DEVELOPMENTAL GENETICS OF THE DROSOPHILA EGG I. IDENTIFICATION OF 59 SEX-LINKED CISTRONS WITH MATERNAL EFFECTS ON EMBRYONIC DEVELOPMENT

J. DAWSON MOHLER[1]

Department of Zoology, University of Iowa, Iowa City, Iowa 52242

Manuscript received June 26, 1975
Revised copy received November 9, 1976

ABSTRACT

Sex-linked mutations to recessive female sterility were induced, sorted for egg-laying, mapped within broad regions and grouped by complementation tests into cistrons. The mutations have also been partially characterized for their temperature sensitivity and pleiotropic effects. Altogether 59 cistrons have been identified, including five allelic with previously known loci: *cin*, *fs(1)N*, *mk*, *sn*, and *r*.

All of the genes make maternal contributions to developing embryos. In some instances mutant defects are recognized in the egg envelopes; in the remainder the defects are presumably in the egg cytoplasm. For mutations in twenty-two genes, including *cin*, *mk*, and *r* alleles, the lethality of the maternal effect is reversed and the embryo is "rescued" by the action of a wild-type, paternal allele. The mutant strains are potentially important material for the study of developing egg envelopes and for furthering the analysis of causation in embryogenesis and its origins in oogenesis.

OOGENESIS is a particularly interesting developmental process. It leads to the simplest form that an entire metazoan can take: an egg. Complex though an egg is, each subsequent stage acquires more complexity in emergent levels of organization. To investigate oogenesis, genetic analysis is one of the most powerful methods available. Events of oogenesis can be singled out by observation and related to the unit causal factors in organisms, *i.e.*, genes. Different forms of a particular process and different forms of a particular resultant step can be related to different forms of a particular gene.

A genetic analysis necessarily begins with the collection of relevant mutations, which for most aspects of oogenesis should be those producing female sterility. The mutant alleles are used to identify and catalogue the genes normally functioning in the development of the egg, and the mutant phenotypes are used to suggest the aspect of oogenesis for which the normal gene is important. Female sterility occurs among the pleiotropic effects of mutations at several loci in *Drosophila melanogaster*. The initial catalogue of genes affecting steps in oogenesis (KING 1970) was based on such pleiotropic mutations. More recently the

[1] The paper was prepared in part while the author was on a research leave from the University of Iowa and in residence at the Department of Biological Science of the University of Arizona.

catalogue has been extended (KING and MOHLER 1975) by systematic collections of female-sterile mutations (BAKKEN 1973; GANS, AUDIT and MASSON 1975; MOHLER 1973; RICE 1973; ROMANS 1973). The genetic details of mutations in 59 cistrons, including some previously mentioned in abstract (MOHLER 1973), are presented in this paper. Since the proteins are unknown, the cistrons are operationally defined by complementation tests (BENZER 1957) that were scored on the basis of fertility *vs.* sterility.

All of the genes in this paper make maternal contributions to developing embryos. In all mutant strains, heterozygous females lay normal, fertile eggs, but homozygous females lay eggs that fail to develop and hatch. In some instances, mutant defects are recognized in the structure of the egg envelopes; in the remainder, the defects are presumably cytoplasmic and result in death of the embryo at different times in different mutant strains. Thus, the mutant alleles of these genes are "maternal effect lethal mutations" (KAPLAN, *et al.* 1970). For mutations in twenty-two genes, the lethality of the maternal effect is reversed and the embryo is "rescued" by the action of a wild-type, paternal allele.

MATERIALS AND METHODS

The origin of female-sterile mutations. The collection of sex-linked, female-sterile mutations in *Drosophila melanogaster* was obtained by the method outlined in Figure 1. A mass-mated

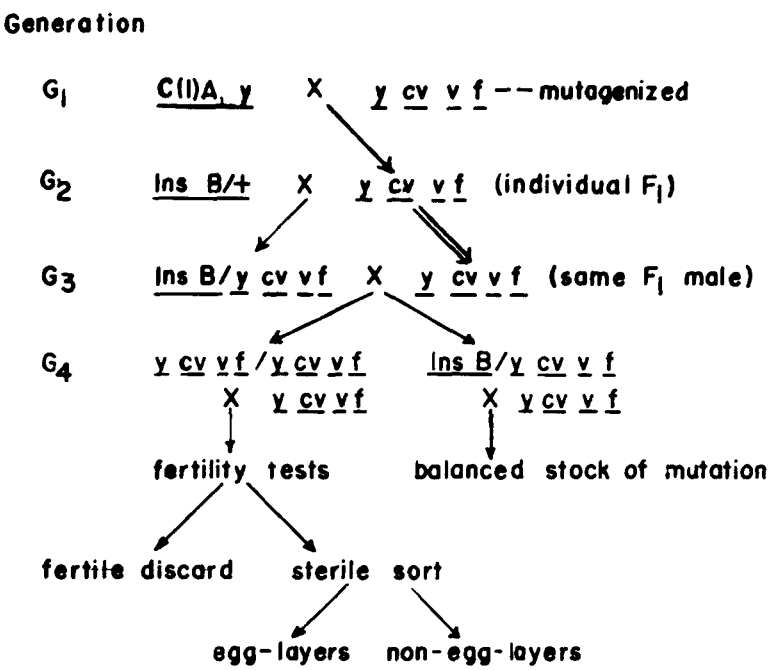

FIGURE 1.—Production of lines with sex-linked female-sterile mutations. *C(1)A*, *y* is an attached *X*; *y*, *cv*, *v*, and *f* are recessive markers in the mutagenized *X* chromosome; *Ins B* is a generic term for balancer chromosomes marked with *Bar*. Additional explanation is made in the text.

