Mosaic Analysis Gives an Estimate of the Extent of Genomic Involvement in the Development of the Visual System in *Drosophila melanogaster*

Harshwardhan M. Thaker and Douglas R. Kankel

Department of Biology, Yale University, New Haven, Connecticut 06511-8112

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ABSTRACT

To investigate the role of vital loci in the development of the visual system of Drosophila, we induced mitotic recombination in individuals heterozygous for recessive organismal lethals and selected for analysis the resulting mosaics with homozygous mutant eye clones. Heads bearing clones were serially sectioned, silver-stained and examined for aberrations in the ommatidia and the neural structures to which they project. In our screen of 68 lines bearing diepoxybutane-induced *X*-linked lethals, 26 yielded few or no homozygous mutant clones (putative cell-lethals). Of the rest, 20 lines produced individuals with morphologically abnormal eye clones showing various degrees of aberrations in the ommatidial architecture. In 14 of these 20, the laminar cartridges innervated by the mutant clones were also disorganized. Clones with normal structure were found in 18 of the lines, and three lines were resistant to the induction of mitotic recombination. In a single line, comparatively normal clones in the eye projected to a lamina with subtle but consistent abnormalities. To the extent that we have a representative sample, these results suggest that about two-thirds of all vital genes may be essential for the normal assembly and neural connectivity of the eye. This points to a high degree of pleiotropy in the manner in which information in the genome of the fly is used in development.

THE genetic dissection of a biological process involves the identification of mutations affecting that process and the subsequent analysis of the corresponding genes; it is hoped that every molecular participant will eventually be identified and understood. This approach has met with considerable success in the investigation of a number of developmental problems in *Drosophila melanogaster*, especially those dealing with earlier events in embryogenesis proper (AKAM 1987); mutants disturbing these early developmental events can be recovered and analyzed independent of their status as viables or lethals. However, when searching for genes affecting later developmental events, it is difficult to assess the role of lethal mutations in processes which begin or continue after the effective lethal phase of the mutation under consideration. Thus, when studying the development of the compound eyes and their interaction with the optic lobes, one has been largely limited to the screening of adults for morphological abnormalities (*e.g.*, HEISENBERG and BÖHL 1979; MEYEROWITZ and KANKEL 1978) or for defective visual behaviors (*e.g.*, BENZER 1967; HARRIS, STARK and WALKER 1976). While vision is not essential for life, it is possible that a gene participating in visual system development has vital functions at other spatiotemporal points in the fly's life cycle. For instance, genes affecting the connectivity of photoreceptor cells may also affect similar processes in the embryonic or larval nervous system where such effects may well be lethal. Indeed, several such vital genes affecting the development of the eye are already known, *e.g.*, Notch [a neurogenic gene (CAGAN and READY 1989)], *Ellipse* [encoding a homolog of the epidermal growth factor receptor (BAKER and RUBIN 1989)], *seven-up* [encoding a steroid-receptor homolog (MLODZIK *et al.* 1990)], *l(1)myospheroid* [encoding an integrin subunit homolog (ZUSMAN *et al.* 1990)] and several dominant enhancers of sevenless [including a *ras* homolg and a putative guanine nucleotide exchange factor (SIMON *et al.* 1991)].

Lethally mutable loci constitute a large fraction of the entire genome of Drosophila. Estimates of the size of this fraction have ranged from as high as 90% (JUDD, SHEN and KAUFMAN 1972) to as low as 30% (BOSSY, HALL and SPIERER 1984). The variation in this estimate has been mostly due to the difficulty in estimating the size of the nonlethal fraction of the genome rather than the total number of vital genes, which is generally accepted as 5000–7000 (RIPOLL and GARCIA-BELLIDO 1979; YOUNG and JUDD 1978). Lethality certainly remains one of the most unambiguous and common phenotypes observed in any mutagenesis, and it would be useful if this class of mutants could be made amenable to phenotypic analysis in adult tissue.

One of the ways in which vital loci reveal their function in adult structures is through phenotypes manifested in hypomorphic viable alleles (*e.g.*, LIPSHITZ and KANKEL 1985) of which conditional lethals, such as temperature sensitive ones (*e.g.*, SUZUKI 1970), constitute a special class. However, it may not be possible to obtain viable hypomorphs for all loci, and

lethality is often the only recognized outcome of mutation at a locus. Another strategy to identify the function of vital loci involves scoring the impact of lethal mutations on the very earliest recognizable stages of the process under study before the effective lethal phase (HADORN 1961). This has been successfully employed for imaginal discs (*e.g.*, SHEARN *et al.* 1971; STEWART, MURPHY and FRISTROM 1972) and for the central nervous system (*e.g.*, DATTA and KANKEL 1992), but this approach requires a recognizable phenotype in often undifferentiated precursor cells before the onset of lethality; such a condition may not always be met. Studying the patterns of gene expression by monitoring the spatial distribution of monoclonal antibody binding (*e.g.*, FUJITA *et al.* 1982; ZIPURSKY *et al.* 1984), differential RNA expression (*e.g.*, PALAZZOLLO *et al.* 1989) or of expression in so-called "enhancer traps" (*e.g.*, O'KANE and GEHRING 1987; WILSON *et al.* 1989) can identify potentially interesting loci, including lethal ones, but these methods still do not surmount the problem of phenotypic analysis in lethal null mutations that may eventually be generated at loci discovered by these procedures.

A more direct way to screen recessive lethals for imaginal phenotypes is to examine clones of homozygous mutant tissue in mitotic recombination mosaics in which the vast majority of cells are of a viable heterozygous genotype (STERN 1936). This approach has been previously used to identify zygotic lethals affecting cuticle morphogenesis (FERRÚS and GARCIA-BELLIDO 1976; RIPOLL 1977) and oogenesis (GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984). Phenotypic effects of specific lethal mutations in the eye (CAMPOS-ORTEGA 1980; MLODZIK *et al.* 1990; ZUSMAN *et al.* 1990) and other imaginal disc derivatives (WOODS and BRYANT 1991) have also been described in mitotic recombination clones. In these latter cases, however, the genes had been identified by other criteria, and mitotic recombination was not itself used as a screening procedure.

The visual system of Drosophila has been one of its most thoroughly investigated imaginal structures, particularly in reference to its development. This system consists of a pair of compound eyes projecting to a pair of optic ganglia (or lobes) which perform the function of processing visual information. Analysis of mutant material has provided a great deal of data regarding the cell-cell interactions that are involved in the development of the ommatidia, which are the basic units of the compound eyes (reviewed by READY 1989; RUBIN 1989). The development of the optic lobes has also been the subject of mutational analyses (reviewed by FISCHBACH *et al.* 1989; KANKEL 1984). One aspect of the formation of this system is the dependence of the developing optic lobe on a normal interactive influence exerted by the eye (FISCHBACH 1983; MEINERTZHAGEN 1973; MEYEROWITZ and KANKEL 1978; POWER 1943). The nature of the signal/s involved is unknown, but recent work suggests that triggering of mitotic activity in the laminar precursors occurs under the influence of retinal innervation (SELLECK and STELLER 1991). In spite of the many tens of known mutants that affect the eyes and/or the optic lobes, relatively few of them are lethals; some known to affect eye development were mentioned above, and several with prominent affects on the optic lobe are also available [*e.g.*, *l(1)ogre* (LIPSHITZ and KANKEL 1985), *elav* (CAMPOS, GROSSMAN and WHITE 1985), *l(1)trol* and *l(1)devl* (DATTA and KANKEL 1992)]. The relative paucity of lethals probably reflects the fact that most screens seeking mutants with an impact on the visual system have examined only adult flies (*e.g.*, HARRIS, STARK and WALKER 1976; HEISENBERG 1974; HEISENBERG AND BÖHL 1979) and consequently have excluded all lines which die before that stage.

