ISOLATION AND CHARACTERIZATION OF SEX-LINKED FEMALE-STERILE MUTANTS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The purpose of the experiments described was to identify *X* chromosome genes functioning mainly or exclusively during oogenesis. Two mutagenesis experiments were carried out with ethyl methane sulfonate. Following treatment inducing 60% lethals, 9% of the treated *X* chromosomes carried a female sterility mutation which did not otherwise seriously affect viability. Among —95 isolated mutants, 19 were heat-sensitive and 5 cold-sensitive. The mutants have been classified as follows: I (16 mutants; 12 complementation groups): the females laid few or no eggs; the defect concerned either ovulation or oogenesis. II (37 mutants; 18 complementation groups): the female laid morphologically abnormal eggs, often with increased membrane permeability. III A (13 mutants; at least 8 complementation groups): the homozygous females were sterile if mated to mutant males; their progeny (homo- and hemizygous) died at a late embryonic stage (11 mutants), at the larval stage (1 mutant) or at the pupal stage (1 mutant). However fertility was partly restored by breeding to wild-type males as shown by survival of some heterozygous descendants. III B (29 mutants; 22 complementation groups): the fertility of the females was not restored by breeding to a wild-type male. Most of the eggs of 13 of the mutants died at a late stage of embryogenesis. The eggs of the others ceased development earlier or, perhaps, remained unfertilized. The distribution of the number of mutants per complementation group led to an estimation of a total of about 150 *X*-linked genes involved in female fertility. The females of three mutants, heat-sensitive and totally sterile at 29°, produced at a lower temperature descendants morphologically abnormal or deprived of germ cells. Three other mutants not described in detail showed a reduction in female fertility with many descendants lacking germ cells. A desirable mutant which was not recovered was one with normal fertile females producing descendants which, regardless of their genotype, bore specific morphological abnormalities. The value of the mutants isolated for analysis of the complex processes leading to egg formation and initiation of development is discussed.

IN higher organisms, the cytoplasm of the zygote is almost exclusively a product of the female gamete; its content is prepared under the direction of the genome of the mother. The zygote is thus a very special cell in which the gene products of one generation coexist with the not-yet-functional genome of the next generation. It is here that development begins. The potential usefulness of female-sterile mutants for the analysis of the role played by maternally synthesized products

in both oogenesis and initiation of development has been pointed out by authors (KING 1970; SCHNEIDERMAN and BRYANT 1971; GEHRING 1973).

A large number of female-sterile mutations have been described (LINDSLEY and GRELL 1968). However, the majority of these were not selected specifically for this characteristic and have pleiotropic effects. Procedures designed to permit the recovery of mutations primarily causing female sterility have been recently undertaken by several authors (GILL 1963; BAKKEN 1973; RICE 1973; KING and MOHLER 1975). However, mutations isolated in this manner may affect many different processes, such as differentiation of nurse cells, yolk production, formation of egg membranes and oviposition. Mutations affecting each of these stages are already known (see review by KING 1970). In addition, mutations of genes involved in various "house-keeping" functions necessary for the survival of all cells may only be lethal in egg cells because of the very intense activity occurring during oogenesis (BISCHOFF and LUCCHESI 1971).

A more interesting type of mutation would be that concerning genes specifically involved in the build-up of cytoplasmic components of the egg, which may be responsible for the initiation of cell differentiation (morphogenetic substances). Some observations suggest that the first stages of differentiation take place before the genome of the zygote becomes functional, and are therefore dependent upon factors under maternal control (see review by DAVIDSON 1968). In *Drosophila melanogaster*, U.V. irradiation or removal of posterior pole plasm before the migration of nuclei into this region leads to embryos devoid of germ cells and, consequently, to sterile adults (GEIGY 1931; HATHAWAY and SELMAN 1961). This defect can be corrected by injecting polar plasm from unirradiated eggs into the posterior pole of irradiated eggs (OKADA, KLEINMAN and SCHNEIDERMAN 1974). Furthermore, when posterior polar plasm is transferred to the anterior tip of the embryo, it is able to induce the formation of primordial germ cells in this region (ILLMENSEE and MAHOWALD 1974). However, at this stage no clear indication of a functional activity of the zygotic genome has been obtained (ZALOKAR 1976). In the grandchildless mutant of *Drosophila subobscura*, the progeny of mutant females are devoid of germ cells whatever the genotype of the zygote (SPURWAY 1948; FIELDING 1967). Recently, mutants characterized by the formation of an abnormal blastoderm uniquely under control of the maternal genome have been described in *Drosophila melanogaster* (RICE and GAREN 1975). The former mutation (grandchildless) possibly concerns a gene controlling maternal factors responsible for differentiation of pole cells, and the latter ones concern genes essential for blastoderm cell formation.

The determination of anterior and posterior parts of the embryos is perhaps also maternally controlled. Irradiation of the anterior pole of Chironomous eggs prior to nuclear migration gave a high yield of embryos with double abdomens in mirror-image symmetry (KALTHOFF 1971). Centrifugation of Chironomous eggs led, in addition to bicaudal embryos, to embryos with double heads, also in mirror-image symmetry (YAJIMA 1960). Anterior and posterior fragments of Drosophila blastoderm embryos, cultured separately led to the formation of imaginal structures, derived from anterior and posterior respectively (CHAN and

GEHRING 1971). Eggs laid by females of the mutant bicaudal of *Drosophila melanogaster*, even when the flies were crossed to wild-type males, yielded some embryos with two abdomens (BULL 1966).

Genes controlling discrete factors involved in determination, if they exist, should be obtainable in mutant form. Such mutations would lead not only to the production by mutant females of inviable eggs, but also to abnormal progeny.

The present article describes two mutagenesis experiments aimed at identifying the *X*-chromosome genes involved in female fertility and the correct initiation of development. The main thrust of the paper is to ask how many genes affect oogenesis and maternally controlled aspects of development, and how they may be classified.

MATERIALS AND METHODS

Description of strains used:

ClB/asc, bb^K ♀♀; *asc, bb^K/B^SY* ♂♂: the *asc* chromosome carries a lethal bobbed mutation, bb^K, induced by ethyl methane sulfonate (EMS) (isolated by M. MARRAKECHI in our laboratory). The *Y* chromosome is labelled so that flies resulting from nondisjunction of the sex chromosomes in the mother and father can be detected.

FM3/asc, bb^K ♀♀; *asc, bb^K/B^SY* ♂♂.

C(1)DX,yf ♀♀; *Ore RC/Y* ♂♂: these attached-*X* females possess no nucleolar organizer on the *X*.

C(1)RM,ywf ♀♀; *w sn/Y^{bb-}* ♂♂.

C(1)DX,yf ♀♀ v^{24} ♂♂ (v^{24} EMS-induced vermilion allele, (isolated by D. LOCKER in our laboratory)).

All the strains were tested in M. L'HERITIER's laboratory to ensure that no sterilities connected with recently discovered non-chromosomic factors (PICARD 1971) would be likely to appear during cross breeding.

All *X* chromosomes containing v^{24} were made isogenic before the second mutagenesis experiment, but not before the first.

