COMPLEMENTATION ANALYSIS OF LATE LETHAL MUTANTS OF *DROSOPHILA MELANOGASTER*[1]

ALLEN SHEARN

Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218

Manuscript received November 19, 1973

ABSTRACT

Late larval and pupal lethal mutants of Drosophila define those gene functions which are essential for the development of pupae (metamorphosis) but not for embryonic or larval development. In a previous report the isolation of a large number of such mutants was outlined, and a description of the imaginal disc defects in those mutants was described. This report concerns genetic analysis of those mutants. 3746 different pairwise combinations of mutants have been tested for complementation. Only 10 pairs fail to complement. In all of the cases tested, the lethal mutation in each member of a non-complementing pair has a similar map location. In addition to the non-complementing pairs one group of seven partially-complementing mutants has been identified.

Comparisons of the imaginal disc defects within the non-complementing pairs and the lethal hybrids formed by the respective pairs were made to test for uniformity of phenotype. No significant qualitative differences were detected between any non-complementing pairs or their respective hybrids.

THE imaginal discs of Drosophila as a developmental system have received much recent attention. Several comprehensive reviews have appeared (URSPRUNG and NÖTHIGER 1972; GEHRING and NÖTHIGER 1973). The imaginal discs in insects such as Drosophila are groups of cells which are present in larvae and which, during metamorphosis, give rise to adult integumental structures. In a preliminary report (SHEARN *et al.* 1971) a procedure was described for screening among third chromosome lethal mutants for those which die after the third larval instar. These mutants are defective in functions which are essential for the development of pupae and adults but not necessary for normal embryonic and larval development. The finding of mutants with no imaginal discs among these late lethals suggests that as a class the late lethals can include mutants that affect any essential function that is specific for imaginal disc development. Imaginal disc defects were detected in 47% of third chromosome, recessive, late larval, pre-pupal, and pupal lethal mutants. By limiting the screening to late larval and pre-pupal lethals, STEWART, MURPHY and FRISTROM (1972) detected imaginal disc defects in 81% of *X*-chromosome mutants.

Genetic studies of the third chromosome mutants are described in this report. Complementation analysis was undertaken in order to determine how many different functional units were identified by the 142 lethal mutants and to estimate

[1] Research supported by American Cancer Society Grant VC-6, Contribution 770 of The Biology Department, Johns Hopkins University, Baltimore, Md. 21218

how many additional units remain to be identified. This kind of estimate can be compared to a different estimate of the same number based on the "one complementation group: one salivary gland chromosome band" hypothesis which has been revitalized by the data of LIFSCHYTZ and FALK (1969), of HOCHMAN (1971) and of JUDD, SHEN and KAUFMAN (1972).

A procedure is described for determining the genetic location of lethal mutants on the third chromosome. This procedure was used to map all of the non-complementing mutations in order to determine whether they were the result of single mutations and to confirm that the non-complementing mutations have similar genetic locations.

The work of SHANNON *et al.* (1972) suggests that non-complementing lethals in a limited region of the *X* chromosome, at least, have a nearly uniform phenotype. In the work reported here an even more stringent test of uniformity has been applied to non-complementing third chromosome late lethal mutants. Imaginal discs from each member of each non-complementing pair were dissected, injected into metamorphosing hosts, and examined for differentiated structures. In addition, lethal hybrids formed from each non-complementing pair of mutants were similarly analyzed.

MATERIALS AND METHODS

Stocks: Each of the lethal mutations used had been induced in a third chromosome marked with multiple wing hairs (*mwh*,3-0.0) and ebony (*e*,3-70.7). Some chromosomes were marked as well with red Malpighian tubules (*red*,3-53.6). The mutants discussed here were described in a previous report (SHEARN *et al.* 1971); they are maintained as balanced lethal stocks with *TM1* as the balancer. For a description of markers and balancers used see LINDSLEY and GRELL (1968). All stocks and crosses were kept in shell vials on a medium of cornmeal, molasses, yeast, and agar at 20° unless stated otherwise.