strain marked with *yellow* (*y*, *1*–0.0), *crossveinless* (*cv*, *1*–13.7), *vermilion* (*v*, *1*–33.0), and *forked* (*f*, 1–56.7) was newly established from 38 isogenic lines that had demonstrated female fertility. Adult, virgin *y cv v f* males were fed ethyl methanesulfonate (EMS) according to the method of LEWIS and BACHER (1968) and mass-mated with attached-*X* *(C(1)A, y)* females. Surviving G_2 males were mated individually with females heterozygous for a sex-chromosome balancer (*Ins B*) and mated again with their heterozygous daughters so as to establish isogenic lines that could be tested for recessive, female-sterile mutations in the homozygous females of G_4. Two different balancers were used: *FM3*, which is described by LINDSLEY and GRELL (1968), and a chromosome having *Ins (1)sc^8, dl-49*. Both balancers are marked with *Bar* (*B*, *1*–57.0) and the *y^{81d}* allele of *y*, but differ in a number of other, recessive markers. *FM3* is lethal, the second balancer is female-sterile.

The initial mating was used to reduce the number of established lines carrying recessive lethal and male-sterile mutations. Because genetic mosaics are produced in EMS mutagenesis (ALDERSON 1965), a repeat mating with attached-*X* females at G_2 would be needed to eliminate all such mutations. In order to evaluate the effectiveness of the treatments, the sex ratio in the G_2 progeny of treated males in one series was compared with that of progeny from untreated males. In the treated series, there were 4,633 females and 1,745 males from 64 cultures. In the control there were 770 females and 848 males from 8 cultures. The lower proportion of males in the treated series implies that 66% of the treated *X* chromosomes carried a new lethal mutation or inactivation that was expressed in the loss of G_2 males. If the distribution of lethal hits is random, the average number of hits at the dosage used (0.24% EMS) was 1.08 per treated *X* chromosome.

Four series of treatments were made, yielding altogether 5,524 fertility-tested lines. There resulted 320 with confirmable, sex-linked mutations to female sterility. In 225 female-sterile lines, homozygous females lay eggs that fail to hatch. The mutations that are the subject of this paper are from this group of female-sterile strains.

Genetic mapping. Because the *fs* mutations are linked in *cis* with recessive markers, mapping is possible with testcross progeny. For each line, heterozygous *Ins B/y cv v f (fs)* females from the balanced strain were mated with wild-type males from a strain with an isogenic Ore-R chromosome *1*, and the F_1 *+/y cv v f (fs)* females and *y cv v f (fs)* males were mated *inter se*. F_2 females were selected and mated with wild-type males to test fertility. In general, the practice for each mutant line was to mate individually two to three females of each of the six single crossover classes (*y, cv v f, y cv, v f, y cv v, f*) and, as a control, to mass-mate *y cv v f* nonrecombinants. In this way each mutation was localized within a limited region of the *X* chromosome.

Genetic complementation. Pairwise tests of complementation were made for mutations that localized to the same region of the *X* chromosome. In the cases that localized near one of the markers, pairwise tests were also made with the mutations in both adjacent regions. For each pair, at least four doubly heterozygous (*trans*) females were mass-mated in a test of their fertility. If these were sterile, the test was repeated to confirm the result. Independent mutations that were confirmed to be noncomplementing by repeated testing are concluded to be allelic. All pairwise tests were made, except that in many cases a mutation that complemented two members of an allelic group was presumed to complement all others of that group.

Culture conditions. The standard culture and testing temperature was 25° C. This condition was maintained rigorously in refrigerated incubators for studies of localization and complementation; but because the mutagenesis experiment exceeded our incubator capacity, the temperature conditions in that case were less well maintained in a room at 23 ± 2° C.

The culture medium is a standard formulation including agar, yeast extract, milk solids, cornmeal, corn syrup and propionic acid described by FRANKEL and BROSSEAU (1968).

RESULTS

Localization of mutations to region. The mapping procedure allows each mutation to be identified with a particular region of the *X* chromosome. 191 mutations

TABLE 1

Localization of mutations and cistrons by region

	Region						
	y—cv	near *cv*	*cv—v*	near *v*	*v—f*	near *f*	*f—sfa*
Mapped mutations	49	17*	12	17*	48	34*	13
Complementation tested	39	13	8	12	33	30	10
Number of cistrons	17	4	7	4	18	5	4
Map distance (cM)†	13.7		19.3		23.7		13.3
Salivary map sections†	1 to 4F		4 F to 10A		10A to 15F		15F to 20F

* Many of these mutations become identified with cistrons in the adjacent regions in the course of progeny testing.

† From LINDSLEY and GRELL (1968). G. LEFEVRE (personal communication) has localized *cv* to section 4F.

in 188 female-sterile (egg-laying) strains are distributed as shown in Table 1. Three strains (Table 2) appear to be mutated at two recombining loci.

