ISOLATION AND CHARACTERIZATION OF SEX-LINKED FEMALE-STERILE MUTANTS IN *DROSOPHILA MELANOGASTER* WITH SPECIAL ATTENTION TO EGGSHELL MUTANTS

KATIA KOMITOPOULOU,*† MADELEINE GANS,† LUKAS H. MARGARITIS,* FOTIS C. KAFATOS,*‡ AND MICHELE MASSON†

** University of Athens, Panepistimiopolis, Kouponia, Athens, 621, Greece; † Centre de Génétique Moléculaire CNRS, 91190 Gif-sur-Yvette, France; and ‡ Cellular and Developmental Biology 16 Divinity Avenue, Harvard University Cambridge, Massachusetts 02138*

Manuscript received November 12, 1982
Revised copy accepted August 1, 1983

ABSTRACT

To study genes that function mainly or exclusively during oogenesis, we have isolated and analyzed female-sterile mutations, with special emphasis on those that affect eggshell formation. Following treatment that induced 61 to 66% lethals, 8.1% of the 1071 *X* chromosomes tested carried recessive female sterility mutations (87 isolates), and 8.0% carried partial female-sterile mutations (86 isolates), respectively. In addition, three dominant female steriles were recovered. Some of the mutants had very low fecundity, and others laid morphologically normal eggs that failed to develop. A third category included 29 mutants that laid eggs with morphological abnormalities: 26 were female steriles, two were partial female steriles and one was fertile. Mutants of this third category were characterized in some detail and compared with 40 previously isolated mutants that laid similarly abnormal eggs. Approximately 28–31 complementation groups with morphological abnormalities were detected, some of which were large allelic series (11, 9, 7, 6 and 5 alleles). Twenty-four groups were mapped genetically or cytogenetically, and 21 were partially characterized by ultrastructural and biochemical procedures. Of the latter, one group showed clear deficiency of yolk proteins, and nine showed prominent ultrastructural defects in the chorion (at least eight accompanied by deficiencies in characterized chorion proteins). At least six groups with clear-cut effects were found at loci not previously identified with known chorion structural genes.

OOGENESIS is an important and complex developmental process, which sets the stage for subsequent fertilization and embryonic development. In *Drosophila melanogaster*, in which both sophisticated genetic analysis and molecular studies are feasible, identification of genes with essential functions during oogenesis represents an important first step toward understanding the regulatory mechanisms that operate in oogenesis and embryogenesis. We and others have previously approached this problem by generating and characterizing collections of female-sterile mutants (BAKKEN, 1973; GANS, AUDIT and MASSON 1975, MOHLER, 1977). Through a new series of ethylmethane sulfonate (EMS) mutagenesis experiments, we have recently expanded our collection of female-sterile mutants that map on the *X* chromosome. We are especially interested in one aspect of oogenesis, formation of the eggshell (chorion) by

the follicular epithelial cells that surround the developing oocyte. Since not all defects of choriogenesis lead to sterility, in these new experiments we also collected partially female sterile or even fertile mutants, if they showed visible egg abnormalities. In the present report we summarize the results of the new mutagenesis experiments and present a preliminary characterization of all mutants with visible egg abnormalities.

MATERIALS AND METHODS

Strains

C(1)DX,y f/Y ♀♀; v^{24}*/Y* ♂♂: v^{24} is an EMS-induced vermillion allele, isolated by D. LOCKER, (GANS, AUDIT and MASSON 1975). This strain was used for mass production of the v^{24}*/Y* ♂♂ used for mutagenesis.

C(1)DX,y f/Y ♀♀; *w v* l^{44TS}*/Y* ♂♂: This strain was used for mass production of virgin *C(1)DX,y f/Y* ♀♀ (symbolized as $\widehat{XX}$*/Y* ♀♀ in Figure 1) at 29°.

FM3/w v l^{44TS} ♀♀; *w v* l^{44TS}*/B*^{S}*Y* ♂♂: This strain was used for mass production of virgin *FM3/wvl*^{44TS} ♀♀ at 29°. The *Y* chromosome was labeled so that flies resulting from nondisjunction of the sex chromosome in the father or mother could be detected.

Two previously described breeding media (GANS, AUDIT and MASSON 1975) were used.

Mutagenesis and mutant isolation

Prior to mutagenesis, 15 isogenic lines of the v^{24} *X* chromosome were generated by crossing a single v^{24} male to attached-*X* females. Males from these lines were treated with EMS, and mutants were isolated as diagrammed in Figure 1. The experiments were performed at 23° to 25° and at 29°. EMS was applied to v^{24}*/Y* ♂♂ according to the methods of LEWIS and BACHER (1968). The mutagenized males were mated to *C(1)DX,y f/Y* ♀♀ for 1 to 2 days (cross 1), and the mated females were transferred to new flasks.

Four mutagenesis experiments were performed. The frequency of induced *X*-linked lethals was estimated as 61 to 66%, from the sex ratio of the progeny of cross 1, as compared with the progeny of control males, in agreement with previous experiments (GANS, AUDIT and MASSON 1975, MOHLER 1977).

A total of 2070 male progeny of the mutagenized males (cross 1) were individually mated with two to three virgin *XX/Y* females (cross 2). This second cross eliminated male steriles and germ line mosaic males (ALDERSON 1965) and also permitted some isogenization, eliminating a fraction of the autosomes subjected to mutagenesis treatment. The resulting 1573 stocks were assigned sequential isolation numbers preceded by K (for KOMITOPOULOU): K1-252 for the first experiment, K253-K628 for the second, K629-K1144 for the third and K1145-K1573 for the fourth. Of these stocks, 80 subsequently gave no male progeny and were eliminated.

Cross 3 involved individual male progeny of cross 2, crossed in the same tube with two types of females and permitted transfer of the mutagenized *X* chromosome, (v^{24})* to females in the heterozygous state, while also maintaining it in males. In this cross, after 3 days at 23°, the temperature was increased to 29°; this eliminated all male progeny except for the desired carriers of (v^{24})* and also made possible detection of some lethal temperature-sensitive mutations. The female progeny required for cross 4 were easily identified phenotypically.

Cross 4, a brother-sister mating involving the heterozygous (v^{24})* female and hemizygous (v^{24})* male progeny of the previous cross permitted construction of homozygous females carrying the (v^{24})* chromosomes. The cross was performed at 29°; if no progeny were recovered, the mutant could be temperature-sensitive male sterile or dominant female sterile. These two possibilities could be distinguished by the absence of progeny in crosses 5a and 5b, respectively. Cross 4 also permitted calculation of the viability of (v^{24})*/(v^{24})* and (v^{24})*/*Y* individuals: the frequency of these genotypes among the young progeny of cross 4 should equal twice the frequency of *FM3*/(v^{24})* if the recessive mutation does not affect viability.

In cross 6, another brother-sister mating, (v^{24})* homozygous females were checked for female sterility, egg morphology, fecundity and partial female sterility. Female sterility was detected by

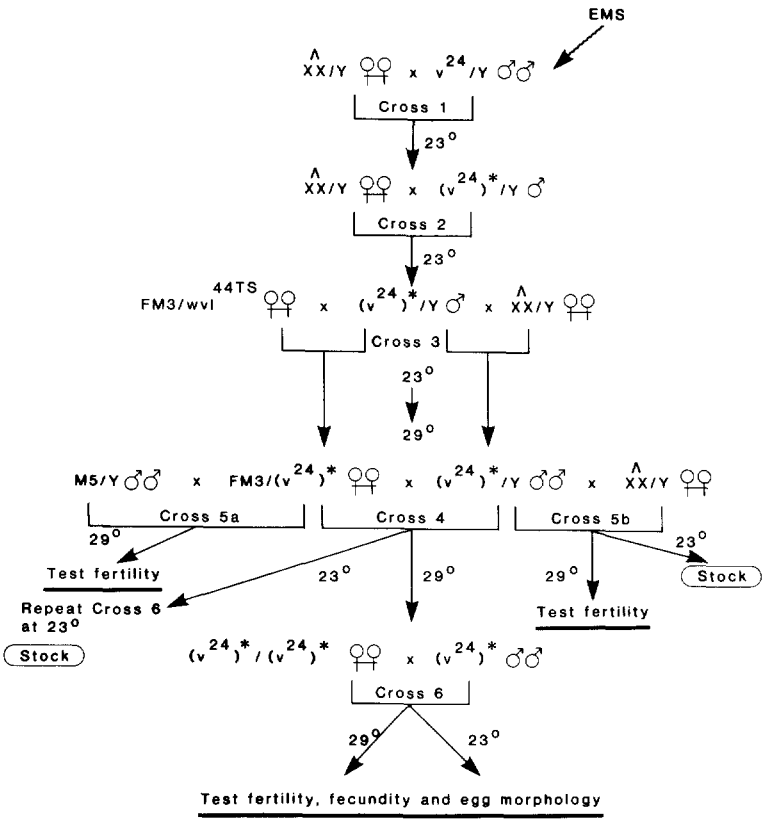

FIGURE 1.—Sequence of crosses used to screen for and classify female-sterile mutants. Crosses 2 and 3 were performed with single males. In cross 3 the temperature was raised from 23° to 29° after 3 days. Chromosomes treated with mutagen are described as $(v^{24})^*$ and attached-*X* chromosomes as $\widehat{XX}$.

absence of progeny and confirmed by repetition of the cross using a larger number of flies (ten females and 15 of their brothers per bottle). For convenience, each female-sterile strain was assumed to correspond to a single mutant, which was identified by the numerical designation of the strain, omitting *fs(1)*. Egg morphology was checked at both 23° and 29° for eggs deposited on both types of breeding media; the laying medium (GANS, AUDIT and MASSON 1975) contained neutral red for detection of abnormally high permeability. Fecundity was estimated from the number of eggs laid per 24 hr on laying medium. For detection of partial female sterility, two females aged 0–4 days and five of their brothers were mated for 4 days at 29° and the number of progeny compared with that of control crosses; progeny numbering less than 25% and more than 1% of the control values were considered evidence of partial female sterility.

