A FINE-STRUCTURE GYNANDROMORPH FATE MAP OF THE DROSOPHILA HEAD

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ABSTRACT

A gynandromorph fate map of the head of D. melanogaster was produced using 28 landmarks derived from one imaginal disc. An examination of the meaning of fine-structure mapping discloses that the sturt value observed between one pair of landmarks within a disc may approximate the relative physical distance of their progenitor cells at blastoderm, but for another pair of landmarks (assuming no directed cell movements), the sturt value may simply reflect their close geographic location at the time the cells are specified for their particular differentiation, a time much later in development when most cell division within the disc has come to an end. The formation of early developmental compartments has little effect on fate-map distances. Our analysis of the data suggests there are approximately ten cells present at the blastoderm stage that are head progenitors. Each blastoderm cell is likely to be the progenitor of a particular array of landmarks, but there is overlap between arrays from different blastoderm cells.

GYNANDROMORPH fate mapping has been used extensively within recent years to determine the relative location of progenitor cell of particular landmarks of adult morphology. In most of these studies, many landmarks have been used, but only a small number of these landmarks arise from cells of the same imaginal disc. Few studies have attempted fine-structure mapping by using a relatively large number of landmarks that arise from the same disc (Ripoll 1972; Wieschaus and Gehring 1976). This type of mapping raises some interesting questions about the physical interpretation of the “distance” observed between a particular pair of landmarks. At the same time it also exposes to view some of the characteristics of the process whereby the landmarks are specified, an event occurring rather late in imaginal disc development.

MATERIALS AND METHODS

Fate mapping was accomplished by using the technique devised by Garcia-Bellido and Merram (1969), which utilizes gynandromorphs produced by somatic loss of the X chromosome. We generated gynandromorphs through somatic loss of a ring chromosome in R(1)2, In(1)w^{107}ln(1)dl-49, γ w f^{36a} heterozygotes. These flies were examined under the dissecting

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Figure 1.—Gynandromorph fate map of the head. Gynandromorphs formed by loss of ring in \( R(1)2, \text{In}(1)w^{sc}/\text{In}(1)\text{dl-49}, y^w f^{yeg} \) females. The distances given are in sturts. Only the lines used in triangulation to form the map are shown. The POc to POv and POc to Vp distances connect the posterior dorsal and ventral landmarks and are drawn shorter than measured in order to make this connection. The locations of the landmarks on the adult cuticle are shown at the bottom of the figure.
microscope to see if any head region showed \( yf \) bristles (forked can be scored in both trichomes and bristles, yellow only in bristles) or if any area of the eye was clearly white and thus caused by ring loss rather than by position-effect variegation of the \( w^+ \) locus in the ring. Only heads of such flies were mounted (in euparal after boiling in KOH) for closer examination. Since this mounting procedure removes eye pigment, a mosaic eye was outlined on standardized two-dimensional drawings of the head prior to boiling. Subsequently, the remainder of the clone was drawn in upon examination of the mounted head with a compound microscope. Sturt distances based only on flies in which part of the head cuticle is mosaic would not affect the internal consistency of the map, but would be about four times too large, since Garcia-Bellido and Merriam (1969) found that only 24% of the gynandromorphs are mosaic on one side of the head. Therefore, we have divided our distance values by four solely for comparison with other fate maps. Of the 28 landmarks used, 19 refer to particular bristles, three to virtual spots on the posterior surface of the head, one to a virtual spot on the prefront where trichome markers must be used, and five to virtual spots in the compound eye where pigment markers were used (see Figure 1). The map was constructed by first using pairs of landmarks with short distances. This allowed the construction of a self-consistent map of the dorsal head regions and of most of the ventral regions. Only a few ambiguities arose during the triangulation process and these were in making the connections between the dorsal and ventral regions. In these cases, we chose the triangulation that made some topological sense, i.e., the orientation most like the spatial orientation of the landmarks in the adult. It will be shown later that this bias did not distort this region of the map.

RESULTS

The fate map pictured in Figure 1 is based on 214 sides of heads of gynandromorphs in which at least one, but not all, of the 28 landmarks was mutant and thus hemizygous. The striking thing about this fine-structure map is the general congruence between the relative location of landmarks in the adult and the “location” of their progenitor cells. This congruence is of importance in our later discussion of the interpretation of such fine-structure fate maps, and this fact will be described by the statement that the map “makes sense.”