Complementation: For each pair of mutants tested for complementation of the lethal phenotype, 3–5 virgin females of one mutant were mated to 3–5 males of the other mutant. Each test can be symbolized as: *mwh e lethal(1)/TM1* × *mwh e lethal(2)/TM1*. Four genotypes result from such a cross. Two of the four are parental-type heterozygotes which are phenotypically indistinguishable. One of the four is homozygous for the balancer chromosome and is lethal as an embryo. The fourth class is a hybrid of the two mutations but homozygous for the recessive markers; it is the diagnostic class. The following criteria were used to classify the results. If 1/3 to 1/30 of the adult progeny were hybrids, then the pair was said to exhibit complementation. If less than 1/30 were hybrids, then the pair showed partial complementation. If no hybrids were detected, the pair was said to not complement. The actual number of progeny counted in the cases of non-complementation ranged from 300 to over 3000.

Mapping: The scheme used for determining the genetic location of third-chromosome lethal mutations is shown in Figure 1. Each determination began with a single male from the mutant stock. As a result, all of the lethal-bearing chromosomes segregating in the second cross of a single determination are co-isogenic. This procedure is capable of detecting possible heterogeneity in a stock and leads to quite reproducible results, considering the large distances between the markers.

In addition to determining the locus of non-complementing lethal mutations, the mapping was used to detect possible multiple mutations and/or chromosome rearrangements. For each determination, the observed recombination frequency between adjacent markers was calculated and compared to published map distances in LINDSLEY and GRELL (1968). A program, written in the BASIC language, aided in the handling of the mapping data. This program converted raw data into position of lethal(s), standard deviation, percent recovery of the markers, observed