To extend the mutational analysis of visual system development to include lethal mutations, we performed an analysis on mitotic recombination induced mosaics carrying clones of homozygous lethal tissue in the compound eye. In this report, we describe the result of screening a sample of *X*-linked lethals for abnormalities in the histology of the eye and the optic ganglion, reflecting a phenotypic effect of homozygous mutant eye clones. The purpose of this study is to estimate the extent to which vital genes participate in the normal assembly and connectivity of the compound eye. Our results suggest that a large fraction of the lethally mutable genome is directly or indirectly involved in this process.

MATERIALS AND METHODS

Stocks and culture: Fly stocks were maintained on cornmeal, molasses, yeast, agar medium (DOANE 1967) at 22–25°. All mutations are abbreviated as described elsewhere (LINDSLEY and GRELL 1968; LINDSLEY and ZIMM 1985, 1987) or as explained in the text. Lethals were induced on a *w sn*^{3} chromosome which had been carried in the laboratory as a homozygous stock. The *FM6, l*^{69a}*/y ewg*^{1} *cho sn*^{3} stock was obtained from R. FLEMMING, and the *w*^{1118}, *P[(w*$^+$*ry*$^+$*)E]1* stock was from G. RUBIN.

Mutagenesis: Two- to four-day-old males of the genotype *w sn*^{3} were isolated from females for 24 hr and starved for 6 hr before being fed a 5 mM solution of diepoxybutane (DEB, Sigma) in 1% sucrose for 24 hr according to the method originally described (LEWIS and BACHER 1968) for ethyl methanesulfonate (EMS). Mutagenized males were crossed to virgins that were heterozygous for a lethal-bearing balancer *FM6, l*^{69a} and either the lethal-bearing chromosome *y ewg*^{1} *cho sn*^{3} (51 lines) or *Binsinscy*, a viable balancer chromosome (17 lines). In both cases, virgin F_1 females carrying the mutagenized *w sn*^{3} chromosome over *FM6, l*^{69a} were crossed to *Binsinscy* males and individual lines set up in small plastic vials. Each vial was inspected in the F_2 generation for the presence of a lethal mutation as indicated by the absence of any males. Balanced lethal lines were set up from lethal-bearing *w sn*^{3}*/Binsinscy* individuals by crossing them to *Binsinscy* males.

Mosaic generation and scoring: Balanced heterozygous virgins of each lethal line were crossed to *w*^{1118}, *P[(w*$^+$*ry*$^+$*)E]*

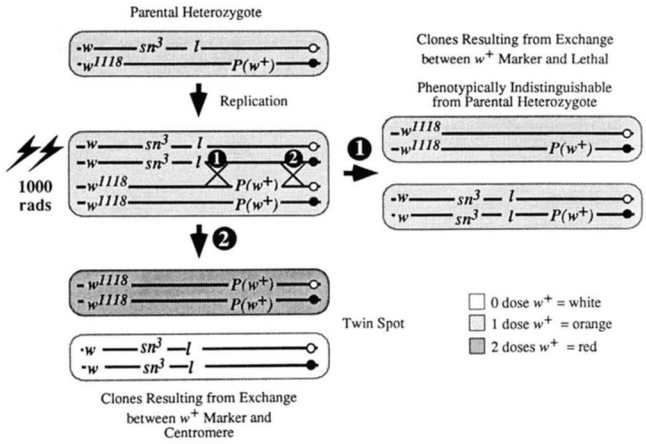

FIGURE 1.—Schematic representation of the cross used to generate mitotic recombination clones. The symbol *l* is used to designate a recessive lethal of undefined chromosomal position; the only relevant positional information is that both the lethal and $P(w^+)$ should be distal to the point of somatic exchange. Given the relatively proximal insertion point of $P(w^+)$, most lethals should also be distal to it. There are two classes of mitotic recombination events that are relevant with respect to $P(w^+)$; these are indicated as *1* or *2* in black circles. The only event which generates a *white* clone (*2*) also ensures homozygosity for the recessive lethal, providing the lethal is distal to the point of somatic exchange.

1 males and allowed to lay eggs in plastic vials (Figure 1). Progeny were X-irradiated (1080 rad delivered in 20 min from a Torrex 150 X-ray inspection system, Scanray Corporation, set at 150 kv and 5 mA using 0.63-mm aluminum and 1-mm plexiglass filters) at the late embryo and first instar larval stages (18–48 hr) to induce mitotic recombination. Eclosed adults were examined under a dissecting microscope using transmitted illumination from a dual fiber-optic source (Dyonics) for the presence of twin, white single, and wild-type single mitotic recombination clones in the eyes. Only discrete patches of size greater than 2 ommatidia were scored as clones. The entire cuticle of the fly was also examined for the presence of *singed* bristles marking mutant cuticular clones. All clones were examined for obvious irregularities on the eye surface. Individuals (aged 1–10 days after eclosion) of each line with larger clones were selected for histological analysis, the size and position of each clone plotted on a scoresheet, the flies decapitated and the heads immersed in fixative.

In some cases, a small number of gynandromorphs were also made. This entailed mating males hemizygous for the unstable ring-*X* chromosome $In(1)w^{vc}$ (HINTON 1955) to females heterozygous for the lethal bearing chromosome over a balancer. Adult gyanders were recognized on the basis of both secondary sexual characteristics and by the exposure of the recessive *w* sn^3 markers on the lethal-bearing chromosome in hemizygous male tissue.

Histology: Heads were fixed overnight in alcoholic Bouin's fixative, dehydrated through an ethanol series, cleared in xylene and embedded in paraffin by standard methods (HUMASON 1972). Serial sections (5–7 μm thick) in the horizontal plane were obtained using a Dupont/Sorvall JB-4 microtome with razor blades (Schick Platinum Plus) and were stained by reduced silver methods described earlier (BLEST 1961; HARTE and KANKEL 1983). Sections were examined for structural aberrations in the ommatidial or optic lobe organization on a Zeiss Universal compound microscope.