Although the general congruence is obvious, one should not ignore the discrepancies because they may point to interesting morphogenetic movements. Although the progenitors of the landmarks in anterior orbital and vertex region dorsal to the eye are very close to each other, and the progenitor cells of the landmarks on the gena ventral to the eye are very close as well, these two regions are quite far apart. In fact, one of the striking features of the map when compared with the adult head is the long distances between the dorsal and posterior regions of the eye and its bordering bristles such as the orbitals, verticals and postorbital bristles. Perhaps this region contains the cells that will give rise to the ptilinum (see fate map of Ouweneel 1970). There are two additional regions whose positions are quite different from those in the adult. The cells that are progenitors of the vibrissae are too posterior and too dorsal in relation to the eye. Presumably there must be later cell movements of this area in relation to the remainder of the head. But most striking is the long distance (sturt = 3.8) between the bristles on the outer surface of the second antennal segment (Ao) and the row of bristles on the inner surface (Ai) of this segment. They are located on the edge of the map at some distance. A more careful mapping of the struc-
tures of the antennal segments may provide evidence of even more extensive morphogenetic movements in the formation of this organ than envisaged by Postlethwait and Schneiderman (1971). Perhaps there is relatively little cell division in the marginal areas, or perhaps the mitotic orientation is mainly perpendicular to the margin, but either event would result in the marginal cells becoming relatively closer together as the disc develops. This would provide both for a sac-like disc and for the close proximity of adult structures whose progenitor cells were far apart and on the margin of the disc (e.g., the dorsal, central and ventral postorbital bristles, PO).

Are these “discrepancies” in the map the result of the difficulties in triangulation (mentioned previously) in connecting the dorsal and ventral regions through the eye? This is probably not correct since they are also apparent in a map produced by Flanagan (1976) from the same data using computer fitting to minimize the overall difference between observed distances and that predicted on the basis of a nonlinear mapping function. You will note (Figure 2) the general similarity of the two maps, but also the striking difference in location of the eye landmarks, and this is the region that raised difficulties in connecting the dorsal and ventral regions of the head by triangulation. Of the 28 landmarks, the seven with the largest errors in the computer-based map are, in decreasing

![Figure 2](image-url)

**Figure 2.**—Comparison of the fate map based on triangulation (left, circles) and the one derived from same data by Flanagan using his computer program (right, triangles). The solid symbols indicate the seven landmarks with the highest errors according to the computer fit.
magnitude: Ed, Ep, Ai, PF, Ao, Ev, and Ec. Perhaps the progenitor cells for each of these adult structures may be specified from a larger number of localized cells than the progenitors of other landmarks, leading to a greater uncertainty (see DISCUSSION following).

**DISCUSSION**

*Meaning of fine-structure fate maps*

It has not been generally appreciated by many workers in the field (including myself) the caution that one must use in interpreting gynandromorph fate maps in terms of relative physical location of progenitor cells of adult landmarks. Recently, Lawrence and Morata (1977) have pointed out some pitfalls of detailed fate mapping, and they arrived at the conclusion that “sturt distances . . . are strongly dependent on compartmentalization and other aspects of postembryonic development.” This problem is deserving of re-examination at somewhat more depth. In reality, it turns out that compartmentalization has little effect on the detailed fate map, and that fine-structure fate mapping assists in elucidating some important aspects of the events intervening between blastoderm and final cell differentiation.

The basic problem is deciding the stage of development that is relevant to the sturt value for a particular pair of landmarks. Consider 10 cells of the blastoderm that will be the progenitors of a given imaginal disc, a reasonable number as shown by the recent work of Wieschaus and Gehringer (1976) and Lawrence and Morata (1977). A group of 10 such cells from a gynandromorph in which

![Diagram](image-url)

**Figure 3.**—Diagramatic view showing the relation between specification of landmarks, compartmentalization, and distances between progenitor cells of landmarks as measured by fine-structure fate mapping using gynandromorphs. See text.
three of the cells are male and the other seven female is pictured diagramatically in Figure 3. Now if it is assumed that each blastoderm cell is the progenitor of a unique and nonoverlapping group of differentiated cells, then 10 is the maximum number of landmarks that can be separated from each other by the boundary separating male from female tissue. Therefore, if one used more than ten landmarks in mapping a given disc, there should be some pairs of landmarks that are never of opposite sex. In mapping the Drosophila head, we used 28 landmarks, and we found that every single landmark was found with the opposite sex from every other of the 27 landmarks in at least one gynandromorph. It is apparent that the assumption that each blastoderm cell is the progenitor of a unique and nonoverlapping set of landmarks is incorrect. Each of the 28 landmarks cannot arise from separate blastoderm cells, since the clonal analysis of head compartmentalization (BAKER 1978) showed that a number of different landmarks are often included in the same clone, and these clones were initiated after blastoderm formation.

