# MOSAIC ANALYSIS OF LETHAL MUTATIONS IN DROSOPHILA*

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ABSTRACT

The effects of various lethal mutations were studied when they were present in the hemizygous state as sectors in genetic mosaics (gynandromorphs) of *Drosophila melanogaster*. Sixty percent of the lethals studied can survive to adulthood in combination with normal tissue in mosaics, whereas the remainder cause the death of such mosaic animals. Some of the gynandromorph-viable lethals appeared to be locally active, in that they allowed the survival of only those mosaics in which the male tissue was present in the abdomen. The regions affected by these lethals were identified more precisely by analysis of the tissue distributions in the surviving gynandromorphs.

THE most common type of genetic mutation in eukaryotes is that which leads to lethality at some stage of development. Such mutations have been known for over 60 years and they represent a potentially rich source of experimental material for developmental biology since "each newly arisen lethal mutant sets up a highly specific experiment, shedding light on the functional relationships between individual mutational states and the processes leading to the formation of characters" (HADORN 1961). However, the useful information obtained from studies of lethal mutants has usually been of a rather general nature, such as the numbers of genes necessary for normal progress through various developmental stages. It should be possible to obtain information on more specific aspects of development by observing the secondary consequences of the primary defects in lethal mutants; this would be the "highly specific experiment" mentioned by HADORN. It would involve the use of genetics to create developmental lesions which otherwise would have to be produced mechanically, chemically, or with radiation. Then the developmental consequences of such lesions would be expected to throw some light on normal cellular and tissue interactions. Although in principle this is an excellent strategy, it must be admitted that it has, on the whole, failed. The reason for its failure is that, while it has been possible to describe a host of complex, pleiotropic syndromes of damage wrought by lethal mutations, the primary defects have, almost without exception, remained elusive at both the gross and the biochemical levels. The "classic approach" to developmental genetics (WRIGHT 1970) has been to describe the pattern of defects found at the time of developmental arrest, and then to trace the abnormalities to the

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earliest stage at which any of them can be discerned, using histological procedures, time-lapse cinematography, and so on. The main difficulty with this approach is that the earliest visible defect is not necessarily, in time or place, the primary functional defect; for example an endocrine mutant might produce the first histologically detectable defect in the target tissues rather than in the affected endocrine organs.

Theoretically, the primary functional defect in lethal mutants could be localized through the use of genetic mosaics. The principle of such a procedure would be to create a number of mosaic animals containing both lethal and normal tissues with various distribution patterns. These mosaics would then show a normal or lethal phenotype depending upon whether the cells at the site of action of the lethal were normal or lethal in genotype. So a study of the phenotypes of a number of mosaics in relation to their mosaicism patterns should allow the precise localization of the primary functional defect caused by the lethal mutation. HOTTA and BENZER (1972) have used an analogous method for mapping the sites of action of behavioral mutations in Drosophila. The method could, of course, be used to determine whether the pleiotropic effects of a lethal gene are due to multiple primary sites of action of that gene, or represent secondary consequences of a single primary functional defect. This kind of analysis has been applied to some non-lethal pleiotropic mutations by STERN and TOKUNAGA (1968). In this paper we explore the use of gynandromorph mosaics of Drosophila in the study of lethal mutations.

## MATERIALS AND METHODS

Hemizygous yellow males were fed 0.025 M ethyl methane sulfonate according to the method of LEWIS and BACHER (1968). Sex-linked lethal mutations were then isolated in their progeny by the standard *Basc* technique (ABRAHAMSON and LEWIS 1971). Thirty-four lethals were obtained from 100 tested chromosomes, and these were balanced over a female-sterile chromosome named *Binsinscy* ($y\ sc^{S1L}\ B\ In(1)dl\text{-}49\ sn^{x2}\ w\ sc^{8R}$) for gynandromorph tests, and over $sc^{1S}\ In(1)dl\text{-}49\ v$ for lethal phase determinations. Five sex-linked lethals from other experiments were included in the analysis.

For the gynandromorph tests, use was made of the unstable ring-*X* chromosome ($R(1)2\ w^{vc}$) ($=In(1)X^{c2}\ w^{vc}$; HINTON 1955) which was balanced over *Binsinscy*. The mitotic instability of $R(1)2\ w^{vc}$ was maintained at a high level by breeding from gynandromorphs (with female abdomens) and *Binsinscy* males in every generation. For each lethal studied, about ten $y\ l/$ *Binsinscy* females were mated with a single $R(1)2w^{vc}$ male and all of the progeny were collected and scored. Any lethal-bearing gynandromorphs were mounted in water-mounting medium between cover-slips, and examined under the compound microscope.