Figure 2 schematically indicates possible results of this sequence of crosses and the types of corresponding mutations.

The chromosomes carrying mutations of interest were kept in males and females using the *FM3* balancer and/or in males only that were mated with attached-*X* females (self-propagating crosses 4 and 5b, respectively.)

Genetic and cytogenetic localization: Figure 3 shows the methods used for gross genetic localization of the mutations to one of six intervals of the *X* chromosome. For the cases indicated in Table 4, more precise localization was accomplished by recovering additional recombinants with the two markers that define the respective interval or with other nearby markers. In addition, cytogenetic localizations were undertaken, by placing mutations against deficiencies selected according to the

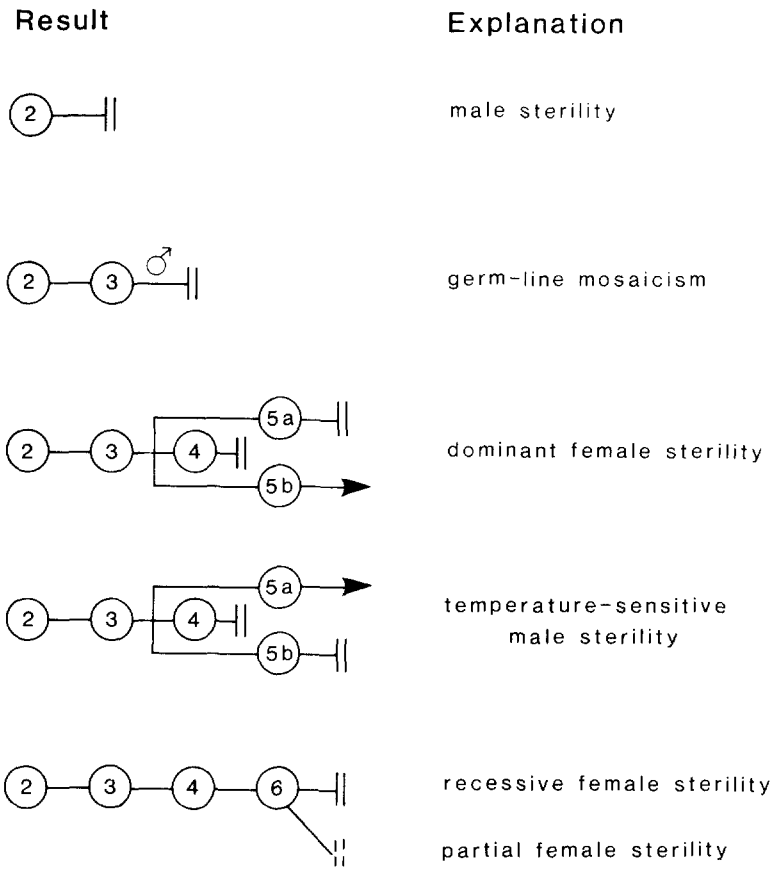

FIGURE 2.—Classification of mutants on the basis of the presence (→) or absence (| |) of progeny from sequential crosses, numbered as in Figure 1.

genetic location of the mutant. The deficiencies and some duplications used in this analysis are summarized in Table 1.

Complementation tests: Allelism was ascertained by performing reciprocal crosses between two mutant stocks, $FM3/(v^{24})^{*a}$ ♀♀ × $(v^{24})^{*b}/Y$ ♂♂ and testing the vermillion female progeny for female sterility, and for egg abnormalities.

Mutants from previous studies: The category II mutants from our previous study (GANS, AUDIT and MASSON 1975) were reexamined as part of the present work. These mutants are identified by plain numbers. The ICR series of mutants were obtained at Gif by B. JARRY and M. GANS using ICR 170 mutagenesis. A systematic study of egg morphology was not attempted in this case, but three mutants with morphological egg abnormalities were identified.

DESCRIPTION OF NEW MUTANTS

The mutagenesis and isolation of mutants is described in MATERIALS AND METHODS and Figure 1. Table 2 summarizes the frequencies of observed mutations. Since the males were isogenized before mutagenesis, all mutations should be independent; all lines yielded some homozygous female-fertile stocks, eliminating the possibility that the single male that founded the line carried a spontaneous female-sterile mutation on the *X*. For some mutants derived from

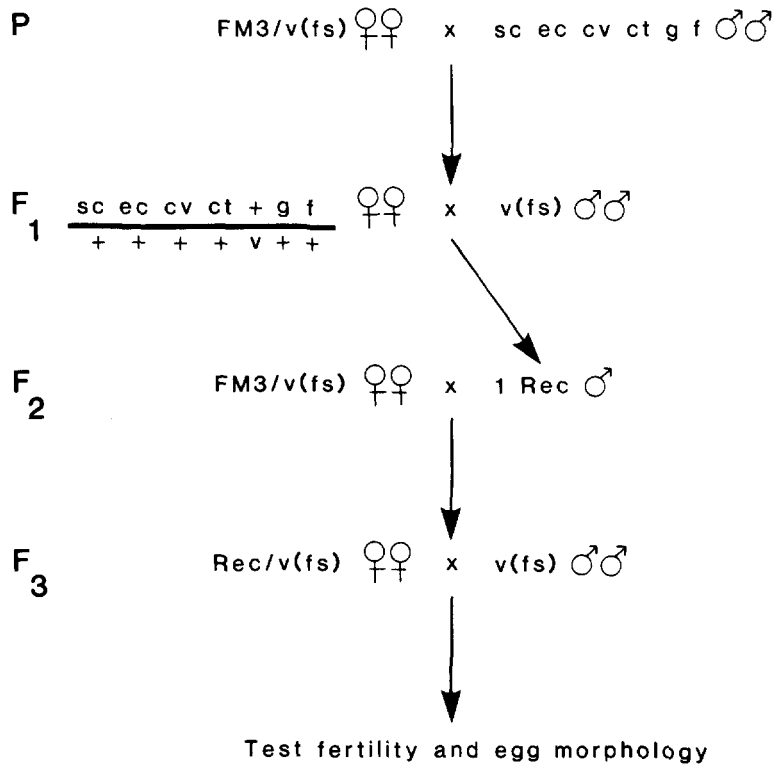

FIGURE 3.—Scheme for assigning female-sterile mutations (*fs*) to map segments defined by the markers *sc*, *ec*, *cv*, *ct*, *v*, *g* and *f*. Cross F_2 was performed with individual recombinant males.

the same isogenic line, nonindependence is conceivable but very unlikely (spontaneous mutation occurring in the germ line between isogenization and mutagenesis). Independence of the mutations was also ensured by allowing cross 1 to proceed for only 1–2 days, *i.e.*, by ensuring that the fertilizing spermatozoa were either mature or at the spermatid stage during EMS treatment (CHANDLEY and BATEMAN 1962). The various types of female-sterile mutants, and a fertile mutant affecting choriogenesis, can be classified as follows.