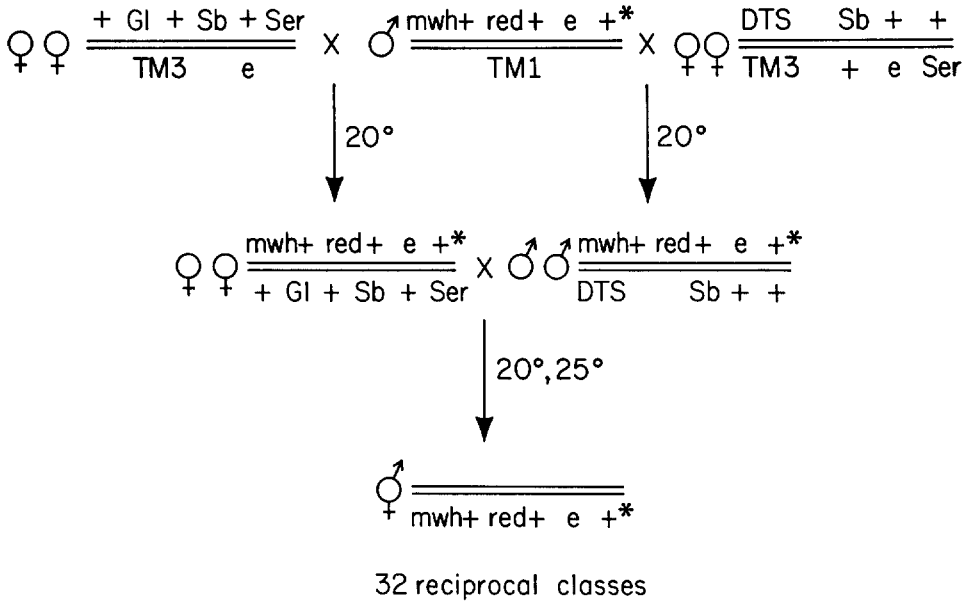

FIGURE 1.—Procedure for genetic localization of third-chromosome recessive lethal mutations For each determination a single male from a balanced lethal stock is mated in a vial with females of two genotypes. Six of the eight genotypes produced by these crosses are viable; Stubble, *(Sb)/TM1* is lethal because TM1 contains stubbloid-lethal (*sbd*[1]). From these progeny, virgin females heterozygous for the lethal-bearing chromosome (indicated by asterisk) and the chromosome bearing three dominant markers and males heterozygous for the lethal-bearing chromosome and a chromosome containing a dominant temperature-sensitive mutant (DTS) are selected and mated to each other. This latter cross is placed at 20° for four days, to ensure good expression of Serrate (*Ser*) (SHEARN, unpublished) and then shifted to 25° to kill all larvae carrying the DTS. This cross generates 32 reciprocal genotypic classes. The position of the six markers is: multiple wing hairs (*mwh*, 3-0.0); Glued (*Gl*, 3-41.4); red Malpighian tubules (*red*, 3-53.6); Stubble (*Sb*, 3-58.2); ebony (*e*, 3-70.7); Serrate (*Ser*, 3-92.5). The DTS mutant used in this scheme is DTS-2 of HOLDEN and SUZUKI (1973) and was generously supplied by T. R. F. WRIGHT.

distance between markers and a chi-square value for the deviation between observed and expected marker distances.

Hybrid phenotypes: The imaginal disc defect in lethal hybrids was examined by dissecting the discs from mature third instar larvae in Drosophila Ringer's solution and injecting single discs into wild-type hosts (Oregon-R strain). The details of the technique have been presented by URSPRUNG (1967). Differentiated implants resulting from such injections were cleared and mounted in Faure's solution and examined with the light microscope.

RESULTS

Complementation tests: For the purpose of testing complementation the mutants were divided into three groups according to the mutagen which was used to induce them. One group included 72 ethyl methanesulfonate (EMS)-induced mutants; the second included 35 N-methyl-N′-nitro-N-nitrosoguanidine (NG)-induced mutants; the third group included 35 mutants induced by the acridine compound, ICR-170. The total number of mutants is eight larger than previously

reported because eight of the original 134 mutants contained two lethal mutations. These eight double mutants have now been resolved by recombination into stocks each with a single lethal mutation. All of the mutants in each of the three groups were tested for complementation in all pairwise combinations. By testing the mutants in three groups the total number of pairwise combinations was reduced from 10,011 to 3,746. Each group contains a similar distribution of mutant phenotypes and is assumed to represent comparable samples. The results are presented in Table 1.

A complementation group includes either a single mutant which complements all other mutants or several mutants which fail to complement with each other but do complement all others. By this definition, the 72 EMS mutants could be placed into 61 complementation groups: there were five non-complementing pairs and one group of seven partially-complementing mutants. The NG mutants are distributed into 32 groups including three non-complementing pairs; the 33 ICR-complementation groups include two non-complementing pairs. Homozygotes from each of the seven mutants in the only complementation group with more than two mutants are lethal at the pupal stage. The marked hybrids from all pairwise combinations of these seven mutants yield a significant frequency of adults which are morphologically normal but which die shortly after eclosion. Because the hybrid individuals develop further than the homozygotes, this group is described as partially-complementing. The complementation results for these seven mutants and their genetic map positions are given in Table 2. These mutants may identify a gene which is a mutational hot-spot and whose product is capable of partial intracistronic complementation. No further examination of these seven has been done.

The lack of complementation between two lethal mutants is generally believed to result from each one containing a lethal mutation in the same cistron. This situation implies that the map location of the lethal mutation in each member of a non-complementing pair should be nearly identical. To confirm this prediction the position on the genetic map of each of the ten pairs of non-complementing mutations was determined (Table 3). In each case tested the position of one member of a non-complementing pair is not significantly different from the position of the other. The probabilities of getting deviations by chance as large as those observed ranged from 9% to 50%. Three of the 20 mutants (*l(3)e26LR*,

TABLE 1

Complementation among three groups of late lethal mutants

Mutagen	Number of mutants	Number of complementation groups	Number of complementation groups containing		
			1 mutant	2 mutants	> 2 mutants
EMS	72	61	55	5	1*
NG	35	32	29	3	0
ICR-170	35	33	31	2	0

* This refers to a group of seven partially-complementing mutants described in the text.