For each lethal phase determination, an egg collection was taken from the $y\ l/sc^{1L}$ $In(1)dl\text{-}49\ v$ stock. One day later, when most of the embryos had developed to larvae and hatched, the collection was examined for the presence of larvae with yellow mouth hooks. Such larvae are hemizygous lethal males and are expected to arrest development at the lethal phase characteristic of that mutant. This sample of larvae was segregated, observed daily, and the stage of developmental arrest was determined. If no larvae with yellow mouth hooks were present after a second examination two days after egg laying, the mutation was recorded as an embryonic lethal.

The approximate genetic locus for some of the lethals was determined by standard mapping procedures using a $w\ m\ f$ (white-miniature-forked) mapping chromosome.

*Control*

The control experiment for these studies involves the production of gynandromorphs from a cross of *y/Binsinscy* ♀ × *R(1)2 w*$^{vc}$ ♂, no lethal being present on the *y* chromosome. The progeny from this cross are shown in Table 1. The first point to notice is the vast excess of males over females + gynandromorphs. This excess of males can be accounted for since *R(1)2 w*$^{vc}$ causes varying amounts of dominant lethality, and since *R(1)2 w*$^{vc}$ males are known to produce a high frequency of matroclinous *XO* progeny, due either to meiotic ring loss or to complete mitotic ring loss (HINTON 1955). Although it is not possible at present to distinguish the latter two alternatives, it seems clear that the matroclinous male progeny are produced as a result of the same chromosomal instability that is responsible for the generation of gynandromorphs. The justification for the latter statement is the direct correlation, in the progeny from ring-X males, between the degree of distortion of the sex ratio and the frequency of mitotic ring loss (Figure 1; see also HINTON 1957 and PASZTOR 1971).

The second point of interest in Table 1 is the inequality in numbers of progeny bearing the *y X*-chromosome as compared to those carrying *Binsinscy*. For the purposes of the following experiments the reason for the inequality is not relevant, but we shall assume that it reflects low viability caused by the *Binsinscy* chromosome. If this is the case then the lowered viability must behave autonomously in mosaics, since *Binsinscy*-bearing gynandromorphs are less frequent than *y*-bearing gynandromorphs. The main purpose of the control experiment, however, is to show that the number of *Binsinscy*-bearing gynandromorphs is approximately 47% of the number of *y*-bearing gynandromorphs. This figure will be used in interpreting the crosses involving lethal mutations.

*Experimental*

The female parents for the experimental crosses were from balanced lethal stocks *y l/Binsinscy* where *l* represents one of a series of 39 EMS-induced sex-linked lethals. The male parents were *R(1)2 w*$^{vc}$, as in the control. These crosses are identical to the control except for the presence of the lethal, and the *Binsinscy*-bearing gynandromorphs which are produced provide an internal control for the frequency of ring-X elimination. The lethals can be divided into the following two classes according to their behavior in these crosses:

*Class 1. Lethal-bearing gynandromorphs do not survive.* An example of this

TABLE 1

*Progeny from* y/Binsinscy ♀ × R(1)2w$^{vc}$ ♂

| ♂ | | ♀ | |
|---|---|---|---|
| *y* | 645 | *y/R(1)2 w*$^{vc}$ | ♀ 74 |
| | | | gyn. 72 |
| *Binsinscy* | 260 | *Binsinscy/R(1)2 w*$^{vc}$ | ♀ 60 |
| | | | gyn. 34 |

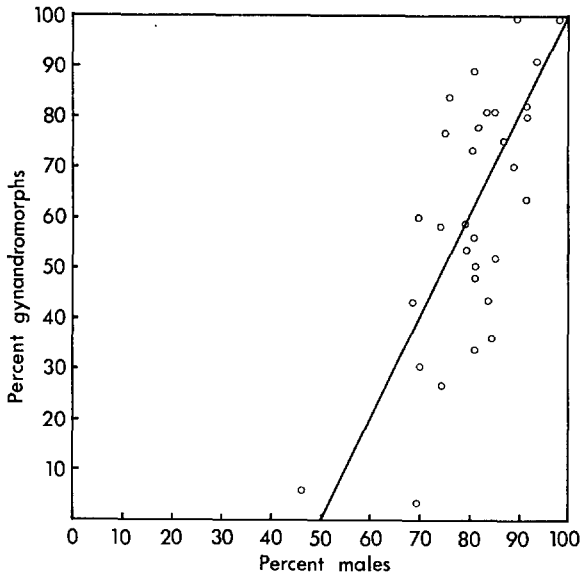


FIGURE 1.—Relationship between sex ratio (males as percent of total) and frequency of gynandromorphs (as percent of XX zygotes) in the progeny of individual *R(1)2 $w^{vc}$* males. Sample sizes 69–648.

class of results *(l(1)M22)* is shown in Table 2. No lethal-bearing gynandromorphs were observed even though it can be calculated from the data of Table 1 and from the number of *Binsinscy*-bearing gynandromorphs that approximately 42 should have been produced. Hence it can be concluded that those gynandromorphs in which this lethal is uncovered in the male tissue invariably die at some stage in their development. An alternative possibility is that the male tissue in these gynandromorphs degenerates and is replaced by regulation from the female tissue. However, this would be reflected as an increase in the number of heterozygous lethal female progeny relative to the number of heterozygous *Binsinscy* female progeny, and no such increase is evident in the data for any of the sixteen lethals in this class.