Dominant female steriles

Three dominant female steriles were isolated. Mutants of this type are rare (KING and MOHLER 1975). Their phenotypes were the same at 23° and 29°. Mutant *FS(1)K1237* has atrophied ovaries. In *FS(1)K1103*, oogenesis usually stops at early stages; occasionally, it proceeds to immature stage 14, with production of few flaccid eggs. In *FS(1)K155*, mature but, in general, morphologically abnormal eggs are formed, with apparently normal chorion. The genetic analysis of these mutations, which are apparently allelic, will be reported elsewhere (D. BUSSON, M. GANS, K. KOMITOPOULOU and M. MASSON, in preparation). They are localized in region 4DE of the *X* chromosome, and their functional defect apparently concerns only the germ line. Mutations of this type are genetically very useful, especially for detecting mitotic clones in the

TABLE 1

Deficiencies and duplications used for cytogenetic localization

Deficiencies and duplications			
Symbol	Extent	Origin	Nomenclature
$*y^{74k24.1}$	tip; 1B9-10	Pasadena	$Df(1)y^{74k24.1}/y^{2}Y^{61e}/y^{2}Y^{61e}/C(1)DX,yf$
*A94	1E3; 2B15	Pasadena	DF(1)A94/FM6
*S39	1E4; 2B11-12	J. CAMPOS-ORTEGA	Df(1)S39/FM6
$**w^{wco}$	2B17-C1; 3C4-5	Davis	$ClB;T(1-3)w^{wco}vf$
$**w^{258.11}$	3A2-3; 3C3-5	Pasadena	$Df(1)w^{258.11},y/In(1)dl49,yHwm^{2}g^{4}$
$*N^{71k24.5}$	3C4; 3D5	Pasadena	$C(1)DX,ywf/Df(1)N^{71k24.5};Dp(1-2)51b$
$*dm^{75e}$	3C11; 3E4±	Pasadena	$Df(1)dm^{75e}/FM7$
*A113	3C6-E1; 4F7-8	Pasadena	$Df(1)A113/C(1)DX,ywf;Dp(1-2)w^{+6rb}$
*RC40	4B1; 4F1	Pasadena	Df(1)RC40/FM7a
*JC70	4C15-16; 5A1-2	G. LEFEVRE	Df(1)JC70/FM7c
*C149	5A8-9; 5C5-6	Pasadena	Df(1)C149/FM6
*N73	5C2; 5D5-6	Pasadena	Df(1)N73/FM6
*HA32	6E4-5; 7A6	Pasadena	Df(1)HA32/FM7c
$*ct^{J6}$	6E1; 7C1	G. LEFEVRE	$Df(1)J6,dl49f/FM6$
$**ct^{268.42}$	7A5-6; 7B8-C1	Bowling Green	$Df(1)ct^{268.42},y/FM4$
$**ct^{4b1}$	7B2-4; 7C3	G. LEFEVRE	$Df(1)ct^{4b1},oc\ ptg/C(1)DX,ywf;Dp(1-3)sn^{13a1}/Ubx$
		Bowling Green	$Df(1)ct^{4b1},y\ sc/B\ in\ sn$
$*sn^{C128}$	7D1; 7D5-6	G. LEFEVRE	$Df(1)sn^{C128}/FM6$
*RA2	7D10; 8A4-5	G. LEFEVRE	$Df(1)RA2/C(1)DX,ywf;Dp(1-2)FN107/bw^{D}$
*C52	8E; 9C-D	D. SEARS	Df(1)C52/FM6
$*v^{-L15}$	9B1; 10A1	D. SEARS	$Df(1)v^{-L15}/FM6$
$*m^{259-4}$	10C2-3; 10E2-3	Pasadena	$C(1)DX,ywf/Df(1)m^{259-4},ras\ f;v^{+}B^{s}Y$
*JA26	11A1; 11A7	Pasadena	Df(1)JA26/FM7
*HA92	12A6-7; 12D3	J. POSTLETHWAITE	Df(1)HA92/FM7
$**g^{l}$	12A; 12E	Bowling Green	$Df(1)g^{l},fB/In(1)AM$
*KA9	12E1; 13A5	Pasadena	Df(1)KA9/FM7
*N19	17A; 18A2	Pasadena	Df(1)N19/FM6
*JA27	18A5; 18D1-2	Pasadena	Df(1)JA27/FM7
*HF396	18E1-2; 20	Pasadena	Df(1)HF396/FM7
$*Dp(1-3)sn^{13a1}$	6C11 to 7C9; 79E	G. LEFEVRE	$Df(1)ct^{4b1},oc\ ptg/C(1)DX,ywf;Dp(1-3)sn^{13a1}/Ubx$

* CRAYMER and ROY (1980).
** LINDSLEY and GRELL (1982).

germ line. In particular, *FS(1)K1237* was used to determine by clonal analysis the tissue (germinal or somatic) that is responsible for the egg abnormality in 15 of the mutants described in the present paper (N. PERRIMON and M. GANS, unpublished results).

TABLE 2

Relative frequencies of sex-linked mutations

	No.	%
Total no. of mutagenized male progeny used in cross 2	2070	100
Sterile males	497	24
Fertile males	1573	100
Males without male progeny (mosaic lethals)	80	5.1
Males with sterile male progeny	90	5.7
Temperature-sensitive male steriles	20	1.3
Dominant female steriles	3	0.2
Stocks lost	309	
X chromosomes examined	1071	100
Recessive female steriles	87	8.1
Category I: low fecundity	14	1.3
[a] Category II: morphologically abnormal eggs	26	2.4
Category III: morphologically normal eggs	47	4.4
Recessive partial female steriles	86	8.0
[a] Category IV: morphologically abnormal eggs	2	0.2
Category V: other partial female steriles	84	7.8
[a] Category VI: Fertile morphologically abnormal eggs	1	0.1

[a] Mutants with morphologically abnormal eggs were genetically localized.

Recessive female steriles

Mutants were classified as female steriles if each female gave on average less than one descendant in 4 days' breeding at 25° or in 10 days' breeding at 16° (approximately 1% of the wild type). For consistency, we classified these mutants into three categories, as defined by GANS, AUDIT and MASSON (1975).

Mutants with low fecundity (category I): Fourteen mutants were assigned to this category but have not been studied fully as yet. Of these, three were lost and six were not studied after the initial tests. In one mutant, *K1221*, oogenesis was shown to be normal: mature eggs are retained, causing enormous distension of the abdomen. Additional mutants of a similar phenotype may have been discarded.

The occurrence of developmental abnormalities was confirmed in four mutants, as follows:

K524: At 29° the ovaries are extremely atrophied, resembling ovaries without germ cells, but very rarely stage 14 follicles are observed. Possibly as a result of a second mutation, at 29° homozygous and hemizygous individuals have rugose eyes and short, fine bristles; they have normal viability, however. At 20°, additional abdominal abnormalities appear (etched abdomen).

K741^{TS}: Viability is good but ovaries are very atrophied at 29°. The mutant is fertile at 23° and is localized between *cv* and *ct*, region 5D-6E.

K1134: At 29°, follicular development is blocked at stage 8. It also appears that the transformation of the larval fat body to adult fat body does not take

TABLE 3

Characteristics of mutants with morphological egg abnormalities

New mutants	Mutants from GANS, AUDIT and MASSON (1975)	Characteristics
K79		[a] Fertile. Short, fragile dorsal appendages.
K93		Very flaccid eggs, strong uptake of dye.
K163		Delayed flaccidity. Delayed development, weak viability. Embryogenesis blocked at various stages. Wings short and thin. Female progeny rescued by wild-type father.
K184, *K294*, *K621*	*148, 305, 332, 1061, 1081^{TS}, 1130, 1186, ICR^{756}*	[c,d] Embryogenesis blocked at various stages. *K621* and especially *K184* show very flaccid eggs, no embryogenesis. In *K294* development occasionally proceeds to larval or pupal stages. *1081* partially fertile at 16°.
K254^{TS}		[a] Short, thin dorsal appendages. Low viability (especially females) at 29°, moderate at 23°.
K313	*59, 1163*	[b] Limited flaccidity, slightly short dorsal appendages. *59* shows low fecundity. *1163* semidominant.
K418, *K743, K1421*	*1057, ICR581*	[c] Alleles of *sn*. Small eggs, incompletely formed dorsal appendages.
K451		[a] Short, thin dorsal appendages. Reduced viability, especially females. Abnormalities of posterior legs, especially in females.
K467, K718, K1090 K1124, K1232, K1511	*267*,* ***384, 1336***	[a] Very thin and fragile chorion, often lost during oviposition.
K499		[a] Abnormal dorsal appendages.
K575^{TS}	*1059*	[b] Dorsal appendages variable in size.
K646		Very flaccid eggs. Embryogenesis blocked early.
*K811**		[c] Dorsal appendages may be fused. Low fecundity.
K1075	*273*	[b] Size of eggs and dorsal appendages small and variable. *K1075* is partial female sterile.
K1193		Limited flaccidity. Development rarely proceeds to pupal stage.
K1214		[a] Dorsal appendages thin and short.
K1281, K1347, K1540	***371***, *379, 1038, ICR398*	[b] Development initiated. *K1540* is partial female sterile.
K1563^{TS}		[a] Dorsal appendages short and thin, eggs very flaccid.
	117	Embryogenesis blocked at various stages. Low fecundity.
	120, *434, 489, 571, 1190, 1198*	Embryogenesis blocked at various stages. Fertility depends on culture medium.

TABLE 3—*Continued*

New mutants	Mutants from GANS, AUDIT and MASSON (1975)	Characteristics
	125^{TS}	Dorsal appendages fused. Low fecundity. Partially fertile at 16° and 23°.
	147, 1369	[c] Development not initiated.
	180^{TS}	[c] Fertile at 16° and 23°.
	321	Short bristles.
	*336**	
	456	[c]
	473^{TS}	[a] Chorion partially missing. Fertile at 16° and 23°.
	508	Delayed development. Egg size variable, often small. Bent bristles. Viability reduced.
	1268	
	1501	Eggs occasionally slightly flaccid.
	1561	Low fecundity.