Effective lethal phase: The lethal phase of each line was determined by identifying the last stage at which mutant males could be identified in cultures of a balanced lethal stock of the line. This was done by using the larval and pupal marker *y* on the balancer chromosome to distinguish between mutant and balancer males. This permitted reliable scoring for all stages after the late second instar, at which points the sexes can be distinguished easily. For the purpose of staging, flies were allowed to lay eggs on well yeasted apple juice agar plates, and a specific number (about 40) of freshly hatched y^+ first instar larvae were transferred to plates with regular medium. Early larval lethality was inferred when approximately half of them failed to survive into the third instar, while embryonic lethality was inferred when nearly all the transferred larvae survived, and all were scored as females.

Mapping: Meiotic mapping was carried out using a multiply marked *X* chromosome, *y cv v wy car* or *y cv cm v f*. Duplications used in the mapping have been described elsewhere (LINDSLEY and ZIMM 1987).

Chromosome cytology: Salivary glands from female third instar larvae heterozygous for the lethal and a cytologically wild-type *y w* chromosome were dissected, squashed and stained by standard methods (ASHBURNER 1989), and examined using phase optics on a Zeiss Universal compound microscope.

Scanning electron microscopy: This was performed on etherized and desiccated flies using an ETEC Autoscan U-1 scanning electron microscope after mounting on pegs and coating with gold-palladium .

RESULTS

Mutagenesis: From a total of 1549 mutagenized chromosomes scored, 111 lines were initially identified in the screen as lethal. Many of these lines, however, were subvital or subfertile even as heterozygotes and were lost before they could be completely analyzed. Apart from this inadvertent selection for fitness in culture, no other selection procedure was performed on the lines. Three lines lost their lethal nature after a few generations and were discarded. A total of 68 lines survived long enough to be analyzed in our screen.

Survival of clones: To enable identification of mutant clones in the eye, all the lethals in this study were generated on a chromosome marked with the eye color marker *white*. Since the map locations of the lethals on the *X* chromosome were unknown and since the marker in this case was located near the tip of the chromosome, we were concerned that mitotic recombination between the marker and a lethal might result in nonmutant *white* clones. To minimize this, we generated mosaics using an insertion of a functional *white* gene located close to the base of the *X* chromosome (LEVIS, HAZELRIGG and RUBIN 1985). See Figure 1 for a schematic of the procedure used. This ensured that all *white* clones were the products of recombination very close to the base of the chromosome, and hence, were almost certainly homozygous for the lethal (see Figure 1). Since cells carrying one dose of the insert were lighter in color than cells carrying two doses, twin spots and wild-type singles could be scored in addition to the *white* mutant spots. This provided a

TABLE 1

Data summary for analysis of lethal lines

Line	No. eyes scored	No. twins	No. white only	No. wild only	Total no. clones	Mitotic rec. freq.	Normalized survival ratio (%)[a]	Effects seen in eye/lamina[b]	Presence of sn^3 clones?[c]	Effective lethal stage[d]	[Meiotic map] or cytogenetic map position[e]
wsn^3	938	132	35	55	222	0.20	100.01	N/N	Yes		
*l*101	260	35	5	12	52	0.18	95.30	A/N	Yes	P	[29]
*l*102	384	33	3	28	64	0.16	66.09	A/A	Yes	E	
*l*103	292	30	3	27	60	0.20	64.83	A/A	Yes	P	
*l*104	434	63	20	17	100	0.18	116.18	A/N	Yes	EL	
*l*105	386	39	39	39	117	0.20	111.98	A/A	Yes	EL	8CD-9CD
*l*106	270	1	4	59	64	0.22	9.33	CL	Yes	LL	
*l*107	316	25	7	16	48	0.13	87.40	A/A	Yes	EL	
*l*108	336	50	10	20	80	0.21	95.98	N/N	Yes	E	
*l*109	142	28	6	19	53	0.33	81.01	A/N	Yes	LL	
*l*110	254	29	4	8	41	0.15	99.88	A/A	Yes	E	
*l*111	230	38	8	34	80	0.31	71.54	N/N	Yes	P	
*l*112	90	0	0	37	37	0.41	0.00	CL	Yes	EL	
*l*113	222	27	5	13	45	0.18	89.59	A/N	Yes	EL	
*l*114	380	7	5	59	71	0.17	20.36	CL	Yes	EL	[31]
*l*115	360	1	0	118	119	0.33	0.94	CL	Yes	E	
*l*116	136	13	4	8	25	0.15	90.65	N/N	Yes	EL	
*l*117	242	14	4	31	49	0.19	44.79	CL	Yes	E/EL	
*l*119	200	0	1	0	1	0.00			Yes	E	
*l*121	140	1	0	21	22	0.16	5.09	CL	Yes	E	
*l*123	218	14	7	6	27	0.09	117.58	N/N	Yes	LL	
*l*125	430	0	1	40	41	0.0	2.81	CL	No	EL	
*l*127	256	29	4	18	51	0.18	78.63	N/N	Yes	P	
*l*128	362	50	7	16	73	0.18	96.71	A/A	Yes	LL	3F3-5E8
*l*129	104	3	0	21	24	0.23	14.00	CL	Yes	EL	
*l*131	212	26	8	20	54	0.22	82.77	N/N	Yes	E	
*l*132	180	29	7	17	53	0.26	87.64	N/A	Yes	EL	
*l*134	158	20	2	14	36	0.22	72.46	N/N	Yes	EL/LL	1A1-2B15
*l*135	212	29	4	11	44	0.19	92.39	N/N	Yes	LL	
*l*136	502	50	15	32	97	0.16	88.77	A/A	Yes	P	13F-16A2
*l*202	354	61	8	28	97	0.25	86.82	N/N	Yes	E/EL	[40]
*l*203	226	0	0	40	40	0.18	0.00	CL	No	LL/P	
*l*205	188	28	8	22	58	0.27	80.63	N/N	Yes	E	[3.3] 3A5-3D1,2
*l*206	200	0	0	0	0	0.00					
*l*207	256	25	5	8	38	0.13	101.80	A/A	Yes	LL/P	[36] 12A6,10-13A25
*l*214	390	1	6	66	73	0.17	11.70	CL	No	E/EL	
*l*218	196	0	0	25	25	0.13	0.00	CL	Yes	P	[0.7] 2D1,2-3A5
*l*219	424	31	9	10	50	0.10	109.25	N/N	Yes	E/EL	
*l*222	226	0	0	47	47	0.21	0.00	CL	No	E/EL	
*l*223	264	0	1	54	55	0.20	2.07	CL	No	LL	
*l*226	312	1	1	52	54	0.17	4.23	CL	Yes	EL	
*l*228	268	23	8	7	38	0.11	115.71	A/A	Yes	P	
*l*229	206	0	0	50	50	0.24	0.00	CL	No	E/EL	
*l*232	200	19	5	7	31	0.13	103.37	N/N	Yes	EL	
*l*233	200	0	1	1	2	0.01					
*l*234	400	46	13	31	90	0.19	85.80	A/N	Yes	P	
*l*235	244	31	4	14	49	0.18	87.10	A/A	Yes	EL	13F-16A2
*l*236	290	7	4	47	58	0.19	22.81	CL	Yes	E/EL	
*l*237	158	0	0	28	28	0.18	0.00	CL	No	E/EL	
*l*238	96	11	1	4	16	0.16	89.59	N/N	Yes	E	
*l*239	128	0	1	14	15	0.11	8.00	CL	No	E	
*l*243	132	9	1	27	37	0.27	31.11	CL	Yes	P	
*l*302	288	32	8	18	58	0.17	89.59	A/A	Yes	E	
*l*303	212	33	4	21	58	0.25	76.73	A/N	Yes	EL	
*l*305	232	25	10	22	57	0.20	83.39	N/N	Yes	LL/P	
*l*307	342	1	2	69	72	0.20	4.80	CL	No	E/EL	
*l*308	170	18	7	4	29	0.13	127.25	A/A	Yes	E	[0.0] 2B15-2C1
*l*309	244	45	11	25	81	0.29	89.59	N/N	Yes	P	
*l*310	266	1	3	40	44	0.15	10.93	CL	Yes	EL	
*l*313	292	51	19	10	80	0.21	128.50	A/A	Yes	LL	[50] 13A2-13F

