X-LINKED FEMALE-STERILE LOCI IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

We have examined the number of X-linked loci specifically required only during oogenesis. Complementation analyses among female-sterile (fs) mutations obtained in two mutagenesis screens—GANS' and MOHLER's—indicate that any fs locus represented by two or more mutant alleles in GANS' collection are usually present in MOHLER's collection. However, when a locus is represented by a single allele in one collection, it is generally not present in the other collection. We propose that this discrepancy is due to the fact that most "fs loci" represented by less than two mutant alleles are, in fact, vital (zygotic lethal) genes, and that the fs alleles are hypomorphic mutations of such genes. In support of this hypothesis we have identified lethal alleles at 12 of these "fs loci." The present analysis has possibly identified all maternal-effect lethal loci detectable by mutations on the X chromosome and has allowed us to reevaluate the number of "ovary-specific fs" loci in the Drosophila genome. Finally, germline clone analysis of a large number of fs mutations was performed in order to estimate the relative contribution of germline and somatic cell derivatives to oogenesis and to embryonic development. All the maternal-effect lethal loci tested are germline-dependent.

KNOWLEDGE of the number of genes for which activities are limited to a specific developmental process is a key initial step toward understanding the genetic control of the process. In Drosophila, oogenesis is one such developmental process for which such knowledge is of interest [see KONRAD *et al.* (1985) for recent review]. The genetics of oogenesis has been extensively analyzed following screens for mutations causing female sterility. Many such experiments have been performed on the X chromosome (GANS, AUDIT and MASSON 1975; MOHLER 1977; KOMITOPOULOU *et al.* 1983; MOHLER and CARROLL 1984; L. ENGSTROM *et al.*, unpublished results) where the number of X-linked female-sterile (fs) loci has been estimated to be around 100, or approximately 10% of the genome (GANS, AUDIT and MASSON 1975; MOHLER 1977; KING and MOHLER 1975).

It is possible that these female sterility mutations actually identify genetic

loci of two different types: (1) gene functions that are only utilized during oogenesis and (2) genes that function both during oogenesis and at other times but do not cause inviability when mutated. Because of the size of the mutagenesis experiments performed on the *X* chromosome (GANS, AUDIT and MASSON 1975; MOHLER 1977; KOMITOPOULOU *et al.* 1983; MOHLER and CARROLL 1984; L. ENGSTROM *et al.*, unpublished results), it is possible that all of the mutable genetic loci relating to female sterility have already been discovered. However, most of the loci are only represented by one allele, even when complementation tests are carried out between mutations in different screens. In this paper, we propose a new type of categorization of female sterility loci on the *X* chromosome, including the hypothesis that most fs loci represented by one or only a few alleles are hypomorphs of lethal genes. These observations allow us to suggest a more definitive list of the "ovary-specific" fs genes on the *X* chromosome.

MATERIALS AND METHODS

Stocks: Two major female-sterile stock collections were used, here referred to as GANS' (GANS, AUDIT and MASSON 1975; KOMITOPOULOU *et al.* 1983) and MOHLER's (MOHLER 1977; MOHLER and CARROLL 1984) collections. Some other fs mutations were obtained from ENGSTROM's screen for fs and grandchildless mutations (L. ENGSTROM *et al.*, unpublished results), others from the Bowling Green stock center and various sources listed by MOHLER and CARROLL (1984).

Complementation analysis: Fertility tests of females heterozygous for two fs mutations were performed on standard Drosophila media at 25° (when a nonthermosensitive mutation was analyzed) as described by GANS, AUDIT and MASSON (1975) or MOHLER (1977). Mutant ovarian phenotypes were examined by Feulgen staining or by direct bright field optics. Embryonic phenotypes of maternal-effect lethal fs mutations were examined using HOYER's cuticular preparations (VAN DER MEER 1977).