In the case of one of the lethals in this class, *l(1)M47*, the lethal-bearing female progeny included a large number of flies in which certain imaginal disc

TABLE 2

*Progeny from* yl(1)M22/Binsinscy ♀ × R(1)2 $w^{vC}$ ♂

| ♂ | | ♀ | |
|---|---|---|---|
| *y l(1)M22* | 0 | *y l(1)M22/R(1)2 $w^{vC}$* | ♀ 18<br>gyn. 0<br>(estimated total=42) |
| *Binsinscy* | 150 | *Binsinscy/R(1)2 $w^{vC}$* | ♀ 17<br>gyn. 20 |

derivatives were absent or defective. Out of 42 flies there were six with some abdominal tergites missing, three with a half-thorax and wing missing, six with one or more legs missing and two with a missing haltere. We interpret these defective flies as being lethal-bearing gynandromorphs in which the male parts of the presumptive adult have died but where the animal as a whole survived. A possible explanation is that this mutation is a cell-lethal for imaginal discs but not for larval structures.

*Class 2. Some lethal-bearing gynandromorphs survive.* For the mutations in this class, some lethal-bearing gynandromorphs survive to adulthood and show the presence of yellow-lethal tissue which we know leads to lethality when the entire organism is of this genotype. An example of this kind of result is shown in Table 3, and in this case the number of surviving gynandromorphs is approximately 13% of the number estimated to have been produced on the basis of the control data. We shall refer to this percentage as the *gynandromorph viability* for the lethal concerned. For the 23 lethals which belong to this class, the gynandromorph viabilities ranged from 1% to 82% (see Table 5). That is, none of the lethals were compatible with regular survival of gynandromorphs, and some allowed only very rare survival. Several of the mutations in this class produced a visible abnormal phenotype in the male tissue of gynandromorphs (Table 5). Seven produced bristle abnormalities (small, sparse, twin, or abnormally oriented bristles), one produced an extreme curly-wing phenotype, and one caused apparent degeneration of ommatidia.

*Quantitative Distribution of Lethal Tissue in Gynandromorphs*

For controls and for the mutations in class 2 above, we studied the distribution of male tissue in the gynandromorphs, in order to obtain some indication of the reasons for survival of lethal tissue in the mosaic situation. For all of these samples we estimated the percentage of male tissue in a number of gynandromorphs, and a histogram of the control data is given in Figure 2. A wide variety of mosaics was found, where the amount of male tissue varied from less than 20% to more than 90%, the mean being 64%. The data for the lethals are presented in Figure 3. In these histograms, the frequencies of the various classes of lethal-bearing gynandromorphs are expressed as a percentage of the total lethal-bearing gynandromorphs estimated to have been produced, rather than of those that survived. Hence the distributions can be compared directly with the controls; the area be-

TABLE 3

*Progeny from* y l(1)M77/Binsinscy ♀ × R(1)2 $w^{vC}$ ♂

| ♂ | | ♀ | |
|---|---|---|---|
| *y l(1)M77* | 0 | *y l(1)M77/R(1)2 $w^{vC}$* | ♀ 33<br>gyn. 17<br>(13% of estimated total of 127) |
| *Binsinscy* | 355 | *Binsinscy/R(1)2 $w^{vC}$* | ♀ 26<br>gyn. 60 |

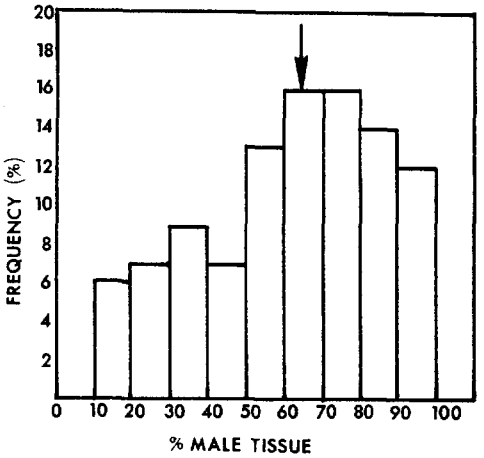


FIGURE 2.—Frequency of gynandromorphs with different sized areas of male tissue in the control series (100 gynandromorphs). Percent male tissue was determined by counting the number of male imaginal disc and histoblast derivatives out of the total of 37, with mixed derivatives scored as 0.5. The arrow indicates the position of the mean (64%). This method differs slightly from that of some previous authors (e.g., GARCIA-BELLIDO and MERRIAM 1969; HOTTA and BENZER 1972) who have scored individual bristles as representing particular regions of the adult.

tween the two curves (experimental and control) in each case represents those mosaics which are missing from the population as a result of the effects of the lethal mutation.