TABLE 1—Continued

Line	No. eyes scored	No. twins	No. white only	No. wild only	Total no. clones	Mitotic rec. freq.	Normalized survival ratio (%)[a]	Effects seen in eye/lamina[b]	Presence of sn^3 clones?[c]	Effective lethal stage[d]	[Meiotic map] or cytogenetic map position[e]
*l*401	330	24	9	27	60	0.15	72.46	A/A	Yes	E	
*l*402	348	18	5	25	48	0.12	59.90	A/N	Yes	LL	[13.7]
*l*403	122	0	0	45	45	0.37	0.00	CL	No	EL	
*l*404	116	1	1	36	38	0.32	6.05	CL	Yes	LL	
*l*407	154	22	5	5	32	0.18	111.98	N/N	Yes	E	
*l*409	104	0	0	20	20	0.19	0.00	CL	Yes	E/EL	
*l*410	330	0	0	50	50	0.15	0.00	CL	Yes	E	
*l*411	250	33	7	18	58	0.20	87.83	N/N	Yes	LL	[15.3]
*l*412	208	0	0	13	13	0.06	0.00	CL	No	E	
Total	17074	1281	350	1791	3422	0.18	59.51				

[a] The numbers in the "Normalized survival ratio" column are derived by dividing the survival ratio for each lethal line by the survival ratio for the control (0.89); see Figure 2 and text for more details.

[b] In the "Effects seen in eye/lamina" column CL = cell-lethal, A = abnormal, N = normal; the identifier to the left of the slash (/) refers to the phenotype in the eye and the identifier to the right of the slash to that of the lamina. Cell-lethal is operationally defined as the inability to generate clones at ≥50% of the wild-type rate.

[c] In these instances, the failure to recover sn^3 clones outside of the eye was consistent with the failure to recover clones within the eye as well. Note that no line which gives clones in the eye fails to give sn^3 clones. Note also that not all lines that we have defined as cell lethal fail to give sn^3 clones; see DISCUSSION for details.

[d] In the "Effective lethal stage" column E = embryo, EL = early larva, LL = late larva, P = pupa; where two abbreviations are separated by a slash (/), the time of lethality could not be resolved to only a single category.

[e] Actual data not shown for either meiotic or cytogenetic mapping. In those cases where mutants have mapped to the same region, additional tests have shown them to be nonallelic.

good internal control against which the survival and phenotype of mutant clones was compared.

The main results of the analysis are presented in Table 1. The frequency of mitotic recombination varied somewhat among the lines but was generally between 0.15 and 0.20 events per eye. The presence or absence of cuticular mutant clones marked with *singed* bristles was also noted for each line. Three of the lethal lines failed to yield even wild-type control clones with any significant frequency. Salivary gland chromosome squashes revealed that each of these lines had a translocation between the *X* chromosome and one of the autosomes. Mitotic recombination in such cases would produce partial aneuploidy in both resulting daughter cells and would thus prevent recovery of clones of either type. These lines were hence unclassifiable in this scheme and were excluded from subsequent analysis. The lethal effective stages and estimated map locations for the lethals, where known, are also indicated in Table 1.

The relative survival of each line in mosaic eye-patches was measured by calculating a viability (survival) ratio for that line. This was a ratio of the total number of *white* clones (twins and singles) to the number of wild-type clones (twins and singles) observed in the same set of eligible flies. This value was then normalized by dividing it by the viability ratio (0.89) observed for the unmutagenized parental stock ($w\ sn^3$). Figure 2 is a graph showing the distribution of lethals according to their viability ratios. Inspection of the graph clearly shows a bimodal distribution of the lines into two groups. One peak occurs near 0.0 and another closer to 0.9, with a decreasing distribution around the two peaks. Twenty-six lines had viability ratios less than 0.5, of which only six lines had ratios exceeding 0.1. Besides the numbers of clones reported in Table 1, several additional rounds of irradiation were performed on these lines with the aim of obtaining suitable numbers of adequately sized mutant clones for histology. Although no quantitative records were maintained regarding clone sizes, it was strikingly evident that mutant clones in the low viability group were not only infrequent but also much smaller on average than wild-type controls. This was in contrast to the lines in the high viability group where the mutant and wild type clones were of similar sizes. The differences in clone sizes were most easily observed in twin spots where a *white* clone from the low viability group would be much smaller than its wild-type sibling clone. All these lines were considered to yield clones that were too infrequent and too small

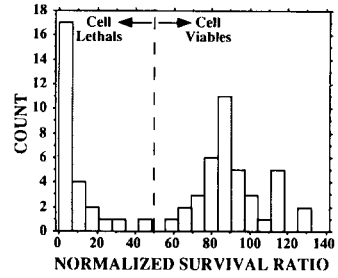

FIGURE 2.—Graphic display of frequency by normalized survival (viability) ratio (expressed in percentages). It is largely on the basis of the inspection of this graph that the population of organismal lethals was divided into cell viable and cell-lethal categories.

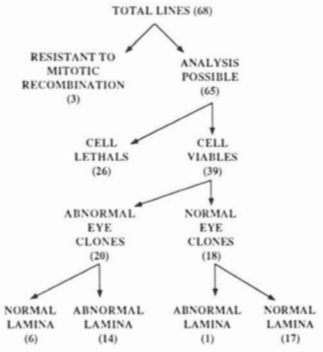

FIGURE 3.—Schematic representation of the division of the population of organismal lethals into the various phenotypic classes referred to in this study. The numbers in parentheses are the numbers of lethal lines in each class.

to permit a thorough examination of their histological phenotype; however, a few clones were sectioned from five of the six lines with viability ratios between 0.1 and 0.5. Four of these lines gave morphologically aberrant clones, while no defect was obvious in a single line. These results suggest that even when mutant clones in this group survive, they are quite unlikely to have a normal phenotype. The combination of reduced frequency of clones and smaller relative sizes for clones in this group of lethals strongly suggested that the predominant effects of these mutations was to inhibit the survival and/or proliferation of individual cells of the eye-imaginal disc. These lines were therefore, operationally categorized as "cell-lethal" for the purposes of this analysis. As will be discussed later, not all the lines in this group would qualify as truly "cell-lethal" as the term is classically defined.