11–73—one mutation is near *cv* and the other is between *v* and *f*, because *y* recombinants and most *f* recombinants are fertile and all other combinations are sterile.

14–743—one mutation is near *v* and the other is near *f*, because both *y cv v* and the *f* recombinants are sterile. The alternative conclusion, *i.e.*, one mutation at some point between *v* and *f*, is unlikely because the probability of no fertile females among the six recombinants would be 1/64 in such a case.

12E–90—one mutation is near *y* and the other is near *f*, because all recombinants are sterile. These mutations are distinguished also in that the yellow-linked mutation is rescued (see below) and the forked-linked one is not. (The double mutant combination is not rescued.)

There are two remarks to make about the overall distribution. First, most of the mutations near *v* and near *f* become identified in subsequent complementation-testing as alleles of other mutations that mapped in the *v—f* region. Second,

TABLE 2

Doubly mutated strains: genetic localization

	Recombinant classes tested					
Strain	*y*	*cv v f*	*y cv*	*v f*	*y cv v*	*f*
11–73						
Fertile	6	0	0	0	0	7
Sterile	0	9	9	12	11	6
14–743						
Fertile	3	0	3	0	0	0
Sterile	0	3	0	3	3	3
12E–90						
Fertile	0	0	0	0	0	0
Sterile	5*	8	8*	9	6*	7

* Rescued, *i.e.*, female zygotes survived.

though the female-sterile mutations are identified over the entire chromosome, the frequencies are not proportional to either genetic map length or cytological map length. The regions *cv—v* and *f—sfa* together account for half of the salivary chromosome map and nearly half of the genetic map (LINDSLEY and GRELL 1968); together they account for about one fourth of the female-sterile genes.

Besides localization, the mapping procedure incidentally gives information on two genetic conditions for expression of the genetic defect. First, because the matings for fertility-testing are made to + males, the mutations that can be rescued become recognized. For all genes, the mutant females are identified as sterile because their homozygous progeny do not complete development; for rescued mutants heterozygous progeny survive, presumably because function of the + allele during embryogenesis replaces the defective maternal factor. In such cases the heterozygous (+/*fs*) daughters, but not the hemizygous (*fs*/—) sons, survive. These mutations are identified in Table 3. Second, because the outcross may introduce new alleles into the autosomal background genotype, many of these mutations that are subject to genetic modification or suppression of their effects will be recognized. For most of the mutations, no genetic suppression was observed. In 26 cases, however, a small number of progeny would survive in the tests of individual recombinant females. Though the survivors are a nuisance for mapping, valid regional localization could be obtained in all but one case (12–521) and the mutations could be included in the complementation-testing. For 14 additional strains, the suppression was complete, that is, all F_2 classes including *y cv v f* females were fertile, so that the map positions are not known. For one other, 12–323, the recombinant classes were all fertile, but the *y cv v f* nonrecombinant females were sterile. Finally, in one other case, 11–1118, the *y cv v f* nonrecombinant females were all sterile, but all recombinant classes included some fertile and some sterile flies. These 17 strains await further analysis of the basis for suppression as well as their individual genetics.

Identification of fs *cistrons.* In general, females heterozygous for two mutations in *trans* were clearly fertile or clearly sterile. Furthermore, most cistrons showed the sterotyped pattern of allelism: noncomplementation among all alleles and complementation with all nonalleles. Inconsistencies and ambiguities did arise in the course of the study; but, for the most part, the confusion was cleared up with careful retesting. The present study began with complete testing among mutations from one series (12–). Subsequently, mutations in the other series were tested with one or two, if available, members of each cistron in the same region of the chromosome. In each case of a noncomplementing pair of mutations, the test was repeated and more tests were made with all other known alleles of that cistron. Whenever the testing was inconclusive because of low-grade fertility in the *trans* heterozygote, ambiguous patterns of allelism, or inconsistent results, new reciprocal crosses were made and fertility tests were repeated on the new F_1 progeny.

Complementation testing has been completed with 146 mutations in 144 female-sterile strains and 59 cistrons have been identified. (A list of the cistrons and the mutant strains in each is to appear in *Drosophila Information Service.*

TABLE 3

Cistrons with temperature-sensitive and rescued alleles

Region	Cistron	Number of alleles	Number of alleles that are Cold-sensitive	Heat-sensitive	Rescued
y—cv	*fs(1)M7* (=*mk*)	1		1	1
	M8	1			1
	M9	3		1*	
	M10	8			7
	M38	2		1*	
	M40	2		1	1
	M41	1			1
	M50 (=*cin*)	1			1
near *cv*	*M13*	5		5*	2*
cv—v	*M15*	2			2
	M16	1			1
	M47	1			1
near *v*	*M43*	1		1	
v—f	*M20*	2			1
	M21	1			1
	M22	2		1	
	M24	3	1		2
	M25	5		1*	
	M27	1			1
	M28	1			1
	M29	6			6
	M34 (=*r*)	15			5
	M37	7		2	4
	M63	2		1	
near *f*	*M32*	1	1		1
	M56	1		1	1
f—sfa	*M57*	4			1*
	M58	1		1	1

* The egg envelopes are defective at all temperatures. It is conceivable that temperature sensitivity and rescue are physiological consequences of that defect in these cases.

