BEHAVIOR OF SOMATIC CELLS HOMOZYGOUS FOR ZYGOTIC LETHALS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The behavior in genetic mosaics of 86 EMS-induced sex-linked lethals has been studied. Seventy-five percent of them are autonomous in gynandromorphs. Forty-three lethals nonviable in sex mosaics have been analyzed in X-ray-induced spots in the abdominal tergites and the imaginal wing derivatives. Of the lethals, 90.7% are homozygous viable in mosaic spots, and only 9.3% have been classified as epidermal cell lethal. Thus, the fraction of the Drosophila genome essential for cell viability has been estimated to be about 420 genes. The phenotypes at the cellular level of some cell-viable mutations altering cell parameters (mitotic orientation, differentiation, etc.) are described.

LETHAL mutations represent a vast majority of the inducible mutation in Drosophila. Mutagenesis studies in which small regions of the Drosophila genome have been saturated show that visible mutations account for a small fraction (about 10%) of all induced mutations (LIFSCHYTZ and FALK 1969; HOCHMAN 1971; JUDD, SHEN and KAUFMAN 1972). Undoubtedly, mutations in genes controlling important developmental processes are expected to be lethal, and the extensive study of this class of mutations has been essential to our understanding of Drosophila development (for review see HADORN 1955; WRIGHT 1970). Lethal mutations can be analyzed either histologically, prior to their effective lethal phase (see HADORN 1955) or later under permissive conditions such as temperature (SUZUKI 1970), *in vivo* transplantation (SHEARN *et al.* 1971) or in sex mosaics (NOVITSKI 1963; STEWART, MURPHY and FRISTROM 1972; BRYANT and ZORNETZER 1973).

One way to study the effect of lethal mutations directly at the cellular level is by means of mitotic recombination (STERN 1936) to induce the appearance of clones of cells homozygous for lethal mutations in otherwise heterozygous flies. The aim of this work is twofold: first, we would like to know what fraction of the zygotic lethals are viable in epidermal cells. Second, we wish to determine whether the analysis of lethals in genetic mosaics can be used as a way to screen for mutations altering the phenotypes of clones. The study of these new mutants should allow us to obtain a better insight into the genetic basis of fundamental developmental processes such as growth, morphogenesis and differentiation. The

approach is in essence similar to the pioneering work of DEMEREC (1934, 1936) and takes advantage of the possibility of inducing point lethal mutations, controlling mitotic recombination, using suitable cell marker mutants, and applying current knowledge on the proliferation dynamics of Drosophila imaginal structures.

Three different types of lethal mutations are expected to be found *a priori*: mutations which do not interfere with the normal behavior of epidermal cells (at least in the developing systems studied), mutations which affect cell viability (called "cell lethals" by DEMEREC), and mutations which do not affect cell viability but do alter in some way the normal behavior of epidermal cells. Some preliminary results on the viability of cells made homozygous for zygotic lethals have been previously published (RIPOLL and GARCIA-BELLIDO 1973).

MATERIALS AND METHODS

Sex-linked lethal mutations were induced with 0.25% EMS (LEWIS and BACHER 1968). In order to recover the mutations coupled with cell marker mutants at both ends of the euchromatic segment of the *X* chromosome, the treated males were *y Hw* f^{36a} (cell markers: yellow, *y*: *1*-0.0, bristle color marker, and forked *36a*, f^{36a}: *1*-56.7, bristle and trichome shape marker. Hairy wing, *Hw*: *1*-0.0, was used to help detect individuals heterozygous for the lethal mutations). Treated chromosomes were isolated over *FM7a*, $y^{31d}sc^{2}w^{a}v^{o}B$ (MERRIAM 1968) and later balanced over *FM6*. The meiotic localization of the induced lethal mutations (*l*) was done by classifying and counting the male progeny of *y Hw l* f^{36a}*/sc ec cv* ct^{6} v^{o} g^{2} *f* females. This procedure does not easily allow the detection of either chromosomal aberrations or double mutations restricted to the region defined by two contiguous markers. But since EMS infrequently induces chromosome breakage (LIFSCHYTZ and FALK 1969), all the lethal mutations localized within one of these regions were considered point mutations. All lethals will be referred to as *l(1)M* (*M* standing for Madrid) followed by the identification number assigned when they were isolated.

The effective lethal phase was determined by counting: (1) eggs laid during 18 hours by 30 *y Hw l* f^{36a}*/FM7a* females crossed to males from a wild-type strain (*Vallecas*) (2) eclosed eggs 25–26 hours after removal of the parents (3) individuals that developed up to puparium formation; and (4) adults emerged from these pupae. The stage of development during which the death of the males hemizygous for the lethal mutations preferentially takes place was thus determined from the fraction of each class which reached the subsequent stage. No attempt was made to subdivide embryonic, larval or pupal stages.

Gynandromorphs were obtained among the progeny of *y Hw l* f^{36a}*/FM7a* females crossed to *R(1)2*, w^{vC}*/y+Y* males. The ring chromosome had been previously selected for high instability. The mean frequency of *FM7a*/Ring control gynandromorphs obtained in the different crosses was 68% of the *FM7a*/Ring progeny.

Mitotic recombination was induced in larvae of the genetic constitution *y Hw l* f^{36a}*/+*; *mwh jv/+*. The cell markers in the third chromosome (multiple wing hairs, *mwh*: *3*-0.1, trichome marker, and javelin, *jv*: *3*-19.2, bristle shape marker) provided an internal control. *y Hw l* f^{36a}*/FM7a* females were crossed to *mwh jv* males and were allowed to lay eggs for 5 days in split bottles (MORATA and RIPOLL 1975). The larvae were then X irradiated in the lower part of the split bottle (Philips MG 15/Be, 100 Kv, 15mA, 2mm Al filter) at a rate of 330 roentgens per minute to a final dose of 1,000 r. Larvae irradiated at different ages were collected as pupae at daily intervals for 5 days. Ages will therefore be expressed in hours between irradiation and puparium formation. The adults were allowed to age 2–3 days and then cooked in 10% KOH, dissected and mounted in euparal for examination under the compound microscope. Lethal-carrying gynandromorphs were also mounted and studied in the same way.

All the experiments were carried out at 25 ± 1° using standard Drosophila medium. For a description of the mutants and chromosomes used see LINDSLEY and GRELL (1968). The growth

parameters of the cellular systems in which the epidermal mosaics were studied can be found in BRYANT (1970) and GARCIA-BELLIDO and MERRIAM (1971a) for the imaginal wing disc, and in GARCIA-BELLIDO and MERRIAM (1971b) and GUERRA, POSTLETHWAIT and SCHNEIDERMAN (1973) for the abdominal histoblasts.