In every case, the average fraction of male tissue in lethal-bearing gynandromorphs is lower than that in the controls, and ranges from 6% to 44%. There is some correlation between gynandromorph viability and the average fraction which is male (Table 5); that is, when lethal-bearing gynandromorphs survive only rarely, the survivors tend to have only small areas of lethal tissue. In one case we were able to classify both the dead and surviving gynandromorphs, since the mutation *(l(1)M25)* caused lethality in the pharate adult stage (Figure 4). Here it can be seen that those gynandromorphs which survive tend to be predominantly female, whereas those which die tend to be predominantly male.

*Spatial Distribution of Lethal Tissue in Gynandromorphs*

The data given above show that the lethal-bearing gynandromorphs which survive to adulthood may be a non-random sample of the lethal-bearing gynandromorphs which were generated, in the sense that they are those containing small amounts of male tissue. We have also determined whether they are a non-random sample in the sense that they might include only those mosaics where a certain part of the animal contains or does not contain male tissue. To answer this question, we considered the imaginal disc derivatives individually and determined the frequencies with which they were male or female in the controls and in the lethal-bearing gynandromorphs of class 2. Some of these data are presented in Table 4. In the controls, the percentage of male structures is roughly equal for all of the imaginal disc derivatives; this is because of the indefinite orientation of the