TABLE 2

*Percent marked hybrid adults recovered among progeny from pairwise matings of seven partially complementing mutants**

Map position	Mutation	*e21R*	*g30R*	*g60R*	*g131*	*n3*	*o51R*	*q6*
71.9 ± 0.6	*e21R*	. .	13(11)	25(1)	11(0)	23(1)	17(1)	17(4)
79.8 ± 0.5	*g30R*			25(5)	19(10)	25(3)	21(3)	24(1)
76.3 ± 1.5	*g60R*				15(0)	19(0)	24(0)	27(0)
74.7 ± 1.8	*g131*					23(3)	20(0)	18(0)
76.5 ± 0.8	*n3*						23(1)	25(0)
72.4 ± 0.8	*o51R*							23(0)
74.6 ± 0.7	*q6*							

* An average of 800 progeny were recovered from each cross. The results are presented as percent dead hybrid marked adults and, in parentheses, percent live hybrid marked adults. For comparison, the average recovery of dead and live marked hybrids when each of these seven was tested against a complementing mutant was 0 (31).

TABLE 3

Map position of non-complementing pairs of mutants

Mutation	Mutagen	Map position ± standard deviation*
l(3)e26L	EMS	41.4 ± 0.2
l(3)g26	EMS	42.9 ± 0.8
l(3)g49	EMS	39.4 ± 1.0
l(3)m75	EMS	41.9 ± 0.5
l(3)j5	EMS	49.2 ± 1.2
l(3)j83	EMS	48.4 ± 2.4
l(3)l23	EMS	65.3 ± 0.8
l(3)l36	EMS	62.4 ± 0.9
l(3)s61	EMS	45.9 ± 1.4
l(3)w89	EMS	49.8 ± 1.3
l(3)703	NG	78.6 ± 1.1
l(3)1803R	NG	81.2 ± 0.9
l(3)1307L	NG	10.7 ± 1.3
l(3)2813	NG	12.4 ± 1.0
l(3)1509	NG	29.3 ± 1.6
l(3)1905	NG	28.1 ± 1.2
l(3)III-10	ICR-170	46.6 ± 0.6
l(3)XVI-18	ICR-170	47.6 ± 0.5
l(3)IX-11	ICR-170	81.7 ± 0.6
l(3)XVI-3	ICR-170	†

* From the pattern of classes recovered in the mapping procedure, a lethal may be placed in one of the regions defined by adjacent pairs of markers. The position is calculated from the relative recombination of the lethal with its adjacent markers. In order to estimate the accuracy of this method for determining map positions, the recessive marked *red* was localized. The standard map position of *red* is 53.6; this method localized *red* at 53.9 ± 0.9.

† Stock lost.

l(3)1803LR, and *l(3)1307LR*) proved to be double mutants. In each of these three cases the two mutations were separated by recombination. The suffix, *L*, was appended to the symbol of the single mutant stock containing the left-most mutation and *R* to the right-most; the symbol of the original double mutants carry the suffix, *LR*. The single non-complementing mutation, in each case, was remapped and used for further testing.

Imaginal disc defects: The phenotype with respect to imaginal disc defects of each of these mutants has already been described (SHEARN *et al.* 1971). Four pairs of non-complementing mutants (*l(3)j5* and *l(3)j83*, *l(3)l23* and *l(3)l36*, *l(3)s61* and *l(3)w89*, *l(3)1307L* and *l(3)2813*) have no identifiable imaginal disc defects. Both members of another three pairs have identical defects. The leg and antenna discs differentiate abnormally in each member of two pairs *l(3)g49* and *l(3)m75*, *l(3)1509* and *l(3)1905*. Only the leg discs differentiate abnormally in a third pair *l(3)703* and *l(3)1803R*. In the remaining three pairs of non-complementing mutants different phenotypes have been attributed to each pair member. This result apparently conflicts with the generalization that noncomplementing lethal mutants have similar phenotypes, which has been used to support the idea that each complementation group corresponds to a single structural gene (SHANNON *et al.* 1972). In order to explore this apparent conflict further, the imaginal disc phenotypes of the lethal hybrid larvae formed from the mating of non-complementing mutants were examined.