Phenotypes of clones: The 39 lines reflecting relatively higher cell viability were analyzed histologically for phenotypic irregularities in the structure of the mutant eye tissue or the underlying optic lobes (Figure 3). We were concerned that nonspecific tissue damage caused by X-rays and unmarked mutant clones arising within the optic lobes could potentially confound our histological analysis. We therefore considered as significant only those structural defects that were confined to the mutant eye-clone and the part of the optic lobe to which it projected. The appearance of the heterozygous background as well as wild-type sibling clones in the case of twin spots served as internal controls for comparison, while clones for the nonlethal parental stock provided an external control. Twenty lines in which the mutant clones or the underlying lamina were repeatedly shown to have distinctly abnormal structure were classified as phenotypically abnormal. Clones from the other 19 lines were indistinguishable from controls at the level of resolution employed in this study and were classified as normal.

Abnormalities in clone structure were often apparent on the surface of the eye as roughness of the mutant patch compared to the regularity of the ommatidial array in the heterozygous background (Figure 4). Since eyes were examined by transillumination under the dissecting microscope, even subtle defects in the rhabdomeric pattern could be noticed as a disturbance of the deep pseudopupil (FRANCESCHINI 1975). We also observed striking disruptions in the morphology of the eye tissue underlying rather regular-appearing corneal surfaces so that definitive classification was only possible after histological examination was complete.

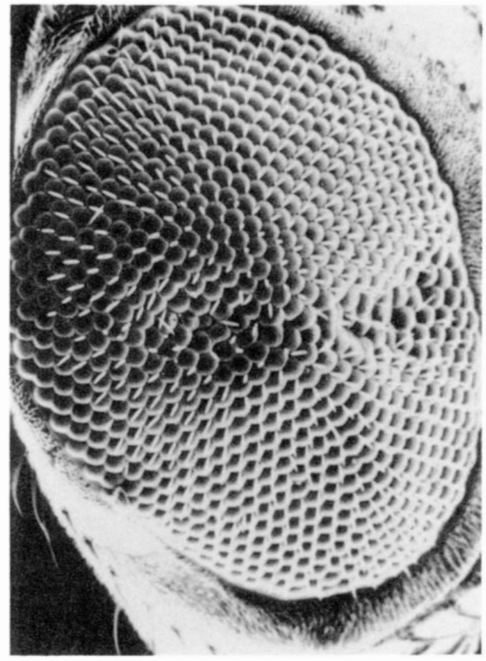

FIGURE 4.—Scanning electron micrograph of an eye containing a clone homozygous for one of the recessive lethals. The clone is near and parallel to the equator of the eye and is recognized by its slight "roughness."

The phenotypes observed in histological preparations of the eyes ranged from mild disorganization of some ommatidia in the clone to large empty spaces through the entire extent of the clone (Figure 5). The mutant clones usually lacked the highly regular ommatidial array, and recognizable photoreceptor cells were either missing or abnormal in morphology. In several cases, there were ectopically located cells of aberrant appearance. This phenotype was most persistent and pronounced in line *l*136 where there were many ectopic cells in the distal portion of the retina. These cells had a rounded rather than the usual elongated shape, had relatively large nuclei and had no distinct cellular specializations. The rest of the white patch consisted of large empty spaces with a few strands of supporting tissue and occasional cells of a similar morphology scattered in more proximal locations.