Solid underlining indicates alleles examined by electron microscopy, dashed underlining alleles examined by electrophoresis of oocyte contents. Boldface indicates alleles used to name the respective complementation group, if two or more alleles exist.

* Mutants lost.

[a] Biochemical and ultrastructural chorion defect observed.

[b] Chorion examined by electron microscopy, somewhat abnormal.

[c] Chorion examined by electron microscopy, normal.

[d] Deficiency of yolk proteins.

place. This mutant might have a juvenile hormone deficiency. Examination of this hypothesis and genetic localization of the mutation are currently underway.

K1274^{TS}: At 29°, viability is good, but ovaries are very atrophied, probably with tumorous follicles. The mutant is fertile at 23°, and at the semipermissive temperature of 25° follicles are observed with 16 nurse cells and no oocyte. They are localized between *cv* and *ct*.

Mutants *K524*, *K741* and *K1274* were not allelic to each other or to the previously isolated oogenesis mutants, *116* and *1621* (GANS, AUDIT and MASSON 1975).

Mutants with morphologically abnormal eggs (category II): This category includes 26 mutants with moderate to normal fecundity but visible egg abnormalities. Since the latter criterion was emphasized, some of these mutants could also be classified in the first category. The appearance of the laid eggs was evaluated under the dissecting microscope, soon after oviposition. In most cases, eggs from homozygous females were flaccid, either immediately after oviposition or within a few hours. The flaccidity resulted either from oocyte defects (*e.g.*, reduced amount of yolk) or from increased permeability of the eggshell. In a number of cases, abnormal permeability was suggested by the uptake of neutral red contained in the laying medium. In a few cases, the egg abnormality was minor (see Table 3). Empty eggshells of all category II mutants were examined under a phase contrast microscope, and shell abnormalities were evident in many but not all cases. These abnormalities were also frequently seen under the dissecting microscope (Figure 4). In particular, the dorsal appendages were

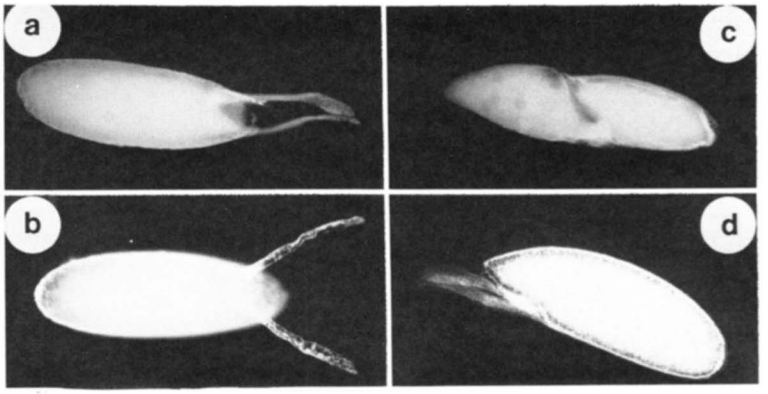

FIGURE 4.— Typical examples of morphological egg abnormalities in female-sterile mutants. Mature (stage 14) follicles produced by the indicated female genotypes are shown in each case. a, Wild-type v^{24}/v^{24}. b, *K499/K499*. Note the misformed dorsal appendages. c, *K418/K418*. Note that the nurse cells have not degenerated; their removal reveals fully formed although misshapen dorsal appendages (not shown). d, *K575/K575*, maintained at the nonpermissive temperature (29°). Note that the chorion is very fine and the dorsal appendages abnormal.

frequently short or thin and unusually fragile. The mutants of category II were assigned to complementation groups (Table 3) and characterized further, as will be described.

Female steriles with morphologically normal eggs (category III): These 47 mutants lay eggs normal in number and appearance but produce no progeny. The sterility might result from failure of fertilization (due to abnormality of the micropyle or of the oocyte itself), or from embryonic lethality. The mutants have not been studied further as yet.

Recessive partial female steriles

Because of the possibility that some chorion mutants might not be fully sterile, we paid special attention to partial female steriles, *i.e.*, mutants yielding progeny under our conditions at less than 25% but more than 1% of the normal number (see MATERIALS AND METHODS). A total of 86 such mutations were recovered, two of which showed egg abnormalities (category IV). As summarized in Table 3, both were allelic with category II female-sterile mutants which showed chorion defects. The remaining 84 partial female-sterile mutants (category V) showed no obvious egg abnormality and were not studied further.

Fertile mutants with morphologically abnormal eggs

A total of 898 *X* chromosome stocks showed no significant female sterility (progeny recovered in numbers of at least one-quarter of normal). The eggs laid by the homozygous females of these stocks were also examined under the compound microscope for chorion abnormalities. One recovered mutant, *K79*, showed clearly short and fragile dorsal appendages and, thus, was classified as a fertile mutant with abnormal eggs (category VI). More limited abnormalities were evident in eggs of *K79/+* heterozygotes, whereas eggs of *K79/Df(1)RA2* and *K79/K79* were identical in terms of morphology.

CHARACTERIZATION OF MUTANTS SHOWING EGG ABNORMALITIES

Genetic localization

As explained in MATERIALS AND METHODS and Figure 3, gross genetic localization was undertaken for most of the mutants showing morphological egg abnormalities (categories II, IV and VI, Table 2). Mutants from our previous collection (GANS, AUDIT and MASSON 1975) were similarly localized. Recombinants were recovered between the chromosome of interest (which also carried the vermillion allele, v^{24} at *1*-33.0) and a multiply marked *X* chromosome carrying the markers *sc*(*1*-0.0), *ec*(*1*-5.5), *cv*(*1*-13.7), *ct*(*1*-20.0), *g*(*1*-44.4) and *f*(*1*-56.7). Both reciprocal types of recombinants between any two markers were recovered (a total of two to six per mutation) and were tested for the presence or absence of the female-sterile mutation. Table 4 shows the assignment of mutations to the seven possible chromosomal intervals.

Mutants of special interest were mapped more precisely by recovering a larger number of recombinants with the two markers that define the interval to which the mutation is assigned; in a few cases additional markers were used for more precise localization. Results are presented in Table 4.

Cytogenetic mapping

Most mutants showing morphologically abnormal eggs were also mapped cytogenetically, against the deficiencies or duplications listed in Table 1. Results are included in Table 4. Two mutants, *K811* and *336*, were lost and not mapped. Four other mutants, *117*, 125^{TS}, *321* and *1268*, were only mapped to the extent of ascertaining that they are not uncovered by the following deficiencies: *Df(1)C52*, *Df(1)HA92* and *Df1(1)KA9*. Localization of *K499* and 473^{TS} is uncertain, as will be explained.

Complementation analysis

Complementation analysis was performed only on mutants showing morphologically abnormal eggs (categories II, IV and VI), from both the new and old collections. All of the mutants from the old collection had already been subjected to complementation tests in all combinations (GANS, AUDIT and MASSON 1975). Furthermore, all combinations of the following new and old mutants were tested: *K93*, *K163*, *K184*, *K254*, *K313*, *K418*, *K451*, *K467*, *K499*, *K621*, *K1075*, *59*, *117*, *120*, *125*, *147*, *148*, *185*, *384*, *1501*, *273*, *321*, *336*, *371*, *456*, *473*, *508*, *1059*, *1268*, *1561*. Both fertility and egg morphology were assayed in these tests. As a result, 25 complementation groups were defined (Table 3). To assign the remaining 17 new mutants to complementation groups, more limited tests were performed among mutants that showed similar phenotypes and also those that genetically mapped to the same chromosomal interval and were not separated cytogenetically when tested against deficiencies. These tests were largely based on fertility testing, although eggs were also examined for visible abnormalities in cases of infertility. These additional tests clearly identified a number of alleles and four new complementation groups, represented by *K646*, *K1193*, *K1214* and *K1563*. No allelic complementation was observed, although this generalization is limited by the fact that, from most of the com-

