GENETIC ANALYSIS OF MUTATIONS AT THE *GLUED* LOCUS AND INTERACTING LOCI IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

A genetic analysis of the dominant mutation *Glued* that perturbs the development of the normal axonal architecture of the fly's visual system was undertaken. Ten new alleles at this locus were identified and characterized. Two complementation groups that were identified failed to complement the original allele, suggesting that it is a double mutant or that it resides at a complex locus. Several of the new alleles display visual-system abnormalities similar to those of the original mutation. Seven of the eight members of one complementation group are embryonic/early larval lethals, like the original mutation. The other allele in this group is temperature sensitive. Homozygous mutant adults exhibit a temperature-sensitive female sterile phenotype. Unsuccessful attempts to recover genetic mosaics carrying clones of cells homozygous for some of these mutations revealed that they are either essential for the viability of individual cells or that they affect some other fundamental cellular function, such as mitosis or the ability to participate in tissue level organization, which prevents them from being recovered in adult mosaics. This also indicates that these mutations do not specifically affect neural cells. A number of X-ray- and EMS-induced partial and complete phenotypic "revertants" of the original allele have also been isolated as material for a comparative analysis of visual system development. All "revertants" that alter the abnormal eye phenotype towards the wild type have similar impact on the organization of the optic lobe.

SEVERAL mutations that disrupt the normal axonal organization of the adult optic lobes were identified in this laboratory by anatomical screening of a large number of existing mutations that disrupt the normal number, form or hexagonal arrangement of ommatidia (MEYEROWITZ and KANKEL 1978). Some of these mutations were already known to be associated with axon disarray in the optic lobes (JOHANNSEN 1924; PILKINGTON 1941; POWER 1943a,b, 1950; PAK, GROSSFIELD and WHITE 1969; HANSON 1971). However, most of the mutations screened were not associated with any gross abnormalities in the organization of the optic ganglia. This observation, in itself, suggests that not all morphological abnormalities in the eye are capable of causing gross optic lobe disorganization.

Genetic mosaics in which eye and optic lobe differed in genotype were constructed for three of the optic lobe mutants (*Glued*, *glass*^{3} and *rough*) identified

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in this screen. These experiments showed that the abnormal optic lobe phenotype associated with each of them is caused not by autonomous expression of the mutation in the optic lobe cells, but by the presence of mutant tissue in the compound eye. Thus, optic lobe phenotype corresponded to the genotype of the eye tissue innervating it. From this result, it was inferred that information transmitted from retina to optic lobe is important for the normal differentiation of the optic lobe (MEYEROWITZ and KANKEL 1978). More recently, it has been demonstrated that the gross disarray of axons found in these mutants is also accompanied by defects in the morphology of individual lamina and medulla neurons and in individual retinula axon terminals in these neuropils (S. GAREN and D. R. KANKEL unpublished). This further strengthens the conclusion that the interaction of retina and optic lobe cells controls important aspects of cellular differentiation in the optic lobe.

Some general questions raised by these experiments concern the nature of this information, how it is transmitted and how it functions during development. These questions are not likely to yield easy answers, but a starting point is to study how mutations at these loci affect visual system development.

We have undertaken a genetic analysis of the *Glued* locus with the following aims: (1) to determine whether the visual system defects associated with the original allele are a common feature of mutations at this locus; (2) to obtain new alleles with varying degrees of eye and/or optic lobe abnormalities. These will provide material for comparative analyses, which may allow us to identify the components of the mutant phenotype responsible for abnormal optic lobe development and those which are not; (3) to determine whether this locus plays a specific role in visual system development or displays a more global spectrum of phenotypic effects; (4) to obtain temperature-sensitive (ts) alleles to determine when the function of this gene becomes essential for normal visual system development. This may provide clues as to what events in the development of the visual system are affected by mutations at this locus.

This report presents the results of a genetic analysis of new mutations at the *Glued* locus and at a number of other loci, which interact with *Glued* to modify its phenotype.

The original mutation at the *Glued* locus (which will be designated *l(3)Gl*^{1}) arose spontaneously among the progeny of heat-treated females (PLOUGH and IVES 1935). *l(3)Gl*^{1} has dominant eye and optic lobe phenotypes (Figure 1) and is also a recessive lethal. It maps to 41.4 on the third chromosome in the *Ly-fz* interval. Meiotic recombination is slightly inhibited in the *Ly-st* interval by *l(3)Gl*^{1}. Cytological examination of the polytene chromosomes of the larval salivary glands of *l(3)Gl*^{1}/+ heterozygotes reveals no visible structural abnormalities in this region. This does not, however, rule out the possibility that *l(3)Gl*^{1} is a double mutant or a more complex genetic event generated by a cytologically invisible rearrangement and that the eye and lethal phenotypes are therefore unrelated. Complementation analyses among new *Glued* alleles may provide information to help resolve this issue. In addition to new lethal alleles, we have isolated a number of revertants of the dominant eye and optic lobe pheno-

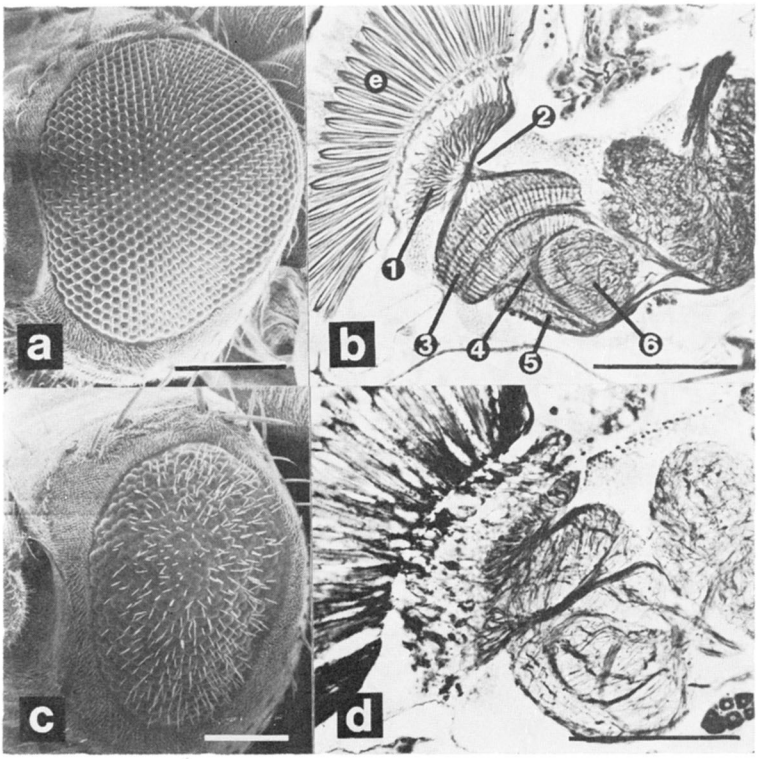

FIGURE 1.—Eye and optic lobe phenotypes of wild-type and *l(3)Gl*^{1} flies. (a) is a scanning electron micrograph of a wild-type eye. (b) shows a horizontal, silver stained section of a wild-type optic lobe. *e*: eye; 1: lamina neuropil; 2: first optic chiasma; 3: medulla neuropil; 4: second optic chiasma; 5: lobula plate; 6: lobula. The cell bodies of the lamina neurons lie between the basement membrane of the eye and the lamina neuropil. The cell bodies of medulla neurons occupy the region surrounding the first optic chiasma. (c) shows a *l(3)Gl*^{1} eye. (d) shows a horizontal silver stained section of a *l(3)Gl*^{1} optic lobe. The lamina cell body layer is thicker than in wild type and contains small darkly staining bodies that resemble rhabdomeres. The retinula fiber bundles in the lamina cell body layer follow twisting paths to the lamina neuropil instead of projecting directly as in wild type. The lamina neuropil contains a disorganized mass of nerve fibers rather than orderly cartridges and the nuclei of the epithelial cells do not form a regular row. In place of a normal first optic chiasma there are large fiber tracts between the lamina and medulla. The medulla neuropil is rotated 45° to 90° clockwise in the horizontal plane from the wild-type position. Some of the fiber tracts from the lamina enter the medulla neuropil at its posterior edge, which is now adjacent to the lamina, and project to the anterior medulla. Columns of transverse fibers are present in the medulla, but vary in thickness and spacing. Many medulla tangential fibers appear to be missing. The lobula and lobula plate appear more normally organized, but the second optic chiasma, instead of forming a single triangular region between medulla, lobula and lobula plate, is divided into several fiber tracts. Some of these fiber tracts from the medulla cross through the lobula and terminate in the lobula plate. Anterior is to the left in (a) and (c), at the top in (b) and (d). The bars represent 100 micrometers.

type and suppressors at other loci, which modify the eye and optic lobe phenotype. The cell viability of several *Glued* lethals has been tested and a number of new phenotypes associated with these mutations have been identified.

MATERIALS AND METHODS

Fly Culture: *Drosophila melanogaster* were kept in standard half-pint milk bottles or in 29 × 80 mm cylindrical vials with foam stoppers. They were grown on cornmeal–molasses–agar–yeast medium (DOANE 1967) supplemented with fresh yeast and were maintained at 25° unless otherwise noted. Wild-type flies were of the Canton-Special (Canton-S) strain.

Scanning electron microscopy: This was done using an ETEC Corp. (Hayward, Calif.) Autoscan U-1 microscope. Prior to viewing, flies were simply etherized and mounted on metal stubs, since adequate pictures were routinely obtainable without prior critical-point drying or gold-coating.