Our interpretation as to why any pair of landmarks may be of opposite sex is set forth in the main part of Figure 3. For the sake of simplicity, we show a stage in imaginal cell development only five cell divisions after blastoderm formation. As you will note, we have assumed that the 32 cells derived from each blastoderm cell are contiguous, each blastoderm cell divides the same number of times, and the relative position of the 10 groups of cells derived from each blastoderm cell is the same as the arrangement of the blastoderm cells themselves. (The validity of these assumptions will be commented on as we consider the actual fate map of the head.) Now, in order to accommodate the fact that most, if not all, pairs of landmarks may be of opposite sex, one must assume either that more than one cell has a finite probability of being the progenitor of a given landmark within a single individual, or that a specific cell in one individual may be the progenitor, but that a different cell may be the progenitor of this landmark in another individual. We will assume the first to be true for illustrative purposes. A probability surface for a group of cells to be the progenitor of a landmark is pictured in Figure 3 (i.e., some cells have a greater probability of being the progenitors of a specific landmark than do others). You will note that the progenitor of landmark 1 will most certainly come from cells derived from blastoderm cells c or a, and thus be male. The progenitor of landmark 3 may be derived from blastoderm cell c, but there is a reasonable probability that it will come from blastoderm cell f, and thus be female. Let us call this process whereby positional information is used to specify once and for all which cell among a group will be the progenitor of a given landmark “specification.”

Let us now examine the process of specification and ask what is involved and when it occurs. First consider the fine-structure fate map that would result if, within any large group of cells, one cell was just as likely to be specified for a given landmark as any other cell. It would be unlikely that one would be able to construct a consistent, detailed fate map since stochastic processes (noise) and not physical distance would be mainly responsible for the small sturt values observed. In fact, our ability to construct a topologically sensible fate map of the
head means that only one rather small group of contiguous cells may be specified for a given landmark. It is apparent that the particular stunt value between any two landmarks within a disc will be a function of the compound probability that the two landmarks are derived from different blastoderm cells and the probability that, if they do not share a common blastoderm ancestry, they have opposite sex. The first probability depends on the spread of the probability surface, i.e., if a given postblastoderm cell is specified from landmark 1, then the probability that landmark 2 will come from a cell of different blastoderm origin will be a function of the spread of its probability surface. Now if this spread is too broad, then there will be so much noise in the system of fine-structure mapping that, in view of the small number of events leading to opposite sex of neighboring progenitor cells, no sensible map will be obtained for these close landmarks. Since we do obtain a sensible (as previously defined) fine map, we conclude that specification involves a small number of neighboring cells.

Specification must occur after most of the cell divisions in the imaginal disc have been completed. We know that often a M+ cell dividing in the presence of a background of M cells (M = Minute bristles) will divide more rapidly and often almost fill a compartment (Morata and Ripoll 1975), and if specification of progenitor cells occurred before these divisions were mostly completed, then M+ clones would produce gross developmental abnormalities, which they do not. Thus landmarks are not specified until late in development, yet certain stunt distances are a reflection of the blastoderm arrangement of cells. How can this be?

It can be seen from Figure 3 that the relative distances between landmark 1 and 4, 1 and 3, and 3 and 4 are a measure of blastoderm distance, since there are distinct nodes in the specification probability surfaces.

Let us now consider how compartmentalization affects fate mapping, a problem discussed at some length by Lawrence and Morata (1977). The matter is not complicated even in fine-structure fate mapping because specification occurs after compartmentalization is completed (at least the type of compartments established by studies to this date). Pictured in Figure 3 are four compartments: an anterior and a posterior compartment formed one cell division after blastoderm, and dorsal and ventral compartments formed after the next postblastoderm cell division. It is instructive to look at the result if the group of cells that can specify a given landmark transcends the border between two compartments, for example, landmark 2 in Figure 3. Since we know that landmarks do not switch from one compartment to another, the process of specification must be able to read the compartment to which each cell belongs. This in turn implies that the genes responsible for compartments (the "selector" genes in the terminology of Garcia-Bellido 1975) must be active at the time of specification. We assume for illustrative purposes, that landmark 2, Figure 3, cannot be specified by cells in the dorsal compartment; thus, the probability surface goes to zero along the dorsal-ventral boundary. Therefore, one effect of compartmentalization on fine-structure fate mapping will be a reduction in the spread of the specification surface when it covers a compartment boundary. This will cause little or no map expansion, as we illustrate in the inset to Figure 3. Map distances are affected
only when two groups of cells, each of which specifies a different landmark, have
different blastoderm origins, and when the compartment border differentially
divides the two types of cells. Landmark 2 in this figure is an example. If we
assume that it can arise only from ventral cells, then it must be specified by the
one \( f \) cell in this compartment of the group of four that can specify the land-
mark. Thus, landmark 2 will of necessity have a different blastoderm origin
from that of landmark 1 (blastoderm cell \( c \)); but, if there were no compartment
boundary, then it would be equally likely that landmark 2 would come either
from a cell derived from blastoderm cell \( c \) or from \( f \). Therefore if landmark 2 is
ventral, the distance to landmark 1 is expanded. However, in the complementary
case where landmark 2 is restricted to the dorsal compartment, the distance
would be shrunk by the compartment border since it would now be twice as
likely that it would be specified by a \( c \) cell rather than an \( f \) cell. In summary, the
concern of Lawrence and Morata (1977) that “measured map distances will
depend, to a considerable extent, on compartmentalization rather than on rela-
tive position of blastoderm cells” is unfounded.