Breeding medium

Standard medium: 83g corn-flour; 83g dry brewer's yeast; 11g agar; 50 ml of a 10% solution of methyl p. hydroxybenzoate in alcohol; 1000 ml water; 75 ml of this medium were distributed in milk bottles, or 10 ml in test tubes.

Laying medium: 500 cc vinegar; 500 g fresh yeast; 10 g saccharose; 50 g agar; 1,000 ml water—neutral red added to color the medium.

Mutagenesis

The mutagenic agent was ethyl methane sulfonate (EMS). The treatment was applied according to the method of LEWIS and BACHER (1968). Males aged 0 to 2 days were distributed in flasks containing "Kleenex" impregnated with 10 cc of a freshly prepared 0.025 M EMS solution in 1% sucrose, left for 24 hr at 23°. These male (non-etherized) were mated to *C(1)DX,yf* females. The males were discarded after three days in the first experiment. In the second they were left for only one day in the presence of females, so that females were fertilized only by spermatozoids exposed to the mutagenic agent at maturity or at the spermatid stage (CHANDLEY and BATEMAN 1962). After elimination of the males, the females were transferred to new flasks.

The frequency of *X* lethals induced by this treatment can be roughly estimated from the sex ratio (male to female) of the progeny of mutagenized males over that of untreated flies. This frequency was approximately 0.6.

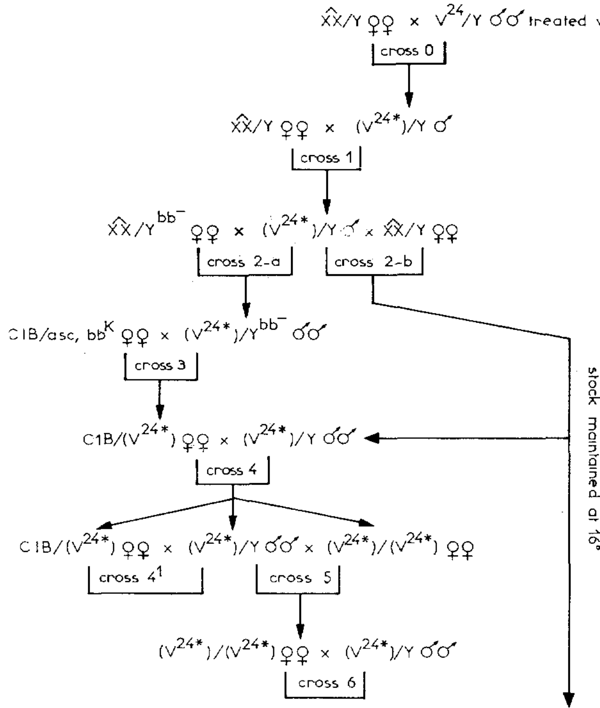


FIGURE 1.—Sequence of crosses used to screen for female-sterile mutants. Cross 1, 2a and 2b were performed with single males; cross 0, 1 and 2a were carried out at 23° ± 1° in the first experiment, at 28.5° ± 1° in the second one; cross 3, 4, 4^1, 5 and 6 were carried out at 16° ± 1° and 28.5° ± 1° in the two experiments. Chromosomes treated by mutagen are described as (v^{24*}) and attached-*X* chromosomes as $\widehat{XX}$.

Isolation of mutants

The procedure used for the two mutagenesis experiments is shown in Figure 1. Cross 1 involved many matings, each with individual males. Since the germ-line of the males in cross 1 could be mosaic, a second mating with single males (cross 2) was carried out. These two successive crosses also allowed isogenization which eliminated a fraction of the autosomes subjected to the mutagenic treatment.

In cross 2a the male already used in cross 2b was mated to attached-*X* females containing a *Y* chromosome bearing the bb^- mutation. The purpose of this cross was to obtain males in which the *X* chromosome exposed to the mutagenesis agent, symbolized by (v^{24*}), was associated with Y^{bb-}. As a result of cross 2a, all of the viable progeny from cross 3 were females. In this way, time-consuming virgin collection prior to cross 4 was avoided.

Crosses 3, 4, 5 and 6 were carried out at two temperatures: 28.5 ± 1° and 16° ± 1°.

Cross 4 permitted the detection of dominant female sterility. However sterility of cross 4 could also be due to temperature-sensitive male sterility: cold- or heat-sensitive male sterility in the first experiment, since crosses 0, 1 and 2 were carried out at 23°; cold-sensitive male sterility only, in the second experiment, since crosses 0, 1 and 2 were carried out at 29°. This ambiguity was removed by mating $ClB/v^{24*})$ females to wild-type males and $(v^{24*})/Y$ males to wild-type females.

Sterility of cross 5 could arise from recessive female sterility, or from a dominant mutation governing defects restricted to polar cells and causing a sterility of the F_1 progeny. To distinguish

between these two possibilities crosses 4^1 and 5 were carried out simultaneously. In the case of recessive female sterility, only cross 5 would be sterile, whereas a dominant mutation causing pole cell defects would result in sterility of both crosses. When cross 5 alone was sterile, cross 4^1 provided an estimation of the viability of mutant flies, and a sterility check.

In order to detect partial sterility of mutant females, experimental conditions of cross 5 were standardized as much as possible. Two females aged 0 to 4 days were mated with 5 of their male sibs and allowed to lay eggs for 4 to 5 days at 29°, or 10 to 14 days at 16° in vials. Under these conditions, the number of offspring from control females of the non-mutagenized v^{24} strain was the same at both temperatures, and did not exceed the nutrient capacities of the tubes. Progeny amounting to less than a quarter of the usual quantity were considered an indication of partial female sterility. Partially sterile mutants could represent "leaky" biochemical mutants, detectable only by a drop in fertility due to a reduction of the high metabolic activity typical of oogenesis (BISCHOFF and LUCCHESI 1971).

When cross 5 was fertile, the external morphology of vermilion flies from crosses 4^1 and 5 were examined for the presence of abnormalities (recessive maternally influenced abnormalities).

Finally, cross 6 allowed the detection of recessive mutations governing a delayed sterility (maternally influenced sterility).

The possible types of mutants which could be isolated from this experimental procedure are summarized in Figure 2.