Histology: For routine light microscopic examination of nerve fiber arrays in the optic lobes, a modification of HOLMES' (1947) silver strain for axons was used. This is identical to the procedure of MEYEROWITZ and KANKEL (1978) with the following exceptions: GREGORY's (1980) modified synthetic "aged" Bouins fixative was used in place of simple alcoholic Bouins fixative, since the former produced superior staining of axons. Following fixation for 4 to 24 hr at 4°, the heads were dehydrated in a graded series of aqueous ethanol solutions (70%, 95%, 2 × 100% (v/v) for 15 min each), cleared in toluene twice for 15 min and then embedded in Fisher Paraplast Plus. Horizontal 10 μm serial sections were cut on a Sorval JB-4 microtome and transferred to "subbed" slides (HUMASON 1972). The sections were deparaffinized in xylene, rehydrated and placed in 20% (w/v) silver nitrate, where they were left for 24 hr in the dark. The impregnating bath contained 1 ml of 20% silver nitrate and 5 ml of 2,6-lutidine. The slides were left in the impregnating bath for two to four days. These prolonged incubation times and the higher concentration of silver in the impregnating bath resulted in superior staining of axons in material processed in paraffin.

Nomenclature: We have followed the standard nomenclature in naming of new alleles at the *Glued* locus and at interacting loci. The original allele has been renamed $l(3)Gl^1$, and all new lethal alleles have been named *l(3)Gl* with distinguishing superscripts. All new phenotypic revertants of the dominant eye phenotype of $l(3)Gl^1$ have been named Gl^{+R} with additional distinguishing superscripts, if they were *inseparable* from $l(3)Gl^1$ by recombination. If they were *separable* from $l(3)Gl^1$, they were designated *Su(Gl)* with distinguishing numbers following the names, as is customary for dominant suppressors. An X-ray-induced deficiency of $l(3)Gl^1$ has been designated $Df(3)Gl^{+R2}$.

Mutations: A list of mutations used in this study is provided in Table 1. Descriptions of mutations not listed can be found in LINDSLEY and GRELL (1968).

TABLE 1

Mutations used in this study

Abbreviation	Complete name	Meiotic map location	Relevant phenotype
ca	claret	3–100.7	eyes brownish
Cy	Curly	2–6.1	wing curled upward
D^3	Dichaete-3	3–40.7	homozygous lethal
$Df(1)mal^{10}$	—	—	an *X* chromosome deficient for 19A5–6 through 19E1–2
Df(1)HF396	—	—	an *X* chromosome deficient for 18E1–2 through 20
$Dp(1;3)sc^{J4}$	—	—	a duplication of the *X* chromosome region bearing y^+ inserted into chromosome 3

TABLE 1—Continued

Abbreviation	Complete name	Meiotic map location	Relevant phenotype
Dp(3;Y)G145	—	—	a *Y* chromosome containing a piece of chromosome 3 that includes the wild-type *Glued* locus
DTS4	Dominant temperature-sensitive *4*	—	dominant ts lethal
e	ebony	3–70.7	dark body
fz	frizzled	3–41.7	thoracic hairs disarrayed
Gl	Glued	3–41.4	ommatidia and optic lobe axons disarrayed
LVM	Balancer of L. V. Morgan	—	chromosome *3L* balancer
Ly	Lyra	3–40.5	wing margins excised, homozygous lethal
Me	Moire	3–19.2	irridescent eye
ot	outheld	1–65.7	wings held out; ocellar bristles missing; male sterile
red	red Malpighian tubules	3–53.6	larval Malpighian tubules red
Sb	Stubble	3–58.2	bristles short, homozygous lethal
sbd^{l}	stubbloid-lethal	3–58.2	lethal over *Sb*
Sco	Scutoid	2–51.0	some bristles absent
Ser	Serrate	3–92.5	wings notched, homozygous lethal
st	scarlet	3–44.0	eyes red-orange
th	thread	3–43.2	arista thread-like
TM1	Third multiple-1	—	chromosome 3 balancer
TM3	Third multiple-3	—	chromosome 3 balancer
TM8	Third multiple-8	—	chromosome 3 balancer
T(Y;3)H156	—	—	a reciprocal translocation with a *3L* breakpoint at 70C
T(Y;3)J132	—	—	a reciprocal translocation with a *3L* breakpoint at 70C
T(Y;3)A60	—	—	a reciprocal translocation with a *3L* breakpoint at 70C
T(Y;3)R91	—	—	a reciprocal translocation with a *3L* breakpoint at 70C
T(Y;3)B170	—	—	a reciprocal translocation with a *3L* breakpoint at 70C
T(Y;3)R83	—	—	a reciprocal translocation with a *3L* breakpoint at 70C
T(Y;3)H156^{D}*/J132*^{P}	—	—	a third chromosome that is deficient for the *Glued* locus
T(Y;3)B170^{D}*/R83*^{P}	—	—	a third chromosome with an extra wild-type copy of the *Glued* locus

Mutagenesis and Mapping: *Glued* phenotypic revertants were isolated by treating three- to five-day-old *l(3)Gl*^{1} males with either 4,000 to 5,000 R of X rays or with ethyl methanesulfonate (EMS) according to the procedure of LEWIS and BACHER (1968) and mating them as shown in Figure 2. This mating scheme only permits the survival of progeny bearing the *l(3)Gl*^{1} chromosome, since all of the other normal progeny classes die due to the lethality of *Sb/sbd*^{l} and *Ser/Ser* genotypes. These *l(3)Gl*^{1} progeny were screened visually with a dissecting microscope at 25X magnification for the presence of exceptional individuals, *i.e.*, those

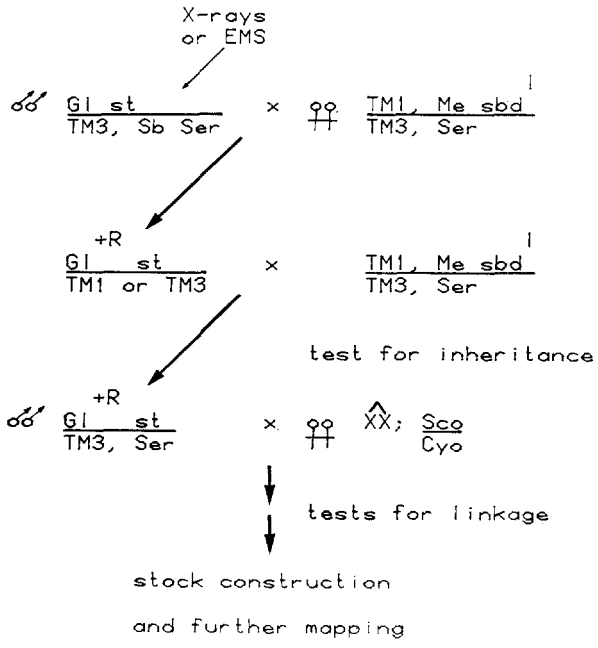

FIGURE 2.—Scheme for obtaining phenotypic revertants of *l(3)Gl*^{1} (*Gl*). *Gl st* males were mutagenized with X rays or EMS and mated to females bearing third chromosome balancers. The particular configuration of mutations on the balancers employed allows only flies carrying the *Gl st* chromosome to survive, since *Ser/Ser* and *Sb/sbd*^{1} genotypes are lethal. These *Gl* progeny were screened visually for flies with phenotypically wild-type or less *Glued* eyes. Such putative revertants were further tested for inheritance, linkage to *Gl*, and map position if unlinked (as indicated in the text).

with wild-type or less extreme *Glued* eye phenotypes. The presence of other markers in the cross usually allowed us to distinguish putative revertants from other exceptional progeny types. Putative F_1 revertants were tested for inheritance by backcrossing each one to *TM1/TM3* flies. Since this screen also permits the recovery of dominant second-site suppressors of *l(3)Gl*^{1}, which are indistinguishable from revertants, F_2 males from positive test crosses were mated to appropriate females bearing attached-*X* chromosomes and dominant autosomal markers, to determine if mutations segregating with other linkage groups were involved. Further mapping was carried out as follows: *X*-linked suppressors were mapped with the multiply marked first chromosome *sc ec ct*^{6} *v g f*. Third chromosome-linked suppressors were mapped with the "*rucuca*" chromosome. Nonsegregating, putative *l(3)Gl*^{1} revertants were mapped within the *Ly-st* interval and the presence of a normal recombination frequency in this region was tested.

Attempts were also made to obtain "true" revertants of the lethality associated with *l(3)Gl*^{1} by using the selection scheme outlined in Figure 3. *l(3)Gl*^{1} males were mutagenized with EMS according to the method of LEWIS and BACHER (1968) and mated in bottles to a newly isolated lethal allele, *l(3)Gl*$^{1-3}$. The balancer for both these mutations carries a dominant temperature-sensitive (DTS) lethal that results in the death of all progeny at 29°, except for *l(3)Gl*^{1}/*l(3)Gl*$^{1-3}$ flies that survive due to reversion of the lethality of *l(3)Gl*^{1}. Estimates of the number of chromosomes screened by this method were made by performing the cross at 18°, the permissive temperature for the DTS, using unmutagenized flies and counting the progeny produced. The number of parents and the duration of the egg collection were the same in these tests as in the mutagenesis.