RESULTS*

I *Induction and characterization of lethals*

Of the 657 EMS-treated *X* chromosomes tested, 94 (14.3%) were found to carry lethal mutations. Five of them were later discarded since in subsequent generations some *y Hw* f^{s6a} males appeared, indicating that sub- or semilethal mutations (see HADORN 1955) had been induced. The remaining 89 strains (13.5%) were kept as carriers of *X* chromosomes with at least one lethal mutation.

The next step was to localize these 89 lethal mutations by meiotic recombination. Eight of them could not be properly mapped for various reasons: (1) Three were either double mutations or inversions (the meiotic behavior is similar in both cases and no cytological analysis was undertaken). (2) Four were either mutant or deficient for one of the markers used for the meiotic location, i.e., one failed to complement with cross-veinless, one with vermilion and two with garnet. (3) For one of them many escapers appeared in that particular cross. For further analysis, all the mutations, except those 3 included in the first class, were handled as if they were lethal point mutations. The distribution along the chromosome of the 81 lethals that could be mapped is similar to previously published distributions concerning both chemically and X-ray-induced sex-linked lethals. (RUDKIN 1965; SUZUKI 1970; STEWART, MURPHY and FRISTROM 1972).

The determination of the effective lethal phase of the 86 lethals was based on an average of 385 eggs. The distribution in effective lethal phases of these mutations is shown in Table 4. 65 out of the 86 mutations (75.6%) appear to be monophasic, 18 (20.9%) diphasic and 3 (3.5%) triphasic, which is consistent with the results obtained by HADORN and CHEN (1952). As a rule the distribution of the effective lethal phases is similar to those published by these and other authors, for both spontaneous and induced lethal mutations in the *X* chromosome (OSTER 1952; EDE 1956) as well as in the autosomes (RIZKI 1952).

II *Autonomy in gynandromorphs*

The loss of Catcheside's ring chromosome in a *y Hw l* f^{s6a}/Ring female zygote leads to the appearance of two genetically different cell lineages, one of which is hemizygous for the lethal mutation as well as for the cell markers. Since the loss of the ring chromosome takes place during the first zygotic divisions (RIPOLL 1972), the initiation of mosaicism must be prior to any phenocritical phase and the hemizygous tissue must embrace about one half of the mosaic embryo. Such a situation has an effect upon the viability of the mosaic individual, depending on the nature of the induced mutation. If the normal allele of a gene is respon-

* Due to the number of mutations studied here, a large amount of data has been collected, particularly concerning the clonal analyses. It was deemed unnecessary to include all the data in this paper since only a presentation of the general characteristics of these classes of mutations was desired. The data, however, are available upon request from the author.

sible for the production, somewhere in the metabolic chain, of substances that can diffuse through cell membranes (a nonautonomous gene expression), in a genetic mosaic the lethal phenotype of the lethal allele can be rescued by the presence of diffusible products made in the tissue of viable genotype, and the viability of all the mosaic individuals will be normal. Nonautonomy has been the classical interpretation for the recovery of lethal-bearing gynandromorphs (NOVITSKI 1963; STEWART, MURPHY and FRISTROM 1972). On the other hand, the lethal effect of an autonomous mutation will be restricted to the tissue or organ where the presence of the normal gene product is essential. In this case the viability of each gynandromorph will depend on the tissue specificity of the mutation, the size of the area of the blastoderm giving rise to the tissue where the mutation is essential and the distribution of the hemizygous tissue in that particular mosaic embryo. As a result of autonomy, the probability of recovery of gynandromorphs for a particular lethal should decrease (BRYANT and ZORNETZER 1973). In those cases when the normal gene product is needed in widely distributed cell types, all the gynandromorphs will die. Thus, complete lack of viability is not the only reflection of autonomy, but a particular case.

In order to have an estimate of the approximate fraction of lethals which are autonomous in gynandromorphs, a "coefficient of viability" (ratio of lethal-bearing gynandromorphs to control gynandromorphs recovered in the same cross) has been calculated for each of the 86 mutations. A given lethal was classified as nonviable in gynandromorphs when no mosaic flies were found among $y\ Hw\ l\ f^{s6a}$/Ring females while at least 25 mosaics were found among control sister females (*FM7a*/Ring). Figure 1 shows the distribution (in percent of the total number of lethals) of these coefficients of viability. Figure 1 also shows the distribution of coefficients of viability found for females heterozygous for the same 86 mutations ($y\ Hw\ l\ f^{s6a}$/+) relative to control sisters (*FM7a*/+) resulting from crossing $y\ Hw\ l\ f^{s6a}$/*FM7a* females to wild-type males (Vallecas). This variation in viability found for lethal-carrying non-gynandromorph flies has been used as a control. The coefficients of viability of females heterozygous for lethals seem to follow a normal distribution (mean 1.07) corroborating previous observations of STERN *et al.* (1952) that heterozygosity for lethal mutations does not affect overall viability. A similar normal distribution (mean 0.94) was also found for the coefficients of viability of *FM7a* males relative to their *FM7a*/+ sisters. Comparison of the distributions shown in Figure 1 reveals that in a large fraction of the lethals the presence of hemizygous tissue leads to the death of either all or a large fraction of the mosaic flies.

In order to evaluate the fraction of mutations that decrease the viability of gynandromorphs, and therefore are autonomous, a limit of the coefficient of viability has been defined. The class corresponding approximately to the point where both distributions shown in Figure 1 seem to cross each other has been taken as an arbitrary limit. If according to this criterion, one takes as an arbitrary limit the class 0.30–0.35 and defines as autonomous mutations those with coefficients of viability equal to or lower than 0.30, this type of mutation would account for 75.5% of the induced lethal mutations. Out of the 65 mutations