TABLE 4

Localization of mutants with abnormal eggs

Interval	Complementation group	Genetic localization[a]		Cytological localization	
		Genetic location	Mapping markers	Cytological location	Mapping deficiencies
sc-ec, map 0-5.5	*371*	0.3	*y-fs(1)K10(6)*	1E4–2B12	*+Df(1)S39*
bands 1B3–3F1-2	*147*	Near *sc*	*sc-ec* (3)	1B9-10–1E3?	*−Df(1)y*$^{74k24.1}$
					−Df(1)A94
					−Df(1)w^{vco}
					−Df(1)w$^{258.11}$
					−Df(1)N$^{71k24.5}$
		Near *sc*			*−Df(1)A113*
	K93		*sc-ec* (4)	3C4–3C11	*+Df(1)N*$^{71k24.5}$
					−Df(1)dm^{75e}
ec-cv, map 5.5-13.7	*K575*^{TS}	NT		4F1–5A1-2	*+Df(1)JC70*
bands 3F1-2–4F9-5D2					*−Df(1)RC40*
	456	NT		4F1–5A1-2	*+Df(1)JC70*
					−Df(1)RC40
cv-ct, map 13.7-20	*K646*	15	*cv-ct* (6)	5C5-6–5D5-6	*+Df(1)N73*
bands 4F9-5D2–7B3-4					*−Df(1)C149*
	K254^{TS}	17	*cv-ct* (21)	5D5-6–6E1	*−Df(1)N73*
					−Df(1)C149
					−Df(1)ct^{J6}
	K163	Near *ct*	*cv-ct* (13)	NT	
	K1214	18.7	*cv-ct* (44)	5D5-6–6C12	*−Dp(1-3)sn13a1*
					−Df(1)ct^{J6}
ct-v, map 20-33	*508*	18	*w-sn* (42)	7C3–7D1?	*−Df(1)sn*^{C128}
bands 7B3-4–10A1-2				5D5-6–6E1?	*−Df(1)ct*^{4b1}
					−Df(1)ct$^{268.42}$
					−Df(1)HA32
	384	20.5	*ct-oc* (51)	7B8-C1–7C3	*+Df(1)ct*^{4b1}
					−Df(1)ct$^{268.42}$
	1501	20.7	*ct-oc* (19)	7C3–7C9	*−Df(1)ct*^{4b1}
					−Df(1)sn^{C128}
					−Df(1)ct$^{268.42}$

	K418	21 (allele of *sn*)		7D1-2	$+Df(1)sn^{C128}$
				7D10–8A4-5	*+Df(1)RA2*
	473^{TS}	NT		7D10–8A4-5	*+Df(1)RA2*
	K79	NT		8E–9B1	*+Df(1)C52*
	K313	NT			$-Df(1)v^{-L15}$
				9B1–9C-D	*+Df(1)C52*
	1561	NT			$+Df(1)v^{-L15}$
v-g, map 33-44.4 bands 10A1-2–12B9-C7	180^{TS}	36	*v-g* (36)	?	$-Df(1)m^{259-4}$
					−Df(1)JA26
	K451	Near *g*	*v-g* (3)	12A6-7–12D3	*+Df(1)HA92*
					−Df(1)KA9
g-f, map 44.4-56.7 bands 12B9-C7–15F1-3	$K1563^{TS}$	44.7	*g-f* (42)	12D3–12E1?	*−Df(1)HA92*
					$-Df(1)g^{J}$
					−Df(1)KA9
	K184	NT		12E1–13A5	*+Df(1)KA9*
					−Df(1)HA92
	120	47	*g-f* (44)	?	*−Df(1)HA92*
					−Df(1)KA9
					$-Df(1)g^{J}$
	K1193	To the right of *g*		?	*−Df(1)N19*
			g-f (3)		*−Df(1)JA27*
					−Df(1)HF396
f-centromere map 56.7-70 bands 15F1-3–20F	*K1075*	Near *car*	*Bx-car* (43)	18E1-2–20A	*+Df(1)HF396*

[a] Numers in parentheses show the number of recombinants recovered between indicated markers. The marker *fs(1) K10*, used for mapping *371*, is the mutant described by WIESCHAUS, MARSH and GEHRING (1978). Genetic localization was accomplished as described in the text, relative to the indicated markers and with the indicated number of recombinants. For cytogenetic localization, all deficiencies tested are listed if none uncovered the mutation. In some cases such a list allows deduction, by exclusion, of the most probable location. If this location is unambiguous in combination with genetic data, it is listed as such. If it is merely the more likely of two or more alternatives, it is listed with a question mark. Thus, *147* could be located either in 1B9-10–1E3 or in 2B14–2B17; the former possibility is preferred, since that region is larger and *147* maps close to *sc*. Two locations are possible for *508*, since it has not been mapped relative to *ct*. In combination with genetic map data, the location of *K1563* is probably in the region of nonoverlap between *Df(1)HA92* and *DF(1)KA9* (*i.e.*, 12D3–12E1), although a location near and to the right of 13A5 is not excluded. For *180*, *120* and *K1193*, the cytogenetic information merely establishes the region in which these mutations cannot be located. In cases in which a deficiency uncovering the mutation was found, only this deficiency is listed unless another deficiency allows the mutation to be more precisely localized.

plementation groups identified in the previous study (GANS, AUDIT and MASSON 1975), only one allele was subjected to testing.

Because of their special interest, *1501* and all of the alleles of the *384* series (from both collections) were tested in all combinations. Invariably, the *trans* heterozygotes laid infertile eggs which, however, resemble those laid by *1501/1501* females in showing no major visible abnormality. By contrast, all combinations of alleles of the *384* series were sterile and produced eggs with very characteristic chorion abnormalities. We chose to consider *1501* and the *384* series as two distinct genes because of the complementation of the chorion abnormality and their physical separability; however, alternative interpretations are possible. Because *K79* is partially codominant with respect to morphology, it was not subjected to complementation testing against *473*, which maps in the same region; the two mutations may be allelic or may correspond to distinct genes. Finally, one mutant, *K811*, was lost before complementation testing. In summary, the 69 mutants included in Table 3 were classified into a minimum of 28 and a maximum of 31 complementation groups. Multiple allelic series were named arbitrarily as shown by boldface in Table 3. Thirteen complementation groups were represented only in our previous collection (GANS, AUDIT and MASSON 1975), 11 only in the new collection and seven in both. Four large groups (11, 9, 7 and 5 alleles, respectively), were represented in both collections, but, surprisingly, another large group (six alleles) was represented only in the former. Three large groups were also represented in the limited ICR series of mutants. Of the maximum of 31 complementation groups, 22 were represented by single alleles. Clearly, the *X* chromosome has not been saturated for mutations of these categories.

Chorion defects

Many of the 31 complementation groups showed various types of eggshell abnormalities when examined under the dissecting and phase microscopes (Tables 3 and 5). These were studied by electron microscopy, as were several others not showing a gross defect, including the large complementation group, *K184*. In total, 18 complementation groups were examined by electron microscopy. The results will be reported in detail elsewhere (K. KOMITOPOULOU, L. H. MARGARITIS, M. GANS and F. C. KAFATOS, in preparation). As summarized in Tables 3 and 5, six complementation groups showed no ultrastructural defects (*K184, K418, K811, 147, 180^{TS}, 456.*) Gross malformation of the dorsal appendages unaccompanied by ultrastructural defects was evident in the *K418* group (alleles of *sn*) and in *K811*; this was apparently due to morphogenetic abnormalities such as failure of migration or degeneration of nurse cells rather than to abnormal construction of the chorion *per se*. Three complementation groups (*K313, K1075, 371*) showed minor defects, such as occasional interruptions of the endochorion roof; an example is shown in Figure 5. Nine complementation groups (*K79, K254^{TS}, K451, K575^{TS}, 384, K499, K1214, K1563^{TS}, 473^{TS}*) showed prominent defects in the main body of the chorion and/or the dorsal appendages; Figure 5 shows one example. Unlike *K575*, its allele, *1059*, only has a mild effect on egg morphology.

TABLE 5

Summary characterization of mutants with abnormal eggs[a]

Interval	Complementation group	No. of alleles	Viability[b]	Fecundity[b]	Fertility[b]	Gross shell defect[c]	Ultrastructural defect[c]	Chorion protein defect[c]	Tissue specification[d]
sc-ec	*371*	7	+++	+++	0,+	(±F)	±		G
	147	2	+++	+++	0		−		
	K93	1	+++	+++	0	(F)			S
ec-cv	*K575*^{TS}	2	+++	+++	0	+	±,+		
	456	1	+++	++	0		−		S
cv-ct	*K646*	1	+++	+++	0	(F)			G
	K254^{TS}	1	ϵ to +++		0 to +++	+	+	+	S
	K163	1	+(D)	+	0				
	K1214	1	++	++	ϵ	+	+	+	S
ct-v	*508*	1	++(D)	+++	0				
	384	9	+++	+	0	+	+	+	S
	1501	1	++	++	0				S
	K418	5	+++	+	0	+	−		G
	473^{TS}	1	+++		0 to +++	+	+	+	S
	K79	1	+++		++	+	+	+	
	K313	3	+++	+++	0	(±F)+	±		
	1561	1	++	+	0	(±F)			S
v-g	*180*^{TS}	1	+++	+++	0 to +++	(F)	−		S
	K451	1	++	+	0	+	+	+	
g-f	*K1563*^{TS}	1	+++	+++	0	(F)+	+	+	S
	K184	11	++	+++	0	(F)	−		S
	K499	1	+++	++	0	+	+	+	
	120	6	+++	+++	0	(F)			S
	K1193	1	+++	+++	0	(±F)			G
f-centro	*K1075*	1	+++	+	+,0	+	±		S

[a] Blank entries indicate no information.