Of the mutants with aberrant eye structure, about

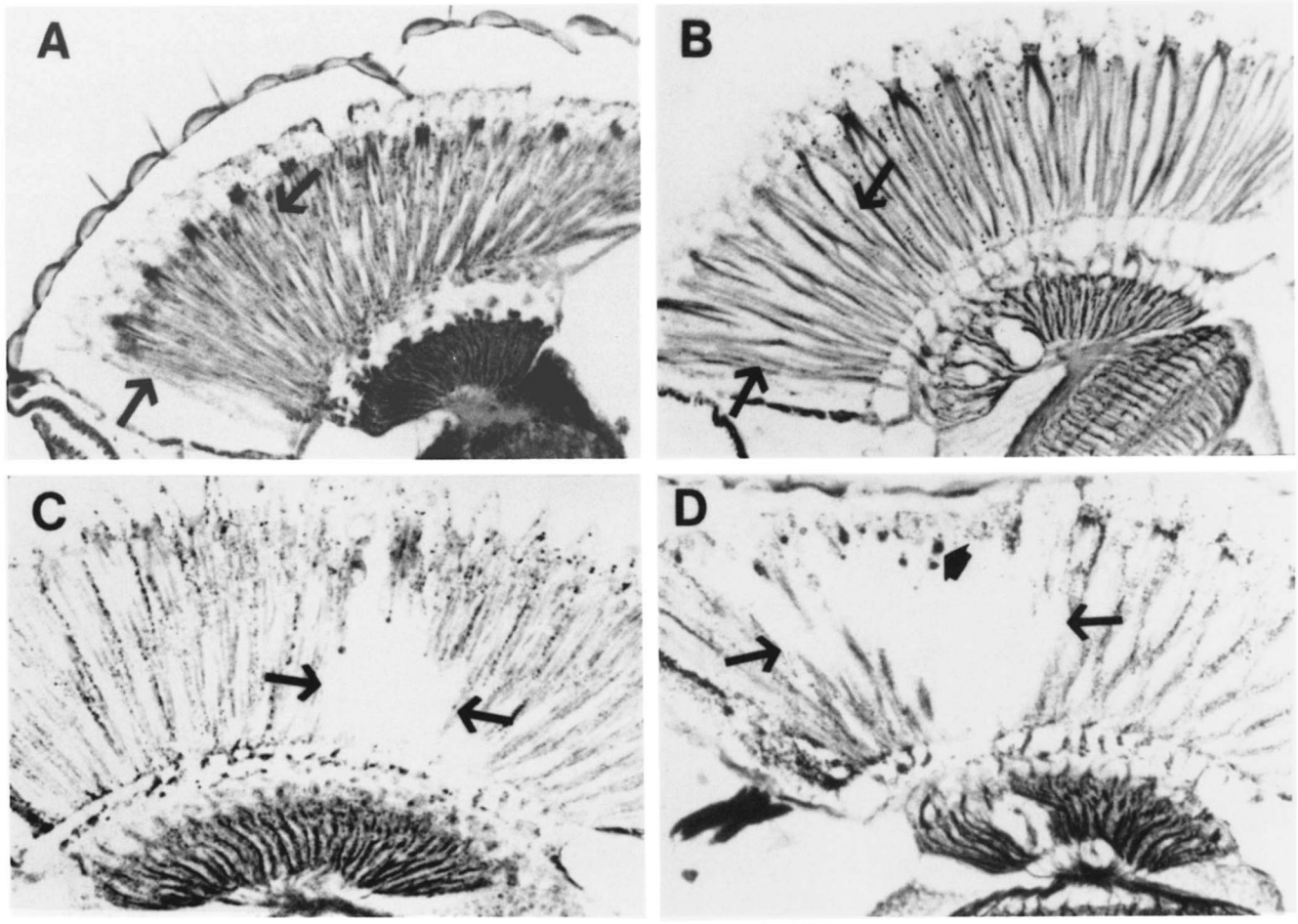

FIGURE 5.—Four different classes of clonal phenotypes in the eye and lamina. A section through the eye and lamina is shown in each panel, and the extent of each clone in the eye is indicated by arrows. A, Line in which the morphology of the clone in the eye as well as of the portions of the lamina contacted by the clone are relatively wild type. B, Line in which the morphology of the clone in the eye is comparatively normal but where the lamina contacted by that clone shows a variety of abnormalities; note the "holes," the incomplete cartridges and the loss of the regular, dense organization. C, Line in which the clone within the eye lacks a great deal of the normally differentiated eye tissue and which has comparatively large cells at the most distal portions of the eye underlying the cornea. The lamina is comparatively normal. D, Line in which the clone within the eye lacks almost all of the normally differentiated tissues but in which are found large, darkly staining cells (broad arrow) under the cornea. The lamina has a variety of abnormalities including "holes" and more subtle aberrations.

two-thirds had significant and frequent disruptions in the part of the lamina where the clone would normally project. In the other lines the phenotype was largely confined to the eye itself, with only an occasional clone overlying defects in laminar morphology. No line was observed which displayed consistent aberrations in the structure of the medulla or the inner optic ganglia, the lobula and the lobular plate. The phenotypes in the lamina ranged from a subtle "waviness" and loss of compaction of the laminar cartridges to a severe disorganization with vacuolar spaces in the neuropil. The affected cartridges often failed to traverse the full thickness of the lamina, as is normally the case. Besides defects observed in the neuropil, the cortical nuclei also appeared inappropriately located in some cases. While a disruption in the architecture of the lamina was usually associated with a disorganization in an overlying eye-clone, in some clones, profound defects in the lamina were seen underlying clones with minimal defects in the architecture of the eye itself. See Figures 5 and 6. In line *l*132, the eye-clones were normal or marginally aberrant in appearance but often displayed dramatic defects in the structure of the lamina (Figure 5).

There was considerable variability in the penetrance and expression of the eye and laminar defects among different lines and also among different clones of the same line. The variation in expression within a given line could range from occasional normal clones to severely disrupted ones. Such variability has been described previously for a population of viable mutants affecting eye and brain phenotypes (MEYEROWITZ and KANKEL 1978). Since the purpose of this study was merely to classify each line as phenotypically normal or abnormal, no attempt was made to quantify these variations within and among the lines.

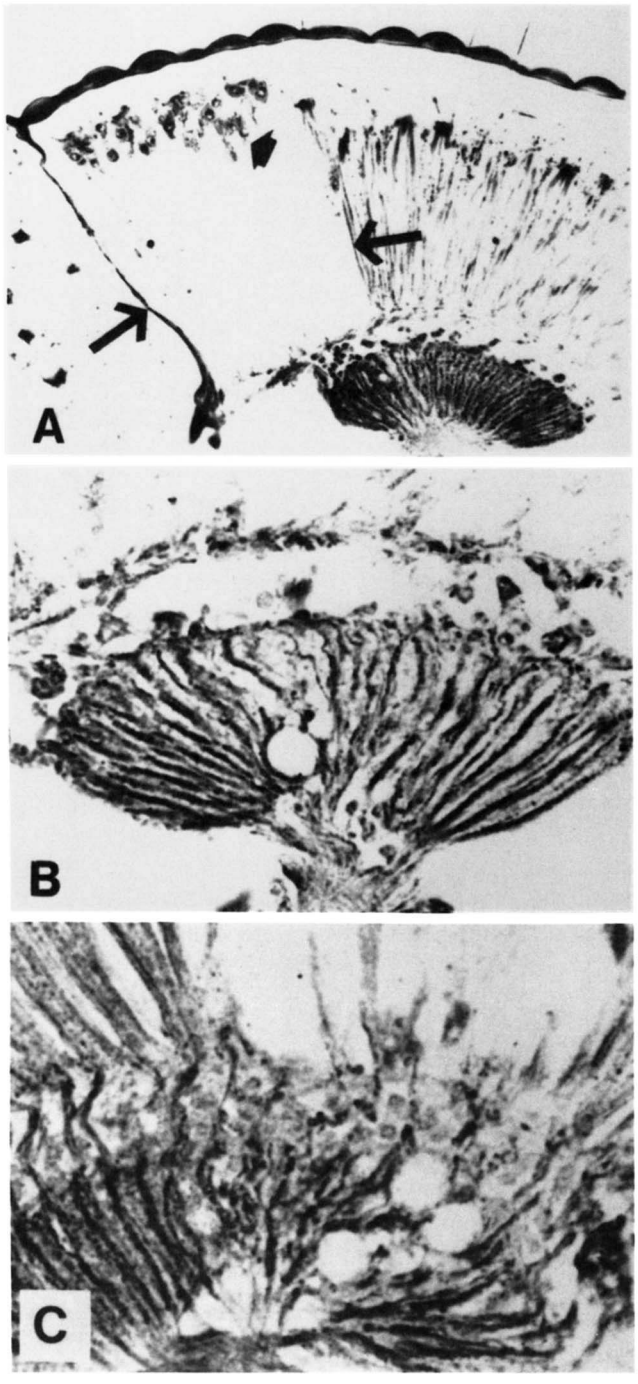

FIGURE 6.—Additional phenotypes with an emphasis on the lamina. A, Section through the eye and lamina; the extent of the clone in the eye is indicated by arrows. Example of a clone in the compound eye with a pronounced effect on the structure of the eye itself. Clones in this line show large empty spaces proximal to the basement membrane of the eye and a population of comparatively large, abnormal cells more distally located (broad arrow). The portion of the lamina that normally receives a projection from this clone is clearly distorted. B and C, Higher magnification micrographs of sections through the laminas of two different lines. Both have "holes" and larger nuclei among the fibers of the neuropil. A number of cartridges are both distorted and less compact than in normal laminae. Normal cartridges are seen both at the left and right extrema in B and at the left in C.

Effects of mutant tissue outside of the eye: While the main focus of this study was the examination of effects of organismal lethals in clones in the eye and of the effects of such clones on the optic lobe, we also wished to know if a mutant genotype in cells of the optic lobe itself might give rise to a phenotypic abnormality as well. Technical considerations made it much more difficult to mark and identify mitotic recombination patches in the interior of the organism, and we did not pursue that line of investigation. However, one of the lines with abnormal eye clones *l*207 yields occasional "escaper" mutant males, and we exploited this fact to examine the phenotype of mutant optic lobes in this line. They showed rather consistent and dramatic disorganization of the normally regular architecture of the neuropil throughout the optic lobe (Figure 7). For a second line *l*105 we generated a small number of gynandromorphs in which large segments of the head were of mutant genotype and therefore for which there was a significant probability that the optic lobes contained mutant tissue as well; we noticed a number of abnormalities in the structure of the optic lobe. While the observed phenotypes resembled those found in known optic lobe mutants, it must be pointed out that in neither of the above two cases can one rule out the possibility that the morphological aberrations are actually a consequence of effects of the mutations on the compound eye itself. Some of the lethals in this study have also been screened directly for morphological abnormalities in the developing optic lobes of third instar larvae (DATTA and KANKEL 1992), and a number of lines with various aberrations have been identified.