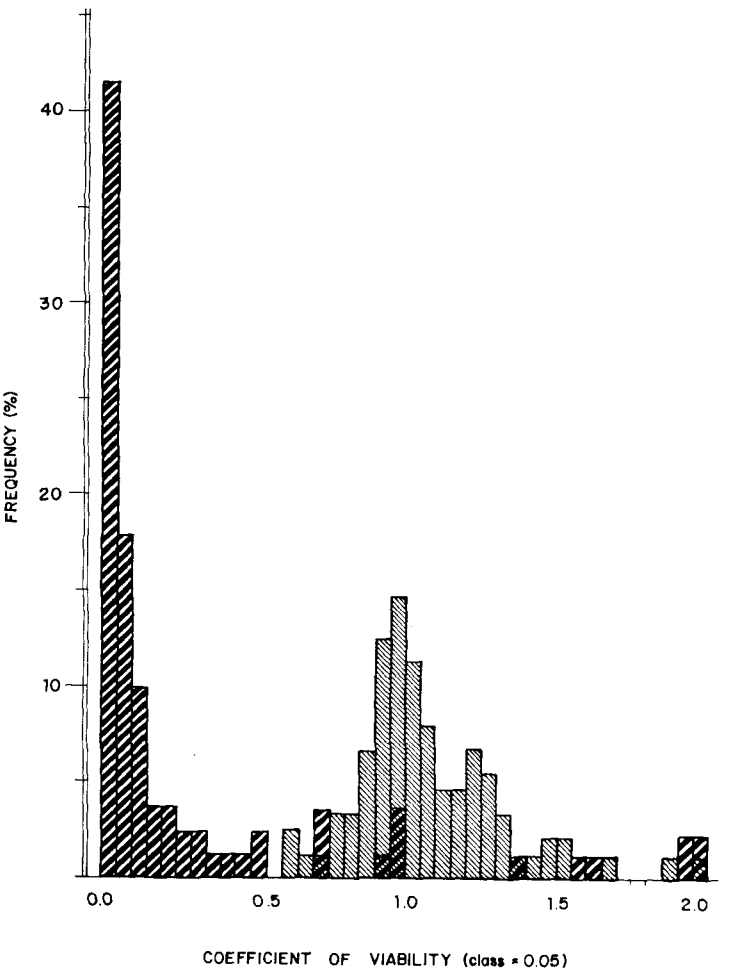

FIGURE 1.—Distribution of the coefficients of viability in gynandromorphs (wide strips) and heterozygous females (thin strips) found for 86 lethals.

thus defined as autonomous 37 (56.9%) were not viable at all in gynandromorphs, 6 (9.2%) were viable only when the hemizygous tissue affected abdominal structures and 22 (33.9%) exhibited marked tissue both in abdominal and dorsal thoracic structures. No attempt was made to classify autonomous mutations according to either their mosaicism in other regions or the distribution and size of male patches, although dfferences in both were found as some other authors have already reported (STEWART, MURPHY and FRISTROM 1972; SHANNON *et al.* 1972; BRYANT and ZORNETZER 1973).

III *Homozygosis of zygotic lethals in epidermal cells*

Mitotic recombination was induced in 43 experiments for the analysis of 37 lethals which were nonviable in gynandromorphs and 6 in which lethal tissue in gynandromorphs was restricted to abdominal structures.

In order to evaluate the behavior of cells made homozygous for lethals, the frequency, size, distribution and differentiation of their clones have been compared with the same characteristics of control clones arising in the same individuals after mitotic recombination in the left arm of chromosome 3, and therefore under identical experimental conditions. Except for those mutations proximal to forked, and even then as rare exceptions, all those clones marked simultaneously with yellow and forked must be homozygous for the corresponding lethal. Clones marked only with forked, arising from double mitotic recombination (STERN 1936; GARCIA-BELLIDO 1972) have not been taken into consideration. All the internal control clones (always heterozygous for the lethals) are marked with multiple wing hairs and a fraction of them also with javelin. Clones marked only with javelin have not been taken into account.

A. *Dorsal abdominal histoblasts:* Although the response of the abdominal histoblasts to radiation-induced mitotic recombination does not change throughout larval development because these cells do not divide during this period (GARCIA-BELLIDO and MERRIAM 1971b; GARCIA-BELLIDO 1972; GUERRA, POSTLETHWAIT and SCHNEIDERMAN 1973), only abdomens from flies irradiated 48–72 hours before puparium formation have been studied in order to have all the experimental conditions as uniform as possible. The evaluation of both mean sizes and frequencies has been based on clones embracing more than one bristle since, in normal nonlethal carrying flies, single bristle clones seem to derive primarily from spontaneous mitotic recombination after puparium formation and their frequency is not directly related to the dose of X irradiation during larval stages (GARCIA-BELLIDO and MERRIAM 1971b; GARCIA-BELLIDO 1972). Only tergites II to VI have been scored, and all equally marked bristles in the same hemitergite were considered to belong to the same clone.

Table 1 shows the clone frequencies found for the different cell markers used. Values given for sex-linked markers have been calculated from data obtained with those lethals that do not interfere with epidermal cell viability (see below). Considering the experimental variations that could take place under the present conditions, one expects that in different irradiation experiments the relative frequency of clones marked with two different cell markers in different chromo-

TABLE 1

Frequencies (mean number of clones per abdomen) and mean sizes (in number of bristles) of clones embracing more than one bristle arising in y Hw l f^{36a}/+; mwh jv/+ *females*

Cell* marker	Number of† experiments	Frequency	Size
y	32	1.79	3.9
f^{36a}	32	0.99	3.5
mwh	43	0.75	—
jv	43	0.55	4.1

* For sex-linked cell markers only data obtained from noncell-lethal mutations have been considered (see text).

† Data correspond to 20 abdomens per experiment.

some arms would remain constant unless the presence of a particular lethal had modified such a relationship. In order to have a criterion to evaluate the effect of the homozygosity of lethals on the viability of the abdominal histoblasts (and thus define cell lethality) the ratio of frequencies (*Rf*) of clones homozygous for each mutation ($y\ f^{36a}$) relative to the corresponding control clones (*mwh*) in the same flies has been used as a coefficient of cell viability.

Figure 2 shows the distribution of such coefficients obtained from 42 out of the 43 lethal mutations studied (one lethal had a phenotype that made impossible the evaluation of both frequencies and sizes of $y\ f^{36a}$ clones in tergites). From this distribution 8 mutations ($Rf<0.2$) were defined as nonviable (cell lethal), and 32 ($Rf\geq 0.8$) were considered viable (noncell-lethal) in the abdominal histoblasts. All those lethals whose viability in gynandromorphs was restricted to abdominal structures appeared to be included in the latter class. As discussed later, the intermediate viability of the mutations corresponding to the class $0.4 \leq Rf < 0.6$ is possibly due to causes other than partial cell lethality, and they were therefore classified as noncell-lethal in the abdominal histoblasts. The *Rf* values found for noncell-lethal mutations seem to follow a normal distribution. Data obtained from these mutations were used to calculate the values given in Table 1 for sex-linked cell markers.