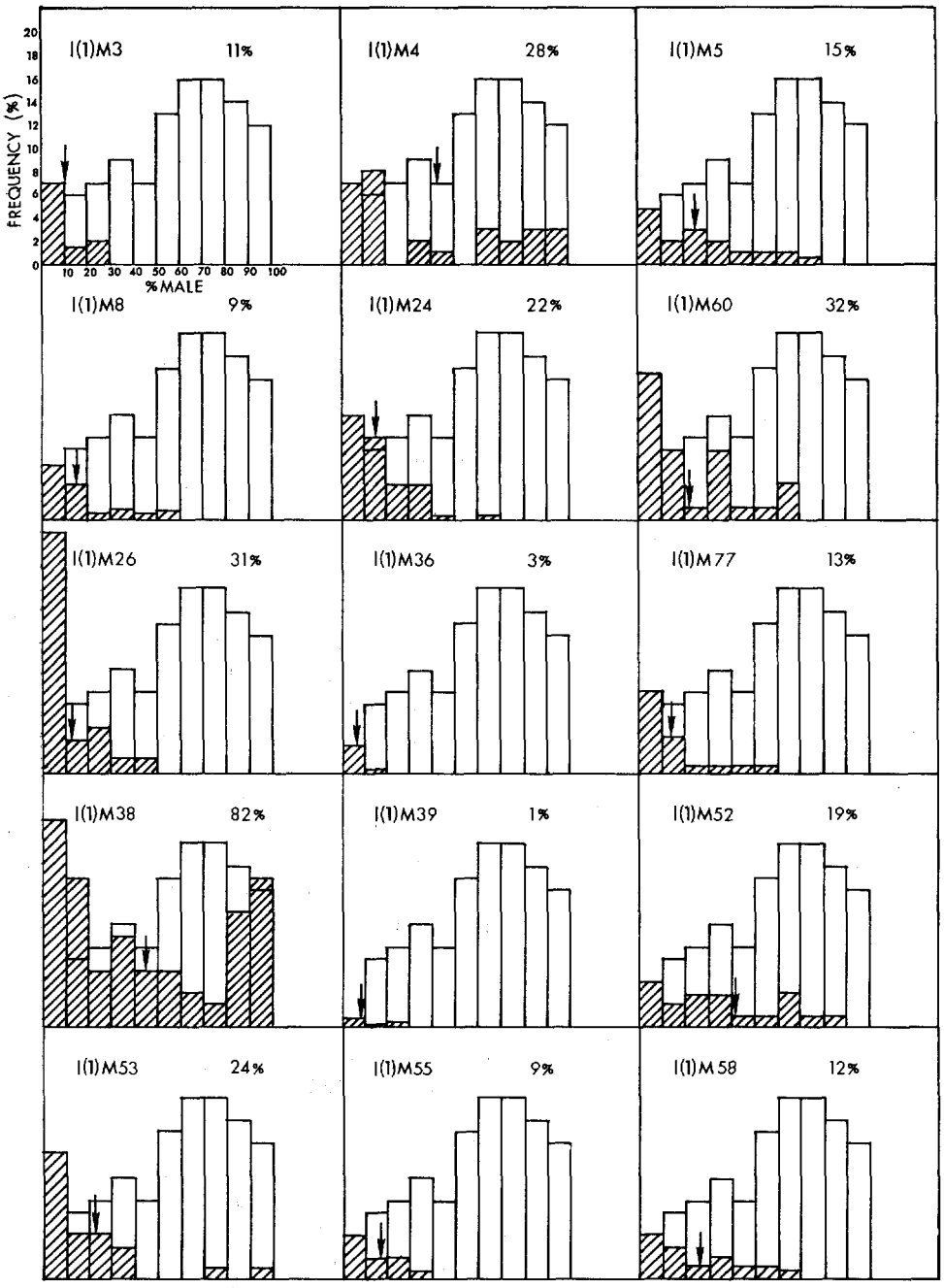


FIGURE 3.—Frequency of gynandromorphs with different sized areas of male tissue in the experimental series, each compared with the control distribution. Frequencies are expressed as a percentage of the estimated total lethal-bearing gynandromorphs, not just surviving animals. Arrows indicate the positions of the means; the percentage figures to the right in each panel are the gynandromorph viability figures.

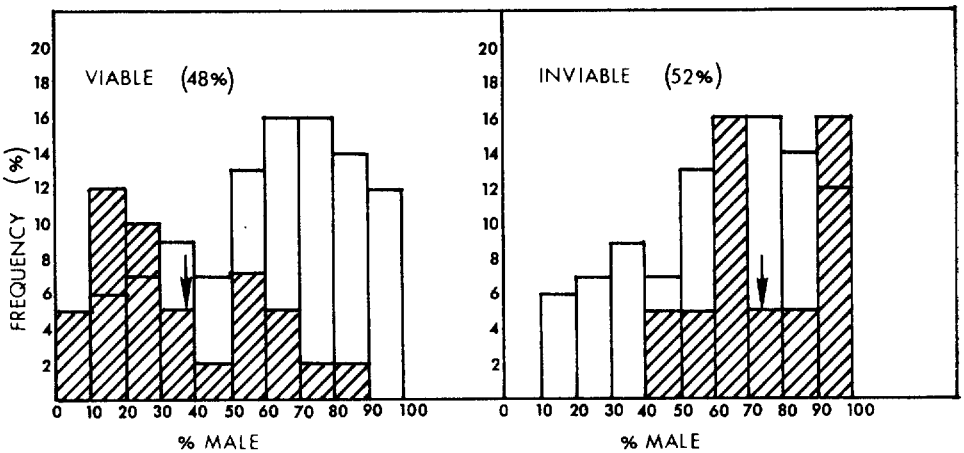


FIGURE 4.—Frequency of gynandromorphs with different sized areas of male tissue in surviving and non-surviving mosaics for *l(1)M25*, a pharate-adult lethal. Each distribution is shown in comparison with the control distribution. Arrows indicate the positions of the means: 36% for viable mosaics, 73% for inviable mosaics.

early cleavage divisions in the embryo, and it has been noted by previous workers (e.g., PARKS 1936; GARCIA-BELLIDO and MERRIAM 1969). For 17 of the 23 class 2 lethals, there is a uniform reduction of male tissue over all regions of the mosaics, and data for four of these mutations are included in Table 4. But for six of the lethals, some parts of the animal are much more likely than others to include male tissue. Table 4 shows four cases where there are greater than fivefold differences in the frequency of appearance of male tissue in different regions. For all six of the mutations showing this kind of result, the abdomen tends to include male tissue more often than does the thorax, indicating perhaps that these lethals produce their deleterious effects somewhere in the anterior part of the animal. In some cases, head structures seem to be precluded from carrying male tissue, whereas in other cases the thorax seems to be affected.

TABLE 4

*Percent of male imaginal disc derivatives in control and in lethal-bearing gynandromorphs*
(Mixed derivatives counted as 0.5)

| Genotype of male tissue | Percent male | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Eye | Antenna | Proboscis | Humerus | Wing | Legs | Tergites | Sternites | Genitalia |
| *y* | 66 | 66 | 64 | 63 | 61 | 64 | 60 | 60 | 51 |
| *y l(1)M4* | 40 | 36 | 34 | 31 | 38 | 42 | 36 | 41 | 42 |
| *y l(1)M8* | 13 | 14 | 13 | 12 | 11 | 12 | 16 | 14 | 20 |
| *y l(1)M24* | 17 | 16 | 14 | 10 | 17 | 17 | 16 | 16 | 9 |
| *y l(1)M55* | 16 | 16 | 12 | 11 | 14 | 14 | 19 | 11 | 9 |
| *y l(1)M26* | 13 | 10 | 3 | 8 | 1 | 3 | 18 | 10 | 13 |
| *y l(1)M36* | 2 | 2 | 4 | 2 | 3 | 1 | 14 | 2 | 9 |
| *y l(1)M39* | 0 | 0 | 0 | 0.5 | 0.5 | 1 | 19 | 13 | 22 |
| *y l(1)Y107* | 11 | 12 | 11 | 5 | 7 | 6 | 27 | 11 | 7 |