The double mutant *l(3)e26LR* has no apparent disc defects. All of the imaginal discs are present in homozygous larvae, and they are capable of normal differentiation when injected into wild-type metamorphosing hosts. This mutant does not complement with *l(3)g26* in which all of the discs are small, morphologically abnormal and incapable of producing characteristic differentiated structures. Thus these two mutants have extremely different phenotypes. Hybrid larvae are phenotypically identical to *l(3)g26* homozygotes, i.e., the discs appear abnormal and do not differentiate. After obtaining this result it was discovered that *l(3)e26LR* is a double mutant with one mutant mapping near *Gl* and the other near *e*. Surprisingly, the phenotype of the non-complementing, left-hand mutant called *l(3)e26L* is identical to that of *l(3)g26*. This means that the genotype of the original hybrid was *l(3)e26L l(3)e26R/l(3)g26* + and that the *l(3)e26R* mutation in the double mutant stock is epistatic to the imaginal disc phenotype of *l(3)e26L*. The nature of this interaction is being pursued. Table 4 summarizes these relationships. However, for purposes of this report the non-complementing pair, *l(3)e26L* and *l(3)g26*, must be added to the list of pairs with identical phenotypes.

Another pair of non-complementing mutants which was reported not to have identical imaginal disc defects includes *l(3)III-10* and *l(3)XVI-18*. Both of these are lethal homoeotic mutants. The mutant *l(3)XVI-18* causes the haltere disc to differentiate wing as well as haltere structures. The mutant *l(3)III-10* leads to a similar transformation but in addition causes the genital disc to differentiate into genital, leg, and antenna structures. Such a difference could be explained by proposing that the latter mutant, *l(3)III-10*, is a double mutant consisting of a lethal

TABLE 4

Genotype and phenotype of combinations of the mutations l(3)g26 *and* l(3)e26LR

Genotype	Imaginal disc phenotype*
$\frac{mwh\ l(3)g26\ e\ +}{mwh\ l(3)g26\ e\ +}$	mutant
$\frac{mwh\ l(3)e26L\ e\ l(3)e26R\ \dagger}{mwh\ l(3)e26L\ e\ l(3)e26R}$	normal
$\frac{mwh\ l(3)e26L\ e\ l(3)e26R}{mwh\ l(3)g26\ e\ +}$	mutant
$\frac{mwh\ l(3)g26L\ e\ +}{mwh\ l(3)g26\ e\ +}$	mutant
$\frac{mwh\ l(3)g26L\ e\ +}{mwh\ l(3)g26L\ e\ +}$	mutant

* The mutant phenotype indicates discs which are small, morphologically abnormal, and not capable of differentiation when injected into metamorphosing hosts.
† This is the genotype of the original *l(3)e26LR* double mutant.

mutation which does not complement *l(3)XVI-18* and another homeotic mutation which would not necessarily be lethal. This explanation is unlikely since the total frequency of homeotic mutations among the lethals previously isolated was 3/142. The probability of one of those three containing two homeotic mutations is exceedingly low. Nevertheless, evidence of more than one mutation has been looked for in three different ways, none of which indicate multiple mutations. (1) *l(3)III-10* maps as a single mutation. (2) By recombination, 12 isolates of the lethal mutation in *l(3)III-10*, which fails to complement *l(3)XVI-18*, have been prepared. The imaginal disc phenotype of each of the 12 is identical to that of the original mutant. (3) Several spontaneous revertants of the lethal mutation have been recovered. Each of these is also free of homeotic defects, although each retains the linked markers.