DISCUSSION

Fraction of vital loci participating in development of the visual system: The purpose of this study was to assess the extent to which vital genes play an essential role in the assembly of the compound eye. We found that about a third of our lethals consistently gave structurally normal eye clones. Approximately another third gave either no clones or infrequent and small sized clones that were often abnormal in structure, and were classified as "cell-lethals." The remaining third was clearly not required for initial cell survival as mutants in this class gave good numbers of adequately sized clones but showed disruptions in the ommatidial architecture, with or without aberrations in the lamina. In other words, the activity of about two-thirds of the vital loci in our sample is necessary for the normal formation of the adult eye. This activity is needed in a cell-autonomous fashion, and its effect may be at any or all times after the early first instar larval stage, when the clones were generated.

To make our sample as representative of the population of vital genes as possible, we had tried to remove some known sources of bias in sampling. We

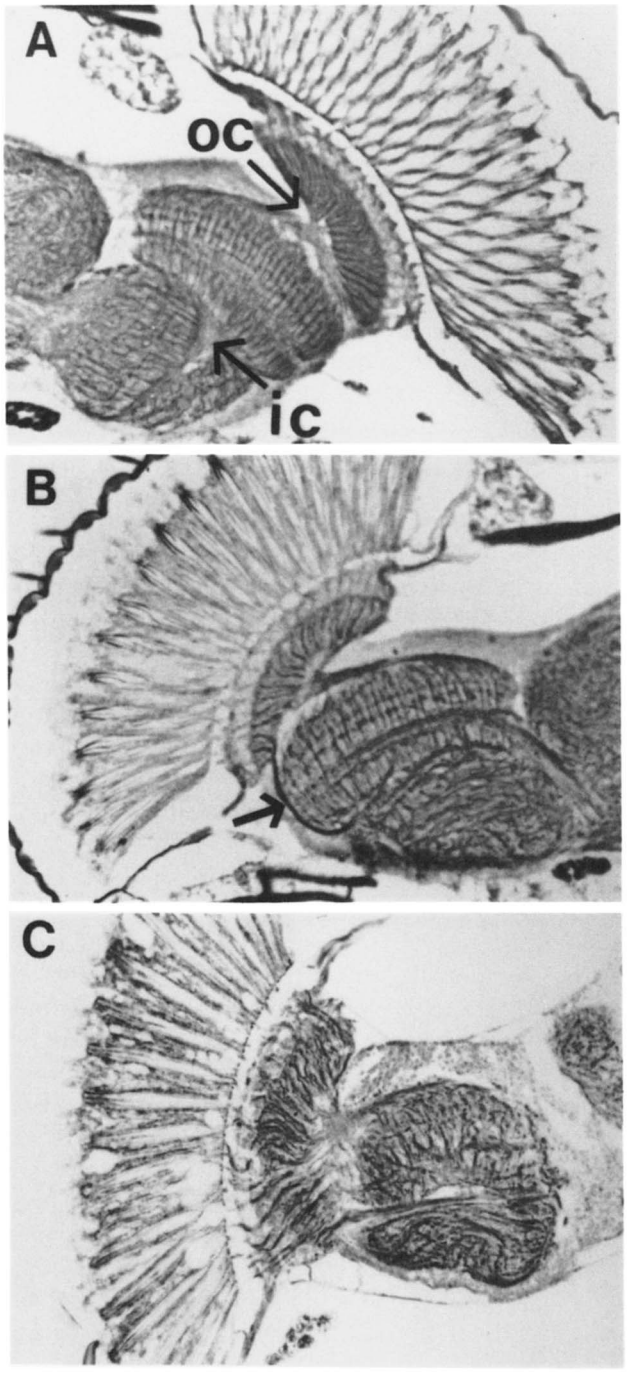

FIGURE 7.—Phenotypes beyond those seen in the compound eye and lamina. A, Wild-type control; oc = outer chiasma, ic = inner chisma. B, Horizontal section through the eye and optic lobe of a gynandromorph in which male tissue is hemizygous for the lethal *l*105. Arrow points to fibers from lamina to medulla which appear misrouted. Medulla is somewhat poorly formed. It is difficult to discern the inner chiasma and to distinguish lobula from lobular plate. C, Section through the eye and optic lobe of an "escaper" male from the *l*207 line. The eye has a number of "holes." The neuropil of all segments of the optic lobe are disorganized although one can discern the lamina. The medulla, the lobula and the lobular plate are not easily distinguished.

avoided the notoriously nonrandom *P* elements for mutagenesis (KIDWELL 1987) and also did not preselect the lethals except for the fact that they were all *X*-linked. Our choice of DEB as the mutagen was influenced mainly by the reported efficiency with which it generates small intragenic deletions in Drosophila (REARDON *et al.* 1987), rather than point mutations that are generated by the more commonly used EMS. We wished thereby to maximize the probability of generating null mutations at the lethal loci, as well as to induce more readily detectable molecular alterations to facilitate subsequent molecular analyses. Considering that only 5–7% of the *X* chromosomes screened carried a lethal, it is unlikely that many of our lines represent more than one mutational event on the *X* chromosome. Genetic mapping of a fifth of the lines has shown them to be scattered at different locations on the *X* chromosome (Table 1). However, inasmuch as mutagenic specificities vary with respect to different loci as well as mutagens, and as there are unintentional sources of bias in any experiment, it is only with caution that we extrapolate from our sample to the lethally mutable genome of the fly.

Having said that, we nevertheless feel justified in estimating that around two-thirds of all vital loci have functions in the eye at some time in its development. Since these are organismal lethals and since the eye is not a vital organ, the genes must have at least one other function at some time in a vital subset of the fly. All these genes are thus by definition pleiotropic in nature, and our findings suggest that pleiotropy is rather commonly seen in development. The compound eye is indeed a complex system, comprising many specialized cuticular and neural structures. It is possible that this complexity is the reason that a large fraction of the genome is needed for its development. However, it is our expectation that other tissues, particularly those of the nervous system, may also need the activity of large fractions of the genome for normal assembly. Consistent with this idea are the observations that a relatively large number of "enhancer trap" lines are expressed at many spatial and temporal points in the fly's life cycle, with a large proportion of total lines being expressed in the nervous system (S. DATTA, K. STARK and D. R. KANKEL, unpublished data). Of particular relevance to this study are recent observations (U. GAUL, T. XU and G. RUBIN, personal communication) that approximately 60% of a population of 600 lethal enhancer-trap lines had expression of the reporter in the eye imaginal disc when examined in the third instar alone. Clearly, the total fraction of lethals active in the eye disc at any time between the first instar and the adult can only be greater than this. While the mere expression of a gene does not imply a function in that tissue, these findings are at least consistent with our estimate that about two-thirds of all vital loci have functions in

the eye at some time during the larval or pupal stages.