[b] Symbols are: +++ = normal, ++ = reduced, + = strongly reduced, ϵ = nearly zero, 0 = zero. (D) indicates delay in development. In the fertility column, the entries for groups *371* and *K1075* reflect differences between alleles (see Table 3). *K254* is temperature sensitive with respect to viability and fertility, *473* and *180* with respect to fertility and *K575* and *K1563* with respect to the chorion defect.

[c] Symbols are: + = presence of defect, − = absence of defect, ± = presence of minor defect, (F) = eggs flaccid, (±F) = eggs slightly flaccid. In the ultrastructural defect column, the entry for group *K575* reflects a difference between alleles (see Table 3).

[d] Symbols are: G = germ line dependent, S = somatic line dependent. Data from WIESCHAUS, AUDIT and MASSON (1981) and N. PERRIMON and M. GANS, unpublished results.

Eight of the complementation groups that showed prominent ultrastructural defects were also analyzed by electrophoresis of chorion proteins. As summarized in Tables 3 and 5, all eight showed biochemical chorion defects (K. KOMITOPOULOU, L. H. MARGARITIS, M. GANS and F. C. KAFATOS, unpublished results; M. LOMBARD and W. H. PETRI, unpublished results).

Figure 7, top, summarizes the localization of eight complementation groups

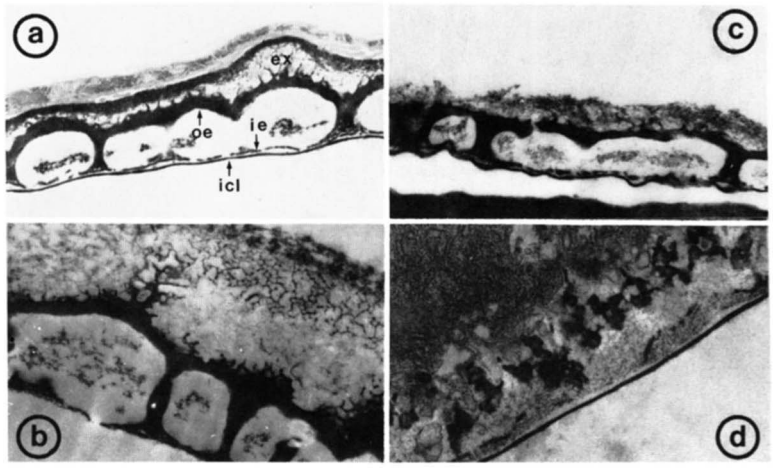

FIGURE 5.—Typical examples of characterization of female-sterile mutants by ultrastructural examination of the chorion. The wild-type or (homozygous) mutant genotype of the mother is indicated. *K1214* is shown as stage 13, wild type as stage 14 and the rest as newly ovulated eggs. a, Wild type. Note the appearance of the innermost chorionic layer (icl), the inner endochorion or "floor" (ie), the outer endochorion or "roof" (oe) and the exochorion (ex). b, In the *K418* (*sn*) series, the chorion appears reasonably normal ultrastructurally, except for unusual proportions which can be ascribed to the small size of the oocyte (see Figure 4c). c, *K313* is a typical example of mutants with minor chorion defects (± in Table 5), in this case slightly thinner roof with occasional interruptions. d, *K1214* is a typical example of mutants with clear chorion defect (+ in Table 5); in this case the innermost chorionic layer is normal, but the endochorion (and especially pillars and roof) is badly disorganized.

with strong chorion defects and three groups with only minor chorion defects. Figure 7, bottom, identifies the locations of 13 other groups that give morphologically abnormal eggs but no chorion defect.

Other defects

The flaccid egg phenotype shown by a number of mutants presumably corresponds to altered eggshell permeability. However, flaccidity varies with time after egg laying, medium composition and other variables and was not studied consistently. Three complementation groups characterized by very flaccid eggs were subjected to electrophoretic analysis of the total proteins in the mature oocyte (Figure 6). The *K184* group (alleles *K184*, *K294*, *K621*) showed clear deficiency of all three yolk proteins, YP1, YP2 and YP3; in parallel with their respective degree of flaccidity, *K184* showed the strongest protein deficiency among these three alleles. Despite their very flaccid appearance, *K93* eggs showed normal yolk protein content; the *K646* pattern was also approximately normal.

Two additional groups, which showed little or no flaccidity, were analyzed for yolk protein content: *K313* because it was localized in the vicinity of the YP1 and YP2 genes (BARNETT *et al.* 1980) and *K418* (alleles of *sn*) because of the small size of the eggs. No major deficiency of yolk proteins was apparent. In the case of *K418*, the small egg size probably results from failure of the

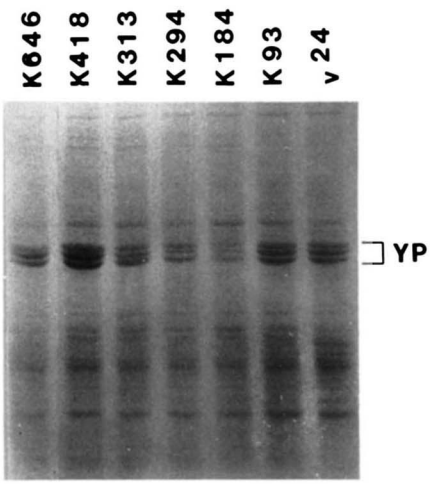

FIGURE 6.—Analysis of oocyte proteins in female-sterile mutations. The proteins were resolved in SDS gels with 10–15% polyacrylamide gradient and stained with Coomassie blue. The content of the three major yolk proteins (YP) can be evaluated relative to the other bands. *K93*, *K313* and *K646* can be seen to be essentially normal, by comparison with the wild type (v^{24}) profile. In *K418* the yolk proteins are overrepresented, presumably as a consequence of normal yolk uptake in the absence of full oocyte growth. By contrast, *K294* and especially its extreme allele, *K184*, show clear deficiency of all three yolk proteins.

nurse cells to make their normal contribution rather than from any defect of the oocyte itself: stage 14 follicles show nondegenerated nurse cells (Figure 4c).

Tests for viability, fecundity and fertility at two temperatures were performed for 25 of the most interesting complementation groups with morphologically abnormal eggs. As summarized in Table 5, viability and fecundity were normal in most cases, although viability was reduced in some and fecundity in a larger number of groups. Three complementation groups, *i.e.*, *K254*, *473* and *180*, were temperature sensitive with respect to fertility and one of these, *K254*, also with respect to viability, especially for females (normal or nearly normal at 23°, zero or nearly zero at 29°). The prominent chorion defects of *473* and *K254* were not temperature sensitive, however. By contrast, *K575* and *K1563* showed temperature sensitivity for chorion (abnormal only at 29°) but not for fertility (zero at both 29° and 23°).

The tissue responsible for the abnormalities of 18 category II mutants was determined by clonal analysis (WIESCHAUS, AUDIT and MASSON 1981; N. PERRIMON and M. GANS, unpublished results). As summarized in Table 5, 14 of these proved to be somatic line dependent, and four were germ line dependent. Of the complementation groups that showed clear-cut ultrastructural and biochemical defects in the chorion, five were tested for tissue specificity, and all were somatic line dependent; presumably, the primary defect in this case is in the follicular cells. Of the complementation groups that showed only minor ultrastructural defects in the chorion, two were somatic line dependent, and, surprisingly, one (*371*) was germ line dependent.

Comments on specific chorion mutants

384 group: In addition to its extremely high mutability, this group is of interest because of its interaction with another mutant, *1501*, which is located nearby. Both the *384* series and *1501* map to the right of *ct* and almost certainly to the left of *sn*. By mapping relative to *ct* (*20*) and *oc* (*23.1*), *1501* was localized to *20.7* (*20.2* to *21.7*) and *384* to *20.5* (*20.2* to *20.9*). Neither *1501* nor *384* (and its tested alleles *1336, K467, K718, K1090*) is uncovered by *Df(1)RA2* or $Df(1)ct^{268.42}$. Localization of the *384* series to the left of *sn* (*21*) is further supported by the fact that *384* is not uncovered by $Df(1)sn^{c188}$ but is uncovered by $Df(1)ct^{4b1}$ obtained from G. LEFEVRE. Localization of *1501* to the left of *sn*, probably in a previously described haplolethal region (LEFEVRE and JOHNSON 1973), is further supported by two observations. In a second experiment performed to localize *1501*, of 68 recombinants between *sn* and *v*, none resulted in crossing over between *sn* and *1501*, indicating that *1501* is either to the left of *sn* or to its right but within 0.7 cM. The latter possibility is extremely unlikely, since *1501* is not uncovered by $Df(1)sn^{c128}$, whereas the *fs(1)h* mutation 0.9 cM to the right of *sn* is uncovered (F. FORQUIGNON, personal communication).

Although *1501* and the nearby *384* series (all alleles) fail to complement their fertility defect, they do complement the morphological egg abnormality. Furthermore, they are cytogenetically separable: the $Df(1)ct^{4b1}$ from G. LEFEVRE (henceforth *LF*) uncovers all alleles of the *384* series but not *1501*. Surprisingly, the $Df(1)ct^{4b1}$ from Bowling Green (henceforth *BG*) uncovers neither *1501* nor any allele of the *384* series. The difference between the two deficiencies is not due to the presence of *oc* in *LF*: not only is *384/oc* fertile with normal eggs, but when *oc* is removed from the *LF* stock, the deficiency continues to uncover all alleles of the *384* series, with respect to both fertility and morphological egg abnormality.