Since ICR-170 is a frameshift mutagen in bacteria (AMES and WHITFIELD 1966) and is believed to act similarly in Drosophila (CARLSON, SEDEROFF and COGAN 1967), perhaps *l(3)III-10* is such a frameshift mutation which causes a defect in two or more adjacent genes. If this were the case, then the phenotype of the hybrid *l(3)III-10/l(3)XVI-18* should be identical to that of *l(3)XVI-18* homozygotes; the genital disc should be normal, and only the haltere disc should manifest a homeotic transformation. Genital and haltere discs from both homozygotes and the hybrid were dissected and injected into wild-type metamorphosing hosts. The results of examining the differentiated implants are presented in Table 5. It was found that the two homozygotes differ slightly in their effect on the haltere disc. The mutant *l(3)III-10* transforms the haltere disc completely to a wing disc; in fact, the dissected larvae appear to have two pairs of wing discs, whereas *l(3)XVI-18* haltere discs differentiate into a mixture of haltere and wing

TABLE 5

Comparison of differentiated structures produced by a pair of non-complementing homeotic mutations and the hybrid formed between them

Donor imaginal disc	Phenotype expressed as disc-specific differentiated structures	Number of discs, of indicated genotype, expressing a given phenotype					
		III-10/III-10		III-10/XVI-18		XVI-18/XVI-18	
Haltere	Haltere	0		0		0	
	Haltere, Wing	0		2		5	
	Wing	10		1		0	
		♀	♂	♀	♂	♀	♂
Genital	Genital	0	0	1	0	5	5
	Genital*	0	0	4	0	5	1
	Genital, Leg	0	0	0	2	0	0
	Genital, Leg, Antenna	3	2	0	0	0	0

* This category denotes presence of small patches of non-genital structures, e.g., fluted bristles surrounded by trichomes which are characteristic of both leg and antenna. Only those structures positively identified as characteristic of leg or antenna are indicated as such.

characteristic structures. The haltere discs of the hybrid larvae express an intermediate phenotype. Two discs gave a mixture of haltere and wing structures and one gave only wing structures, although not so many different wing structures as *l(3)III-10* homozygotes produced.

Both female and male genital discs of *l(3)III-10* homozygotes differentiate into specific leg and antenna structures as well as into some genital structures. The hybrid genital discs also showed homeotic abnormalities. The male discs produced characteristic leg structures (femur) and four out of five female genital discs produced, in addition to normal genital structures, groups of fluted bristles surrounded by trichomes. Neither fluted bristles nor trichomes are characteristic structures of the female genital disc. However, the patches were too small to identify them positively as part of a specific disc. They could be either leg or antenna bristles. This somewhat unexpected result stimulated a re-examination of genital discs from *l(3)XVI-18* homozygotes. In 6 out of 16 discs tested by injection, a small number (1–3) of non-genital disc bristles were present, usually surrounded by a few trichomes.

It thus appears that the phenotypes of *l(3)III-10* and *l(3)XVI-18* are not qualitatively different since both affect the haltere and the genital discs. They are markedly different quantitatively, with *l(3)III-10* causing a more extreme disc phenotype than *l(3)XVI-18*. The phenotype of the hybrid is intermediate between the two homozygotes. A more detailed developmental analysis of this pair of mutants is in progress.

Hybrid larvae of the third pair of non-complementing mutants with different imaginal disc phenotypes (*l(3)XVI-3* and *l(3)IX-11*) unfortunately could not be tested because one of the mutants (*l(3)XVI-3*) was lost sometime after the complementation testing was completed. However, it seems likely that further analysis would have shown this difference to be quantitative as originally proposed (SHEARN *et al* 1971), since additional mutants have now been analyzed

in which different individuals of a single mutant stock can express the range of phenotypes found in *l(3)IX-11* and *l(3)XVI-3*. (SHEARN and GAREN 1974).

Finally, as a control for the validity of comparing hybrid to homozygous discs, hybrids of each of the pairs of mutants with a similar imaginal disc defects were compared to their respective homozygotes. In each of the three cases the defects found in the hybrids were identical to those found in the respective pair of homozygotes.