More detailed information in the form of complementation matrices for each region of the chromosome is available on request.) Altogether, 129 mutations are conclusively assigned to 50 cistrons distributed over all regions of the *X* chromosome; 17 other mutations in 15 strains, including most of those found in the *cv—v* region, are tentatively assigned to 9 cistrons; one more mutation in strain 11–1505 cannot now be identified with a cistron. The reasons for the tentative conclusions and doubt are developed in the following discussion.

Complex patterns of complementation were encountered with several groups of mutations (Figure 2, 3, and 4). In principle such patterns mean either the occurrence of intracistronic complementation in some transheterozygotes of allelic mutations, or else the occurrence of multiple mutations or deletion involv-

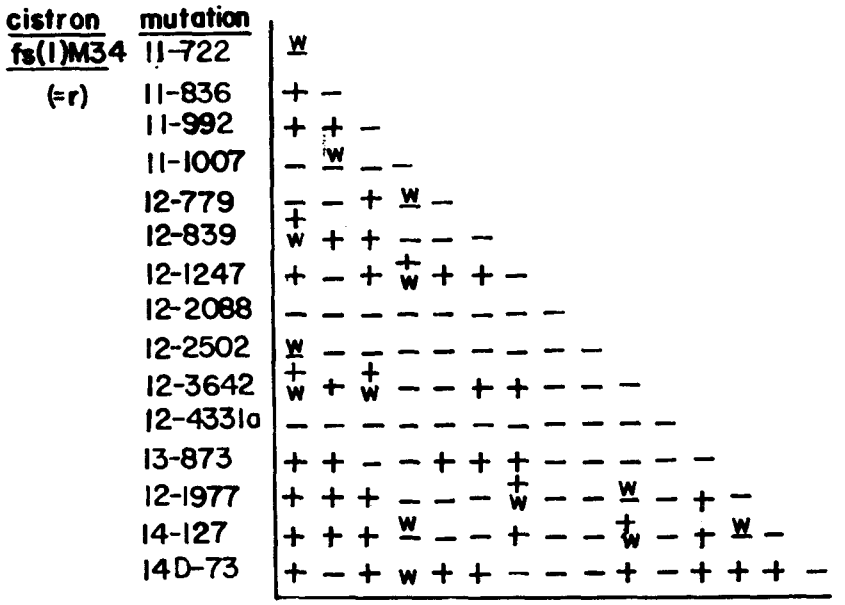

FIGURE 2.—Complex complementation pattern for rudimentary *(fs(1)M34)* mutations. Each symbol summarizes the results of fertility tests of homozygous females from each strain (diagonal) and double heterozygous females resulting from crosses of two strains (below the diagonal). The strains are ordered identically for both rows and columns but, for convenience, are made explicit only for rows. + = fertile test; w = semisterile; — = sterile. $\overset{+}{w}$ and $\underline{\overset{w}{-}}$ indicate where inconsistency is seen in repeated tests.

ing two or more cistrons of an individual female-sterile strain. In the case of *fs(1)M34* (Figure 2), it appears that intracistronic complementation is occurring. First, the alternative interpretation is unlikely because the pattern would require multiple mutations in more than one independently derived strain. Second, the interpretation of intracistronic complementation is supported by the weak productivity of many complementing transheterozygotes. At a different level of perception, the semisterility of transheterozygotes for some pairs would be viewed as noncomplementation. Third, all 15 mutations express the rudimentary phenotype to some degree. Therefore, these mutations are at the *r* locus (1–54.5) and the pattern of complementation recalls similar patterns previously described for this locus by GREEN (1963) and by CARLSON (1971). Intracistronic complementation is recognized also in *fs(1)M12* and *fs(1)M13* (Figure 3) and in *fs(1)M18*, *fs(1)M29*, and *fs(1)M37* (Figure 4). In each of these the alternative interpretation can be rejected because the pattern requires either two mutations in more than one independently derived strain or more than two mutations in a single strain. The interpretation of intracistronic complementation is also suggested in the cases of *fs(1)M15* (Figure 3) and *fs(1)M24* (Figure 4), as well as most of those listed above, by the weak productivity.