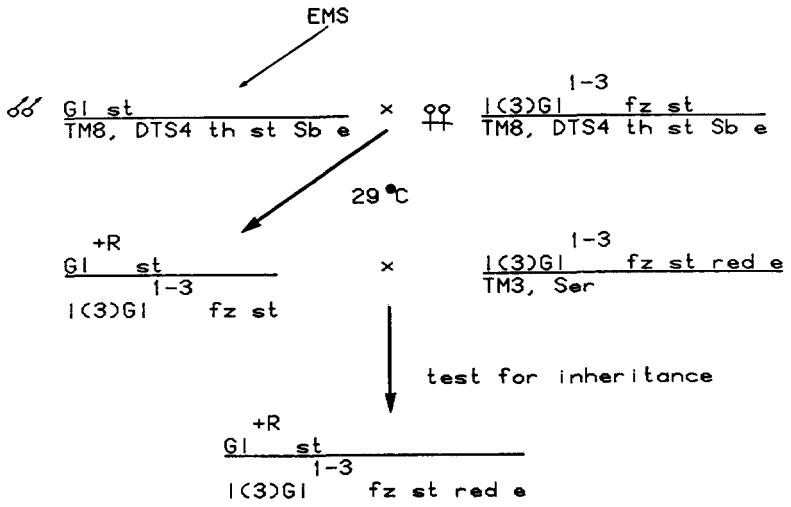

FIGURE 3.—Selection scheme for detecting revertants of the lethality associated with *l(3)Gl*^{1} (*Gl*). *Gl st* males mutagenized with EMS are crossed to *l(3)Gl*$^{1-3}$ females and the progeny raised at 29°. The dominant temperature-sensitive mutation, *DTS4*, carried on the chromosome used to balance both these stocks ensures that all homo- and heterozygotes carrying this chromosome will die when reared at 29°. Since *Gl st/l(3)Gl*$^{1-3}$ flies also die, the only survivors of this cross are exceptional progeny, among which should be the desired revertants, if it is possible for such a reversion event to occur. Putative revertants are then tested further for inheritance.

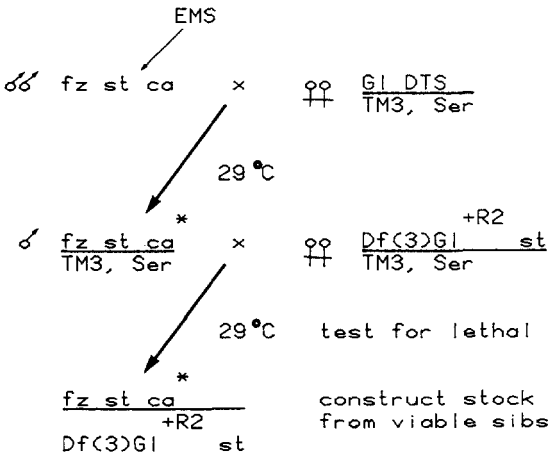

FIGURE 4.—Scheme for obtaining new lethals in the *Glued* (*70C*) region of chromosome 3. Males bearing some closely linked markers in the region are mutagenized and mated to females. F_1 males bearing the mutagenized chromosomes are mated individually to females bearing the small deficiency for the *Glued* region. The progeny from each of these crosses is scored for the presence of progeny carrying the mutagenized third chromosome heterozygous with the deficiency. The absence of this class indicates the presence of a lethal that is uncovered by the deficiency, and from these same crosses balanced stocks of the lethals are constructed from the *fz st ca/TM3* siblings. The 29° temperature regime was employed to allow the recovery of temperature-sensitive lethals.

New lethal alleles of *l(3)Gl*^{1} were isolated by mutagenizing *fz st ca* or *st red e* males with EMS according to the scheme in Figure 4. Individual F_1 males were mated to females bearing a small deficiency for the *Glued* region that was previously isolated as an X-ray-induced *l(3)Gl*^{1} revertant. These matings were carried out at 29° to allow the detection of temperature-sensitive lethals. Stocks were constructed from the viable siblings in crosses that evidenced the presence of a lethal within the region uncovered by the deficiency. New *Glued* alleles were identified by testing all of these lethals for failure to complement the lethality of the original *l(3)Gl*^{1} mutation or by interaction with the dominant eye phenotype.

Complementation tests: Complementation tests were performed between *l(3)Gl*^{1} revertants and *l(3)Gl*^{1}, *Ly*, *D*^{3} and *fz*; between *l(3)Gl*^{1} and all new lethals isolated over *Df(3L)Gl*$^{+R2}$; between newly isolated *Glued* alleles; and between *l(3)Gl*^{1} revertants and representatives of all new complementation groups isolated over *Df(3L)Gl*$^{+R2}$. In all complementation tests, a minimum of 150 flies were counted. Complementation tests involving new *Glued* alleles were done at 18°, 25° and 29°. All other tests were conducted at 25°.

Stage of lethality of Glued *lethals*: The stage of lethality of *Glued* lethals was estimated by crossing balanced stocks of each lethal to *Df(3)Gl*$^{+R2}$. The marker *red* was present on these lethal and deficiency bearing chromosomes to permit the identification of *lethal/Df larvae.* This marker produces a dark red coloration in the Malpighian tubules, which becomes identifiable by the late first instar. Eggs were collected from these crosses for four hr and larvae collected at four hr intervals the next day. Larvae were then observed for the appearance of *red* Malpighian tubules; *red* larvae were separated and their further development observed. At least 200 eggs were observed for each cross.

Viability of Glued *lethals in cells*: To test whether individual cells homozygous for *Glued* lethals can survive in adult tissues, genetic mosaics for *l(3)Gl*^{1} and *l(3)Gl*$^{1-3}$ were constructed by somatic recombination. To detect homozygous mutant cells in the eye, *bw;l(3)Gl*^{1} *st/M(3)i*^{55} and *bw*; *l(3)Gl*$^{1-3}$ *st/M(3)i*^{55} larvae were irradiated with 1000 R of gamma rays 12 hr after the end of a 24-hr egglay (36 ± 12 hr AEL). Somatic recombination between *st* and the centromere can give rise to a clone of cells homozygous for *l(3)Gl*^{1} *st*, which will be phenotypically white in a *bw* background, due to the failure of *bw*; *st* cells to become pigmented (Figure 5). The expected frequency of such somatic recombinants was estimated from a population of similarly irradiated *bw;st/M(3)i*^{55} larvae not carrying the *Glued* mutations.

To test whether cells homozygous for *Glued* lethals can survive in other adult tissues, larvae of the genetic constitution *y*; *Dp(1;3)sc*^{J4}, *y*$^{+}$ *M(3)i*^{55}*/l(3)Gl*^{1} were irradiated with 1000 R of gamma rays at 36 ± 12 hr AEL. Somatic recombination between *l(3)Gl*^{1} and the centromere can give rise to a clone of cells homozygous for *l(3)Gl*^{1} in the cuticle, which will also express the body color mutant *y* due to the loss of *y*$^{+}$ by somatic recombination (Figure 6). The expected frequency of such events was estimated from similarly irradiated control larvae *y;Dp(1;3)sc*^{J4}, *y*$^{+}$/+.

RESULTS

Gene dosage analysis: The construction of individuals with various doses of mutant and wild-type alleles provides an important means of defining a number of formal properties of the alterations produced in a gene product by a particular mutation (*cf.*, MULLER 1932). For example, it is possible to determine whether the phenotype in question results from an alteration in the amount of functional gene product or from the production of an abnormal gene product. It is also possible to determine whether a particular mutation represents the most extreme alteration of a gene product (total disfunction or absence) or produces some residual normal function. In some cases, one may also obtain clues about whether a mutant phenotype results from disruption of normal temporal or spatial regulation of the gene, such that it functions at an inappropriate time

or in an inappropriate tissue during development. The underlying assumption of these tests is that alterations in gene dosage result in alterations in the quantity of gene product made. This assumption is borne out in cases where direct measurement of gene product is possible, but of course may not hold in every case (for review see O'BRIEN and MACINTYRE 1978).

Since our interest in mutations that perturb the visual system is based in part on the assumption that they identify genes whose functions are essential for normal visual system development, before initiating a screen for new alleles, we were interested in discovering whether the *l(3)Gl*^{1} mutation might represent the gratuitous expression of a gene that is not normally active in the eye disc at this time. The existence of dominant mutations such as *Glued* at a locus whose gene product is unknown raises problems for any interpretation of the relationship of the wild-type locus to the process such mutations perturb. There is no general interpretation of dominance and, indeed, there cannot be, although in some cases it can be demonstrated to result simply from alterations in the amount of gene product. The dominant phenotypes of some deficiencies (LINDSLEY *et al.* 1972), and the dominant toxin-resistance phenotypes associated with some mutations that cause an increase in activity of the resistance-conferring enzyme (SHERALD and WRIGHT 1974), are cases in point. However, the possibility that a dominant phenotype results from more complex effects of a mutation must be confronted in each case individually. In at least one case, the dominant phenotype of a well-known homeotic mutation was recently shown to result from expression of the mutant gene in a tissue where it is not normally expressed (STRUHL 1981). The dominance of the *l(3)Gl*^{1} eye phenotype together with its spontaneous origin and singularity raised the possibility that this might be the case.

One way to answer this question is to determine whether adding extra copies of the wild-type gene to genotypes containing the mutant allele effectively "titrates" the dominant phenotype in question (*i.e.*, renders it less extreme). The failure of any number of extra wild-type gene doses to do so is not strictly conclusive, and is consistent with a number of interpretations, including the one that the wild-type gene may not function in the same tissue or at that time. The observation of progressive titration of the dominant mutant phenotype with extra gene doses, on the other hand, implies that the wild-type gene product functions at the same time and in the same tissue as the mutant, and hence can compete with it. To determine some of these properties of the *l(3)Gl*^{1} mutation, genotypes with varying doses of mutant and wild-type alleles were constructed. These genotypes and the phenotypes associated with them are outlined in Table 2.