Two explanations are possible for the difference between the two deficiency stocks. One is that the respective deficiencies are identical but that a mutation allelic to *384* has arisen in the *LF* stock. This seems unlikely, since females heterozygous for $Df(1)ct^{4b1}$*(LF)* and *1501* (hypothetically $Df(1)ct^{4b1}$, *384*/1501*) are fertile, whereas all known alleles of *384* in *trans* with *1501* are sterile. We consider more likely the alternative hypothesis that the two deficiencies have slightly different breakpoint(s). Indeed, salivary gland squashes of $Df(1)ct^{4b1(LF)}$/ $Df(1)ct^{4b1(BG)}$;DpFN*107* were examined by G. RICHARDS, who confirmed that *BG* is slightly more distal than *LF* (tentative limits for *BG* 7A3-5; 7B8-C1,2). Possibly *BG* is identical with $Df(1)ct^{268.42}$.

In summary, although other explanations are possible, we favor the interpretation that two separate genes are defined by *1501* and by the *384* series and that these genes interact in *cis*, with respect to fertility.

K575: The two alleles of this group are quite different: although both are sterile, *1059* shows only a minor chorion defect, whereas *K575* shows a prominent defect, at 29°. It is not known whether sterility and the chorion abnormality of *K575* are due to the same mutation.

K254: Fertility is temperature sensitive, being approximately 50% of normal at 23° but zero at 25° and 29°. Furthermore, the stock is characterized by temperature-sensitive inviability: at 23° and 25° partial inviability of females only and at 29° complete pupal mortality for both sexes. The chorion abnormality is not temperature sensitive. Genetic and cytogenetic analysis indicates that, if these properties are due to more than one mutation, the mutations are very closely located.

K1214: At all temperatures, the viability of this stock is slightly depressed (80–90% of normal), and its fertility is close to but not zero. The chorion defect is also not temperature sensitive.

K79, 473: These mutants are provisionally classified as nonallelic, on the basis of observations by M. F. LOMBARD and W. H. PETRI (personal communication), which suggests that *473* contains two mutations, one located within the region defined by *Df(1)RA2* and responsible for temperature-sensitive sterility and the other located outside that region and responsible for the chorion defect, which is not temperature sensitive. The *K79* mutation responsible for chorion defect is located within the *Df(1)RA2* region, which also encompasses a cluster of chorion structural genes (SPRADLING 1981). Detailed complementation analysis remains to be performed among *K79*, *473* and the known chorion mutants of the same region, *oc* and *cor-36* (DIGAN *et al.*, 1979); electron microscopy will probably be necessary as part of this analysis.

K451: Viability is reduced at all temperatures, especially for females (33–72% of normal). The mutation responsible for this property is located in the region defined by $Df(1)g^{1}$: indeed, the heterozygous females, $K451/Df(1)g^{1}$, are inviable at all temperatures (in contrast to the hemizygous males).

K1563: This mutation appears to be partially dominant, especially at 29°. The chorion defect is temperature sensitive, whereas fertility is essentially zero at all temperatures. Although the genetic localization of the mutation at 44.7 map units is unambiguous, an interesting interaction is observed with the distal region encompassed within *Df(1)RA2*, which includes a cluster of chorion structural genes: after several hours from the time of egg laying, only 3% of the eggs laid by *K1563/FM7C* individuals are flaccid, whereas 83% of the eggs laid by *K1563/Df(1)RA2* are flaccid under the same conditions.

K499: This stock may contain two or more mutations: in an experiment designed to localize genetically the sterility mutation, in addition to the parental (wild type and *K499*) phenotypes a whole series of new phenotypes were recovered. Our best estimate is that a sterility mutation is located near *g*.

DISCUSSION

Some general comments

The new mutagenesis experiment yielded results broadly similar to those reported earlier (GANS, AUDIT and MASSON 1975). Since the dose of EMS used and the proportion of induced lethals were the same in the two experiments, it is not surprising that the incidence of female steriles was also similar (8.7% of the chromosomes examined in the previous study, 8.1% in the present one).

Furthermore, the proportions of female steriles belonging to the three categories were also similar in the two studies: for category I, 17% previously and 16% now; for category II, 38% and 30%, respectively; and for category III, 45% and 54%, respectively. Finally, alleles were recovered from both experiments for seven out of nine allelic series in categories II, IV and VI.

The protocol for recovery of mutants was somewhat different in the new study. Because of our interest in eggshell mutants, partial female-sterile and female-fertile stocks, in addition to female steriles, were screened for morphological abnormalities of the eggs. Three mutants were thus recovered, but of these the two partial female steriles were allelic to female-sterile mutants with minor chorion defects. The low yield of chorion mutants (and, especially, new complementation groups) among partial female-sterile or -fertile stocks, in contrast to their prevalence among female-sterile stocks, validates the strategy of using female sterility as a guide in the initial screen for chorion mutants. On the other hand, some interesting mutants such as *K79* would be missed by exclusive reliance on this strategy.

In the previous study, mutants that showed a markedly reduced viability were not retained. Two such mutants were recovered in the present study among category II mutants, and one of these (*K254*) was accompanied by a prominent chorion defect. Furthermore, two additional mutants with prominent chorion defects (*K451* and *K1214*) showed more limited effects on viability. These results suggest that it may be inadvisable to reject mutants with reduced viability, if one is interested in all chorion mutants. For example, some mutants affecting amplification (which is known to be important for choriogenesis; SPRADLING and MAHOWALD 1981) may have a weaker effect on general DNA replication, thus reducing viability.

Despite the differences between the previous and present studies, the similarities are sufficient for results to be combined. The observation that a considerable number of complementation groups are represented by single alleles (Table 3) clearly indicates that many *X* chromosome genes which affect egg morphology and, more specifically, chorion construction remain to be discovered.

Chorion mutants

As summarized in Figure 7, top, on the basis of their strong effects on chorion morphology we classify as chorion mutants nine complementation groups which were recovered in this and/or the previous study. Three additional complementation groups are provisionally not considered as chorion mutants, although they have a minor effect on chorion morphology. Such minor effects could easily be produced indirectly by a wide variety of mechanisms; for example (C. LAMOUR and L. H. MARGARITIS, personal communication), limited morphological chorion abnormalities are caused by the temperature-sensitive ecdysoneless mutant, *ecd*^{1} (GAREN, KAUVAR and LEPESANT 1977) at the restrictive temperature, apparently without a concomitant major biochemical effect on chorion protein synthesis (S. BELINSKI, D. BUSSON and C. LAMOUR, personal communication). Similarly, we do not as yet understand

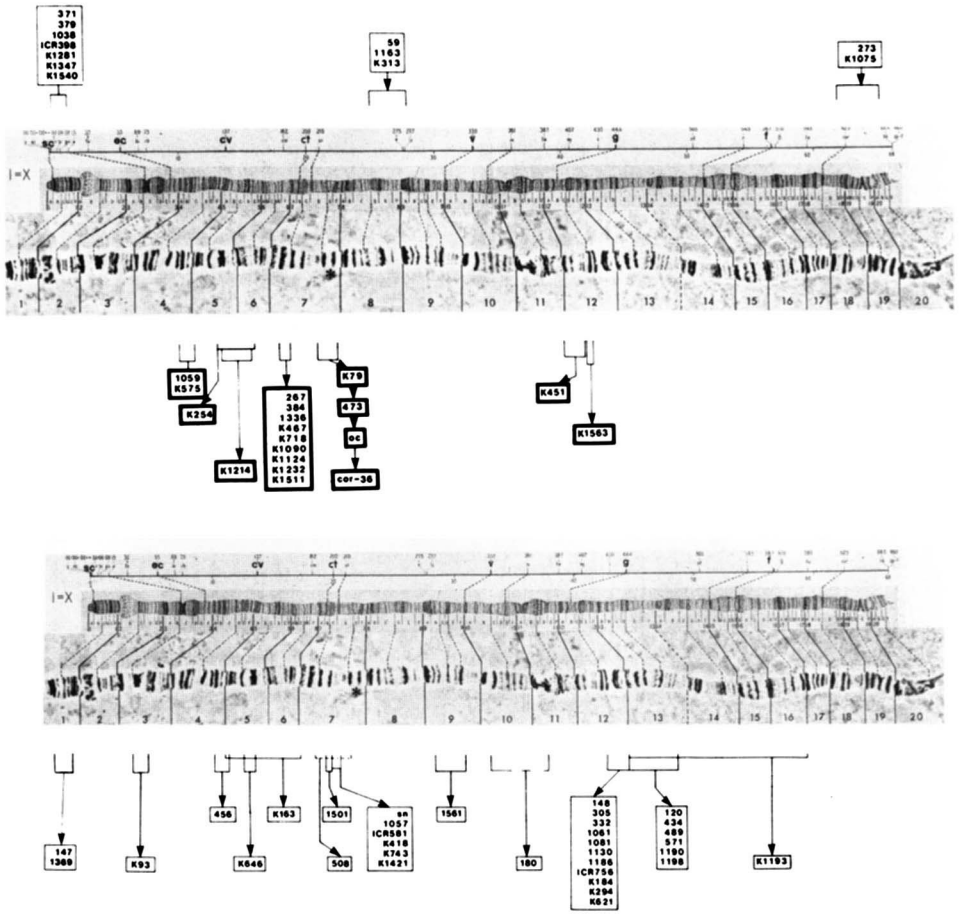

FIGURE 7.—Localization of female-sterile mutants (*cf.* Table 4). Top, Complementation groups that show minor or prominent chorion abnormalities are shown with thin and thick borders, above and below the chromosomal map, respectively. For the mutants (*K79*, *473*, *oc* and *cor-36*) which map in the vicinity of the known *s36-1* and *s38-1* chorion genes (asterisk), complementation analysis has not been performed. *K499* also shows prominent chorion abnormalities but has not been mapped. Bottom, Complementation groups that have no known chorion abnormalities.