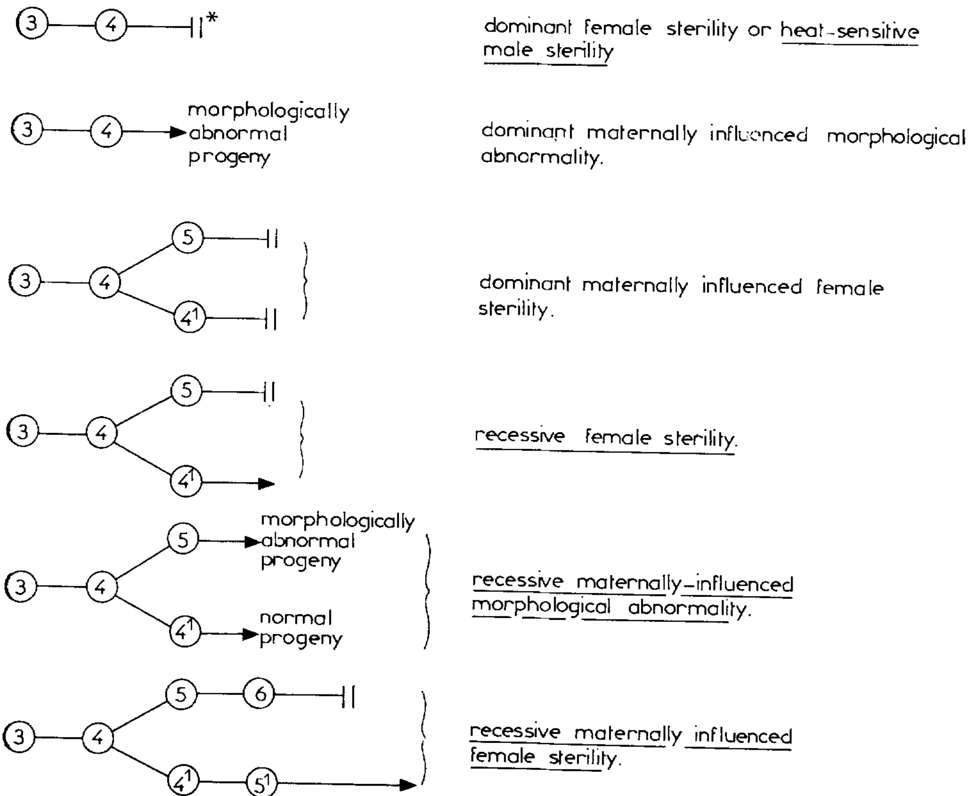


FIGURE 2.—All the kinds of mutants expected from the experimental procedure employed were taken into account. The classes actually recovered were underlined.

Cross-checking and study of mutants

When abnormalities were observed, usually in the form of few or no descendants from mating 5, the study was pursued as follows:

1) Vermilion flies issued from cross 4 were examined and their viability evaluated by the ratio:

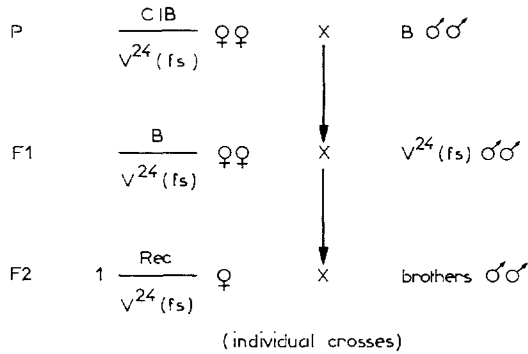
$$\frac{\text{No. of } ♀♀\ v + \text{No. of } ♂♂\ v}{2 \times \text{No. of } ♀♀\ B/+}.$$

In control matings where the v-labelled chromosome is that of the non-mutagenized v^{24} strain, this ratio was close to 1 at both temperatures. The count was made twice, at the beginning and end of the imago emergence, in order to detect an increase in the length of development of homozygotes or hemizygotes (v^{24*}). It should be noted that the count was always made when the flies were relatively young. A reduction in the life expectancy of the imagos therefore would not have been detected.

2) Fertility (% survival of progeny to adult stage) was checked on a larger number of females by the repetition of cross 5: generally 2 tubes with 2 ♀, 2 tubes with 10 ♀♀.

3) The fecundity (number of eggs laid in 24 hrs) was roughly estimated and the aspect of the eggs noted. Browning of the eggs showed that embryo development had begun and had reached at least the gastrulation stage (Mitchell 1966).

MATING SCHEME 1



MATING SCHEME 2

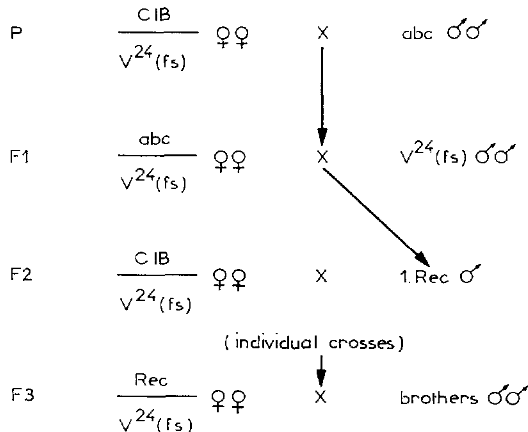


Figure 3.—Mating schemes used to map female-sterile mutants ("fs"). Possible recombinants were symbolized as "Rec".

4) Virgin homozygous females were bred to males not bearing the mutant *X* chromosome (males of the original v^{24} strain, the wild-type strain or $bb^{K}/B^{S}Y$) to see whether the sterility could be corrected by introducing a wild-type genome at the moment of fertilization.

5) When the results obtained at 16° and 29° were different (heat- or cold-sensitive mutants), the time of the sensitive period could be roughly determinel by transferring females from one temperature to the other and studying successive batches of eggs.

The chromosomes considered to be carrying interesting mutations were kept in two forms. Males were bred to attached-*X* females or females were balanced by *FM3*, which provided a better crossing-over inhibitor than *ClB* originally used.

Classification in allelic series and localization

Only mutants characterized by a lack of descendants in crosses 5 (recessive and immediate sterility) are considered here. Complementation tests were carried out among mutants from the first mutagenesis is experiment by the reciprocal matings ♀ $ClB/(v^{24*})^{x}$ to o $(v^{24*})^{y}/Y$ (x and y indicate different mutants) and by studying the fertility of four vermilion females born of each cross. *FM3* was used instead of *ClB* in later experiments.

Mapping was accomplished by two methods, shown in Figure 3. The first used the marker v^{24}, which was present at the outset in the mutagenized strains, and *B*; the second method required an extra generation by means of which the presence or absence of the sterility gene on each recombined chromosome was determined by the study of more than one female. Several multi-mutant strains were used in the second method (*y ct f*, *w cv B*, *y cv B car* or *sc ec* ct^{6} g^{2} *f*). The method recommended by Rice (1973) for the fast localization of a sterility gene in a short chromosome segment was also used.

RESULTS

The presence of larvae in the breeding medium was taken as the working criterion of fertility. No dominant female-sterile mutants, identifiable by the sterility of cross 4 in the experimental procedure, were found. Most of the mutants represented recessive female steriles (cross 5 sterile). The symptoms ranged from defective egg production, morphologically abnormal eggs, to arrested development during embryogenesis.

In cases where cross 5 was fertile (presence of larvae) the study of larval development and of the morphology and fertility of the imagos revealed very few mutants. Only two cases of mortality at the larval or pupal stages were found, and these were corrected by the presence of a wild-type allele in the zygote. Finally three mutants were detected from the sterility of cross 6. They resembled the grandchildless mutant of *Drosophila subobscura* (Spurway 1948) by the presence of specimens devoid of germ cells among the offspring of homozygous females and by the fact that this defect depended only upon the maternal genotype. However, the fertility of homozygous females born of heterozygous mothers was subnormal. The penetrance of the absence of germ cells was not total and in one case, at least, the feature was governed by two sex-linked genes. The properties of these mutants will be dealt with in another article (D. Thierry-Mieg, manuscript in preparation).