In 4 of the eight mutations classified as cell lethal, $y\ f^{36a}$ clones embracing more than one bristle have been found, although with a very low frequency ($0.0 \leq Rf < 0.2$). Two of the four mutations are proximal to forked and the clones detected must be due to mitotic recombination between the locus of forked and that of the lethal; in fact, the mean size of these clones is normal (3.8 and 2.6

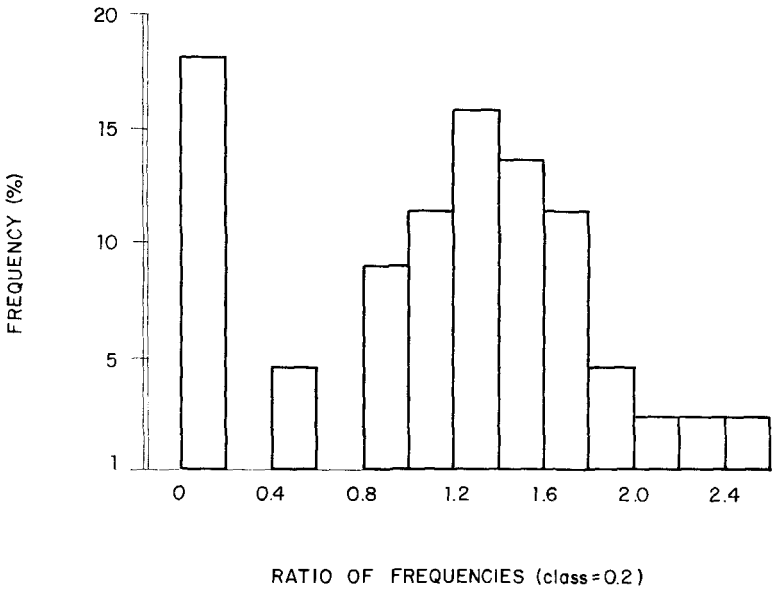

FIGURE 2.—Distribution of the ratios of frequencies (*Rf*) obtained in abdominal tergites for 42 lethal mutations nonviable in gynandromorphs.

bristles, respectively). With the remaining two mutations, very few $y\ f^{36a}$ clones were found. Such clones could be due to either: (a) triple mitotic recombination and thus no longer include the lethal, or (b) to more than one mitotic recombination event in two or more histoblast cells of the same nest, and thus represent several single bristle clones. It seems plausible to think that all these 8 mutations are, in the dorsal abdominal histoblasts, completely cell lethal. The fact that in all of them, $y\ f^{36a}$ clones of only a single bristle were found is compatible with cell lethality and will be discussed later.

Two mutations appeared to be partially cell lethal in the abdominal histoblasts (Figure 2, $0.4 \leq \text{Rf} < 0.6$). Both were localized in the distal part of the *X* chromosome (*sc–ec*). In one of them the frequency of *y* clones (arising from mitotic recombination distal to *forked*) was 99% of that expected according to the frequency of *mwh* control clones in that experiment, showing that the lethal mutation was perfectly cell viable. The other mutation showed, together with a decrease in the frequency of *y* and $y\ f^{36a}$ clones embracing 2 or more bristles, a decrease in the mean size of these spots (0.46 that of the control *jv* clones), and an increase in the frequency of single bristle spots. As will be discussed later such behavior is expected for mutations lowering cell division rate without altering cell viability (MORATA and RIPOLL 1975).

B. *Imaginal wing discs:* In contrast to the abdominal histoblasts, which do not divide during larval development, the cells giving rise to the dorsal mesothorax divide exponentially throughout the larval period, stopping shortly after puparium formation (BRYANT 1970; GARCIA-BELLIDO and MERRIAM 1971a).

As before, the effect of the homozygosis of lethals upon the behavior of wing imaginal disc cells was deduced comparing the frequency, shape and size of clones of cells homozygous for the lethal and those of the control clones arising in the same flies. Since wing trichomes cannot be scored for yellow, a fraction of the f^{36a} clones arising from double mitotic recombination events (one exchange proximal to forked and other between forked and the locus of the lethal mutation) can be erroneously considered as being homozygous for the lethal. The expected frequency of such events is very low (GARCIA-BELLIDO 1972), and it can be detected when the clone embraces bristles; in this sense it was useful that $y\ f^{36a}$ clones were simultaneously homozygous for Hairy wing, which autonomously produces the appearance of extra bristles in the wing surface (GARCIA-BELLIDO and MERRIAM 1971c). Since f^{36a} clones embracing normal colored bristles could be discarded, the frequency of forked clones heterozygous for lethal mutations that could be misinterpreted was low enough so as to not interfere with the results.

Evaluation of frequencies and sizes of clones was restricted to the wing surface, where each trichome corresponds to a single cell (DOBZHANSKY 1929) and where with the cell markers used clones of one single cell can be detected. In the mesonotum, with growth parameters similar to the wing blade (BRYANT 1970; GARCIA-BELLIDO and MERRIAM 1971a; RIPOLL 1972), only those $y\ f^{36a}$ clones embracing bristles can unequivocally be scored, and consequently the evaluation of clone sizes in number of cells is not as accurate as it is in the wing

surface. The analysis of the mesonotum has therefore been restricted to the presence or absence of clones and their phenotype.

As was previously done with the abdominal histoblasts, homozygosis was induced for the 43 lethal mutations that were either inviable or viable only in abdominal structures in gynandromorphs. In order to look for possible differences among different larval stages, individuals irradiated at various developmental stages were studied, grouping them, depending on their age at the moment of irradiation, in 5 classes: 0–24, 24–48, 48–72, 72–96 and 96–120 hours between irradiation and puparium formation. Since irradiation early in development produces a delay in pupariation (GARCIA-BELLIDO and MERRIAM 1971a) these ages approximately correspond to: 0–24 and 24–48 to late and early 3rd instar, respectively; 48–72 and 72–96 to the second and first halves of the 2nd instar, respectively; and 96–120 to the beginning of the 2nd and the end of the 1st instar. For all of the mutations studied, forked clones induced at the end of development (0–24 hours) were found. Since the behavior of clones homozygous for all the lethals tested was almost identical when initiated later than 24 hours before puparium formation, unless otherwise stated only those results obtained with irradiation prior to this period will be considered.

Table 2 shows the mean values of frequencies (mean number of clones per wing disc) and sizes (average number of cells per clone) of control *mwh* clones. The figures represent averages of mean values obtained after 43 irradiation experiments, one for each lethal, and are similar to those previously reported (BRYANT 1970; GARCIA-BELLIDO and MERRIAM 1971a). The frequency (reflecting number of target cells) increases and the size (reflecting number of cell divisions needed to reach final differentiation) decreases exponentially with proceeding development. The analysis of forked clones compared with their internal control (*mwh*) allowed us to classify the 43 lethal mutations into two major groups corresponding to two differential responses to X irradiation during development:

(1) *Mutants viable in clones during the entire disc development.* The cell behavior of 22 out of 43 mutations studied in wing imaginal cells is similar, both

TABLE 2

Frequencies (mean number of clones per disc) and sizes (mean number of cells per clone) of control clones (mwh) *arising in the wing blade after mitotic recombination during larval development*

Age (hours)	Mean no. of discs per experiment	Frequency	Size
24–48	16	3.69	13
48–72	23	1.06	41
72–96	54	0.29	137
96–120	92	0.14	331

Figures represent mean values of 43 irradiation experiments. Ages are given with respect to pupariation.

in frequency and size of clones, to that expected in the absence of the lethal. Figure 3 shows the behavior of these 22 lethals. Each value represents the average of the means obtained for the 22 experiments after irradiation at a given age. As a reference, the values expected in the absence of lethal mutations have been represented as the regression lines obtained for *mwh* internal control clones (actual data in Table 2). The 22 mutations (25.6% of all lethals) showing this cell behavior were classified as nonlethal in wing imaginal cells. All of them happened to have been previously classified as noncell-lethal in the abdominal histoblasts. All those mutations whose viability in gynandromorphs was restricted to abdominal structures were also included in this group.