Germline clonal analyses: Germline clonal analyses were performed using the dominant female-sterile technique described by PERRIMON and GANS (1983) that utilizes the mutation *Fs(1)K1237*. A fs mutation is germline-dependent if eggs derived from homozygous fs germline clones exhibit the same phenotype as derived from homozygous fs flies. Alternatively, if eggs are produced from germline clones that are normal in all respects, then the fs mutation is somatic-dependent. Progeny from the cross *FM3/fs* crossed with *Fs(1)K1237* v^{24}/Y males were irradiated with a constant dose of 1000 rads for 40 sec (gamma-ray machine; Model GR-9 Co-60 irradiator) at the end of the first instar larval stage. Such conditions generate mosaic germlines in 5% of the *fs/Fs(1)K1237* females. The "fs" chromosome usually carries recessive visible markers that allows the identification of distal mitotic recombination events (PERRIMON and GANS 1983). In each experiment about 300 *fs/Fs(1)K1237* females were examined for the presence of germline clones. Flies possessing germline clones were individually analyzed, and the phenotype of the eggs and eventual progeny were examined. If no females possessing germline clones were found, all flies were dissected and their ovarian phenotype examined.

RESULTS

Scope of screens and rationale: Two independent fs screens (GANS, AUDIT and MASSON 1975; MOHLER 1977; MOHLER and CARROLL 1984) have identified 52 (containing one to seven alleles each) and 109 (with one to 16 alleles each) loci, respectively. The frequency distributions of the number of alleles

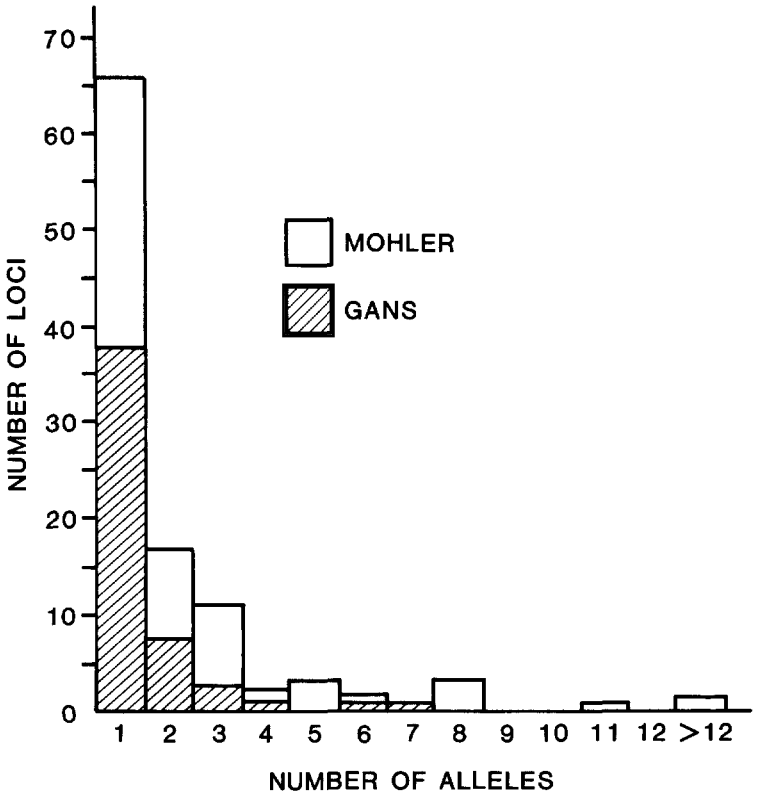

FIGURE 1.—Distribution of the number of alleles per female-sterile locus recovered during two different mutagenesis experiments. Areas in dashed lines refers to GANS' (GANS, AUDIT and MASSON 1975), the other one to MOHLER's (MOHLER 1977; MOHLER and CARROLL 1984).

per locus from the two independent screens are similar (Figure 1). Using the data from these mutagenesis screens, the number of loci on the *X* chromosome has been estimated to be about 100 (KING and MOHLER 1975) (however, see DISCUSSION for a modification of this estimate). If this estimate reflects the total number of *X*-linked fs loci, then MOHLER's screen should have identified all, or nearly all, such loci. Therefore, it is reasonable to postulate that the fs loci in the GANS' collection should be present in MOHLER's.