1) *Frequency of EMS-induced* X *chromosome mutations leading to female sterility*

The results obtained in the two mutagenesis experiments are summarized in Table 1. A strain was classified as "female-sterile" when each female gave on average less than 1 descendant in 4 days' breeding at 29° or 10 days at 16°. The

TABLE 1

Relative frequencies of several types of sex-linked recessive mutations induced by EMS treatment of adult males

	Mutagenesis 1		Mutagenesis 2	
	N	%	*N*	%
Total number of tested males	732	..	781	..
Sterile males	157	21	173	22
Fertile males	575		608	
Males without male progeny (mosaic lethals)	20	3,5	23	3,8
Heat-sensitive lethals	7	1,3	—	
Heat-sensitive sterile males	11	2	—	
Stocks lost in the course of the study	22	4	36	6
X chromosomes examined	515		549	
Non-mutants (examined at 16° and 29°)	391		418	
Non-mutants? (examined only at 16° or 29°)	28		25	
Partial lethals	19		18	
Female lethals	4		1	
Partial female steriles	27		39	
Female steriles	45	8,7	50	9,1

only mutants retained were those with a viability greater than ⅔ that of the reference strain under optimum breeding conditions. Those labeled with numbers below 1000 were isolated during the first experiment, those above 1000 during the second.

In order to estimate the frequency of EMS-induced mutations causing female sterility, it is important to know whether all the mutants isolated represent independent mutations. In this respect the two experiments were different. In the second experiment the *X* chromosome was isogenized immediately before mutagenesis. The presence of a mutation causing sterility before treatment was therefore unlikely and the isolated mutants were probably of independent origin, assuming that no spontaneous mutation had taken place between the moment of isogenization and mutagenesis (two generations). In the first experiment, on the other hand, the *X* chromosome was not isogenized beforehand. Although the two experiments gave very similar results, it was necessary to examine the complementation groups of the first one to check that no numerically large series, which would point to the pre-existence of sterility mutations in the strain, were present. Functional complementation tests were therefore carried out among 42 mutants. Thirty-one one-mutant groups, two two-mutant groups, one three-mutant group and one four-mutant group were identified. It thus seems safe to assume that no sterility mutations were present in the strain before the first mutagenesis experiment.

A dose of EMS which induced a lethal mutation on the *X* chromosome in 60% of the cases induced a female-sterile mutation in 9% of the cases, in these experiments.

2) *Properties of the mutants*

The isolated mutants had very heterogeneous properties. They were classified according to the number and morphology of the eggs into: I, mutants with no or low fecundity (few or no eggs laid); II, mutants giving morphologically abnormal eggs; and III, mutants giving numerous and apparently normal eggs.

The sterility of the mutant females in the first two categories was found, as expected, to be independent of the genotype of the male to which they were bred. In category three, the genotype of the male partner may or may not affect the sterility.

Among 42 mutants of various types obtained during the first mutagenesis experiment, those belonging to the same complementation group proved to have the same or similar properties. Complementation tests were therefore only performed for all the mutants within each of the three categories.

Category I: mutants presenting little or no fecundity

Since the fecundity (number of eggs laid) varies with many factors (age of the females, breeding temperature, medium, lighting, etc. . . .) which would be difficult to check for a large number of strains, an accurate quantitative overall evaluation was not possible. This category covers mutants showing a very serious reduction in fecundity, detectable without an exact egg count (approximate limit: 1 or 2 eggs per female per 24 hr at 29° during the active laying period, compared with 20 eggs laid by the reference females v^{24}). Table 2 summarizes the characteristics of 16 mutants in this category. Complementation tests revealed 12 groups (nine with one mutant, two with two mutants, one with three mutants).

The viability of the homozygotes was often subnormal and development was frequently retarded. In several cases, sterility was accompanied by morphological abnormalities. With mutants 1057 and 1569, which are respectively alleles of singed and lozenge, it is highly probable that both the morphological abnormalities and the sterility resulted from a single mutation, since most mutations at these two loci have this pleiotropic effect. Mapping of 231 demonstrated that a single pleiotropic mutation is also involved here. However, some doubt still remains in the case of mutant 543. It appears that most of the mutations in category I involved genes with an important function during oogenesis or ovulation, but with other pleiotropic effects.

A morphological study of the ovaries, generally combined with a cytological study, revealed the origin of the sterility in certain cases (Table 2).

When sterility was due to egg retention, old females had enormously distended abdomens. The ovarioles contained a series of mature eggs. Oogenesis seemed to take place normally, although when a string of mature eggs was formed, the immature stages were fewer than in normal ovaries. Several mutants were heat-sensitive (1145, 462, 543, 1001, 1569). Fertility at the permissive temperature was not always completely normal, but the eggs which were produced did develop. The temperature effect was irreversible in both cases studied so far (543 and 1569). These mutants probably correspond to abnormalities of the

TABLE 2

Characteristics of the mutants of class I

Strain number	Viability—29°	Delay in development—29°	Morphology	Fecundity	Abnormality	Other characteristics
116	0,9	+ (⩽ 1 day)	normal	0	ovo.*	
[231]†	0,5	+ (4 days)	short and thin bristles	0	ovo.	normal viability and morphology, but no eggs laid at 16°
[265‡]	0,9	+ (1 day)	short and thin bristles	0	ovo.	
[248]	0,7	+ (1 day)	normal	ε§	ret.¶	
[1145TS]	0,8	—	normal	ε	ret.	partial fertility at 16°
427	0,8	+ (1 day)	normal	ε	ret.	
462TS	0,6	+	normal	0	ret.	partial fertility at 16°
543TS	1	—	short wings	0	ret.	fertile at 16° and 23°
1001TS	0,7		normal	0	ret.	partial fertility at 16°
1057	0,7		curled bristles	ε	ovo.	allele of singed
[1245	0,7	+ (2 days)	normal	0	ovo.	{ovogenesis apparently normal until stage 11–12 of KING; no formation of dorsal appendages
1246	0,7	+ (1 day)	normal	0	ovo.	
1248]	0,4	+ (3 days)	normal	0	ovo.	stage 9 is the last normal phase
1304	1	—	normal	0	ovo.	Most of the egg chambers degenerated after KING's stage 6
1569TS	1	—	ommatidia irregularly distributed and pigmented	0	ret.	no spermathecae at 29°; allele of lozenge, fertile at 16°
1621	1	—	normal	0	ovo.	most of follicles contained many undifferentiated cells; resembles the *fs (2) B* mutant (KING, SANG and LETH 1961)

* Visible abnormalities of oogenesis.
† Stocks in brackets are non-complementing.
‡ Stock lost in the course of the experiment.
§ Very low fecundity ⩽ 2 eggs per female per day.
¶ Egg retention.

genital tract responsible for ovulation. In no case was any absence of communication observed between the ovaries and the vagina.

When sterility was caused by abnormal oogenesis, the anomalies observed were of many kinds, ranging from non-differentiation of nurse cells to lack of formation of dorsal appendages.

The non-complementing mutants 1245, 1246 and 1248, obtained in the same series of crosses, may have arisen from a pre-existing mutation. In strain 1248, the anomalies of oogenesis appeared earlier than in the two others. This difference might be due to the existence of a second mutation on the 1248 chromosome.