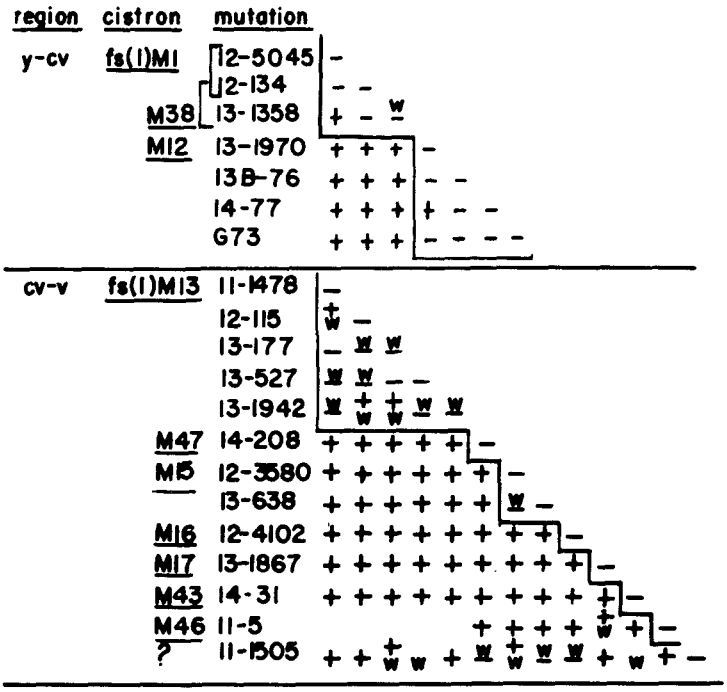

FIGURE 3.—Complex complementation pattern for mutations in *y — cv* and *cv — v* regions. The figure is drawn in the same way as is Figure 2. The mutation G73 was provided by C. AUDIT and M. GANS from their collection.

Some cistrons are less conclusively established. The *fs* mutations in strains 12–5045 and 13–1358 (Figure 3) are tentatively concluded to be in different cistrons, *fs(1)M1* and *fs(1)M38*, respectively, because the possibility that strain 12–134 is mutated or deleted for both cistrons is not yet ruled out.

There are two arguments to make regarding the tentative assignments of mutations in the *cv—v* region of Figure 3. First, the low-grade productivity of 13–1867/11–5 females was unexpected because 11–5 maps close to *v* and 13–1867 appears to map closer to *cv*. However, in the latter case *v f* recombinant females, though always producing offspring, produced less than ten offspring in each of 5/11 tests; *y cv* females were completely sterile in 12/13 tests. Thus, 13–1867 may have two mutations: one near *cv* and effecting complete sterility and a second, semisterile mutation near *v*, allelic to that in 11–5.

The second comment is with respect to the anomalous complementation pattern involving 11–1505. This mutation in heterozygotes with mutations at several sites from near *cv (fs(1)M13)* to near *v (fs(1)M46)* results in semisterile females. It is unlikely that 11–1505 is a deletion for the several cistrons because it is viable when homozygous and because *trans* heterozygotes are never completely sterile; it is also unreasonable that 11–1505 is mutated in that many places. Rather, the other mutations, which are recessive in most heterozygotes, interact cumulatively with that in 11–1505 similarly as many nonallelic cross-

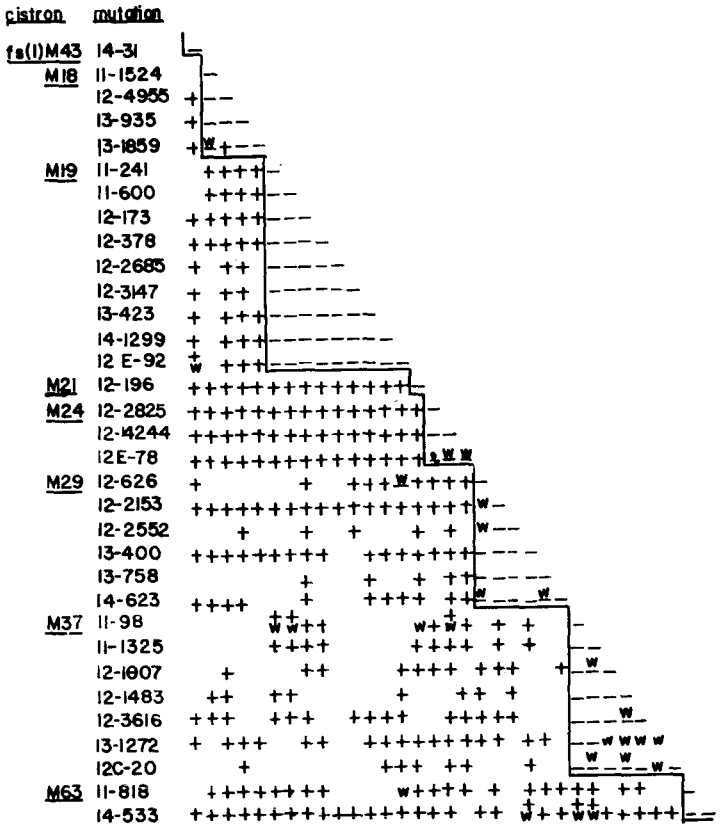

FIGURE 4.—Complex complementation patterns for mutations in the v — f region. The figure is drawn in the same way as is Figure 2. The heterozygote for 12–2825 and 12E-78 is a lethal (l) genotype.

veinless-like genes interact positively with the *cv* mutation in heterozygotes (MILKMAN 1970). The concept of cistrons is not applicable in the case of 11–1505 and the other cistrons are recognized by ignoring it. Three other mutations, 11–98 in *fs(1)M37*, 12E-92 in *fs(1)M19* and 14–533 in *fs(1)M63*, interact cumulatively in transheterozygotes with nonallelic mutations. Each of these, however, are otherwise unambiguously identified with a distinct complementation group (Figure 4).