One could look on specification as the ultimate compartmentalization. After
all, the two processes share common characteristics: they involve segregation
of groups of contiguous cells, the cells in one group do not have to be clonally
related, presumably early compartmentalization is responding to positional
information in the egg cortex and specification is responding to positional infor-
mation available at a later developmental stage.

**Interpretation of the head fate map**

Let us return to a consideration of blastoderm mapping. If postblastoderm
cell divisions were completely indeterminate with regard to the potentiality
of a blastoderm cell being the progenitor of a group of landmarks, one would
not expect the landmarks to fall into groups based on their sturt distances, neither
would one expect the frequency distribution of the sturt values to fall into nodes.
On the other hand, if there were some determination in these divisions, then one
would expect to find the landmarks falling into approximately the same number
of groups as there are blastoderm cells giving rise to the disc. The latter expecta-
tions are precisely the ones found; thus, there is a degree of determinancy in the
cell divisions. This should not be too surprising for the head in view of Becker’s
(1957) demonstration of a high degree of determination in the cell divisions in
the ventral compound eye. One way of seeing whether the landmarks fall into
groups based on sturts is to tabulate the distances of one landmark to every other
landmark and then see if there is one or more landmarks that show almost the
same distribution of distances to all the other landmarks. Groups so constituted
are based on an overall similarity of distances to other landmarks. Table 1 is a
tabulation of the 378 sturt values that can be formed between the 28 landmarks
of the head. They appear to fall into 10 groups, which we choose to interpret
as an estimate of the number of blastoderm cells giving rise to the head. As can
be seen in the table, the uniformity of values within one group is remarkable,
especially when one considers that landmarks specified near the edge of the
descendants of a single blastoderm cell have a higher probability of being of
opposite sex to another landmark (from one gynandromorph to the next) than
do landmarks specified near the center of the descendant cells. We have averaged
these within-group sturts and thereby assigned values to the distances between