Heterozygotes for deficiencies lacking the *Glued* chromosomal region display completely normal eye and optic lobe phenotypes, indicating that the dominant phenotype seen in *l(3)Gl*^{1}/+ heterozygotes does not result simply from haploinsufficiency, *i.e.*, the failure of the mutant homolog to produce a functional gene product. Likewise, the wild-type phenotype of flies bearing a small duplication of the normal chromosomal region (*i.e.*, +/+/*Dp*) indicates that the

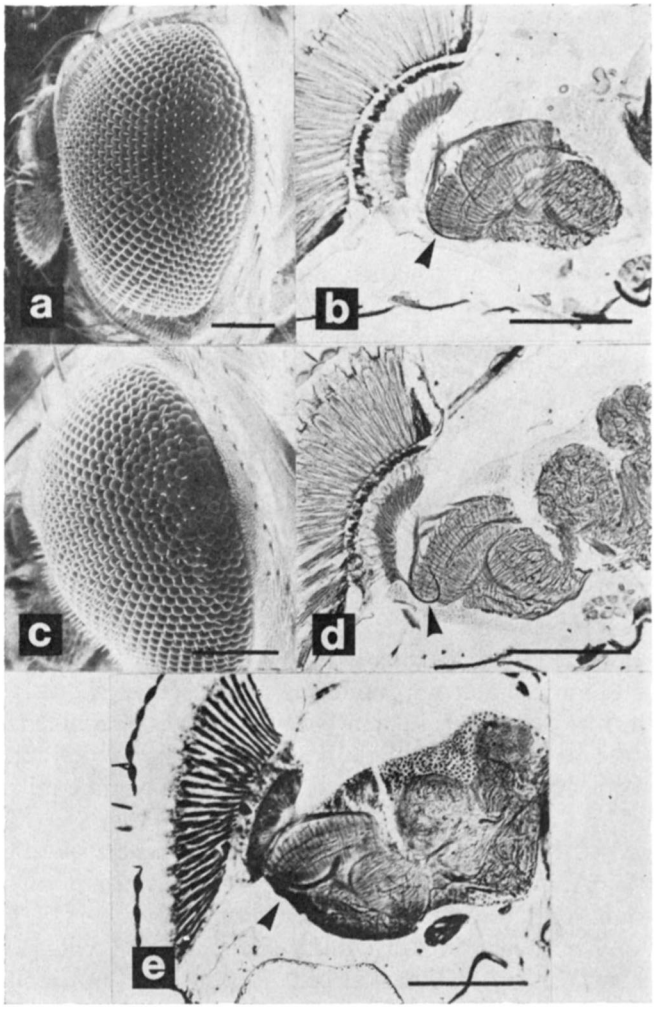

FIGURE 5.—Eye and optic lobe phenotypes of new *Glued* alleles. (a,b) $l(3)Gl^{15\text{-}C}/+$ (c,d) $l(3)Gl^{5\text{-}J}/+$ (e) $l(3)Gl^{7\text{-}6}/l(3)Gl^{29\text{-}1}$. *5-J* and *15-C* both have essentially the same dominant phenotype. The eye is normally organized except for a small cluster of abnormally packed facets of variable size, located at the posterior edge of the eye, straddling the equator. The optic lobes are normally organized except for the region directly beneath these abnormal facets. The only abnormality observable in silver stained optic lobes is the projection of a bundle of fibers from the posterior lamina directly into the medulla instead of crossing the first optic chiasma (arrows) and the distortion of the posterior lamina associated with this aberrant projection. These fibers grow down along the posterior face of the medulla neuropil and then penetrate it either at the level of the medulla tangential fibers (d) or even deeper, at its inner face (b). They then project across the medulla and outwards toward their normal target region in the anterior medulla cortex. The medulla is normally positioned in both these mutants. A small number of Golgi stained specimens of *5-J* indicate that at least the R7 and R8 fibers are involved in this aberrant projection. The only other adult visual system abnormality associated with new *Glued* alleles has been observed in the *7-6/29-1* heteroallelic combination. These are sick, relatively infrequent survivors. They have a uniformly distributed, slightly abnormal

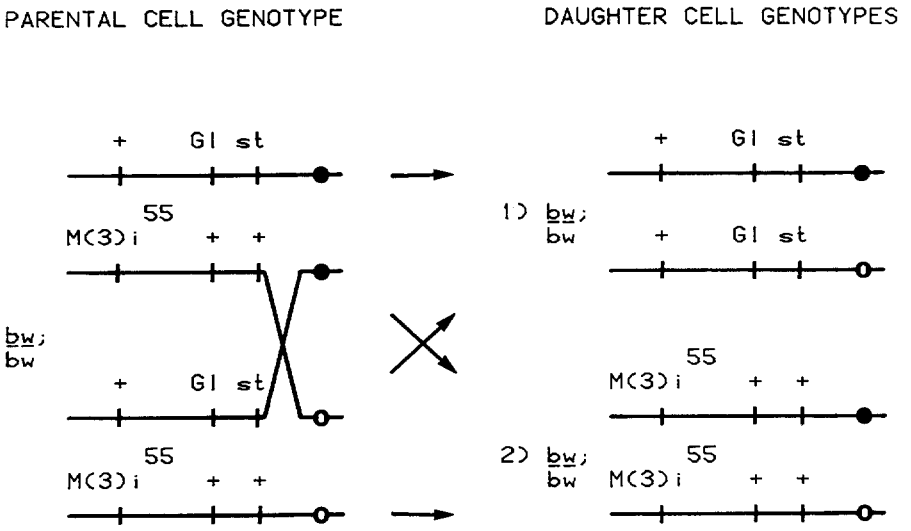

FIGURE 6.—Scheme for making clones of cells homozygous for *l(3)Gl*^{1} or *l(3)Gl*$^{1-3}$ (*Gl*) in the eye via somatic recombination. Only the third chromosome is depicted. Larvae to be irradiated were obtained by crossing *bw*; *M(3)i*^{55}*/TM3* females to *bw*; *Gl st/TM3* males. *M(3)i*^{55} was included in the cross to increase the size of the clones and thereby make them more readily detectable. Homozygous *st* in combination with *bw* produces unpigmented white cells in the eye. The homozygous *M(3)i*^{55} twin clones are never seen.

dominant *l(3)Gl*^{1} phenotype probably does not result from an overproduction of normal gene product by the mutant chromosome, unless it does so at least in excess of the amount produced by *+/+/Dp*.

When a duplication is added to flies heterozygous for *l(3)Gl*^{1} (*i.e.*, *Gl/+/Dp*), a considerable reduction in the severity of the eye and optic lobe phenotype is observed, but complete restoration of the wild-type phenotype is never seen. A few flies carrying two extra wild-type copies of the *Glued* region (*i.e.*, *Gl/+/Dp/Dp*) have been recovered and display an even more normal eye phenotype. This behavior indicates that the *l(3)Gl*^{1} chromosome produces an aberrant gene product that interacts directly or indirectly with the wild-type gene product in an antagonistic manner. MULLER (1932) classified such mutations as "antimorphs." There are numerous hypotheses by which one can interpret this behavior of dominant mutations (*e.g.*, the functional gene product of the locus is multimeric). Whatever the explanation, the observed gene dosage effect implies that the *l(3)Gl*^{1} mutation does not produce a gratuitous perturbation of visual system development but rather that it shares the temporal and spatial domain of the normal product of the locus, which is therefore essential for normal visual system development.

facet array. There are sometimes no detectable abnormalities in the optic lobes beneath such eyes, but others display the familiar lamina-medulla projection abnormality (arrow). Anterior is to the left in (a) and (c) and at the top in (b), (d) and (e). The bars represent 100 micrometers.

TABLE 2

Gene dosage effects at the Glued *locus*

Genotype	Gene dosage	Phenotype
Df(3)Gl+R2/+	0/+	wild type
T(Y;3)H156D/J132P/+	0/+	wild type
Dp(3;Y)G145; +/+	+/+/+	wild type
T(Y;3)B170D/R83P/+	+/+/+	wild type
Gl/Df(3)Gl+R2	*Gl*/0	lethal
Gl/T(Y;3)H156D/J132P	*Gl*/0	lethal
Gl/+	*Gl*/+	"Glued"
Dp(3;Y)G145; Gl/+	*Gl*/+/+	less "Glued"
Gl/T(Y;3)B170D/R83P	*Gl*/+/+	less "Glued"
Dp(3;Y)G145; Gl/T(Y;3)B170D/R83P	*Gl*/+/+/+	much less "Glued"
Dp(3;Y)G145; Gl/Gl	*Gl/Gl*+	lethal

Attempts to rescue the recessive lethality of *l(3)Gl*[1] with a duplication (*i.e.*, *Gl/Gl/Dp*) failed, except for the survival of two adults out of many thousands of progeny. These displayed extreme *Glued* eye phenotypes and extreme reductions in the size of all bristles and of the wings. It is possible that these may have arisen by spontaneous reversions of the embryonic lethality, although this seems unlikely, given the difficulty in obtaining such events by mutagenesis using a direct selection scheme (see below). This behavior indicates that the mutant gene product responsible for the recessive lethality also interacts antagonistically with its wild-type counterpart and suggests that both the recessive lethal and dominant visible phenotypes associated with the *l(3)Gl*[1] mutation are due to alteration of single gene function, although other possible explanations exist.

Isolation of X-ray-induced Glued *revertants*: The most straightforward way to obtain new *Glued* alleles is to screen for lethal mutations over a small deficiency for this region of the chromosome and test them for failure to complement the lethal or dominant visible phenotypes associated with the original *Glued* mutation.

To do this required isolating a small deficiency for the *Glued* region. It is possible to isolate deficiencies for many loci as X-ray-induced phenotypic revertants of their dominant visible alleles (LIFSCHYTZ and FALK 1969; DENELL 1972; DUNCAN and KAUFMAN 1975). By "phenotypic" revertants we mean mutations at the same or different loci which relieve the dominant visible effects of the mutation and restore the wild-type phenotype in heterozygotes, but which do not necessarily restore the product of the gene to its normal functional condition. To determine if this were possible in the case of *l(3)Gl*[1], a series of small adjacent deficiencies uncovering the likely region were synthesized using the segmental aneuploid technique (LINDSLEY *et al.* 1972). Note that these deficiencies are constructed from complex rearrangements, which are not themselves convenient to use in routine screens for new mutations, necessitating the independent isolation of simple interstitial deficiencies for the region of interest.

l(3)Gl^{1} was found to be lethal in heterozygous combination with the synthetic deficiency constructed from the distal fragment (D) of *T(Y;3)H156* and the proximal fragment (P) of *T(Y;3)J132*, which is deficient for third chromosomal material in the *70C* region of BRIDGES' cytogenetic map. *l(3)Gl*^{1} was not lethal in heterozygotes with either of the parental chromosomes from which this deficiency was synthesized, confirming that it is located within the bounds of this deficiency. By similar logic, the closely-linked visible mutation *fz* was localized to the *70C-D;71B* interval, between the breakpoints of *T(Y;3)R91* and *T(Y;3)-A60*, and the right breakpoint of *In(3L)D*^{3} at *70C13-D1* was determined to be lethal over this same deficiency.