Complementation tests: We have tested this postulate by crossing alleles of the 33 complementation groups still available from Gans' collection (GANS, AUDIT and MASSON 1975; KOMITOPOULOU *et al.* 1983) (all of the complementation groups lost had only one allele) to alleles representing every complementation group in MOHLER's. The details of this analysis are presented in Table 1 and plotted in Figure 2. Of the 19 loci remaining from the GANS collection represented by a single allele, only six (31%) were present in MOHLER's collection; whereas, of 14 loci represented by more than one allele in GANS' collection, 12 (86%) were present in MOHLER's. These results indicate that previous estimates of the number of fs loci are inaccurate, because most of the loci with only one allele in the Gans collection are not present in the

TABLE 1

Complementation test results

Mutation from GANS' screen	Locus in MOHLER's	No. of alleles in GANS'	No. of alleles in MOHLER's
73	*M12*	2	3
99		1	0
107		1	0
147	*M5*	2	3
148	*M25*	11	8
180		1	0
231	*M101*	3	8
336		1	0
384	*M102*	9	14
387	*M10*	2	8
456	*M66*	1	1
457	*M22*	1	2
508		1	0
572		1	0
573	*M18*	1	6
1140		1	0
1182		1	0
1187		1	0
1456	*M16*	1	1
1501		1	0
1502	*M44*	2	2
1509		1	0
1561		2	0
1578		1	0
K79	*C2*	1	1
K313		2	0
K418	*M45*	5	3
K499	*M23*	2	2
K575	*M60*	3	1
K646	*M3*	1	4
K1075	*M114*	2	1
K1214		1	0
K1540	*M6*	7	3

Thirty-three different mutations from GANS' were examined for alleles in MOHLER's collection. fs loci in GANS' collection with the letter "*K*" are from KOMITOPOULOU *et al.* (1983); others are from GANS, AUDIT and MASSON (1975). The number of fs mutation tested and the corresponding allelic series number from MOHLER's are shown. The respective number of alleles in each allelic series is given. Note that, when only one allele has been isolated in GANS' collection, there is almost no homology inside MOHLER's.

MOHLER collection. In total, the 109 fs complementation groups from MOHLER and the 33 from GANS identify 124 fs complementation groups, 80 of which are represented by one allele and 44 by more than one.

Some fs loci are alleles of zygotic lethals: At 12 fs loci, many lethal alleles have been recovered by other workers and by us (see Table 2 for references)

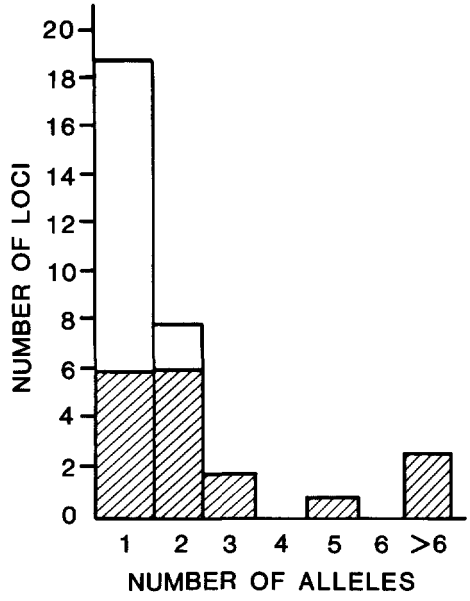

FIGURE 2.—Thirty-three different mutations at 33 different loci (see Table 1 for details) from GANS' screen were examined for allelism among MOHLER's. In this graph, the number of loci common to GANS and MOHLER has been plotted as a function of the number of alleles present in GANS' collection (unhatched: loci in GANS collection; hatched: loci in GANS collection also found in MOHLER collection). Note that very few new alleles are recovered in class 1.

TABLE 2

fs alleles of lethals

Locus	No. of fs alleles		References to lethal alleles
	fs screen	Visible	
deep orange	1	1	1
disheveled	0	1	1
fs(1)h	4	0	1, 3
fused	0	6	2
pentagon	1	1	1
platinum	1	1	1
raspberry	0	1	1
rudimentary	Many	Many	4
Sex-lethal	2	0	5
107	1	0	2
K451	1	0	6
goggle	1	0	7

To date, lethals alleles at 12 fs loci have been identified. The number of fs alleles obtained during fs screens (see Table 3 for details) or for visible (LINDSLEY and GRELL 1968) and references are given for each loci. References on the origin of zygotic lethal alleles: 1, G. LEFEVRE (personal communication); 2, N. PERRIMON (unpublished observations); 3, DIGAN *et al.* (1985); 4, NORBY (1970); 5, CLINE (1984); 6, ORR, KOMITOPOULOU and KAFATOS (1984); 7, J. D. MOHLER (unpublished observations).

indicating that these gene functions are also required for viability. Most of these loci are represented by only one female-sterile allele; however, there are three exceptions: *fused*, *rudimentary* and *fs(1)h*, in which multiple alleles have been isolated. In the case of *fused* and *rudimentary*, most mutations were first isolated based on their visible phenotype. These mutations are associated with both a visible and a female sterility phenotype, and are hypomorphic. In the case of *fs(1)h*, many alleles were obtained following an extensive genetic analysis of the locus (DIGAN *et al.* 1986). There are also a large number of fs loci that show poor viability (*i.e.*, *diminutive*, *tiny*, *refringent*, *Hairy-wing*), even though no lethal alleles have yet been characterized. It is possible that most of the loci with rare alleles are actually essential genes (*cf.* DISCUSSION).