the basis of most of the morphological egg abnormalities, which are unaccompanied by gross shell defect. Such abnormalities, chiefly flaccidity, may be due to subtle eggshell defects, such as increased permeability of the vitelline membrane or the wax layer, or they may result from lesions in any one of the three cell types in the follicle, including defects in yolk accumulation, nurse cell function or degeneration, follicular cell migration, etc. Further analysis of these mutations, including both germ line and somatic line defects, should be of considerable interest for understanding the cellular functions and interactions involved in oogenesis.

Needless to say, the molecular defects leading to prominent chorion abnormalities also remain to be determined. We cannot yet exclude the possibility

that even some of the putative chorion mutants act quite indirectly, although the data so far suggest otherwise. Thus, *K451* and *K1214* appear to act in *trans* to depress amplification of all major chorion genes (K. KOMITOPOULOU and W. ORR, unpublished observations), whereas the large allelic series, *384*, appears to affect minor chorion protein(s) (KOMITOPOULOU 1982). In general, we expect that genetic approaches such as the one used here, which are not narrowly targeted, will help identify genes that encode quantitatively minor but functionally important chorion proteins, as well as *trans*-acting genes that regulate chorion gene amplification and expression. The molecular approach used previously is best suited to identification of genes for major chorion proteins (SPRADLING and MAHOWALD 1979; GRIFFIN-SHEA *et al.* 1980; SPRADLING *et al.*, 1980; YANNONI and PETRI 1980, 1981; SPRADLING 1981; GRIFFIN-SHEA, THIREOS and KAFATOS 1982). On the other hand, genetic analysis targeted to the vicinity of chorion structural genes is most likely to yield *cis*-acting regulatory mutants such as ocelliless, which is a breakpoint that depresses amplification from a presumed nearby origin (SPRADLING, WARING and MAHOWALD 1979; SPRADLING and MAHOWALD 1980, 1981).

Once chorion mutants are precisely localized cytogenetically, they can be used for molecular analysis, such as cloning and characterization of the respective genes by transcriptional mapping, hybrid-selected translation, transformation, etc. Caution is necessary, however, because of the possibility that mutant chromosomes carry more than one mutation: the EMS dose used was so high that multiple hits per chromosome can be expected. Ideally, parts of the mutagenized *X*-chromosome other than the one that includes the mutation of interest should be recombined out before final molecular characterization; a limitation is that it is extremely difficult to separate very closely linked mutations.

In conclusion, the new mutagenesis experiment has yielded a number of interesting mutations. In addition to other types (dominant female steriles and category I and III mutations), the new mutants which were obtained, together with similar mutants from the previous study (GANS, AUDIT and MASSON 1975) help define 28–31 genes that affect egg morphology, including nine genes that affect chorion formation.

We are grateful to M. LEFUR, curator of stocks at Gif, for excellent technical assistance. We thank N. PERRIMON, S. BELINSKI, D. BUSSON, C. LAMOUR, M. LOMBARD, W. H. PETRI and W. ORR for permission to quote unpublished results, G. RICHARDS for evaluation of salivary gland chromosome squashes and C. FROTHINGHAM for secretarial assistance. We thank the individuals and stock centers identified in Table 1 for sending us various mutant stocks. This work was supported by a fellowship from the European Molecular Biology Organization (K. K.) and by grants from C.N.R.S. and the University Pierre et Marie Curie (M. G.), N.A.T.O. (L. H. M.) and the National Institutes of Health and the American Cancer Society (F. C. K.).

LITERATURE CITED

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

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

BARNETT, T., C. PACHL, J. GERGEN, and P. C. WENSINK, 1980 The isolation and characterization of *Drosophila* yolk protein genes. Cell **21:** 729–738.

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

CRAYMER, L. and E. ROY, 1980 New mutants: *Drosophila melanogaster.* Drosophila Inform. Serv. **55:** 200–204.

DIGAN, M. E., A. C. SPRADLING, G. L. WARING and A. P. MAHOWALD, 1979 The genetic analysis of chorion morphogenesis in *Drosophila melanogaster.* pp. 171–181. In: *Eucaryotic Gene Regulation,* ICN-UCLA Symposium, Edited by R. AXEL, T. MANIATIS, and C. F. FOX. Academic Press, New York.

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

GAREN, A., L. KAUVAR and J. A. LEPESANT, 1977 Roles of ecdysone in *Drosophila* development. Proc. Natl. Acad. Sci. USA **74:** 5099–5103.

GRIFFIN-SHEA, R., G. THIREOS and F. C. KAFATOS, 1982 Organization of a cluster of four chorion genes in *Drosophila* and its relationship to developmental expression and amplification. Dev. Biol. **91:** 325–336.

GRIFFIN-SHEA, R., G. THIREOS, F. C. KAFATOS, W. H. PETRI and L. VILLA-KOMAROFF, 1980 Chorion cDNA clones of *Drosphila melanogaster* and their use in studies of sequence homology and chromosomal location of chorion genes. Cell **19:** 915–922.

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.

KOMITOPOULOU, K., 1982 Oogenesis mutants and genetic, biochemical and morphological characterization of choriogenesis mutants in *Drosophila melanogaster.* Ph.D. Thesis, University of Athens, Greece.

LEFEVRE, G. and T. K. JOHNSON, 1973 Evidence for a sex-linked haplo-inviable locus in the cut-singed region of *Drosophila melanogaster.* Genetics **74:** 633–645.

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, 1982 Genetic variations of Drosophila melanogaster, 1967, Ed. 2. Carnegie Inst. Wash. Publ. 627.

MOHLER, J. D., 1977 Developmental genetics of the *Drosophila* egg. I. Identification of the 59 sex-linked cistrons with maternal effects on embryonic development. Genetics **85:** 259–272.

SPRADLING, A. C., 1981 The organization and amplification of two chromosomal domains containing Drosophila chorion genes. Cell **27:** 193–201.

SPRADLING, A. C., M. E. DIGAN, A. P. MAHOWALD, M. SCOTT and E. A. CRAIG, 1980 Two clusters of genes for major chorion proteins of *Drosophila melanogaster.* Cell **19:** 905–914.

SPRADLING, A. C. and A. P. MAHOWALD, 1979 Identification and genetic localization of mRNAs from ovarian follicle cells of *Drosophila.* Cell **16:** 589–598.

SPRADLING, A. C., and A. P. MAHOWALD, 1980 Amplification of genes for chorion proteins during oogenesis in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **77:** 1096–1100.

SPRADLING, A. C. and A. P. MAHOWALD, 1981 A chromosome inversion alters the pattern of specific DNA replication in Drosophila follicle cells. Cell **27:** 203–209.

SPRADLING, A. C., G. L. WARING and A. P. MAHOWALD, 1979 *Drosophila* bearing the ocelliless mutation underproduce two major chorion proteins both of which map near this gene. Cell **16:** 609–616.

WIESCHAUS, E., C. AUDIT and M. MASSON, 1981 A clonal analysis of the roles of somatic cells and germ lines during oogenesis in *Drosophila.* Dev. Biol. **88:** 92–103.

WIESCHAUS, E., J. L. MARSH and W. J. GEHRING, 1978 The development and function of the female germ line in *Drosophila melanogaster*: a cell lineage study. Dev. Biol. **68:** 29–46.

YANNONI, C. Z. and W. H. PETRI, 1980 Characterization by isoelectric focusing of chorion protein variants in *Drosophila melanogaster* and their use in developmental and linkage analysis. Wilhelm Roux Arch. **189:** 17–24.

YANNONI, C. Z. and W. H. PETRI, 1981 *Drosophila melanogaster* eggshell development: localization of the S19 chorion gene. Wilhelm Roux Arch. **190:** 301–303.

Corresponding editor: A. CHOVNICK