(2) *Mutants viable in clones during only part of disc development*: The remaining 21 lethal mutations show as a whole a common cell behavior characterized by the absence of forked clones induced during early stages of development and, when clones are found, by a decrease in both their frequency and size as expected on the basis of their respective control clones. As an example, Figure 4 shows the kinetics of three mutations classified within this group. The cell behavior of the rest of the lethal mutations included in this class was either similar to one of these three, depending on the developmental stage after which clones could be found, or no clones were detected prior to 24 hours before puparium formation. The stage of larval development when the first clones of cells homozygous for a given lethal mutation can be found will be referred to as the *viability limit* of that lethal.

The proliferation dynamics shown by these mutations are similar to the kinetics observed for two different biological phenomena: *perdurance* (GARCIA-BELLIDO and MERRIAM 1971c) and *cell competition* (MORATA and RIPOLL 1975). In the first case, after a specific time in development, cells made homozygous for a recessive allele show the dominant phenotype even though prior to that moment the recessive trait behaved cell autonomously. *Perdurance* of a trait can be interpreted either as a reflection of the time of development when gene activity stops or is not needed, or as due to the presence in the cytoplasm of the mother cell of gene products in sufficient amount to last for several cell divisions. *Cell competition* has been observed for mutant cells that are unable to maintain the same pace as normal cells growing in the same tissue, and was described for Minute mutations, characterized by an increased length of the cell cycle (MORATA and RIPOLL 1975). It is a characteristic of these mutations that the mutant cells are viable in the abdominal histoblasts, but clones induced early in development in the wing disc are not found. Any mutations lowering mitotic rate will probably exhibit such cell competition even though in the absence of normal cells it does not necessarily interfere with epidermal cell viability. Since both perdurance and cell competition are reflected by the same proliferation kinetics, in the absence of a better criterion we have defined as epidermal cell-lethal mutations those mutations which are lethal in abdominal histoblasts and exhibit a distinct viability limit in the wing disc. In the wing the presence of cells made homozygous for lethal mutations late in development could be due in these cases to perdurance of the effect of the wild-type gene. The remaining 13 lethals

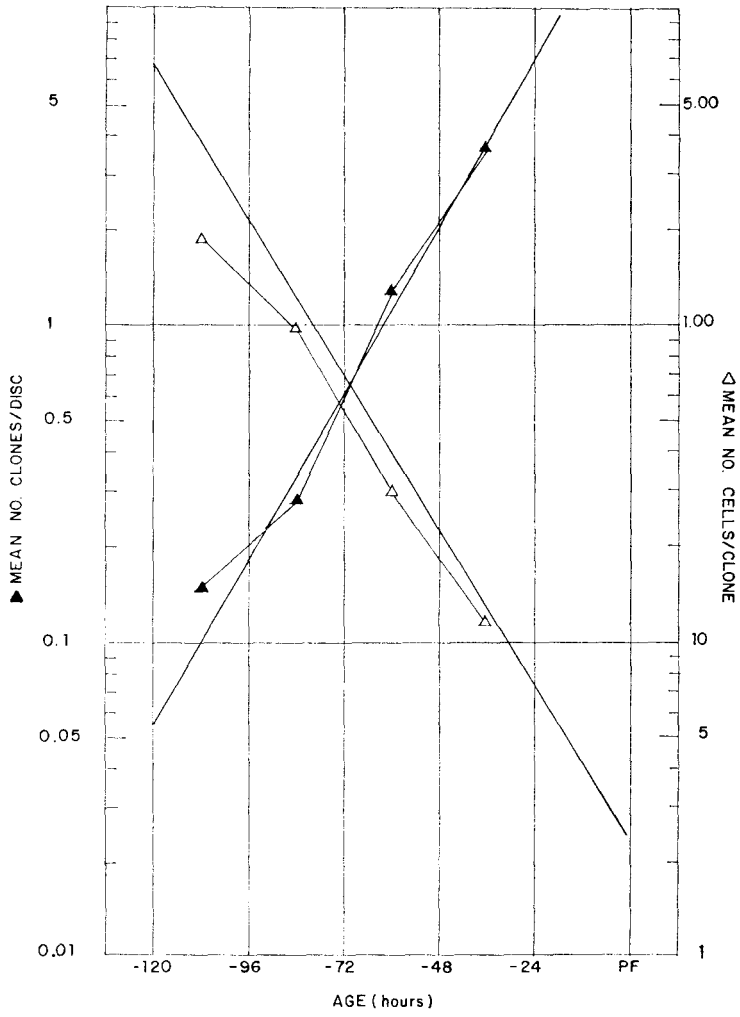

FIGURE 3.—Behavior of imaginal wing cells homozygous for lethals not affecting cell viability. Values represent means of 22 different lethals. The regression lines represent control *mwh* clones (Table 2). Semilogarithmic plot. ▲ Mean frequencies; △ Mean sizes. PF, puparium formation.

which show this type of kinetics in the wing disc but which are viable in the abdominal histoblasts may diminish the capacity of the homozygous cells to compete with the surrounding normal cells without directly interfering with cell viability.

IV *Lethal mutations altering cell behavior*

Those mutations that in genetic mosaics showed abnormal phenotypes in the abdomen or dorsal mesothorax are described below. Among the lethals viable in gynandromorphs, tissue hemizygous for the lethal frequently showed lighter and