In addition to localizing *l(3)Gl*^{1} to a small region of the third chromosome, we also found that the synthetic deficiency *T(Y;3)H156*^{D}*/J132*^{P} displayed no dominant visible eye phenotype when heterozygous with a wild-type third chromosome (see Gene dosage analysis, above). This fact suggested that the simplest way to obtain *Glued* deficiencies would be to isolate X-ray-induced phenotypic revertants of the dominant eye phenotype and examine the polytene chromosomes of their larval salivary glands for deficiencies of the *70C* region.

X-ray-induced phenotypic revertants of *Glued* were isolated as outlined in the MATERIALS AND METHODS and Figure 2. The mating scheme employed permitted only progeny bearing the *l(3)Gl*^{1} chromosome to survive. These were screened for revertants of the eye phenotype. The results are outlined in Table 3. No partial revertants were found, and no temperature-sensitive revertants were found. To identify deficiencies, each revertant was crossed to the closely flanking mutants *Ly*, *D*^{3} and *fz* as well as *l(3)Gl*^{1}. The polytene chromosomes of the larval salivary glands were examined in *Gl*$^{+R}$/+ heterozygotes for structural abnormalities in this region. The results of these tests are presented in Table 4. Subsequently, these phenotypic revertants were also crossed to representatives of each of the new lethal complementation groups isolated over *Df(3)-Gl*$^{+R2}$. All those revertants which complemented *Ly*, *fz*, and *D*^{3} in the previous tests were also found to complement all these lethals, except ones in the *Glued* complementation group itself (A. GAREN, personal communication). The failure of all these revertants to complement the lethal phenotype of *l(3)Gl*^{1} is not surprising and indicates that none of these are true revertants. On the contrary,

TABLE 3

Results of screen for X-ray-induced Glued *revertants*

Sperm stage sampled	Flies scored	F_1 revertants	F_2 revertants	F_1 mosaics	F_2 mosaics
Post-meiotic	11,284	19*(0.0017)	7(0.0006)	3†(0.16)	1†(0.14)
Pre-meiotic	2,185	3 (0.0014)	3(0.0014)	0	0
Totals	13,469	22 (0.0016)	10(0.0007)	4 (0.18)	

* Twelve of these produced no offspring in the F_2.

† Three of the phenotypic "revertants" detected in the F_1 were mosaic for *Gl* in one or both eyes. An examination of the ten revertant lines recovered in the F_2 showed that an additional F_1 individual had been mosaic but had gone undetected as such.

TABLE 4

Complementation behavior and cytology of X-ray-induced Glued *revertants*

Revertant	Mutations tested				Cytology
	Gl	*Ly*	D^3	*fz*	
Gl+*R1**	—	+	—	—	*Df(3)70C-71B*
Gl+*R2*	—	+	—	—	*Df(3)70C-70E-F*
Gl+*R3*	—	+	+	+	normal
Gl+*R9*	—	+	+	+	normal
Gl+*R17*	—	+	+	+	NT
Gl+*R18*	—	+	+	+	NT
Gl+*R22*	—	+	+	+	NT
Gl+*R24*†	—	+	+	+	NT

Key: +: complements lethal phenotype (*Gl*, *Ly*, D^3) or visible phenotype (*fz*);
—: fails to complement lethal phenotype (*Gl*, *Ly*, D^3) or visible phenotype (*fz*);
NT: not tested.
* Lost; two others were lost before testing.
† Female-sterile.

given the "revertant" phenotype of heterozygous deficiencies for $l(3)Gl^1$, these revertants are likely to be genetic events, which completely or almost completely eliminate the function of the $l(3)Gl^1$ gene product.

Isolation of EMS-*induced* Glued *revertants and suppressors*: In addition to new lethal alleles, we were also interested in obtaining additional alleles with variations in the eye and optic lobe defects for comparative analysis. The most direct way to do this is to screen for partial revertants of the original $l(3)Gl^1$ allele using the scheme outlined in Figure 2. Ethyl methanesulfonate (EMS) was chosen as a mutagen since no partial revertants were found using X rays and since EMS is known to produce hypomorphic (leaky) and temperature-sensitive mutations. The scheme used for this purpose also allows the recovery of dominant second-site suppressors of $l(3)Gl^1$. These are also of interest since they may alter the eye and optic lobe phenotypes in different ways than do partial revertants and may help us to identify aspects of the eye phenotype crucial for the induction of normal optic lobe development.

The results of this screen are presented in Table 5. All the closely linked revertants were found to map within the *Ly-st* interval and probably represent

TABLE 5

Results of screen for EMS-induced Glued *revertants*

Flies scored	F_1 revertants	F_2 revertants	F_1 mosaics	F_2 mosaics
15,127	161 (0.011)	21 (0.0014)*	124† (0.77)	12† (0.074)

* Eighty-six of the F_1 revertants were sterile or died. Six of the 21 recovered were subsequently lost due to low viability.
† Many of the F_1 individuals evidence mosaicism for the reversion within the compound eye. Additionally, 12 individuals not detected as mosaics in the F_1 proved to be mosaics in the germ line when the F_2 was examined.

second mutational events at the *Glued* locus. None behaved as deficiencies in the same genetic tests used with the X-ray-induced revertants, and all failed to complement the lethal phenotype of *l(3)Gl*^{1}. Three of the second site suppressors map to the same region on the left arm of the third chromosome between *ru* and *h*. Another, *Su(Gl)27*, maps to the *X*-chromosome to the right of *f* and appears to fall within the bounds of *Df(1)HF396* and *Df(1)mal*^{10}, indicating a cytogenetic location of 19A5-E2. Furthermore, *Su(Gl)27* may be an allele of the existing mutation *outheld* (*ot*), based on its similar phenotype when heterozygous with the above deficiencies.

Attempts to revert the lethal phenotype of Gl: We attempted to isolate true revertants of *l(3)Gl*^{1} using a selection scheme outlined in MATERIALS AND METHODS and in Figure 3. None of the normal progeny resulting from this cross survived; among the exceptional survivors should be "true" revertants of the lethality associated with *l(3)Gl*^{1}. The ability to obtain such revertants would suggest that *l(3)Gl*^{1} is most likely not a complex mutation (*e.g.*, deletion, rearrangement, fusion). Simultaneous reversion of the visual system phenotype along with the lethal phenotype would constitute fairly strong evidence that both these phenotypes result from a lesion in a single gene. Approximately 200,000 mutagenized chromosomes were screened, but no revertants were found. Inability to revert a mutation is generally interpreted to mean that the mutation results from a deletion, rearrangement or other complex event and not from a simple base substitution. However, a rigorous interpretation along these lines depends on screening a much greater number of chromosomes and employing a spectrum of mutagens which are known to act in diverse ways. This is currently feasible only in prokaryotes, where mutagen action at the molecular level is well characterized and large-scale selection procedures can be carried out rapidly. Hence, these negative results are inconclusive with respect to this question.

Isolation of new lethal alleles of Glued: *Df(3)Gl*$^{+R2}$ was used to screen for new lethals in the *70C* region of the third chromosome as outlined in MATERIALS AND METHODS and in Figure 4. This screen was carried out in ALAN GAREN'S laboratory in the course of a search for mutants at other loci uncovered by this deficiency. Fifty new lethal mutations were recovered within this region. New *Glued* alleles were identified by testing all of these lethals for failure to complement the recessive lethality of the original *l(3)Gl*^{1} mutation, interaction with the dominant eye phenotype or appearance of new phenotypes in these double heterozygotes. Ten new *Glued* alleles were identified in this manner. The details of the pair-wise complementation tests among all the other lethals isolated in this screen will be published separately by A. GAREN. The pertinent results of those tests are that no other alleles reflecting more complex complementation patterns at the *Glued* locus were discovered besides those identified by their failure to complement the original *l(3)Gl*^{1} mutation. All of these new lethal alleles are also lethal when heterozygous with the small synthetic deficiency for *70C*, *T(Y;3)H156*^{D}*/J132*^{P}. All those tested (six) also mapped meiotically to within the *Ly-fz* interval.

Complementation patterns of Glued *lethals*: The results of all possible complementation tests among new *Glued* alleles are presented in Table 6. Initial complementation tests between *l(3)Gl*^{1} and the newly isolated lethals revealed two complementation patterns. Eight lethals failed to complement the lethal phenotype of *l(3)Gl*^{1} at all temperatures tested. One other lethal, *l(3)Gl*$^{32\text{-}2}$, complemented the lethality of *l(3)Gl*^{1} at all temperatures, but enhanced the *l(3)Gl*^{1} eye phenotype and also displayed small-bristle and outheld-wing phenotypes in combination with *l(3)Gl*^{1} at 29°. The other, *l(3)Gl*$^{34\text{-}3}$, was lethal over *l(3)Gl*^{1} at 29°, but produced a more extreme eye phenotype, small-bristle and outheld-wing phenotypes at 18°. These latter two mutations complement the lethal phenotype of all the other lethals tested, suggesting that *l(3)Gl*^{1} might be a double mutant or that *Glued* might be a complex locus.