The extent to which these 59 cistrons are allelic with known sex-linked female-sterile mutations was examined in detail. There are mutations allelic with female-sterile (1) Nasrat (*fs(1)N*, *1*–0.0), murky (*mk*, *1*–0.8), singed-36a (sn^{36a}, *1*–21.0), and rudimentary (*r*, *1*–54.5), which are listed in LINDSLEY and GRELL (1968), and cinnamon (*cin*, *1*–0.0) which is described by BAKER (1973). None of the cistrons are allelic with deep orange (*dor*, *1*–0.3), deflected wings (*dfw*, *1*–21.6), almondex (*amx*, *1*–27.7), dishevelled (*dsh*, *1*–33.3), slimma (*sla*, *1*–48.6), fused (*fu*, *1*–59.5), or refringent (*rfr*, *1*–67.9), which also are listed by LINDSLEY and GRELL.

Temperature sensitivity. Mutants that are temperature independent in their expression are expected to be useful for developmental analysis (SUZUKI 1970). For that reason the fertility and fecundity of most strains has been measured from homozygous females raised, mated and maintained at 18° as well as 25°. Because the temperature control during the original operations that screened these mutations was not rigorously held at 25°, cold-sensitive mutations as well as heat-sensitive mutations are found in the collection. The fecundity was measured as the mean number of offspring per female in a standard unit of time adjusted for the effect of temperature. The mutant strains that are fertile at 18°, but not at 25°, or that have a four-fold or greater fecundity at the lower temperature are classed heat-sensitive. Two mutant strains that are sterile at 18°, but produce a few offspring at 25°, are classed cold-sensitive. The cistrons with temperature-sensitive alleles are listed in Table 3.

Phenotypic aspects. The systematic description of the maternal effects of these mutations on the developing embryo will be presented in a later paper. It is, however, useful to point out that the homozygous females in all strains mate and store sperm successfully, so that the blocks are at steps subsequent to those events. In addition, these mutations do not seem to cause gross abnormalities in the appearance of the yolk. The only obvious defects are in cases of mutations that affect the egg envelopes. Often such eggs seem to have collapsed under their own weight. In two cases, *fs(1)M9* and *fs(1)M45* (*sn*), the size and shape of the dorsal appendages are abnormal as well.

The mutations that can be "rescued" are counted in Table 3. In only a few cases are there substantial proportions of rescued individuals. The mutations in *fs(1)M41* and *fs(1)M50* (*cin*) are especially remarkable for nearly 100% rescue of female zygotes. The phenomenon is not really characteristic of maternal effect lethal phenotypes.

Other pleiotropic effects of the female-sterile mutations have been sought in morphology and viability. Except that more subtle differences may have been overlooked, the +/*fs* and *fs*/*fs* females in nearly all strains are morphologically alike as adults. The murky mutation (*fs(1)M7*) is an example of a subtle effect that is actually overlooked in this strain. The mutations in *fs(1)M50* (*cin*), *fs(1)M45* (*sn*), and *fs(1)M34* (*r*) show the appropriate eye color, bristle and wing effects respectively. Homozygotes for *cin*, which must come from +/*cin* females, rarely show the eye color effect, however, because the eye phene is also subject to maternal influence. Homozygotes for the allele discovered here have abnormal eye color more often than do those with the original allele described by BAKER (1973). Besides these mutations in known genes, the female-sterile mutation in *fs(1)M11* is closely linked or identical with a mutation producing small body size. Four other cases of an association of the female-sterile mutation and a morphological or behavioral effect have been separated by crossing over.

Viability effects were looked for in segregating progenies by scoring the relative numbers of the two classes of females. In all cases the sample sizes were large enough ($N = 50$ or more females) to give reasonable resolution of differences at the 5% level of significance. The mutant females in only 27 cistrons

manifest marked reductions in viability. Though we cannot assign in a rigorous way the various effects to a single gene difference, we can presume that the strains that do not have effects on morphology or viability and that are not "rescued" have mutations that are expressed only in oogenesis. Such strains characterize 23 cistrons.

DISCUSSION

A general goal of this line of research is to identify the gene products and genetic controls that contribute to normal development of the egg. The genetic catalogue of cistrons provides the initial identity in terms of genes that presumably code for specific macromolecules or function as controlling elements. The information of use here to catalogue distinguishable cistrons is found only in the results of the many complementation tests. In most cases the results are quite unambiguous and lead to simple conclusions. Some results (Figures 2, 3, and 4) are more complicated and lead to conclusions only if additional assumptions are made. If the assumptions are held to a minimum, the complex patterns, with the exception shown in Figure 3, are each interpreted to identify a single cistron. We cannot contend, however, that the unit cistrons at this level correspond to single gene products in every case. For the fifteen rudimentary mutations, a single cistron at the ultimate level of resolution is unlikely. Different mutations at this locus are known to affect the activity of one or another of the first three enzymes in the pyrimidine synthetic pathway (JARRY and FALK 1974; RAWLS and FRISTROM 1975). Furthermore, the mutations affecting the same enzyme are contiguous in CARLSON's (1971) fine structure map. Thus the "rudimentary gene" must include at least three cistrons at the level of enzyme activity. Yet in the absence of the molecular information, a single cistron is a fair conclusion, because the linearly overlapping array of several complementation groups (CARLSON 1971) gives no clues to the three enzymatic units of function. Similarly, each cistron in the present list is a fair conclusion, appropriate to the information about it. We have yet to learn how these units of function correspond to gene products.

