles apparently fail to respond to these developmental environments, leading to a failure to regulate the production of the supracellular factor and thus to abnormal tissue movement.

It is concluded that *dp* is not a prepattern gene with respect to the distribution of thoracic bristle organs, since it does not have any effect on the determination of bristle organ anlagen. The differentiation of doubled chaetae, however, is due to a physical change in the cellular environment, indirectly brought about by *dp*; in this minor respect *dp* can be considered a prepattern gene.

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