Subsequent complementation tests among these new lethal alleles suggested that these two complementation classes might be related. Two of the new lethals, *l(3)Gl*$^{5\text{-}J}$ and *l(3)Gl*$^{15\text{-}C}$ have dominant abnormalities in the arrangement and size of facets not unlike the original *l(3)Gl*^{1} allele, although these defects are found only in the most posterior region of the eye. Beneath these abnormal ommatidia are optic lobe abnormalities similar in detail to those of partial revertants of *l(3)Gl*^{1} (Figure 5). The dominant eye phenotype of these two alleles is apparently slightly enhanced in both cases in double heterozygotes with *l(3)Gl*$^{34\text{-}3}$. This is similar to the effect of *l(3)Gl*$^{34\text{-}3}$ in double heterozygotes with *l(3)Gl*^{1}, but much less pronounced.

TABLE 6

Complementation patterns of Glued *lethals*

	1	*1-3*	*7-1*	*7-6*	*29-1*	*34-3*	*32-2*	*5-J*	*5-K*	*7-E*	*15-C*
1	X										
1-3	—	X									
7-1	—	—	X								
7-6	—	—	—	X							
29-1	—	—	—	±‡	X						
34-3	±*	+	+	+	+	X					
32-2	++†	+	+	NT	NT	NT	X				
5-J	—	—	—	—	—	+§	NT	X			
5-K	—	—	—	—	—	+	NT	—	X		
7-E	—	—	—	—	—	+	NT	—	—	X	
15-C	—	—	—	—	—	+§	NT	—	—	—	X

Key: +: complements lethal phenotype;
—: fails to complement lethal phenotype;
±: partial complementation (see text for explanation of individual cases);
NT: not tested.

* At 29° *l(3)Gl*$^{34\text{-}3}$*/l(3)Gl*^{1} is lethal; at 18° adults are produced with more extreme *Glued* eye phenotype, reduced bristles and small outheld wings.

† At 29° *l(3)Gl*$^{32\text{-}2}$*/l(3)Gl*^{1} adults have a more extreme *Glued* eye phenotype, reduced bristles and small outheld wings; at 18° adults look just like *l(3)Gl*^{1}/+. NB. Allele *32-2* was lost before complementation tests with it could be completed.

‡ A small number of feeble *l(3)Gl*$^{7\text{-}6}$*/l(3)Gl*$^{29\text{-}1}$ adults survive at 18° and 25° and have reduced wings and bristles and a disrupted facet array.

§ *l(3)Gl*$^{5\text{-}J}$*/l(3)Gl*$^{34\text{-}3}$ and *l(3)Gl*$^{15\text{-}C}$*/l(3)Gl*$^{34\text{-}3}$ adults display a slight enhancement of the dominant eye phenotypes associated with *l(3)Gl*$^{5\text{-}J}$ and *l(3)Gl*$^{15\text{-}C}$ (see text).

Stage of lethality of Glued *lethals*: The stage of lethality of *Glued* lethals was estimated by crossing balanced stocks of each lethal to $Df(3)Gl^{+R2}$ as outlined in MATERIALS AND METHODS. The results are summarized in Table 7. All but two of the alleles produced no *red* larvae in the test cross, indicating that all *lethal/Df* larvae were dying in the embryonic period or early in the first instar. Nearly all $l(3)Gl^{7\text{-}6}/Df$ larvae identified in the late first instar survive at least until the late third instar, while nearly all $l(3)Gl^{34\text{-}3}/Df$ larvae identified in the late first instar survive into the pupal period. $l(3)Gl^{7\text{-}6}$ and $l(3)Gl^{34\text{-}3}$ exhibit some temperature sensitivity. The results also suggest that $l(3)Gl^{7\text{-}6}$ is a leaky or hypomorphic allele and that the gene product of this complementation group becomes essential for the viability of the organism through the later as well as very early stages of development. The pupal lethality of $l(3)Gl^{34\text{-}3}$ is not inconsistent with the possibility that it represents a second genetic unit; however, this could also suggest that $l(3)Gl^{34\text{-}3}$ may in fact be an extremely hypomorphic (very "leaky") allele in the same genetic unit as the other lethals.

Temperature-sensitive maternal effect at Glued *locus*: Balanced stocks of two *Glued* lethals, $l(3)Gl^{7\text{-}6}$ and $l(3)Gl^{34\text{-}3}$, were found to produce viable homozygous mutant adults at all temperatures, although both produced more homozygous progeny at 18° than 29°. It was possible to construct homozygous stocks from these flies at 18° but not at 29°. To test whether this was due to maternal effects of these mutations on viability, males and females homozygous for each mutation were mated to wild-type (Canton-S) females and males. The males were found to be fertile at 18° and 29°, but females mated and maintained at 29° produced no progeny. Females mated at 29° produced normal progeny when left at 18° for several days, indicating that mating success is unaffected at 29° and that these mutations have maternal effects on development. Females produce and lay a reduced number of eggs at 29°, but none hatch, even when shifted to 18° immediately after a four hr egg collection at 29°. This indicates that these mutations affect egg production in the female.

TABLE 7

Stage of lethality of Glued-lethals/Df(3)Gl^{+R2}

Allele	Stage
1	Embryo or early first instar larva
1–3	Embryo or early first instar larva
7–1	Embryo or early first instar larva
7–6	18°: early to mid-pupa
	29°: late third instar to early pupa
32–2	NT
34–3	18°: mid to late pupa
	29°: early to mid pupa
5–J	Embryo or early first instar larva
5–K	Embryo or early first instar larva
7–E	Embryo or early first instar larva
15–C	Embryo or early first instar larva

Viability of Glued *lethals in cells*: An estimated 90% of all mutations in the Drosophila genome that are lethal to the organism when present in every cell are viable in individual cells, as evidenced in mosaics where they are confined to a fraction of the cells (RIPOLL and GARCIA-BELLIDO 1973; RIPOLL 1977). The term "cell lethal" has been applied to lethal mutations which are not recoverable in the homozygous condition in clones of cells in genetic mosaics. This test does not, strictly speaking, identify only genes essential for the survival of individual cells, but may also identify those affecting cell division or the ability of a cell to participate in tissue-level organization. All these effects will prevent the recovery of clones of mutant cells in adult tissues, but are indistinguishable by this operational criterion. To determine the effects of homozygous *Glued* lethals on individual cells and to study their impact on visual system development, attempts were made to recover clones of homozygous mutant cells in several adult tissues. This was done by inducing mitotic recombination in larvae heterozygous for a mutation, as outlined in MATERIALS AND METHODS. We tested the original $l(3)Gl^1$ mutation and one of the newly induced lethal alleles, $l(3)Gl^{1\text{-}3}$, in this manner. The mating schemes for these tests and their controls are outlined in Figures 6 and 7.

Controls for the frequency with which clones are detected involved crosses that are similar to the experimental crosses except for the presence of the lethal to be tested. In an effort to assure that the chromosomes carrying the lethals to

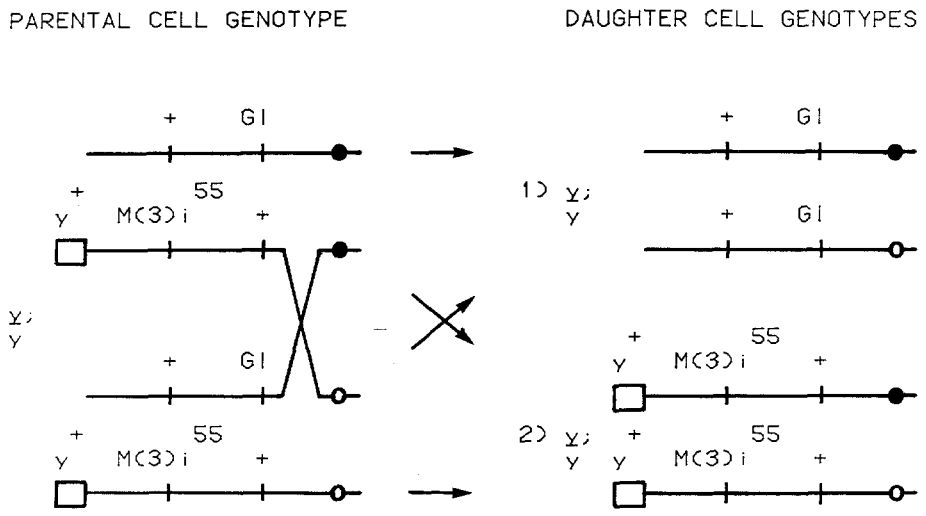

FIGURE 7.—Scheme for making clones of cells homozygous for $l(3)Gl^1$ (*Gl*) in the cuticle via somatic recombination. Only the third chromosome is depicted. Larvae to be irradiated were obtained by crossing *y*; $Dp(1;3)sc^{J4}$, y^+ $M(3)i^{55}/TM1$ females to *y w*; *Gl/TM3* males. The y^+ duplication attached to the tip of the left arm of chromosome *3* permits scoring of cuticular clones. When a homozygous *Gl* clone is generated via somatic recombination, the duplication segregates away, exposing the *y* mutation carried on the *X* chromosomes. $M(3)i^{55}$ was included in the cross to increase the size of clones and thereby make them more readily detectable. The homozygous $M(3)i^{55}$ twin clones are never seen.

be tested were free of other lethals that might affect the results, the chromosomal regions to the left and right of the *Ly-st* interval were replaced with presumably lethal-free chromosomal material. The results are presented in Tables 8 and 9. We failed to recover clones of homozygous *l(3)Gl*^{1} cells in the eye, thorax and abdomen. We recovered a small number of phenotypically white eye clones in the experiment to test the cell viability of *l(3)Gl*$^{1-3}$. However, these clones did not contain abnormal facet arrays, as would be expected based on the phenotype of the *29-1/7-6* survivors and on the assumption of cell-autonomous expression of this phenotype. Furthermore, these clones all occurred in males and could thus be the result of spontaneous or X-ray-induced somatic mutation at the *white* locus. The low frequency of these events relative to control clones also suggests that they are not clones of homozygous *l(3)Gl*$^{1-3}$ cells. These results indicate that the *Glued* locus specifies a function that is essential for cells to survive, divide or participate in tissue-level organization and thereby be recovered in differentiated adult tissues.