The strength of the catalogue is dependent upon how nearly complete it is. Though the present list is limited to sex-linked genes, it provides an opportunity to consider criteria for completeness. A random localization of the genes throughout the genome is a reasonable expectation. A nonrandom pattern could reflect differential localization of female-sterile genes or it could reflect regional bias in the rates of induction and recovery of new mutations. In the present case, mutant sites are underrepresented in the *cv*—*v* and *f*—*sfa* regions (Table 4). Together there are 15 cistrons (averaging 1.6 ± 0.3 mutations) identified in these regions, compared with 44 (averaging 2.8 ± 0.4 mutations) in the remaining 53% of the chromosome. The differential rates of mutation suggest a bias favoring recovery of mutation in the *y*—*cv* and *v*—*f* regions, thus a greater opportunity for detecting the several cistrons.

Arguing from the distribution of female-sterile mutations, KING and MOHLER (1975) calculated that there should be an additional 27 genes with reasonable mutation rates to maternal-effect lethal alleles. They did not, however, take into

TABLE 4

Frequency distributions of female-sterile mutations among cistrons

Number of mutations	Number of cistrons: *y—cv; v—f*	Number of cistrons: *cv—v; f—sfa*
1	19	11
2	10	2
3	5	0
4	2	1
5	3	1
6	1	0
7	1	0
8	1	0
9	1	0
15	1	0

account the apparent bias in the regional locations of the mutations, and their estimate is probably low. Table 4 gives the frequency distribution for cistrons in different parts of the *X* chromosome. If there is bias as suspected, the data for the *y—cv* and *v—f* regions together should be representative of half of the *X* chromosome, or about 1/10 of the whole genome. The first four terms in the distribution are compatible with the Poisson distribution with a mean = 1.0, calculated as follows:

$$\frac{\text{number of cistrons with two mutations}}{\text{number of cistrons with one mutation}} = \frac{Nm^2e^{-m}}{2Nme^{-m}} = \frac{m}{2} \text{ and}$$

$$\frac{\text{number of cistrons with one mutation}}{m} = Ne^{-m} = X ,$$

where N is the total number of relevant genes and m is the average number of mutations in each gene. It follows that X is the number of genes that are not mutated by chance. From the distribution in Table 4, $m = 20/19 \simeq 1.0$ and $X \simeq 19$. Thus, about 63 genes (19 + 44) on that half of the *X* chromosome can be readily mutated to maternal-effect lethal alleles. Similar calculations for the *cv—v* and *v—sfa* regions together result in a total of 45 genes. Being based on a larger sample, the larger number is presumably a better estimate of the numbers of maternal-effect lethals to expect in one-half of the *X* chromosome.

If the rest of the *X* chromosome has a similar density of these genes, though underrepresented in my collection, my catalogue of the sex-linked genes is 3/8–1/2 complete and at least one half of the cistrons in an independently produced, random collection should be nonallelic with those listed in Table 3. In collaboration with C. AUDIT and M. GANS, I am testing the allelism of the two sets of cistrons. Up to date 15 of their cistrons have been completely tested; eight are not allelic with those reported here. Similarly, 7/12 female-sterile genes listed in LINDSLEY and GRELL (1968) are not allelic with those here.

The only part of the *X* chromosome that has been exhaustively examined for maternal effect mutations is the *zeste* (*z*, *1*–0.8) to white region, which repre-

sents approximately 1.5% of the *X* chromosome and has two cistrons that mutate to maternal-effect lethal alleles (JUDD and YOUNG 1974). The average number in such short regions can be calculated from the predicted total and is nearly two.

In using the first two terms of the distribution to estimate the size of the null class, I have assumed that the larger part of the genes are identified by mutation with a probability that is much less than that of the nine genes distributed over the high end. There is no way now to evaluate the possibility that many other genes mutate to maternal-effect lethal alleles at frequencies even lower by an order of magnitude or more. The genes that are counted here are only those that should be mutated at a reasonably high frequency in studies of this kind.

Another general goal of this research is to provide material to use in causal analysis of the mechanisms and controls of different aspects of oogenesis and embryogenesis. Practically all of the present understanding now comes from morphological and physiological analysis. The mutant strains can add new dimensions. Among the present collection of mutations there are expected to be some of interest for studies of egg envelope formation (MARGARITIS 1974; MARGARITIS, PETRI and KAFATOS 1976; TURNER and MAHOWALD 1976), others for studies of the genesis of embryonic determination in the egg cytoplasm and its epigenetic consequences for the embryo (RICE and GAREN 1975; ZALOKAR, AUDIT and ERK 1975), and perhaps some for studies of macromolecular functioning in early embryology (DAVIDSON 1968; GOLDSTEIN and SNYDER 1973; GREEN *et al.* 1975).

Several people have contributed in important ways to the work or the preparation of the manuscript. JOLENE DODDS helped with the mutagenesis and screening of new female-sterile mutations; BOBBY MOHLER worked on all phases of the project; ELLYN MURPHY produced the data on the phenotypes of the mutant strains at 18°; MME. C. AUDIT and MME. M. GANS generously shared their mutations and ideas; DR. R. MILKMAN, DR. R. SEECOF and DR. A. SHEARN shared their ideas, reviewing an earlier manuscript. All of this help is gratefully appreciated.

Special thanks is extended to DR. W. B. HEED who made room for me in his laboratory while I was on research leave.