Category II: Fecund mutants with morphologically abnormal eggs (Table 3)

Functional complementation tests were carried out on 35 mutants of this category. Two mutants, lost during the study, were not tested. Eighteen complementation groups were identified (thirteen with one mutant, 1 with two mutants, one each with three, four, six and seven mutants). The size of these last two

TABLE 3

Characteristics of the mutants of class II

No. of mutant strains grouped grouped by complementation units	Special properties*
59	low fecundity
117	low fecundity; development initiated and blocked at various stages
120, 343, 489, 571, 1190, 1198	development initiated and blocked at various stages; fertility dependent on the culture medium
125^{TS}	low fecundity; dorsal appendage joined together; partially fertile at 16° and 23°
147, 1369	no initiation of development
148, 305, 332, 1061, 1081^{TS}, 1130, 1186	development initiated and blocked at various stages; 1081 partially fertile at 16°
180^{TS}	fertile at 16° and 23°
267†, 384, 1336, 1501	low fecundity
273	
321	short bristles
336	
371, 379, 1038	development initiated
456	
473^{TS}	eggs partially devoid of chorion; fertile at 16° and 23°
508	the size of the eggs was variable, often smaller than normal; twisted bristles; important delay in development
1059	
1268	
1561	low fecundity

* Except when otherwise indicated, the viability, fecundity, morphology and length of development were normal. The eggs were flabby, and removal of the chorion was impossible. Two mutants, 1518 and 1555, lost in the course of this study, are not mentioned in the table.

groups might suggest the pre-existence of sterility mutations in the strain. This is not likely since several mutants of both groups were isolated in each of the two mutagenesis experiments; in the second series the mutagenized *X*-chromosomes were first shown to be free of sterility mutations. It is more likely that the frequency of detectable EMS-induced mutations was high at these two loci.

In most cases the eggs lacked the usual turgidity (flaccid eggs). This abnormality was observed either at the time of egg laying or appeared after several hours, and depended occasionally on the medium on which the eggs were laid: in the case of series 120 the abnormalities were less serious when the eggs were laid on the special vinegar medium than when the standard medium was used; a large proportion reached the imago stage in the former case but very few in the latter. Development was blocked at widely varying times (embryonic, larval or pupal stages).

In certain mutants (180, for example) the eggs very quickly absorbed neutral red from the medium—unlike normal eggs. This suggests a change in their permeability. In other cases it was the formation of the chorion (mutant 473) or the dorsal appendages (mutant 125) which was affected. Finally, the abnormality sometimes affected the size of the eggs (mutants 472 and 508).

Three mutants (180, 473 and 1081) belonging to different allelic series were heat-sensitive, the temperature effect being reversible. The sensitive period of mutant 180, determined by single temperature shifts, covered the last third of oogenesis and ended at the moment of oviposition.

The viability and development time of the mutants in this category were normal, as was the morphology of the imagos except in the case of mutants 321 and 508. It is not yet known whether the morphological abnormalities and the sterility of these last two mutants are governed by the same gene.

In some cases, fecundity was low and therefore the classification of these mutants into categories I or II was largely arbitrary. Nevertheless it is probable that in this category most of the mutations concern genes involved specifically in vitellogenesis or formation of the egg membranes.

Category III: Fecund mutants with morphologically normal eggs

Mutants were called fecund when a hundred eggs were observed in vials where two females had been left for 4 days at 29° or 10 days at 16°. This does not necessarily imply a fecundity equal to that of the reference strain v^{24}.

The mutants were divided into two groups according to the effect of the male partners on the lethality. The first group (category IIIA) covers cases where mutant females were fertile when bred to wild-type males but gave only female descendants; lethality thus depends on the genotype of both the mother and the zygote. The second group (category IIIB) includes mutants where the genotype of the father does not affect the fertility of the females; the lethality of the zygote hence depends exclusively on the maternal genotype.

Mutants IIIA:

The effect of introducing a normal allele at fertilization is probably very variable according to the mutant; in fact, crosses between mutant females and

wild-type males frequently yielded only a small number of daughters. This fact has already been pointed out and studied more precisely in other cases (KAPLAN *et al.* 1970; SHANNON 1972).

Thirteen mutants of this type were isolated. Complementation tests have been carried out only among those with similar properties. These properties, fairly diverse, are summarized in Table 4.

In the two heat-sensitive strains 475 and 1074, mutant females when mated to wild-type males were completely sterile at 29°, but at 23° some daughters were produced. The heterozygous progeny only survived when conditions allowed the embryos to develop far enough for the normal allele to function.

The heat-sensitive mutant 1456 had complex properties. At 16° it was indistinguishable from the original strain. At 29° it behaved as a partial lethal: 1456/1456 or 1456/*Y* zygotes born of 1456/*FM3* mothers generally died at the pupal stage; when imagos did emerge their morphology was normal. Homozygous females obtained in this way, or taken from the stock kept at 16° and transferred to 29° so that oogenesis took place at this temperature, were sterile. They laid eggs which stopped developing during embryogenesis. This sterility depended only on the genotype of the mother and the temperature at which oogenesis took place: when oogenesis occurred at 29°, the eggs of 1456/1456 females failed to hatch, even if they were mated to wild-type males.

At 23° mutant 1456 flies born of heterozygous mothers had a normal viability, morphology and fertility but the progeny of the mutant females was abnormal. In addition to a shortage of females there were morphological abnormalities, the most typical of which was the absence of halteres and legs. These abnormali-

TABLE 4

Characteristics of the mutants of class III A

Mutant strain	Special properties
107, 387, 436, 476, 1559	All these mutants complement each other. Death occurred at an advanced stage of embryogenesis; in mutant 1559 some larvae hatch.
475	Heat-sensitive: mutant females laid eggs at 23°, but not at 29°.
1074	Heat-sensitive: at 29° all the eggs were white; at 23° development stopped later during embryogenesis and somtimes during larval or pupal stage. Rescue of daughter progeny by crossing female mutant with wild-type male only at 23°.
1456	Heat-sensitive: complex properties of 1456 detailed in text.
99	Cold-sensitive. Death occurred during larval stage. Normal at 29°.
1024	Death usually took place at the pupal stage. The yield of imago was only 30%. The flies had abnormalities (wings unfolded) and died in few days.
[1071, 1361, 1630]	Alleles of *r* (rudimentary); 1630 was cold-sensitive.

ties occurred with a frequency o fabout 25% in females and less in males, and their distribution seemed to obey strict rules. Of a sample of 117 abnormal females, 66% lacked a single haltere, 22% a single haltere and the leg on the same side, 11% both halteres and sometimes one or two legs. The absence of legs was highly correlated with the absence of halteres. This distribution suggests certain specificity in the primary lesion, probably located at the blastoderm; the main target would be the region where presumptive haltere cells are formed.

The abnormalities of mutant 1456 at 23° were very similar to those of the *mel-3* mutant described by RICE (1973). Unlike this latter mutant, the morphological defects of 1456 did not depend exclusively on the genotype of the mother. Their frequency was lower in 1456/+ than in 1456/1456 zygotes born of mutant females raised at 23°: 13% and 27% in one experiment, 11% and 25% in another, although the distribution was the same in either cross. Because of this difference 1456 was classified in category III A.