Visual system phenotypes of Glued *mutations*: A brief summary of the eye and optic lobe phenotypes associated with the EMS-induced revertants and suppressors is given in Tables 10 and 11. Although genetic events leading to reversion of the eye phenotype were selected, all complete revertants of the eye phenotype also exhibited wild-type optic lobes. This is not surprising, given the wild-type visual system phenotype of heterozygous deficiencies for the *Glued* region.

The partial revertants and suppressors showed a similar correlation of eye and optic lobe phenotype (Figures 8 and 9). We expected that it might be pos-

TABLE 8

Cell viability of 1(3)Gl^{1} *and* 1(3)Gl$^{1-3}$ *in the eye*

Genotype	Nonmosaics		Mosaics		Frequency*
	M†	F†	M†	F†	
bw; l(3)Gl^{1} *st/M(3)i*^{55}	1304	890	0	0	0.0
bw; l(3)Gl$^{1-3}$ *st/M(3)i*^{55}	2266	1754	0	4	0.00099
bw; st/M(3)i^{55}	1270	966	11	10	0.0093

* Frequency per eye.
† M: males; F: females.

TABLE 9

Cell viability of 1(3)Gl^{1} *in the thorax and abdomen*

Genotype	Nonmosaic		Mosaic				Frequency*	
			Thorax		Abdomen		Thorax	Abdomen
	M†	F†	M†	F†	M†	F†		
y; l(3)Gl^{1}*/y+M(3)i*^{55}	630	463	0	0	3	1	0.0	0.0036
y; +/y+M(3)i^{55}	464	302	14	8	33	22	0.036	0.065

* Frequency per abdomen (T1–T5) and per mesothorax.
† M: males; F: females.

TABLE 10

Visual system phenotypes of EMS-induced Glued *revertants*

Revertant	Eye	Optic lobe
Gl^{R+26}	slightly *Glued*	slightly *Glued*
Gl^{R+32}	less *Glued*	less *Glued*
Gl^{R+33}	slightly *Glued*	slightly *Glued*
Gl^{R+34}	wild type	wild type
Gl^{R+50}	slightly *Glued*	slightly *Glued*
Gl^{R+70}	slightly *Glued*	slightly *Glued*
Gl^{R+72}	wild type	wild type
Gl^{R+73}	wild type	wild type
Gl^{R+80}	wild type	wild type
Gl^{R+86}	wild type	wild type

TABLE 11

Visual system phenotypes of EMS-induced Glued *suppressors*

Suppressor	Map position	Eye	Optic lobe
Su(Gl)27	*1*: 66	wild type (29°)	wild type (29°)
		less *Glued* (18°)	less *Glued* (18°)
Su(Gl)57	*1* ?	less *Glued*	less *Glued*
Su(Gl)77	*3: ru-h*	less *Glued*	less *Glued*
Su(Gl)102	*3: ru-h*	slightly *Glued*	slightly *Glued*
Su(Gl)160	*3: ru-h*	slightly *Glued*	slightly *Glued*

sible to recover partial revertants which altered specific components of the eye phenotype (*e.g.*, abnormal R-cell complements per ommatidium) without altering optic lobe phenotype, or *vice-versa*. This would allow us to identify components of the eye phenotype essential for normal optic lobe development and those which are not. Examples of this have been found among suppressors of *rough* (*ro*) mutations, another locus affecting visual system development in a similar manner to *Glued* mutations. One *ro* suppressor drastically alters the mutant eye phenotype towards wild type, but has no apparent impact on the optic lobe phenotype. Another suppressor of *ro* falls into a complementary class, restoring the optic lobe phenotype toward wild type with no apparent impact on the eye phenotype (P.E. LEWIS, personal communication). All partial revertants and suppressors of the $l(3)Gl^1$ eye phenotype had equivalent impacts on the gross eye and optic lobe phenotypes. The partial revertants have a number of interesting properties. Those that are the most nearly wild type display a single, relatively small region of abnormal facets in the very posterior part of the eye straddling the equator. These facet abnormalities are similar to those found in $l(3)Gl^1$: small facets, nonhexagonal array. The rest of the eye is wild type in appearance and the eye is nearly normal in size. Beneath these abnormal facets are found a relatively normal-looking lamina and medulla except for the projection of a small bundle of fibers from the posterior lamina, which fails

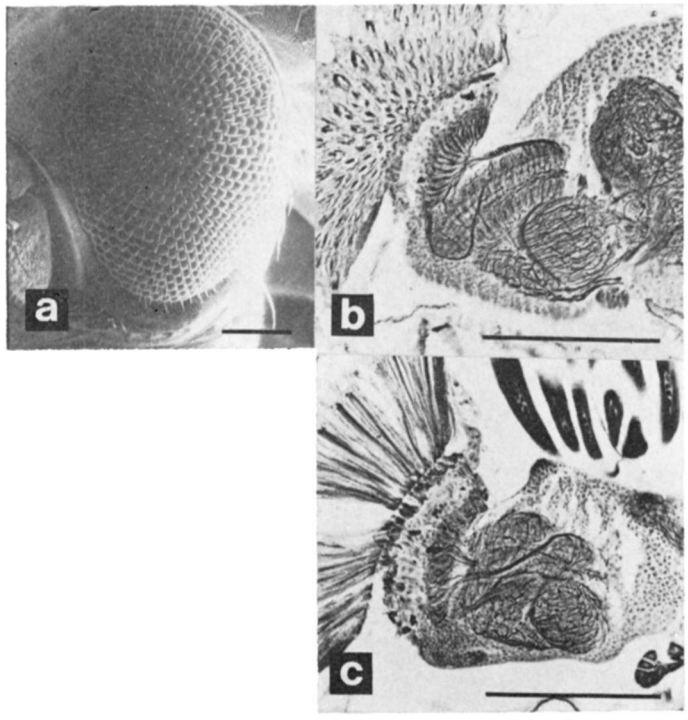

FIGURE 8.—Eye and optic lobe phenotype of partial revertants of *l(3)Gl*^{1} (a) shows the eye phenotype of *Gl*$^{+R26}$. The eye displays a wild-type facet array except for a small number of disarrayed facets in the posterior equatorial part of the eye. The eye is also not quite the size of wild type. (b) shows a horizontal silver stained section of a *Gl*$^{+R26}$ optic lobe. The optic lobe is normally organized except for a fiber tract from the posterior lamina that projects along the posterior face of the medulla and through the medulla neuropil. From a small number of Golgi preparations we know that at least the R7 and R8 retinula fibers are involved in this aberrant projection. (c) shows the optic lobe of a *Gl*$^{+R33}$ fly. This partial revertant is temperature-sensitive. Its eye phenotype ranges from nearly wild-type at 29° to only slightly less normal than *l(3)Gl*^{1} at 18° and its optic lobe phenotype is correspondingly more or less normal. The optic lobe shown here is from a fly raised at 25°. The lamina is considerably more normally organized than in *l(3)Gl*^{1}. The medulla is also more normally organized, but is still rotated at 25° and contains the familiar fiber tracts from the posterior lamina crossing through its neuropil. The second optic chiasma is also abnormal.

to cross the first optic chiasma. This bundle grows down along the posterior lateral face of the medulla, then enters it and projects across it towards its normal target region in the anterior medulla. These abnormal fiber bundles enter the medulla at the level where the medulla tangential fibers divide the medulla or, even deeper, at the medial edge of the medulla. They always project upwards toward the medulla cortex as they traverse the medulla. In the most nearly normal revertants (*e.g.*, *Gl*$^{+R26}$, *Gl*$^{+R33}$, *Gl*$^{+R70}$), this abnormal lamina–medulla projection is rarely, if ever, accompanied by the abnormal medulla "rotation" found in *l(3)Gl*^{1}. These revertants thus simplify the *l(3)Gl*^{1} optic lobe phenotype to an extent, making aspects of it more analyzable. Furthermore, the