Classification of X-linked female-sterile loci: Because of the size of these *X*-chromosomal mutagenesis screens, it is useful to assemble a list of all of the female steriles in the *X* chromosome that have been studied and to provide a brief description of their phenotypes. We have arranged them according to the categories of GANS, AUDIT and MASSON (1975) (Table 3).

Female-sterile loci affecting early oogenesis: Most mutations that disrupt early oogenesis appear to affect interactions of nurse cells, the oocyte and the follicle cells (*e.g.*, *diminutive* and *tiny*). The most thoroughly studied locus is *ovarian tumor* (*otu*) (KING *et al.* 1981; KING and RILEY 1982; KING *et al.*, unpublished results) for which many fs alleles exist without the existence of a lethal allele. Wild-type activity of the *otu* locus is required for establishment of ovarian germ cells, for correct division of the germ cells and for normal development within the 15 nurse cell-oocyte syncytium. All of the available 18 alleles express ovarian tumors to some degree. Some alleles produce agametic ovarioles, pseudonurse cells with polytene chromosome or abnormal oocytes.

The only screen for dominant female steriles on the *X* chromosome was carried out by GANS, AUDIT and MASSON (1975), and the only fully penetrant dominant fs mutation forms are alleles, (called Ovo^D), in spite of different phenotypes for the three alleles (PERRIMON 1984). The effects are thought to be antimorphic in nature (BUSSON *et al.* 1983). Revertants of the Ovo^D mutations usually exhibit nondissociable recessive fs phenotypes.

Female-sterile loci affecting late oogenesis: In this category, we have identified 30 loci that have activities that are required during late oogenesis. Females homozygous for these mutations usually produce collapsed eggs. During late oogenesis two major processes occur, vitellogenesis and choriogenesis. One locus that disrupts a fundamental step of vitellogenesis has been characterized. Oocytes in females homozygous for null alleles of *fs(1)yolkless* appear to lack coated vesicles which are involved in receptor-mediated endocytosis (WARING, DIORIO and HENNEN 1983; DIMARIO 1985).

Some of the loci involved in the production of the vitelline membrane and chorion have been studied in detail. Mutations of at least two loci, *defective chorion*[1] (*dec 1*) (A. CARROLL and D. MOHLER, unpublished results) and *cor 36*, (DIGAN *et al.* 1979), affect choriogenesis. A complex complementation pattern is observed between mutations at the *cor 36* locus (A. CARROLL and D. MOHLER, unpublished results). There are four alleles, *cor 36*, *dec 2*, *K79* and *ocel-*

liless (*oc*), all of which produce female sterility and affect the chorion in similar ways. Molecular characterization of *oc* has indicated that the pleiotropic phenotype (eye defect and female sterility) is a result of a small inversion disrupting two genes, each responsible for one component of the phenotype. The distal breakpoint is within the region of gene amplification associated with two major chorion genes and results in the underproduction of these two chorion proteins (SPRADLING and MAHOWALD 1981). Analysis of the pattern of protein synthesis in ovaries of *cor 36* indicates that only one of these major chorion proteins is missing (DIGAN *et al.* 1979). *cor 36* and *oc* weakly complement each other, presumably because there is sufficient chorion protein to make a functional chorion (DIGAN *et al.* 1979). The relationship of *dec 2* and *K79* to these two mutations is not yet clear. Both *dec 2* and *K79* fail to complement *oc*. *K79* fails to complement *cor 36*, whereas *dec 2* complements both *cor 36* and *K79*. Some of the complexity of this locus is probably due to the presence of two chorion genes at the site and the requirement of adequate amplification for normal chorion production. Some mutations that disrupt late oogenesis affect normal amplification of all chorion genes (*e.g.*, *fs(1)K1214*, *fs(1)K254*, and *fs(1)K451* ORR, KOMITOPOULOU and KAFATOS 1984). It is interesting that *fs(1)K451* has been shown to be allelic to *l(1)mus101*, a mutation which affects an essential mitotic function (ORR, KOMITOPOULOU and KAFATOS 1984). Oocytes produced by homozygous *fs(1)Nasrat* females produce eggs with vitelline membrane defects (KERN 1979) as well as some embryos with defective development (COUNCE and EDE 1957). Recently, a new allele of *fs(1)Nasrat* ($fs(1)N^{211}$) has been described that produces the "torso" embryonic phenotype (DEGELMANN *et al.*, unpublished results).