DISCUSSION

The complementation results shown in Table 1 indicate that within each of the three groups most of the mutations are in different functional units. By the Poisson method these data may be used to estimate the total number of genes on the third chromosome which are capable of yielding recessive late lethal mutants. BENZER (1961) used this method to estimate the degree of saturation of the *rII* locus in T4 phage. This method depends on the assumption that all genes are equally mutable, although that is rarely observed. However, it is possible to discard from the calculation what appear to be mutational hot-spots. The group of seven partially-complementing EMS-induced mutants appears to be such a hot-spot. From the data in Table 1, the three estimates for the number of genes on the third chromosome which are capable of yielding late lethal mutants are 364, 172 and 273 for the EMS, NG and ICR mutants, respectively.

HOCHMAN (1971) has nearly saturated the tiny fourth chromosome of Drosophila by inducing a large number of lethal mutants. His work is relevant to this discussion in the following two ways: (1) The frequency distribution of the number of mutations he recovered fits a Poisson distribution if three loci are not considered. His results support the idea that the probability of recovering a lethal mutation in most genes is similar but that the probability is much higher in a few others. These results tend to validate the use of complementation data from Drosophila to estimate total numbers of vital genes with the Poisson distribution. (2) HOCHMAN found that the total number of complementation groups he identified on the fourth chromosome was similar to the number of bands detectable on the fourth chromosome in salivary gland chromosome squashes. The one band-one vital complementation group hypothesis has received additional support from LIFSHYTZ and FALK (1969) and from JUDD, SHEN and KAUFMAN (1972). Each of these groups has shown, by saturating small portions of the *X* chromosome with lethal mutations, that there is one vital complementation group per salivary gland chromosome band.

The third chromosome contains about 2030 bands (40% of the 5072 bands in the genome). If we assume that the one band-one complementation group relationship holds for the entire genome, the third chromosome should contain 2030 vital complementation groups. About 23% of all lethal mutations on the third chromosome are late lethals (SHEARN *et al.* 1971). Thus the one band-one vital gene hypothesis predicts 465 late lethal genes on the third chromosome. This estimate is the same order of magnitude as the estimates based on the complementation data presented above.

JUDD, SHEN and KAUFMAN (1972) have proposed the idea that each complementation group defines a single structural gene plus cis-dominant regulatory elements. One prediction of this model is that the phenotype of different lethal mutants within a single complementation group should be nearly uniform. Heterogeneity of phenotypes would suggest more than one function per group as is found in bacterial operons, for example. To test this prediction SHANNON *et al.* (1972) compared different mutants in each of the 13 adjoining complementation groups of the zeste to white region of the *X* chromosome. They observed stage of lethality, morphology, and autonomy in gynandromorphs and concluded that similarity of phenotype is the rule among non-complementing mutants. One possible flaw in this argument is that most of the mutants tested were induced by x-rays and may be small deletions. The data presented here, however, using chemically-induced mutants, also support the conclusion that the phenotypes of non-complementing lethal mutants tend to be uniform. The criterion of uniformity applied was still more stringent than those used by SHANNON *et al.* (1972). Two non-complementing late lethal mutants were considered to show a uniform phenotype if their imaginal discs manifested the same pattern of defects when tested by injection into metamorphosing hosts and if the lethal hybrid formed by the two mutants also had the same phenotype. There were three apparent exceptions to the rule of uniformity. One resulted from the presence of a second lethal mutation which was epistatic. The two other cases appeared to be quantitative differences. Only the non-complementing homeotic mutants, however, were studied in detail. Both of these mutants, *l(3)III-10* and *l(3)XVI-18*, affect the haltere and genital discs, but *l(3)III-10* causes more extreme changes. The hybrid *l(3)III-10/l(3)XVI-18* has an intermediate phenotype.

One additional test was made of the uniformity of phenotype among non-complementing mutants. Thirty-five EMS-induced mutants were selected (GAREN, unpublished) on the basis of their failure to complement *l(3)k43*, an EMS-induced mutant which has no imaginal discs (SHEARN *et al.* 1971). Each of the 35 has a phenotype identical to that of *l(3)k43*; i.e., no imaginal discs are detectable.