LITERATURE CITED

ALDERSON, T., 1965 Chemically induced delayed germinal mutation in Drosophila. Nature **207:** 164–167.

BAKER, B. S., 1973 The maternal and zygotic control of development by cinnamon, a new mutant of *Drosophila melanogaster*. Devel. Biol. **33:** 429–440.

BAKKEN, A. H., 1973 A cytological and genetic study of oogenesis in *Drosophila melanogaster*. Devel. Biol. **33:** 100–122.

BENZER, S., 1957 The elementary units of heredity. pp. 79–93. In *A Symposium on the Chemical Basis of Heredity*. Edited by W. D. MCELROY and B. GLASS. Johns Hopkins Press, Baltimore.

CARLSON, P. S., 1971 A genetic analysis of the rudimentary locus of *Drosophila melanogaster*. Genet. Res. **17:** 53–81.

DAVIDSON, E. H., 1968 *Gene activity in early development*. Academic Press, New York.

FRANKEL, A. W. K. and G. E. BROSSEAU, JR., 1968 A Drosophila medium that does not require dried yeast. Drosophila Inform. Serv. **43:** 184.

GANS, M., C. AUDIT and M. MASSON, 1975 Isolation and characterization of sex-linked female sterile mutants in *Drosophila melanogaster*. Genetics **81**: 683–704.

GOLDSTEIN, E. S. and L. A. SNYDER, 1973 Protein synthesis and distribution of ribosomal elements in ovarian oocytes and early developmental stages of *Drosophila melanogaster*. Exp. Cell Res. **81**: 47–56.

GREEN, L. H., J. W. BRANDIS, F. R. TURNER, and R. A. RAFF, 1975 Cytoplasmic microtubule proteins of eggs and embryos of *Drosophila melanogaster*. Biochem. **14**: 4487–4491.

GREEN, M. M., 1963 Interallelic complementation and recombination at the rudimentary wing locus in *Drosophila melanogaster*. Genetics **34**: 242–253.

JARRY, B. and D. FALK, 1974 Functional diversity within the rudimentary locus of *Drosophila melanogaster*. Molec. Gen. Genet. **135**: 113–122.

JUDD, B. H. and M. W. YOUNG, 1974 An examination of the one cistron: one chromomere concept. Cold Spring Harbor Symp. **38**: 573–579.

KAPLAN, W. D., R. L. SEECOF, W. E. TROUT III and M. E. PASTERNACK, 1970 Production and relative frequency of maternally influenced lethals in *Drosophila melanogaster*. Am. Nat. **104**: 261–271.

KING, R. C., 1970 *Ovarian development in Drosophila melanogaster*. Academic Press, New York.

KING, R. C. and J. D. MOHLER, 1975 The genetic analysis of oogenesis in *Drosophila melanogaster*. In *Handbook of Genetics*, Vol. 3. Edited by R. C. KING. Plenum, New York.

LEWIS, E. B. and F. BACHER, 1968 Method of feeding EMS to Drosophila males. *Drosophila Inform Serv.* **43**: 193.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Pub. No. 627.

MARGARITIS, L. H., 1974 Programmed synthesis of specific proteins in cellular differentiation. A contribution to the study of chorion formation in the follicles of *Drosophila melanogaster*. Ph.D. Thesis, Laboratory of Biology, University of Athens. Athens, Greece.

MARGARITIS, L. H., W. H. PETRI and F. KAFATOS, 1976 Three dimensional structure of endochorion in wildtype *Drosophila melanogaster*. J. Exp. Zool. **198**: 403–408.

MILKMAN, R. D., 1970 The genetic basis of natural variation in *Drosophila melanogaster*. Adv. Genetics **15**: 55–114.

MOHLER, J. D., 1973 Female -sterile mutations in *Drosophila melanogaster*. Genetics **74**: s182.

RAWLS, J. M. and J. W. FRISTROM, 1975 A complex genetic locus that controls the first three steps of pyrimidine biosynthesis in Drosophila. Nature **255**: 738–740.

RICE, T. B., 1973 Isolation and characterization of maternal-effect mutants: an approach to the study of early determination in *Drosophila melanogaster*. Ph.D. Thesis, Department of Biology, Yale University, New Haven, Connecticut.

RICE, T. B. and A. GAREN, 1975 Localized defects of blastoderm formation in maternal effect mutants of Drosophila. Devel. Biol. **43**: 277–286.

ROMANS, P. A., 1973 A locus with a maternal effect on embryonic development in *Drosophila melanogaster*. M.Sc. Thesis, Department of Genetics and Cell Biology, University of Alberta, Edmonton, Canada.

SUZUKI, D. T., 1970 Temperature-sensitive mutations in *Drosophila melanogaster*. Science **170**: 695–706.

TURNER, F. R. and A. P. MAHOWALD, 1976 Scanning electron microscopy of Drosophila embryogenesis. I. The structure of the egg envelopes and the formation of the cellular blastoderm. Devel. Biol. **50**: 95–108.

ZALOKAR, M., C. AUDIT and I. ERK, 1975 Developmental defects of female-sterile mutants of *Drosophila melanogaster*. Devel. Biol. **47**: 419–432.

Corresponding editor: D. T. SUZUKI