TABLE 5

*Summary of the characteristics of the lethals studied*

| Lethal | Approx. locus | Lethal stage: Embryo | Larval instar: 1st | Larval instar: 2nd | Larval instar: 3rd | Pupa: Early | Pupa: Late | Gynandromorph viability, percent | Average percent male in gynandromorphs | Phenotype of male tissue in gynandromorphs |
|---|---|---|---|---|---|---|---|---|---|---|
| M1 | ND | + | .. | .. | .. | .. | .. | 0 | .. | ..... |
| M3 | ND | .. | + | .. | .. | .. | .. | 11 | 10 | Abnormal bristles |
| M4 | 44 | .. | + | .. | .. | .. | .. | 28 | 42 | Normal |
| M5 | ND | .. | .. | + | .. | .. | .. | 15 | 23 | Normal |
| M8 | 57 | .. | + | .. | .. | .. | .. | 9 | 15 | Cuticle incompletely tanned |
| M9 | 37 | .. | + | .. | .. | . | .. | 0 | .. | ..... |
| M11 | 56 | .. | .. | .. | .. | + | .. | 0 | .. | ..... |
| M16 | 57 | .. | + | .. | .. | .. | .. | 0 | .. | ..... |
| M19 | ND | + | .. | .. | .. | .. | .. | 0 | .. | ..... |
| M22 | ND | .. | .. | + | .. | .. | .. | 0 | .. | ..... |
| M24 | 52 | + | + | .. | .. | .. | .. | 22 | 16 | Normal |
| M25 | 46 | .. | .. | .. | .. | .. | + | 48 | 36 | Normal |
| M26 | 41 | .. | + | + | + | .. | .. | 31 | 11 | Normal |
| M36 | 41 | .. | .. | + | .. | .. | .. | 3 | 6 | Abnormal bristles |
| M38 | ND | .. | .. | .. | + | .. | .. | 82 | 44 | Normal |
| M39 | 39 | .. | .. | .. | + | .. | .. | 1 | 9 | Abnormal bristles |
| M42 | 57 | + | .. | .. | .. | .. | .. | 0 | .. | ..... |
| M46 | 12 | .. | .. | .. | .. | + | .. | 0 | .. | ..... |
| M47 | 11 | .. | .. | .. | .. | + | .. | 0 | .. | ..... |
| M52 | 44 | .. | .. | .. | .. | .. | + | 19 | 40 | Normal |
| M53 | 34 | .. | + | .. | .. | .. | .. | 24 | 21 | Normal |
| M54 | 15 | .. | .. | .. | + | + | .. | 0 | .. | ..... |
| M55 | 3 | + | .. | .. | .. | .. | .. | 9 | 15 | Normal |
| M58 | 28 | .. | + | + | .. | .. | .. | 12 | 24 | Curly wings, abnormal bristles |
| M60 | ND | .. | + | .. | .. | .. | .. | 32 | 21 | Normal |
| M64 | 13 | .. | .. | + | .. | .. | .. | 41 | 42 | Normal |
| M66 | 35 | + | .. | .. | .. | .. | .. | 0 | .. | ..... |
| M70 | ND | .. | .. | .. | + | .. | .. | 24 | 26 | Abnormal bristles |
| M75 | ND | .. | + | .. | .. | .. | .. | 0 | .. | ..... |
| M77 | ND | .. | .. | .. | ND | .. | .. | 13 | 14 | Normal |
| M82 | 27 | .. | .. | .. | .. | + | .. | 6 | 17 | Abnormal bristles |
| M83 | ND | .. | .. | .. | .. | + | .. | 0 | .. | ..... |
| M84 | ND | + | .. | .. | .. | .. | .. | 0 | .. | ..... |
| M96 | ND | .. | .. | .. | ND | .. | .. | 0 | .. | ..... |
| Y8 | ND | .. | + | .. | .. | .. | .. | 0 | .. | ..... |
| Y12 | ND | + | .. | .. | .. | .. | .. | 3 | 12 | Wings incompletely expanded |
| Y80 | ND | + | .. | .. | .. | .. | .. | 20 | 21 | Normal |
| Y107 | ND | .. | + | .. | .. | .. | .. | 2 | 15 | Abnormal bristles |
| Y110 | ND | .. | .. | .. | .. | .. | + | 6 | 17 | Degenerated ommatidia |
| Total of monophasic lethals | | 8 | 10 | 4 | 3 | 5 | 3 | | | |

ND = not determined.