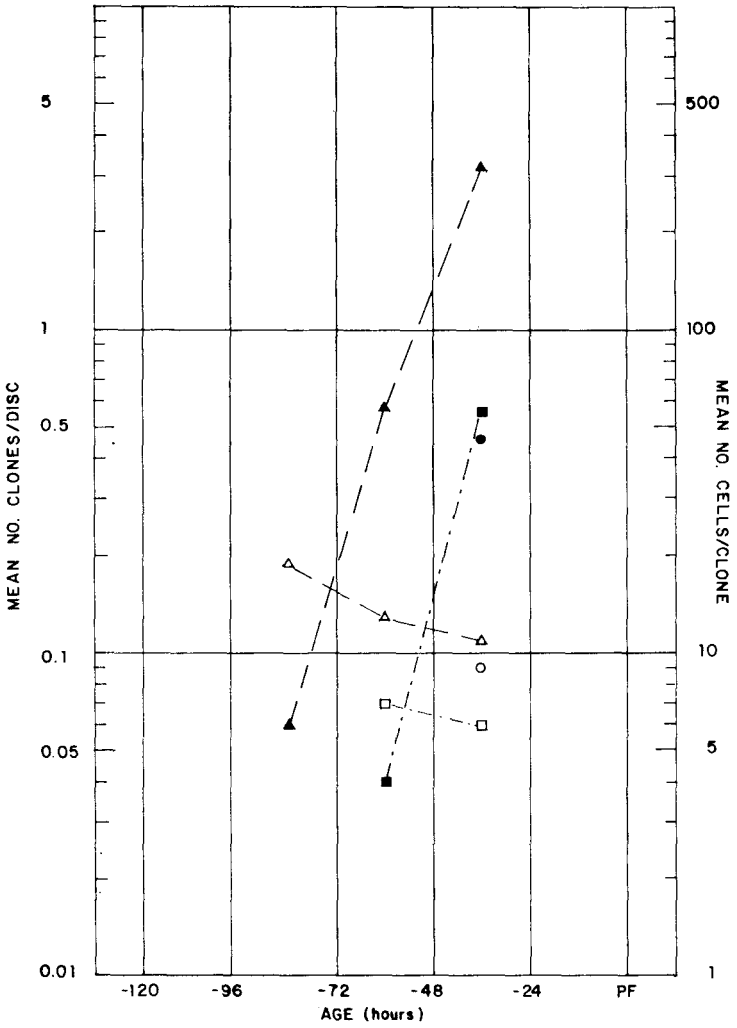

FIGURE 4.—Behavior of imaginal wing cells homozygous for lethals showing different viability limits. ○, *l(1)Mb41*; □, *l(1)Mb48*; △, *l(1)Mb9*. Semilogarithmic plot. Full symbols, mean frequencies; empty symbols, mean sizes. PF, puparium formation.

smaller than normal bristles. Those mutations that presented a more abnormal phenotype are described in Table 3.

Lethals nonviable in gynandromorphs:

l(1)Mc28 *Locus*: 0. *Lethal phase*: embryo-larva. *Cell viability*: decreased frequency and size of abdominal spots. *Viability limit*: 48–72 hours before puparium formation (BPF). *Phenotype*: bristles smaller than normal. Wing trichomes small; occasionally clones of cells without epidermal processes appear. It behaves as expected for a mutation decreasing mitotic rate.

TABLE 3

Mutations found among lethals viable in gynandromorphs

Lethal	Locus	Phase	*c.v.**	N	Phenotype of male tissue
l(1)Mb7	7	E	0.04	5	All bristles transparent, smaller than normal; glassy eyes; aristae almost branchless.
l(1)Mc39	23.5	L	0.04	4	Very thin bristles, shorter than normal; abdomen almost completely devoid of bristles.
l(1)Mc56	25	L	0.19	5	Wings coiled dorsally.
l(1)Mc51	27	P	0.18	5	Several (2–6) trichomes per cell in the wing disc derivatives. Only 75% of the individual cells show this phenotype.
l(1)Mb38	48.5	P	0.22	4	When the external genitalia is male (2 cases) or mosaic (1 case) it does not evaginate; all the apparently normal structures remain within the abdomen. When other structures (wing, head, leg) were male, they evaginated normally.
l(1)Mc1	N.L.	P	1.63	5	Rounded wings, about ⅓ shorter than normal. Rough and bulgy eyes.

* *c.v.*, coefficient of viability in gynandromorphs. *N*, number of specimens studied under the compound microscope. N.L., not located. E, embryo; L, larva; P, pupa.

l(1)Mb16 *Locus*: 0.5. *Lethal phase*: larva. *Cell viability*: normal. *Phenotype*: bristles and trichomes normal. 30% of the clones split into several (3–6) spots of similar sizes which together add up to a total area similar to that of control spots. This phenotype is found only in clones induced prior to 48 hours BPF. (Figure 5a).

l(1)Mb28 *Locus*: 15.5. *Lethal phase*: pupa. *Cell viability*: normal. *Phenotype*: bristles of the wing margin small and unpigmented. Clones in the wing blade extremely elongated. While control clones are 5–10 times longer than wide, with irregular borders, clones of cells homozygous for the lethal are 30–50 times longer than wide, with smooth borders. This phenotype is not found in clones induced later than 48 hours BPF. Shape and size of abdominal spots normal. (Figure 5b).

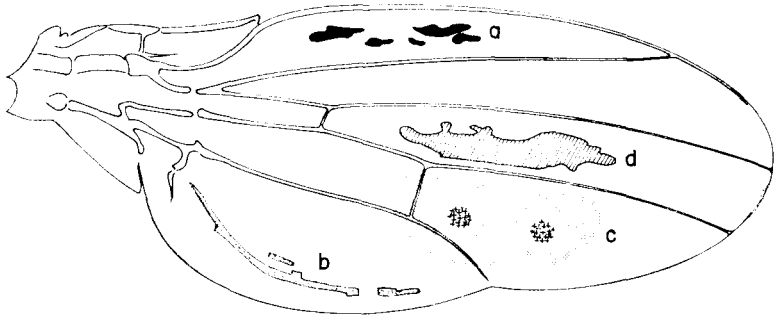

FIGURE 5.—Schematic representation of clones homozygous for some lethals affecting cell behavior. a, *l(1)Mb16*; b, *l(1)Mb28*; c,*l(1)Mc52*; d. control clone.

l(1)Mb24 *Locus*: 22.4. *Lethal phase*: larva. *Cell viability*: normal. *Phenotype*: bristles small and unpigmented. Wing trichomes about 50% shorter than normal. As a consequence of smaller cell size, the area occupied by a clone does not correspond to that expected for its number of cells.

l(1)Mb26 *Locus*: 26.8. *Lethal phase*: embryo. *Cell viability*: epidermal cell lethal. *Viability limit*: 0–24 hours BPF.

l(1)Mb15 *Locus*: 30.1. *Lethal phase*: larva. *Cell viability*: epidermal cell lethal. *Viability limit*: 48–72 hours BPF.

l(1)Mc32 *Locus*: 32.4. *Lethal phase*: embryo. *Cell viability*: epidermal cell lethal. *Viability limit*: 24–48 hours BPF.

l(1)Mb22 *Locus*: 39.7. *Lethal phase*: larva. *Cell viability*: due to its phenotype, it has not been evaluated in abdominal histoblasts. *Viability limit*: 48–72 hours BPF. *Phenotype*: in the abdomen all *y* f^{s6a} and a fraction of the *y* bristles are very small and appear scattered without forming typical clones. Very frequently there are several isolated bristles per hemitergite suggesting that they do not derive from independent recombinational events.