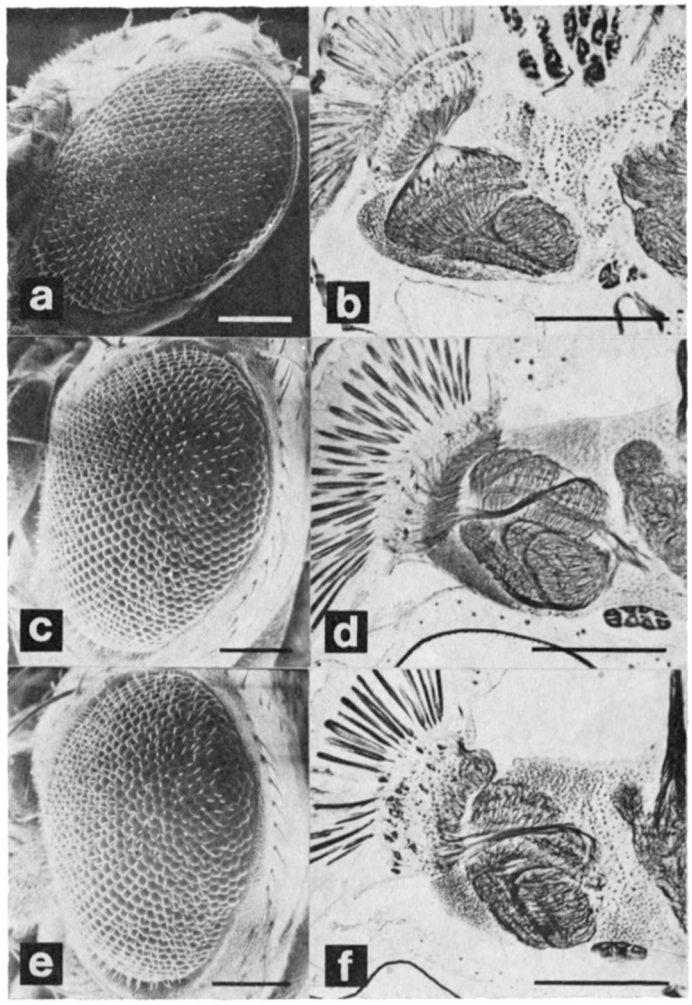

FIGURE 9.—Eye and optic lobe phenotypes of suppressors of *l(3)Gl¹*. (a) shows the eye phenotype of a *Su(Gl)27; l(3)Gl¹/+* male raised at 29°. This mutation is temperature sensitive, the eye and optic lobe appearing less normal at 18°. The eye exhibits an almost normal hexagonal facet array at 29°, but is not as large as a wild-type eye. There are some small facets scattered throughout the eye. The facets are not packed as tightly together as in wild type. Close examination reveals the presence of some smooth pigmented material separating facets from one another. (b) shows a horizontal, silver stained section through the optic lobe of a *Su(Gl)27; Gl/+* male raised at 29°. The optic lobe is very nearly normal in appearance. The lamina cell body region is thicker than wild-type and the lamina neuropil is slightly irregular in contour, but the medulla rotation and gross fiber tract abnormalities of *l(3)Gl¹* are missing. (c) and (e) show the eye phenotypes of *Su(Gl)102 l(3)Gl¹/+ +* and *Su(Gl)160 l(3)Gl¹/+ +* respectively. They are substantially similar, although *102* is generally slightly more nearly wild-type than *160*. Both tend to exhibit more normal facet arrays in the anterior than in the posterior part of the eye. The optic lobe phenotypes of these two suppressors are also substantially similar. (d) shows the most extreme departure from wild-type seen in *Su(Gl)102 l(3)Gl¹/+ +* flies. The lamina cell body layer is thicker than normal in its posterior region and the lamina neuropil is somewhat misshapen, particularly anteriorly. The

phenotypes of these revertants are very similar to the dominant phenotypes associated with the two new lethals *l(3)Gl$^{5\text{-}J}$* and *l(3)Gl$^{15\text{-}C}$*. These two groups of mutations thus suggest that this abnormal lamina–medulla projection is not sufficient to cause failure of the medulla to rotate from the larval to adult position. We do not know why these mutations do not produce abnormal ommatidial organization uniformly throughout the eye, but this phenomenon may reflect something important about the development of the eye rather than a peculiarity of these mutations.

DISCUSSION

The genetic analysis of the *Glued* locus has provided some information about the spectrum of phenotypic effects associated with its mutations and allowed us to draw some general conclusions about their relationship to the specific defects in development of the visual system. The dosage analysis of the original *Glued* allele indicated that it falls into the relatively rare class of "antimorphic" mutations (MULLER 1932). The "titrating" effect of duplications for the *70C* region indicates that the dominant effects of this mutation on the development of the visual system do not result from a failure of the normal temporal or spatial regulation of this locus. This implies that the *Glued* locus specifies a function which is normally required during visual system development. The apparent "antimorphic" behavior of the recessive lethality also provided the first evidence that these two phenotypes probably result from a lesion in a single gene. This suggested that the isolation of additional mutations that failed to complement the lethal phenotype of *l(3)Gl^{1}* would probably identify mutations that would demonstrate the effects of total absence of this gene product on the visual system as well as the full range of phenotypes associated with this locus. While we began by assuming that the lethal and visual system phenotypes might not be related to a lesion in a single genetic function, the results of the accumulation of new lethal alleles suggest that they do result from a single genetic lesion. The strongest evidence for this conclusion is the presence of the typical dominant *Glued* visual system phenotypes in two of the new lethal alleles, *5-J* and *15-C*, and in the heteroallelic combination *7-6/29-1*. While the complementation analysis suggests that the *Glued* locus may be complex, the late lethal phase of *l(3)Gl$^{34\text{-}3}$/Df(3)Gl^{+R2}* heterozygotes and their viability as homozygotes suggests that their complementation behavior may be the result of their extreme "leakiness." This interpretation would imply, however, that none of the eight lethals in the first group is completely lacking in functional gene product, *i.e.*, equivalent to a deficiency for the locus. While this is not entirely unlikely, we cannot resolve this issue at this time.

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medulla is abnormally rotated, its posterior edge directly apposed to the lamina, but it is more normally organized than in *l(3)Gl^{1}*. The familiar abnormal projections from the posterior lamina through the medulla are present. The second optic chiasma is also divided into several tracts. In less extreme cases, the medulla is only slightly rotated and the second optic chiasma is normal.

While the very early lethality of the most extreme alleles was anticipated from the original mutation, we had planned to analyze their impact in homozygous condition on visual system development in somatic recombination mosaics. Attempts to construct such mosaics with the original allele and one of the new alleles failed. Since some care was taken to ensure that these were the only lethals present on these chromosomes, we conclude that imaginal cells homozygous for these mutations are not recovered in mosaics either because they are lethal to the cells or because they prevent such cells from dividing or from participating in tissue-level organization. This result suggests that the wild-type gene product of the *Glued* locus specifies a very fundamental cellular function. Thus, the effects of some *Glued* alleles on visual system development are not the result of their specific effects on neural cells, but a more indirect effect of their impact on cellular patterning in the eye. Nonetheless, these mutations provide useful probes for investigating the rules governing the cellular events involved in the interaction of retinula axons and optic lobe cells during development. The number of loci with dramatic effects on cellular patterning in the eye is large, but those which also perturb neural pattern formation in the optic lobes remain small in number. Thus, comparative analysis of the specific effects of these mutations on cellular patterning in the eye is likely to yield some basic information about the cellular events and interactions involved in assembly of the Drosophila visual system.

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LITERATURE CITED

DENELL, R. E., 1972 The nature of reversion of a dominant gene of *Drosophila melanogaster*. Mutat. Res. **15:** 221–223.

DOANE, W. W., 1967 *Drosophila*. pp. 219–244. In: *Methods in Developmental Biology*. Edited by F. WILT and N. WESSELLS. Thomas Y. Crowell, New York.

DUNCAN, I. W. and T. C. KAUFMAN, 1975 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: mapping of the proximal portion of the right arm. Genetics **80:** 733–752.

GREGORY, G. E., 1980 Alcoholic Bouin fixation of insect nervous systems for Bodian silver staining. II. Modified solutions. Stain Technology **55:** 151–160.

HANSON, T. E., 1971 Neurogenesis in the optic tracts of *Drosophila*. Caltech Biol. Ann. Reprt. 69–70.

HOLMES, W., 1947 Pp. 404–405. In: *Recent Advances in Clinical Pathology*. Edited by S. C. DYKE. Blackiston Co., Philadelphia.

HUMASON, G. L., 1972 *Animal tissue techniques*, 3rd edition. W. H. Freeman, San Francisco.

JOHANNSEN, O. A., 1924 Eye structure in normal and eye mutant *Drosophila*. J. Morphol. **39:** 337.

LEWIS, E. B. and X. X. BACHER, 1968 Method of feeding ethyl methane sulfonate (EMS) to *Drosophila* males. Drosoph. Inf. Serv. **43**: 193.

LIFSCHYTZ, E. and R. FALK, 1969 A genetic analysis of the Killer of prune (K-pn) locus of *Drosophila melanogaster*. Genetics **62**: 353–358.

LINDSLEY, D. L. and E. H. GRELL, 1968 *Genetic variations of Drosophila melanogaster*. Carnegie Institute of Washington Publication No. 627.

LINDSLEY, D. L., L. SANDLER, B. S. BAKER, A. T. C. CARPENTER, R. E. DENELL, J. C. HALL, P. A. JACOBS, G. L. GABOR MIKLOS, B. K. DAVIS, R. C. GETHMANN, R. C. HARDY, A. HESSLER, S. M. MILLER, H. NOZAWA, D. M. PARRY and M. GOULD-SOMERO, 1972 Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71**: 157–184.

MEYEROWITZ, E. M. and D. R. KANKEL, 1978 A genetic analysis of visual system development in *Drosophila melanogaster*. Devel. Biol. **62**: 112–142.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutation. Proc. 6th Intern. Cong. Genetics **1**: 213–255.

O'BRIEN, S. J. and R. J. MACINTYRE, 1978 "Genetics and biochemistry of enzymes and specific proteins of *Drosophila*." pp. 396–552. In: *The Genetics and Biology of Drosophila* Edited by M. ASHBURNER and T. R. F. WRIGHT. Vol. 2a. Academic Press, New York.

PAK, W. L., J. GROSSFIELD and N. V. WHITE, 1969 Nonphototactic mutants in a study of vision of *Drosophila*. Nature (London) **222**: 351–354.

PILKINGTON, R. W., 1941 Facet mutants of *Drosophila*. Proc. Zool. Soc. Lond A **111**: 199–222.

PLOUGH, H. H. and P. T. IVES, 1935 Induction of mutations by high temperature in Drosophila. Genetics **20**: 42–69.

POWER, M. E., 1943a The brain of *Drosophila melanogaster*. J. Morphol. **72**: 517–559. ——, 1943b The effect of reduction in numbers of ommatidia upon the brain of *Drosophila melanogaster*. J. Exp. Zool. **94**: 33–71.

RIPOLL, P., 1977 Behavior of somatic cells homozygous for zygotic lethals in *Drosophila melanogaster*. Genetics **86**: 357–376.

RIPOLL, P. and A. GARCIA-BELLIDO, 1973 Cell autonomous lethals in *Drosophila melanogaster*. Nature, New Biol. **241**: 15–16.

SHERALD, A. F. and T. R. F. WRIGHT, 1974 The analog inhibitor, a-methyl dopa, as a screening agent for mutants elevating levels of dopa decarboxylase in *Drosophila melanogaster*. Molec. Gen. Genet. **133**: 26–36.

STRUHL, G., 1981 A homeotic mutation transforming leg to antenna in *Drosophila*. Nature **292**: 635–638.

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