*Fate Mapping of Lethality*

A more accurate approach to determining the area of the organism affected by a lethal mutation is through the blastoderm fate-mapping procedure (STURTEVANT 1929; GARCIA-BELLIDO and MERRIAM 1969; RIPOLL 1972) which has been used to localize the "foci" or sites of action of behavioral mutants (HOTTA and BENZER 1972). The procedure relies on the fact that the male-female borderline in gynandromorphs lies in various directions due to the variable and apparently arbitrary orientation of nuclear division spindles in the early cleavage embryo (PARKS 1936). Thus, the frequency with which the borderline lies between any two imaginal discs is a function of their distance apart at the time of their origin. Hence these relative distances can be deduced from the relative frequencies with which the corresponding pairs of imaginal disc derivatives are of opposite sex in the adults (cases where one or both of the structures are themselves mosaic are counted as 0.5 in determining these frequencies). The unit of distance in these maps is the *sturt*, which is defined as a distance such that the boundary line in gynandromorphs passes between the derivatives in 1% of the cases (HOTTA and BENZER 1972). A fate map produced by this technique using 100 control gynandromorphs is shown in Figure 5, and it agrees in most respects with the maps put forward by GARCIA-BELLIDO and MERRIAM (1969), HOTTA and BENZER (1972), and HALL, GELBART and KANKEL (1973).

The fate mapping technique can be extended to determining the sites of action (foci) in the animal of lethal mutations. For lethal mutations with localized effects, gynandromorphs are of four types with respect to the lethal focus and a given marker structure:

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| marker: | ♂ | ♀ | ♀ | ♂ |
| focus: | ♂ | ♀ | ♂ | ♀ |

The first and third classes of animals will die since the lethal focus contains male tissue which is hemizygous for the lethal. The marker–focus distance in sturts is therefore given by the frequency of the fourth class in the surviving (classes 2 + 4) gynandromorphs. That is, the percent male for any structure in surviving gynandromorphs represents the distance of that marker from the lethal focus in sturts.

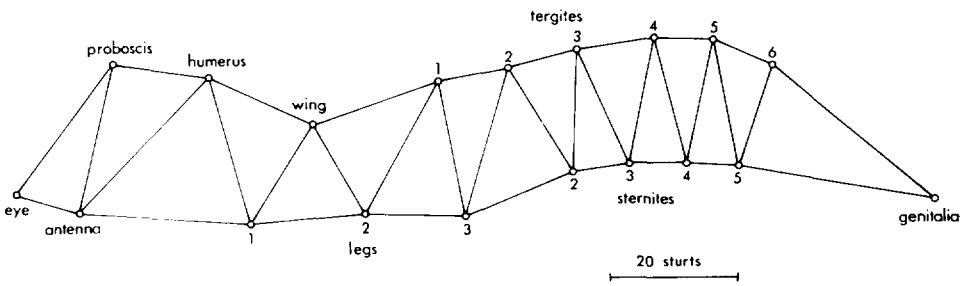


FIGURE 5.—Fate map of the imaginal disc precursors derived from the control gynandromorph data. See text for explanation.

We have carried out this kind of mapping for the six mutations identified in the previous section as having a localized site of action, and the data are presented in Figure 6. In these diagrams, circles are drawn around markers with the radii equal to the marker–focus distances; thus, markers enclosed by small circles are close to the lethal focus, and those surrounded by larger circles are distant from it. It is clear that in each case the lethal focus does not map to a point, but it is possible to recognize certain regions which are characteristic of each mutant. For instance, the foci of *l(1)Y107* and of *l(1)M26* seem to be in the thorax, while *l(1)M39* and *l(1)M36* seem to affect both thorax and head.

*Lethal phases:* The approximate lethal phases (i.e. times of death) for the mutations studied are shown in Table 5. It is clear that the sample includes various kinds of embryonic, larval, and pupal lethals, and that there is no obvious relationship between lethal phase and gynandromorph viability.

## DISCUSSION

These results show that about 40% of lethal mutations are incompatible with survival when about half of the organism has the lethal genotype, irrespective of which part of the organism is affected. Some of the lethals in this group are expected to be "cell lethals"—that is, mutations affecting functions which are necessary to the survival of every cell. This class would also include mutations which blocked cell multiplication, and such defects could be distinguished from cell lethals by studying them in mosaics produced by somatic crossing over. Experiments of the latter type were performed by DEMEREC (1936), who showed that about 40% of X-ray induced lethals would prevent the survival of small homozygous clones generated by somatic crossing over, and by RIPOLL and GARCIA-BELLIDO (1973) who showed that 16.3% of EMS-induced lethals were of this cell-lethal type. It therefore seems likely that many of the gynandromorph-inviable mutants reported here are in fact cell lethals, and we are now testing them by the somatic crossing over technique.