Phenotypic classes and developmental functions of vital loci: What kind of molecules might be encoded by the different categories of genes that we have classified? Clearly, the cell-lethal group is essential to the very survival of the cells and probably includes molecules affecting "housekeeping" functions like intermediary metabolism, membrane permeability, etc. Cell-lethals are classically defined by the inability to recover any mitotic recombination clones homozygous for the lethal (DEMEREC 1934). Because of the operational nature of this definition, genes required for cell division would also fall in this category, since clones larger than one cell would infrequently be obtained. Conversely, some small clones may be recovered in cell-lethal lines because of perdurance, or persistence of the maternal cell's protein or transcript in the mutant daughter cells (GARCIA-BELLIDO and MERRIAM 1971). Occasional white single spots in the eye may also be generated by means other than mitotic recombination; in this case, by a somatic excision of the *white*$^+$-bearing P insert. We have in this study grouped all organismal lethals with a viability ratio of 0.5 or less in one category; this is a group in which clone size is exceedingly small and for which thorough analysis of clones by histological procedures was impractical. While we have called it the "cell-lethal" group, we wish to point out that they are not all strict cell-lethals as originally defined, since *white* clones have been recovered for some of them. However, we believe that the predominant effect of these mutations is to inhibit survival or proliferation of mutant cells. The recovery of a few clones for some of these lines may be explained by perdurance or somatic excision as described above, or by redundancy or lack of amorphy of the mutations at the loci.

While this is the first estimate of the percentage of all organismal lethals that are cell-lethal in the compound eye, other tissues have been investigated for this property in previous studies (GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984; RIPOLL 1977; RIPOLL and GARCIA-BELLIDO 1973). The fraction of organismal lethals that were cell-lethal in abdominal cuticle was 9.3%, while 26% of them were cell-lethal in the wing when irradiation for the production of clones was performed at more than 96 hr before puparium formation (RIPOLL 1977). The cell-lethal fraction is even greater in germ line clones, having been reported as being 31% in one study (PERRIMON, ENGSTROM and MAHOWALD 1984) and 66% in another (GARCIA-BELLIDO and ROBBINS 1983). In the present study, there were 26 lines (40% of 65) that were cell-lethal (by our liberal definition) in the eye. Since it is difficult to compare across different studies using different populations and experimental conditions, we have also noted the presence or absence of *singed* cuticular clones in each line. Cuticular clones were absent in 11 (17% of 65) of the 26 lines that were cell-lethal in the eye. None of our eye-cell-viables failed in giving *singed* clones. This comparison, when considered along with the previous studies, suggests that the cells of the eye need approximately twice the number of genes for viability as do those of the general imaginal cuticle. Whether this last speculation is true or an artifact of our particular experimental conditions is unknown.

The cell-viables that displayed no phenotype may or may not participate in eye development. No phenotype would be observed for a mutation in a gene that normally participates in eye development but whose function can be substituted by redundant pathways or by surrounding wild-type tissue. Neither would we have detected events earlier in embryogenesis prior to the X-ray induction of clones. The only phenotypes that we could identify were those that were obvious with light microscopy in silver-stained 5-μm horizontal sections of paraffin-embedded heads. This protocol was adopted to facilitate the identification of aberrations in neural connectivity of the clones, reflecting our primary interest in eye-brain interactions and optic lobe development. It is likely that our screen missed more subtle defects in the ommatidial array that may have been picked up if the clones had been observed in other planes of section or at a higher resolution.

Approximately one-third of all lethals give clones with definite abnormalities in morphology. In most of these lines, the actual survival (and size) of the clones is close to control. This means that the mutant cells could survive and proliferate as well as the control cells. However, the products of these loci are obviously necessary for development or maintenance of structure of the eye. It is known that cell interactions play a very important role in the development of the eye (READY 1989; TOMLINSON 1988). Mutations in nonsecreted molecules that participate in such interactions would presumably fall in this class. Other molecules involved in more general functions of the eye-imaginal disc cells including proliferation, cell-cell adhesion, interaction with the extracellular matrix, and response to hormones in metamorphosis would also give phenotypes in this screen. A commonly observed phenotype is the breakdown of ommatidial architecture and large empty spaces in the clone. It is possible that some of these mutants cause cell degeneration after initially supporting proliferation and differentiation. The degeneration could be primary or secondary to some other developmental failure caused by the mutation. Another observation in many cases is the presence of abnormal cells, often at ectopic locations, as seen in line *l*136. These may reflect a block at some point in the differentiation of the photoreceptors or accessory cells.

Of particular interest to us was the observation in

many cases of a disruption in the neural architecture of the part of the lamina where the clone would normally project. It should be pointed out that the different cell lineages of the eye and optic lobe rule out the possibility of a mutant eye clone also including laminar cells. Since the probability of having a separate laminar clone developing in the appropriate cartridges is very small, the lamina can always be assumed to be heterozygous and hence nonmutant. Aberrations detected in the lamina must therefore be a consequence of the influence of the mutant clone innervating the lamina. It has been shown by previous mosaic analyses (MEYEROWITZ and KANKEL 1978) and by other studies (FISCHBACH 1983; POWER 1943; SELLECK and STELLER 1991) that retinal innervation has important influences on the development of the optic lobes in Drosophila. The mosaic screen employed in this study is well suited to identify novel loci that participate in this process. Particularly interesting in this regard would be lethal lines for which the clones in the eye cause minimal or no detectable abnormalities in the eye itself but have disruptive effects in the lamina. These genes would more likely be directly involved in eye-brain interactions rather than exerting mutant effects through a general disruption of eye structure. Our screen has identified at least one line where this is the case. We also identified lines in which clones with severe aberrations of eye structure are associated with minimal or no noticeable defects in optic lobe organization. The sparing of the lamina is similar to that seen when diphtheria toxin is used for ablation of eye cells during late larval and pupal stages (KUNES and STELLER 1991). This class of mutants may therefore represent genes that function relatively late, after the eye-brain interaction has taken place.

The technique of screening lethals by using mitotic recombination has been useful in the identification of mutations with phenotypes in cuticle (RIPOLL 1977), oogenesis (PERRIMON, ENGSTROM and MAHOWALD 1984) and, with this study, the visual system. One important disadvantage of this approach has been the laboriousness of scoring for X-ray induced mitotic recombination mosaics which tend to be too infrequent or too small or both. The recent development of a more efficient technique for mitotic recombination (GOLIC 1991) should relieve this tedium, since it yields a very high frequency of mutant clones. This technical advantage can, however, confound the analysis of mutant phenotypes by making it difficult to attribute a phenotypic defect to one clone. For example, in this study, a much greater frequency of mitotic recombination would have increased the likelihood that an observed defect in the lamina was due to a separate laminar clone rather than due to the overlying eye-clone. Nevertheless, the ability to dramatically increase the incidence of mitotic recombination should facilitate the use of mosaic screens in other imaginal tissues including the central nervous system.

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