Mutant 1456 had two temperature-sensitive periods. The first period began during oogenesis and lasted until a few hours after oviposition. At 29°, it was responsible for the embryonic mortality; at 23° it induced morphological abnormalities of the imago. The second period occurred at the pupal stage and caused pupal mortality.

It is possible that mutant 1456 carries two heat-sensitive mutations, one causing zygotic pupal lethality (second sensitive period), the other an embryonic lethality dependent on the maternal genotype (first period); but localization experiments have not confirmed this hypothesis. If two genes are involved, they both lie at about 17 crossing over units from the free end of the *X*, and it has not been possible so far to separate them.

We should mention that a mutant, *l (1)ER*^{TS}, with properties *fairly similar* to those of 1456 (zygotic pupal lethality and maternally-influenced embryonic lethality; two sensitive periods) and located in the same region of the *X* chromosome (18 c.o.u. on the genetic map) has been described recently (FULLILOVE and WOODRUFF 1974). However, the second phase of the sensitive period is longer and the authors did not mention any morphological abnormality of the imagos at intermediate temperatures. It would be interesting to study the functional complementation between these two mutants. If they prove to be allelic for all the mutant properties, this would argue for the existence of a single gene with a pleiotropic effect.

Mutant III B:

Lethality depended exclusively on the maternal genotype. Among 28 mutants of this group (one cold-sensitive mutant, 1371, has not yet been studied) 22 complementation groups were found (sixteen with only one mutant, five containing two mutants, one containing three mutants). Mutant 1509 complemented two mutants of the 457 series (457 and 1203) but not the third (1168), the simplest hypothesis being that 1168 is a double mutant. The mutations 1509 and 457 were both located between garnet and forked with 1509 at 3,7 crossing over units from garnet; 457 was located between 1509 and forked at 1,5 crossing over

units from 1509. In fact it has been possible to isolate the two mutations presumed to be present on the mutant 1168 chromosome.

The properties of category III B mutants are given in Table 5. The exact characteristics of the abnormalities detected by a cytological study of the eggs and of embryonic development will be described in another article (ZALOKAR and AUDIT 1975).

TABLE 5

Characteristics of the mutants of class III B

	No.	Viability (29°)	Delay in development (29°)	Fecundity† (29°)	Fertility* 16°	Fertility* 29°
white eggs:	73	1	—	+++	0	0
	1526	1	—	+++	0	0
	1528	1	+ (3 days)	++	0	0
	97	1	—	+++	+++	ε
	[214 ‡	1	—	+	0	0
	1031]	1	—	++	+++	0
	1042	1	—	++	ε	0
	1242	0,8	+ (3 days)	+++	0	0
	1578	1	—	++	ε	+++
white and brown eggs:	330	0,8	+ (30 h)	++	ε	ε
	331	AM§ 1	+ (24 h)	++	0	0
	[383	AM§ 0,7	+ (3 days)	++	+	ε
	1162]	1	—	++	+	ε
	426	1	+ (12 h)	++	+	ε
	[457	1	+ (12 h)	++	0	0
	1168	1	—	+++	0	0
	1203]	1	—	++	ε	ε
	1140	1	—	++	0	+
	1371	0,9	—	++	ε	++
	1459	0,9	—	+++	+++	0
mostly brown eggs:	573	1	+ (24 h)	++	0	0
	1182	AM§ 0,9	—	++	++	ε
	1509	1	—	+++	0	0
	572	1	—	+++	0	0
	1187	1	—	++	+++	ε
	[1497	1	—	+++	0	0
	1502]	1	—	+++	0	ε
	[1103	1	—	+	+++	0
	1122]	1	—	+	+++	0

* Fertility (estimated on the basis of number of progeny per female per four days' egg laying at 29°, or ten days at 16°):
ε : < 1
+ : between 1 and 10
++ : between 10 and 30
+++ : > 30

† Fecundity (estimated as the number of eggs laid per female per day at 29°);
+ : between 2 and 5
++ : between 5 and 15
+++ : > 15

‡ The mutants in brackets belong to the same complementation group. For 1509, see text.

§ Morphological abnormalities of the imagos.

The mutant which developed further was 1502: some of the eggs hatched but the larvae displayed abnormalities of the mouth apparatus and died very quickly. In the case of four temperature-sensitive mutants examined (97, 1187, 1459 and 1578), the temperature effect was reversible; the sensitive period occurred during oogenesis (97, 1187, 1578), and during the first hours of embryogenesis (1459).

It is important to know whether the properties of the mutants in this category are consistent with a functional defect limited to oogenesis. Viability was generally normal, but some mutants showed a retarded development, and in three cases (330, 383, and 1182) the imagos from heterozygous mothers presented morphological abnormalities. It has been shown that the delays and abnormalities of 383 are governed by two mutations different from that causing sterility. It remains unknown whether the same is true of mutants 330 and 1182.

It would have been possible to place mutants 1103 and 1122 in category I because at 29° the number of eggs laid by homozygous females was low. At 16° their fertility was normal; the imagos presented a normal aspect except that a certain fraction (20%) had no germ cells. At 23°, homozygous daughters of heterozygous mothers were fertile but all their descendants lacked germ cells. Moreover they often showed morphological abnormalities such as absence or displacement of the abdominal tergites, malformation of the wings, reduction in eye size. The absence of germ cells and the tergite abnormalities did not depend on the genotype of the zygote. The localization experiments showed that both of these properties of the mutants were governed by the same gene or closely linked genes. The temperature-sensitive period appeared to be finished at the moment of egg laying (D. THIERRY MIEG, personal communication).

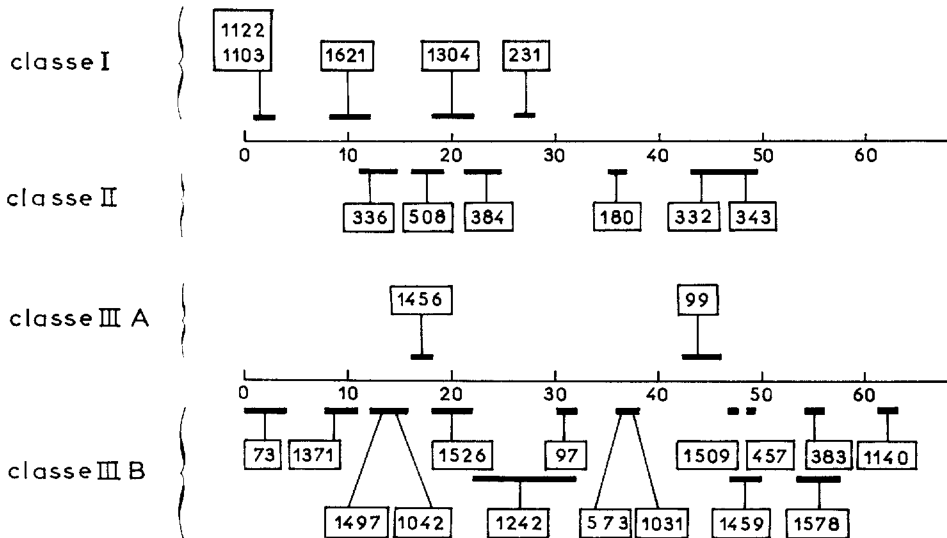


FIGURE 4.—Position of the female sterility mutations on the X chromosome. For the complementation groups represented by more than one mutant, genetic localization was usually performed for a single allele.