The remaining 60% of lethals can survive as male sectors in some gynandromorphs, but it is clear that those gynandromorphs which do survive are predominantly those with a relatively small area of male tissue, as was found to be the case for *lethal (1) melanoma-like* (OSTER and SOBELS 1956). In many cases, surviving gynandromorphs can have a smaller male sector than is ever found in control (non-lethal) gynandromorphs, indicating that the hemizygous lethal in those cases inhibits the growth of male tissue or causes a limited amount of cell death.

There are two possible reasons for the survival of hemizygous lethal tissue in gynandromorphs. The first is that the lethal may be non-autonomous; that is, the lethal cells are deficient in some essential material which can be supplied by the surrounding non-lethal tissue in the mosaic. Thus, only those lethal mutations which block the formation of diffusible materials are expected to be in this class. If a comparison can be made with non-lethal mutants, we might expect this type of mutant to be quite rare, since almost all of the eye-pigment mutants, pattern mutants, and behavioral mutants which have been studied behave autonomously

a
I(1)M3
b
I(1)M26
c
I(1)M36
d
I(1)M39
e
I(1)M82
f
I(1)Y107
proboscis
humerus
wing
legs
eye
antenna
tergites
sternites
genitalia

in genetic mosaics or chimeras (e.g., BEADLE and EPHRUSSI 1936; STERN 1968; HOTTA and BENZER 1972). The alternative reason for survival of lethal tissue in mosaics is that the mutation causes a functional defect only in a certain part of the organism—that is, the mutation would be a locally active lethal. Then, if the site of action (focus) of the lethal mutation were mutant (male) in the mosaic the animal would die, whereas if it were non-mutant (female) the mosaic could survive. This class of mutants would, of course, include cell lethals which had a limited sphere of influence. In the simplest case of a single, small lethal focus we would expect gynandromorph viability to be 50%, whereas if the lethal focus were larger the gynandromorph viability would be either less than or more than 50%, depending on what assumptions were made about the survival of gynandromorphs in which the lethal focus itself was mosaic.

In the case of a locally active lethal the site of the primary functional defect can be determined through the analysis of survival data on a large number of lethal-bearing gynandromorphs, with various distributions of mutant tissue. Ideally, this procedure would involve a precise determination of the genotypic borderline in all of the mosaic animals. Since there are no generally useful genetic markers for the cells of the internal organs of Drosophila, in this study we have relied on cuticular markers, with detailed statistical analysis of the mosaics, to try to pinpoint the lethal foci for six of the mutations. The lethal foci determined in this way turn out to be rather large, encompassing most of the head and thorax in some cases, most of the thorax in others (Figure 6). It should be pointed out, however, that if the lethal causes a general lowering of cell viability or proliferation rate (as many of these appear to do) then this would result in a general lowering of marker-focus distances which would tend to artificially enlarge the apparent lethal focus.

In several other recent studies, the behavior of lethal mutations in gynandromorphs has been observed. RIPOLL and GARCIA-BELLIDO (1973) find that 50% of EMS-induced lethals are gynandromorph-viable, and SHANNON *et al.* (1972) find that lethal mutations in ten of the thirteen zeste-white complementation groups are gynandromorph-viable. STEWART, MURPHY and FRISTOM (1972) find that 11 of 23 pupal lethals with imaginal disc defects can survive in gynandromorphs but only at low frequencies, which "may reflect death of gynandromorphs when large body regions are hemizygous for the lethal." NOVITSKI (1963) noted that only a "small fraction" of lethals can survive as sizable patches of tissue in gynandromorphs, and that those mutations often resulted in abnormal amino-acid content. One interesting feature of several of these studies is the repeated occurrence of lethals which can survive only in the abdomen in mosaic animals. Six cases of this type are reported here; SHANNON *et al.* (1972) found four complementation groups which show this effect; four of the mutants of STEWART, MURPHY and FRISTROM (1972) are of this type; and RIPOLL and

FIGURE 6.—Fate maps for the localization of lethal foci for six of the mutations reported here. The circle around each marker has a radius equal to the distance from the marker to the lethal focus, measured in sturts.

GARCIA-BELLIDO (1973) found three mutants of this type. Furthermore, the maternal effect lethals fused and rudimentary (FAUSTO-STERLING 1971) can survive only in the abdomen of mosaic progeny from homozygous females. These findings emphasize the developmental differences between the abdomen and other structures, which are also demonstrated by the unusual cell proliferation dynamics and hormonal sensitivity of the abdomen (ASHBURNER 1970; GARCIA-BELLIDO and MERRIAM 1971; GUERRA, POSTLETHWAIT and SCHNEIDERMAN 1973).

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