| Va  | - O A B  | C B B A A | E D E D E | D E E E | B B B E E | F F | B E E |
| Ec  | A A - A  | B B C B A | E E E E | D E E E | C B A F E | E E | C E E |
| Ev  | B B A -  | C B C B B | E E E D | D E E E | C C B F E | F F | E C E |
|     | 2.3      | 5.1       | 4.5      | 5.7      | 2.7       | 5.7 | 6.1 |
| Ep  | C C B C  | - B D B B | G F F F F | F F G G | D D C E F | D D | C E F |
| P0v | B B B B B | B - B A A | F F F F F | E F F F F | D C E F E | F E D | A E F |
| Ai  | B B C C D B - B B | F F F F E | E F F | C C D E F | E F | B E F |
| M   | A A B B A B A - A | F F F E E | E F F | C C C E F | E E | B E F |
| PG  | A A A B B A B A - | E E E E | D E E F | C B C E F | F F | A E F |
|     | 5.4       | 6.5       | 3.7      | 6.2      | 5.4       | 2.4 |
|     | 6.0       | 5.3       |
| O   | E E E E G F F F E - O A A A | B A O B | C D D C B G G F | E D |
|     |     | F D E E E F F F E E O - O A A | B A O B | C C D D D B G G E |
| AO  | E E E E F F F F E A O - O A | C B A B C C D D C G G F | E E |
| PD  | D E E D F F F E E A A O - O | C B A B C C D D D B F G F | E D |
| FO  | E E E D F F E E E A A A O - C | B B A B C C C D D F G E | E E |
| Ed  | D D D D F F E E D B B C C | - C C C | C D D C C F F | D D D |
|     |     |     | 3.1      | 4.0      | 3.4       | 6.7 |
|     |     |     | 4.6      | 4.4      |
| PV  | E E E E F F F F E A A B B B | C A - B | D D E B A F F E | D C |
| IO  | E E E E G F F F E O O A A A | C A - B | C D E C B G G F | E D |
| IV  | E E E E F F F F F B B B B B | C B B - | D D E B A F F | F C |
| Ao  | B C C C D D C C C C C C C C C | C D C D | - B B | D D F G | D E E |
| PF  | B B B C D D C C C C | D D D D B B - D D D D | F E C E E |
| Ea  | B B A B C C D D C C C C | D D D D D E E E | B B - E E F F D F F |
|     |     |     | 4.9      | 6.4      | 4.1      | 5.9 |
|     |     |     | 4.1      | 5.9      |
| OCP | E E E F E E E E E E E E E E | C B C B C | D D E - A | D D E B B |
| Tc  | E E E E F F F F F B B C D C | A A B A D D D | A - E E E C B |
| Tb  | F F F F F E E E E G G G G G G | F F E E | F F E E | F B | D C D |
| POc | F F F F F E E E E G G G G G G G | F F F F | G G E F E B - D C D |
|     |     |     | 4.7      | 3.8      |
| Ta  | B B C C C A A B A A | F F F F F F | E F F | D E F D | E D - D E |
|     |     |     | 5.1      |
| POd | E E E E E E E E E E E E | E E E E | D E E E E | B C C C D - A |

The left-lower half of table is redundant, but is presented so that the reader can see more easily the 10 groups into which the 28 landmarks fall. The letters indicate ranges of sturts: $0 = 0-0.9$, $A = 1-1.9$, $B = 2-2.9$, $C = 3-3.9$, $D = 4-4.9$, $E = 5-5.9$, $F = 6-6.9$, $G = 7-7.9$. The numbers in table are the means of the sturts between groups.
each of the putative 10 blastoderm cells. From these values we have constructed the blastoderm fate map portrayed in Figure 4.

Now if one were to plot the frequency distribution of the 378 sturt values, there should be a peak corresponding to the sturts between neighboring cells and additional peaks corresponding to the other possible physical distances between 10 presumably closely packed cells. Such a histogram of the actual values is shown in Figure 5, as well as the frequency distribution of the five physical distances between 10 cells arranged in the manner shown in the figure. (The frequency distribution is little affected by the specific arrangement as long as the 10 cells form a compact group.) There is a peak between 2.5–3 sturts, which we assume to be the distance between neighboring blastoderm cells. According to the physical packing distances, the next two peaks should fall between 5–6 sturts, the spot where the largest frequency of actual sturt values falls. However, the sum of these two peaks should be only a little larger than the 2.5–3 peak (42% vs. 40%), but, in fact, the frequency of the two next-neighbor peaks is much larger, an observation for which we do not have an explanation. The expected frequencies of the two largest distances agree well with the observed frequencies of the largest sturts. Note that one should not expect sharp peaks because “specification” sturts will modify the “blastoderm” sturts, thus partially filling the valleys between the peaks, i.e., two landmarks may be specified from descendants of different—but neighboring—blastoderm cells from one individual to another. Thus, the observed sturt value between them is spread. We conclude that the frequency distribution of sturt values, qualitatively and in part quantita-
Figure 5.—The frequency distribution of the 378 sturt values, using the 28 landmarks. If one assumes 10 blastoderm cells arranged as shown and a distance of 2.5–3 sturts between adjacent cells, the heights of the arrows indicate the expected frequencies of sturt values that refer to the blastoderm stage. The sturt values less than 2.5 are a measure of distance at the time of landmark specification, as is also true of the values falling between the expected peaks (see text).

Let us return to the fate map of the 10 blastoderm cells shown in Figure 4. We have drawn a 2.5 sturt diameter circle around each of the ten points to approximate roughly the distance between neighboring cells. As indicated in this figure, all of the landmarks that we called dorsal and all of those we called ventral on the basis of our compartment analysis (Baker 1978) fall into two appropriate locations on the blastoderm fate map. Thus it would appear that what we have called the dorsal-ventral restriction in the head is present at the blastoderm stage. If it occurred later, after a division or two, then one of two landmarks that are most likely specified from the descendants of a single blastoderm cell might be in the dorsal and the other in the ventral compartment.
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LITERATURE CITED


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