Localization of genes

Apart from the localization of female-sterile mutations on the *X* chromosome, it was possible to check whether segregation is monogenic, and to isolate the female-sterile genes of at least a part of the *X* chromosome subjected to mutagenesis. The work is not yet completed. The positions known at present are shown in Figure 4. It seems that mutations causing sterility are not situated in any particular region of the *X* chromosome.

DISCUSSION

Oogenesis is a stage of intense metabolic activity during which a large quantity and variety of substances is prepared for storage. Everything needed for the first stages of development is probably synthesized during this period under the direction of the maternal genome; the zygote genome is thought to begin to play an active part mainly from the blastoderm stage (LOCKSHIN 1966; ZALOKAR, 1976). The very fast nuclear divisions preceding the formation of the blastoderm must therefore depend on DNA precursors and on enzymes provided before fertilization. With regard to ribosomal RNA, the fact that zygotes deprived of nucleolar organizing regions can complete embryonic development (MARRAKECHI, personal communication) shows that the mature egg contains a large quantity of maternal ribosome RNA. It is therefore to be expected that the functional deficit of a large number of housekeeping genes, not specific to oogenesis, can lead to female sterility. In fact, many mutants detected at the outset by different abnormalities of the imagos and nutritional deficiency also exhibit female sterility (LINDSLEY and GRELL 1968).

When attempts are made to detect genes acting specifically during oogenesis, the mutation of genes not specific to oogenesis form a very troublesome background. It was hoped that this problem could be overcome by keeping only those mutants in which females sterility was not accompanied by a very pronounced loss of viability (viability equal to or greater than 2/3 that of the reference strain v^{24}). It was difficult to demand normal viability since the EMS dose used was high, and it is probable that many of the *X* chromosomes treated carry not only female-sterile mutation but also one or more extra mutations leading to a slight loss of viability or a delay in development. These points could be tested by isolation of sterility mutations from the rest of the mutagenized *X* chromosome.

The information gained from these experiments on the number of *X*-chromosome genes subject to mutation causing female sterility and on the nature of the functional deficiencies responsible for sterility will be discussed in turn, in particular with respect to whether any of the mutations isolated have the expected properties for defects in the morphogenetic potentialities of the egg.

Number of X*-chromosome female-sterile genes*

Altogether 95 of the 1064 mutagenized *X* chromosomes studied carried at least one mutation governing female sterility (< 1 descendant per female per four laying days at 29°, or ten laying days at 16°). The term sterility includes the very rare cases where the females have an abnormal offspring.

Strictly speaking it is impossible to state that all the mutations are independent, in spite of the X chromosome isogenization immediately preceding the second mutagenesis experiment. Spontaneous mutations in the germ cells of the males could be responsible for clones of mutants. This question could have been settled by breeding the mutagenized males individually and keeping only one mutant per issue. This was not done.

The fact remains that the complementation groups detected are numerically small (eight with two mutants, four with three mutants, three with four, six and seven mutants respectively); it should be noted that the functional complementation tests, where all the mutants of both experiments are concerned, were carried out mainly within each category. However when a mutant was difficult to classify in one category or another its complementation was generally studied with the mutants of both categories. It is unlikely that a complete study of the complementation reactions would greatly increase the numbers of the allelic series.

Excluding the mutants of category III A and those lost or not yet studied, the complementation reactions known at present show the existence of 52 loci (38 represented by 1 mutation, 8 by 2 mutations, 3 by 3 mutations, 3 respectively by 4, 6 and 7 mutations).

From this distribution, an estimation can be made of the total number, T, of genes responsible for female sterility and detectable by mutation.

If it is assumed that the frequency of mutations detectable after E.M.S. treatment is the same at all loci, and that the mutations are independent, the distribution should conform to the Poisson law:

$$f(x) = \frac{m^x e^{-m}}{x!}$$

where m represent the mean number of hits per target. The value of T which minimizes the χ^2 can be determined by attributing successively to T a series of values and calculating for each of them the χ^2 of conformity. The value of T estimated by this method is 107.

However this method of analysis probably gives an underestimation of the total number of genes whose mutation could lead to female sterility: on the one hand it is not certain that all of the mutations obtained from a given experiment of mutagenesis are independent; moreover, it is possible that the frequency of mutations obtained by EMS treatment is not the same for all loci.

A second method arriving at an estimation of T (RICE 1973) is based uniquely upon the number of genes mutated one and two times. Thus $N0$, $N1$ and $N2$ are respectively the number of targets hit 0, 1 and 2 times:

$$T = N0 + 52$$

$$N1 = m \times N0$$

$$N2 = \frac{m}{2} \times N1$$

from which

$$N0 = \frac{N1^2}{2N2} \pm \left(\frac{2}{\sqrt{N1}} + \frac{1}{\sqrt{N2}}\right)$$

The estimation of T is thus:

$$T = 142 \pm 67$$

In spite of these uncertainties it appears that a significant fraction of the genes which can lead to female-sterile mutations have been identified by the study of a thousand X chromosomes. It does not seem to be impossible for technical reasons to detect the majority of these genes.

Similar experiments have been carried out by other authors to detect female sterility genes on either the X chromosomes (KAPLAN *et al.* 1970) or the autosomes (BAKKEN 1973; RICE 1973). It is difficult to compare their results with ours, since the experimental conditions under which the mutants were isolated and the criteria of classification were not strictly identical.

It is evident, however, that the genes involved in the fertility of females, whatever phenotype their mutation confers, are distributed over all the chromosomes.

Classification of mutants

The mutants were classified on the basis of visible criteria without considering the real causes of female sterility. It was sometimes difficult to decide whether a mutant should be included in one category or another, but in spite of this, classification greatly simplified the preliminary study of large numbers of mutants.

Faulty housekeeping functions were present in quite a number of mutants in spite of the precautions taken to reduce their frequency. They could be present in all categories but were probably concentrated mainly in I and III A. Defects in the differentiation of nurse cells or the transit of mature eggs were found in class I mutants. Category II was more homogeneous. It included mutants with defects of egg membrane, permeability, perhaps linked to an inadequate functioning of the follicle cells. All the multiple allele series (above three alleles) are found in category II, which contains few heat-sensitive mutants.

The specimens of group III A and III B were distinguished by the presence or absence of daughters in the progeny of mutant females mated to wild-type males. The importance of this criterion should be stressed, since the presence of daughters proves that the gene concerned is not active exclusively during oogenesis, while their absence does not prove the opposite. The death of the zygote can occur before the wild-type allele introduced during fertilization has had time to direct the synthesis of the lacking product. This is illustrated by the different temperature-dependent responses of mutants 475, 1074 and 1456.

Category III B might contain mutants possessing a fault in the morphogenetic potentiality of the eggs, but many other defects can lead to arrested development The subject will be discussed in a separate paper (ZALOKAR and AUDIT 1975).