l(1)Mc23 *Locus*: 43.5. *Lethal phase*: larva. *Cell viability*: normal in abdominal histoblasts. *Viability limit:* 48–72 hours BPF. *Phenotype:* bristles on the notum completely unpigmented, not hardened. Bristles in the wing margin small and unpigmented. Wing blade trichomes very small. Occasionally some of the cells within a clone do not differentiate trichomes. Abdominal bristles slightly lighter in color than normal. A large fraction of the spots in the abdomen are doubly marked (*y* and *y* f^{s6a}, *y* f^{s6a} and *mwh*). Increases the frequency of spontaneous mitotic recombination.

l(1)Mc24 *Locus*: 55.9. *Lethal phase*: larva. *Cell viability*: epidermal cell lethal. *Viability limit*: 24–48 hours BPF.

l(1)Mb46 *Locus*: 56.4. *Lethal phase*: larva. *Cell viability*: epidermal cell lethal. *Viability limit*: 48–72 hours BPF.

l(1)Mb8 *Locus*: 60. *Lethal phase*: embryo-larva. *Cell viability*: epidermal cell lethal. *Viability limit*: 48–72 hours BPF.

l(1)Mc35 *Locus*: 62. *Lethal phase*: embryo-larva. *Cell viability*: epidermal cell lethal. *Viability limit*: 0–24 hours BPF.

l(1)Mc52 *Locus*: proximal to forked. *Lethal phase*: larva. *Cell viability*: normal. *Phenotype*: bristles in the abdomen and wing margin smaller than normal. Wing trichomes small, tightly packed in rounded clones smaller and more frequent than normal probably representing split clones. Frequently these clones appear separated from the surrounding normal cells by a band of cuticle with ridges. Occasionally some clones seem to bulge out of the wing surface (Figure 5c).

l(1)Mc19 *Locus*: noncomplementing with garnet. *Lethal phase*: embryo. *Cell viability*: epidermal cell lethal. *Viability limit*: 24–48 hours BPF.

DISCUSSION

Numerical parameters

After treatment of *y Hw* f^{36a} male gametes with EMS, 89 *X* chromosomes were isolated over *FM7a* as carriers of at least one lethal factor. Since EMS does not frequently induce chromosomal aberrations (LIFSCHYTZ and FALK 1969) the criterion followed to define a lethal as a point mutation was whether or not it could be mapped within one chromosomal interval defined by two adjacent markers. Although 4 lethals could not be properly mapped because they failed to complement with one of the markers, they were handled as point mutations under the assumption that if they are deficiencies, their behavior would correspond to the loss of the gene with the most extreme phenotype. Only one of these 4, non-complementing with *garnet*, was nonviable in gynandromorphs. Crosses *inter se* among the 86 lethals were not made, and therefore they were treated as independent loci.

The distribution of the 86 mutations according to their effective lethal phase and their behavior in genetic mosaics is shown in Table 4. There is a correlation between the behavior in mosaics and effective lethal phases: a large fraction (72%) of the nonautonomous lethals die after pupariation, while lethals with earlier effective lethal phases are more frequently autonomous. Among autonomous mutations, those leading to the death of all the mosaic embryos preferentially cause death before puparium formation, and lethals affecting epidermal cell viability do not survive beyond larval stages.

Autonomy in gynandromorphs might be a reflection of specificity of gene action since it can be used to fate map the focus of the organ where the presence of the normal gene product is essential for survival of the mosaic fly (BRYANT and ZORNETZER 1973). This does not imply that the gene is not active in any other organ or tissue. Most of the mutations described in Table 3 decrease the viability

TABLE 4

Distribution of zygotic lethals according to their effective lethal phases and behavior in genetic mosaics

Phase	Zygotic lethals		Autonomous in gynandromorphs		Nonviable in gynandromorphs		Epidermal cell lethals	
	N	(%)	*n*	%	*n*	%	*n*	%
E	15	(17.5)	15	100	10*	67	3	20
E-L	10	(11.6)	10	100	8*	80	2	20
L	29	(33.7)	22	76	20	69	3	10
L-P	8	(9.3)	5	63	2	25	0	
P	21	(24.4)	12	57	3	14	0	
E-L-P	3	(3.5)	1	33	0		0	
Total	$\Sigma N = 86$		65	(75.5)	43	(50.0)	8	(9.3)

E, embryo; L, larva; P, pupa.

* Three of them restricted to abdominal structures. (%) percent of the total $\left(\frac{N \times 100}{\Sigma N}\right)$; %, percent of the corresponding lethal phase $\left(\frac{n \times 100}{N}\right)$.

of gynandromorphs, showing that the effect of the mutation is probably not restricted to the cuticular structures where the phenotypes have been described. Since the recovery of one single sex mosaic is enough to classify a lethal as "viable in gynandromorphs," the number of progeny scored may affect the extent of the class of mutations defined as nonviable in sex mosaics. Therefore, the estimate (43%) obtained for this class of lethals, although similar to the one obtained by BRYANT and ZORNETZER (1973), is probably too high. By studying a larger number of offspring a much lower estimate is reached (HOTTA, personal communication).

As expected, cell-lethal mutations have early effective lethal phases. Three of the 8 cell lethals found here die during larval development, and in two lethals some individuals are viable as larvae. It is surprising that mutant cells for genes controlling processes essential for epidermal cell viability can undergo many cell divisions to give rise to individuals perfect enough to hatch from the egg. This can be explained in part as due to the presence in the egg cytoplasm of normal gene products laid down by the maternal genotype, which are able to compensate for the lack of the normal allele during part of development. The fraction of lethals that impair epidermal cell viability appears to be very low. Using the criteria outlined above, only 9.3% of the lethals induced in the *X* chromosome proved to be cell lethal. Since lethals represent 89% of the mutations that can be induced in Drosophila (LIFSCHYTZ and FALK 1969; HOCHMAN 1971; JUDD, SHEN and KAUFMAN 1972), 8.3% of the genome is capable of mutating to a cell lethal condition. If each band in the salivary gland chromosomes corresponds to one complementation group (HOCHMAN 1971; JUDD, SHEN and KAUFMAN 1972), Drosophila must have about 420 genes essential for epidermal cell viability. This figure could be an underestimate since it should be kept in mind that some of the mutations that did not behave as cell lethals may be hypomorphic alleles of genes essential for epidermal cell viability and which, in amorphic condition, would lead to cell lethality. DEMEREC (1934, 1936) systematically studied the cell viability of sex-linked lethal factors induced with X rays, and classified a considerable fraction of them as cell lethals (90% of the visible deficiencies and 40% of the lethals not associated with visible chromosome aberrations). Although the differences between DEMEREC's and the present results can be explained in part as being due to the different methods used, they might also be explained by the fact that DEMEREC was dealing with deficiencies equivalent to amorphic mutations.