One group of seven mutants has been found to exhibit partial complementation in all pairwise combinations. Partial complementation has been found among mutants in a single cistron and may result from polymerization of defective subunits into a partially active enzyme (GAREN and GAREN 1963). Unfortunately, nothing is known about the defect in these seven mutants. It is unusual that all of the alleles show some complementation. Ordinarily one would expect some of the alleles to be non-complementing.

I would like to thank CYNTHIA CALABRIA for her valuable technical assistance. I also thank HELEN ELLIS, ANDREA MARTIN, PRESLEY MARTIN, JOSE RAMIREZ, TOM RICE, ANN TIFFORD, and GEORGE UHL for their help in various aspects of this work. This work began while the author was supported by a Helen Hay Whitney Foundation postdoctoral fellowship at Yale University in ALAN GAREN's laboratory.

LITERATURE CITED

AMES, B. N. and H. J. WHITFIELD, JR., 1966 Frameshift mutagenesis in *Salmonella*. Cold Spring Harbor Symp. Quant. Biol. **31**: 211–225.

BENZER, S., 1961 On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **47**: 403.

CARLSON, E. A., R. SEDEROFF, and M. COGAN, 1967 Evidence favoring a frameshift mechanism for ICR-170 induced mutations in *Drosophila melanogaster*. Genetics **55**: 295–313.

GAREN, A. and S. GAREN, 1963 Complementation *in vivo* between structural mutants of alkaline phosphatase from *E. coli*. J. Mol. Biol. **7**: 13–29.

GEHRING, W. and R. NÖTHIGER, 1973 The Imaginal Discs of *Drosophila*. pp. 211–290. In: *Developmental Systems: Insects*. Edited by C. H. WADDINGTON and S. J. COUNCE. Academic Press, New York.

HOCHMAN, B., 1971 Analysis of chromosome 4 in *Drosophila melanogaster*. II: Ethyl methanesulfonate induced lethals. Genetics **67**: 235–252.

HOLDEN, J. and D. T. SUZUKI, 1973 Temperature-sensitive mutations in *Drosophila melanogaster*. XII. The genetic and developmental characteristics of dominant lethals on chromosome 3. Genetics **73**: 445–458.

JUDD, B. H., M. W. SHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the *X* chromosome of *Drosophila melanogaster*. Genetics **71**: 139–156.

LIFSCHYTZ, E. and R. FALK, 1969 Analysis of ethyl methanesulfonate induced lethals. Mutation Res. **8**: 147–155.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations in *Drosophila melanogaster*. Carnegie Inst. Washington Publ. **627**.

SHANNON, M. P., T. C. KAUFMAN, M. W. SHEN and B. H. JUDD, 1972 Lethality patterns and morphology of selected lethals and semi-lethal mutations in the zeste-white region of *Drosophila melanogaster*. Genetics **72**: 615–638.

SHEARN, A. and A. GAREN, 1974 Genetic control of imaginal disc development in *Drosophila*. Proc. Natl. Acad. Sci. U.S. (In press.)

SHEARN, A., T. RICE, A. GAREN and W. GEHRING, 1971 Imaginal disc abnormalities in lethal mutants of Drosophila. Proc. Natl. Acad. Sci. U.S. **68**: 2594–2598.

STEWART, M., C. MURPHY and J. W. FRISTROM, 1972 The recovery and preliminary characterization of X chromosome mutants affecting imaginal discs of *Drosophila melanogaster*. Develop. Biol. **27**: 71–83.

URSPRUNG, H., 1967 *In vivo* culture of *Drosophila* imaginal discs. pp. 485–492. In: *Methods in Developmental Biology*. Edited by F. H. WILT and N. K. WESSELLS. Thomas Y. Crowell Co., New York.

URSPRUNG, H. and R. NÖTHIGER, 1972 (Editors) *The Biology of Imaginal Discs*, Results and Problems in Cell Differentiation. Volume 5, Springer-Verlag, New York.

Corresponding editor: B. H. JUDD