Certain conclusions can already be drawn from the two mutagenesis experiments concerning the genetic control of cell determination at the blastoderm

stage. If cell determination at the blastoderm stage is due to morphogenetic substances specific for one or another organ of the larva or imago, a mutation of the genes governing the production or localization of these substances should cause abnormalities consisting merely of the absence of a particular organ, or its transformation into another one without affecting the rest of development. No mutant conforming strictly to this description was obtained in our experiments, nor, to our present knowledge, in other laboratories working on the subject. The eggs of certain mutants (426, 383) continued to develop beyond embryogenesis, but development did not stop at a precise stage, nor was there a constant pattern of abnormalities.

It is possible that mutant 1502, which produced larvae with local damage of the buccal parts represented a faulty determination of this larval organ. However many embryos died earlier so that a detailed anatomical study will be necessary to show the degree of specificity of the lesions.

For the three heat-sensitive mutants 1103, 1122 (alleles) and 1456, most of the eggs reached the imago stage under certain temperature conditions, and the flies showed typical defects (absence of germ cells in all the descendants of 1103 or 1122 females; absence of halteres in 25% of the daughters of 1456 females). However the damage to the egg was not strictly localized at the posterior pole of the eggs (1103–1122) or in the blastoderm region containing the primordial cells of the imaginal haltere discs (1456). In fact, the first two mutants also showed abnormalities of the abdomen, wings and eyes, although less systematically, while 1456 sometimes lacked a leg as well as a haltere. While these phenomena are only observed at certain temperatures (23°), at 29° the consequences of the mutation are more general and more serious: 1103 and 1122 females produce very few eggs, while the eggs of 1456 females die during embryogenesis.

The sample of strictly maternally-influenced lethal or morphological mutants selected by different authors is at present fairly large. The absence of mutants displaying a very limited change in the morphogenetic pattern of the egg does argue against the existence, in the euchromatic regions of the *X* chromosomes or autosomes, of genes governing the synthesis of specific morphogenetic substances. It should nevertheless be noted that the morphogenetic pattern of the egg is so vital to the survival of the species that the genetic systems which govern it may be redundant or polygenic and new tactics should be invented to identify them.

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LITERATURE CITED

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

BISCHOFF, W. L. and J. C. LUCHESI, 1971 Genetic organization in *Drosophila melanogaster*: complementation and fine structure analysis at the deep orange locus. Genetics **69**: 453–466.

BULL, A., 1966 Bicausal, a genetic factor which affects the polarity of the embryo in *Drosophila melanogaster*. J. Exptl. Zool. **161**: 221–242.

CHAN, L. N. and W. GEHRING, 1971 Determination of blastoderm cells in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **68**: 2217–2221.

CHANDLEY, A. C. and A. J. BATEMAN, 1962 Timing of spermatogenesis in *Drosophila melanogaster* using tritiated thymidine. Nature **193**: 299–300.

DAVIDSON, E. H., 1968 *Gene Activity in Early Development*. Academic Press, New York.

FIELDING, C. J., 1967 Developmental genetics of the mutant *grandchildless* of *Drosophila subobscura*. J. Embryol. Exptl. Morphol. **17**: 375–384.

FULLILOVE, S. L. and R. WOODRUFF, 1974 Genetic, cytological and ultrastructural characterization of a temperature sensitive lethal in *Drosophila melanogaster*. Devel. Biol. **38**: 291–307.

GEHRING, W. J., 1973 Genetic control of determination in the *Drosophila embryo*. pp. 103–128. In: *Genetic Mechanisms of Development*. Edited by F. H. RUDDLE. Academic Press, New York.

GEIGY, R., 1931 Action de l'ultraviolet sur le pole germinal dans l'oeuf de *Drosophila melanogaster*. Rev. Suisse Zool. **38**: 187–288.

GILL, K. S., 1963 Developmental genetic studies on oogenesis in *Drosophila melanogaster*. J. Exptl. Zool. **152**: 251–278.

HATHAWAY, D. S. and G. G. SELMAN, 1961 Certain aspects of cell lineage and morphogenesis studied in embryos of *Drosophila melanogaster* with an ultra-violet micro-beam. J. Embryol. Exptl. Morphol. **9**: 310–325.

ILLMENSEE, K. and A. P. MAHOWALD, 1974 Transplantation of posterior polar plasma in *Drosophila*: Induction of germ cells at the anterior pole of the egg. Proc. Natl. Acad. Sci. U.S. **71**: 1016–1020.

KALTHOFF, K., 1971 Photoreversion of U.V. induction of the malformation "double abdomen" in the egg of *smittia* spee (Diptera, chiromomidae). Devel. Biol. **25**: 119–132.

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. Naturalist **104**: 261–271.

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

KING, R. C., J. H. SANG and C. B. LETH, 1961 The hereditary ovarian tumors of the *fes* mutant of *Drosophila melanogaster*. Exptl. Cell Res. **23**: 108–117.

KING, R. C. and J. D. MOHLER, 1975 The genetic analysis of oogenesis in *Drosophila melanogaster*, pp. 757–791. In: *Handbook of Genetics*. Vol. 3. Edited by R .C. KING. Plenum Press, New York.

LEWIS, E. B. and F. BACHER, 1968 Method of feeding ethyl methane sulfonate (E.M.S.) to *Drosophila* males. Drosophila Inform. Serv. **43**: 193.

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

LOCKSHIN, R. A., 1966 Insect embryogenesis: macromolecular synthesis during early development. Science **154**: 775–776.

MITCHELL, H. K., 1966 Phenol oxidases and Drosophila development. J. Insect Physiol. **12**: 755–765.

OKADA, M., I. A. KLEINMAN and H. A. SCHNEIDERMAN, 1974 Restoration of fertility in sterilized *Drosophila* eggs by transplantation of polar cytoplasm. Devel. Biol. **37**: 43–54.

PICARD, G., 1971 Un cas de stérilité femelle chez *Drosophila melanogaster*, lié à un agent transmis maternellement. Compt. Rend. Acad. Sci. (Paris) **272**: 2484–2487.

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, Yale University.

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

SHANNON, N. P., 1972 Characterization of the female-sterile mutant *almondex* of *Drosophila melanogaster*. Genetica **43**:244–256.

SCHNEIDERMAN, H. A. and P. J. BRYANT, 1971 Genetic analysis of developmental mechanisms in *Drosophila*. Nature **234**: 187–194.

SPURWAY, H., 1948 Genetics and cytology of *Drosophila subobscura* IV. An extreme example of delay in gene action causing sterility. J. Genet. **49**: 126–140.

YAJIMA, H., 1960 Studies on embryonic determination of the harlequin-fly *Chironomous dorsalis*. I. Effects of centrifugation and its combination with construction and puncturing. J. Embryol. Exptl. Morphol. **8**: 198–215.

ZALOKAR, M., 1976 Autoradiographic study of protein and RNA formation during early development of Drosophila eggs. Devel. Biol. In press.

ZALOKAR, M. and C. AUDIT, 1975 Developmental defects of female-sterile mutants of *Drosophila meelanogaster*. Devel. Biol.**47**: In press.

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