When studying the behavior of wing imaginal cells homozygous for lethals, 21 of the mutations studied here (24.3%) did not show up in clones induced early in development. There were differences among the lethals for the stage after which homozygous clones could be found (*viability limit*). Table 5 shows the distribution of these mutations according to their viability limits, effective lethal phases and cell lethality in abdominal histoblasts. Mutations with late effective lethal phases show, as a whole, earlier viability limits, whereas mutations nonviable in abdominal histoblasts present viability limits closer to the end of wing disc development. As was pointed out before, this behavior can reflect either *per-*

durance or *cell competition*. Lethals nonviable in the abdomen, all showing lack of clones early in wing disc development, have been defined here as cell lethals. In a previous report (RIPOLL and GARCIA-BELLIDO 1973) we supposed that some mutations had their lethal effect restricted to wing imaginal cells. However, after studying their developmental behavior and considering the phenomenon of cell competition, (MORATA and RIPOLL 1975), it is no longer possible to attribute to any of them such tissue specificity, although it may exist.

The cell behavior of 13 (15.1%) lethals has been interpreted as being due to *cell competition*. Probably this figure is an underestimate, since mitotic recombination was not induced for lethals viable in gynandromorphs, and cell competition is compatible with viability in gynandromorphs. Individuals mosaic for different Minute mutations, *M(1)Bld*, *M(2)c*, *M(4)*, arising from chromosome loss during the first cleavage, are viable but the mean size of the Minute territory is smaller than the expected 50% (FERRUS and GARCIA-BELLIDO, in preparation). Since Minute mutations are subject to cell competition in the wing disc, the reduced male territories in gynandromorphs may be similarly explained. Lethals showing similar behavior in gynandromorphs (BRYANT and ZORNETZER 1973) would probably also show cell competition if studied in homozygous spots in the wing. The 6 lethals found here, which gave male patches restricted to abdominal structures, cannot be included in this class, since they all behaved normally in the wing disc. Their behavior in gynandromorphs is therefore indicative of their automomy in noncuticular anterior structures.

The survival of clones induced late in development homozygous for the eight mutations defined as cell lethal has been interpreted as *perdurance* of the wild-type allele (GARCIA-BELLIDO and MERRIAM 1971c). If perdurance reflects the time of development after which the gene is inactive or the normal gene product is not needed, cell lethals with early effective lethal phases should exhibit *viability limits* early in development. This is not the case for the 8 cell lethals studied here (Table 5), suggesting that their normal alleles are needed throughout the course of development or at least very early and again very late. This late perdurance may be due to the release in the mother cell of products needed for cell viability that are able to persist in the cytoplasm of the daughter cells in sufficient amount to carry on during several divisions the functions controlled by the wild-

TABLE 5

Distribution of mutations according to viability limits and effective lethal phases

Viability limit	Effective lethal phase E	E-L	L	L-P	P	Total
0–24	1(1)	1(1)	0	0	0	2(2)
24–48	2(2)	3	3(1)	0	0	8(3)
48–72	0	3(1)	6(2)	0	0	9(3)
72–96	0	0	0	1	1	2
Total	3(3)	7(2)	9(3)	1	1	21(8)

E, embryo; L, larva; P, pupa.
( ) Fraction cell lethal in abdominal histoblasts.

type allele. The low number of cell divisions needed to complete development would prevent an excessive dilution of these products. All of the cell lethals proved to be viable in clones embracing one single bristle in the tergites and the mesonotum, and in clones of 6–14 trichomes in the wing blade. This also happens in cells which are extensively aneuploid (MERRIAM and GARCIA-BELLIDO 1972), indicating that either very little genetic information is needed at the end of development or that the cell cytoplasm carries enough products to permit it to divide a few times and differentiate.

Mutations affecting cell behavior

The analysis of lethals in genetic mosaics has led to the detection of several mutations that alter the phenotype of male tissue in gynandromorphs (Table 3) or, if not viable in gynandromorphs, in mitotic recombination clones. These mutations affect a wide variety of processes from differentiation to morphogenesis. A general feature of these lethals is their high degree of pleiotropy even though studied at the cellular level. It is noticeable, for instance, that a majority of the mutations alter in some way cuticular differentiation even if they also affect processes apparently as unrelated to the formation of cuticle as mitotic orientation or mitotic recombination.

One mutation, *l(1)Mc28*, seems to decrease mitotic rate. This might also be true of some other lethals showing cell competition in the wing imaginal disc, but this mutation has been singled out because it exhibits the most extreme phenotype. *l(1)Mc23* increases the frequency of mitotic recombination both when it is heterozygous and when it is homozygous. The increase in the frequency of somatic exchange is a characteristic of the Minute mutations (STERN 1936; KAPLAN 1953; FERRUS 1975), but *l(1)Mc23* does not seem to be a Minute since it does not have the dominant bristle phenotype and is viable in epidermal cells, whereas Minutes are cell lethal (STERN and TOKUNAGA 1971; MORATA and RIPOLL 1975). It is most likely that *l(1)Mc23* represents a defect in some repair enzyme since mutants of this kind are known to increase the frequency of appearance of cuticular spots (BAKER, CARPENTER and RIPOLL, in preparation).

A small fraction of the mutations studied here (4.6%) seem to alter cellular functions essential for normal morphogenesis (see Figure 5). *l(1)Mb28* affects the final shape of the clones and probably represents an alteration in the normal orientation of the mitotic spindle. Preferential orientation of mitoses seem to play a very important role in the final shape of cuticular appendages. Three mutations have been found that probably affect membrane properties: *l(1)Mc52* homozygous cells behave as expected for cells with cell-to-cell recognition properties different than those of the surrounding normal cell population. It is known that cells with different cell affinities segregate from each other in culture (NÖTHIGER 1964), as well as in genetic mosaics (MORATA and GARCIA-BELLIDO 1976). The high frequency of split clones found with *l(1)Mb16* suggests some defect in cell-to-cell adhesivity properties, thought to be an important factor during normal wing morphogenesis (SANTAMARIA and GARCIA-BELLIDO 1976). A similar function could be affected by *l(1)Mb22*.

The analysis of zygotic lethals in epidermal cells has allowed us to estimate the

fraction of EMS-induced mutations affecting cell viability. About 90% of the genome is amenable to study in patches resulting from mitotic recombination in cuticular structures. The method used here has proved efficient enough to permit the description of the effect in the cuticle of new mutations even though they are zygotic lethals. We may hope that with appropriate modifications, the analysis of lethals in genetic mosaics can be focused to screen for mutations altering specific processes both in cuticular and noncuticular structures.

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