Some fs loci within this phenotypic class also exhibit somatic pleiotropic effects. Examples include (1) *dunce* which is involved with cyclic AMP metabolism; flies homozygous or hemizygous for *dunce* are poor learners, and homozygous female are sterile (BYERS, DAVIS and KIGER 1981); (2) homozygous and hemizygous *singed* flies that possess gnarled bristles and reduced nurse cell divisions during oogenesis (KING 1970); (3) homozygous and hemizygous *lozenge* flies that possess reduced eyes; the mutation also results in egg retention during oogenesis (KING 1970), the correlation between the egg retention, fs phenotype and the reduced eye phenotype of *lozenge* has not been elucidated; and (4) *mei 41* appears to disrupt an essential process of meiosis (BAKER and CARPENTER 1972) and belongs to the class of mutagen-sensitive lethal loci (MASON *et al.* 1981).

Female-sterile loci affecting embryogenesis: The 19 loci in the third category exhibit either strict maternal-effect lethal (MEL) phenotypes or paternally rescuable MEL phenotype.

Seven loci exhibit strict MEL phenotype. Embryos derived from homozygous *fs(1)Y2* females are arrested during syncytial cleavage stages because the normal $Y2^{+}$ gene product is necessary for nuclear divisions (YOUNG and JUDD 1978; ZALOKAR, AUDIT and ERK 1975). A similar MEL phenotype is exhibited by *fs(1)1242*. Mutations at two MEL loci result in dorsalized embryonic phenotypes, *fs(1)k10* (*k10*, Figure 3B) and *fs(1)gastrulation defective* (*gd*, Figure 3C).

TABLE 3

Classification of female-sterile mutations

	Location	GANS	MOHLER	Others	Spec.	References	NC
Early oogenesis							
diminutive	3D5	0	NT	1	GLD	1, 14, 15, 37	B2
Ovo^{D}	4C15-F1	*K1237*(4)	0	0	GLD	25, 31	C
1621	4F	*1621*(1)	NT	0	GLD	18, 31	B2
K741	5D5-6E1	*K741*(1)	NT	0	GLD	31	B2
K1274	5D5-6E1	*K1274*(1)	NT	0	GLD	31	B2
Sxl	6E4-7A2	0	*M106*(2)	0	GLD	37, 38	B2
otu	7F1	*231*(3)	*M101*(8)	7	GLD	2, 30, 31, 39	A
tiny	44.5*	0	0	1	GLD	1, 14, 31, 33	B2
Late oogenesis							
Hairy wing	1A-B	0	NT	1	NT	1, 14, 15	B2
147	1B9-E3	*147*(2)	*M5*(3)	0	NT		A
Nasrat	2A4	*K1540*(7)	*M6*(3)	2	GLD	1, 3, 6, 31	A
murky	0.8*	0	*M7*(1)	1	NT	1	B2
dunce	3D4	NT	*M42*(2)	2	GLD	13, 37	B1
K575	4F1-5A2	*K575*(3)	*M60*(1)	0	GLD	37	A
456	4F1-5A2	*456*(1)	*M66*(1)	0	SD	31	B2
pole hole	5C5-D6	*K646*(1)	*M3*(4)	3	GLD	6, 31	A
K1214	5D5-6E1	*K1214*(1)	0	0	SD	20, 31	B2
K254	5D5-6E1	*K254*	NT	0	SD	20, 31	B2
dec 1	7C1	*384*(9)	*M102*(14)	2	SD	30, 37, 39	A
1501	7C2-9	*1501*(1)	0	0	SD	31	B2
singed	7D1-2	*K418*(5)	*M45*(3)	4	GLD	1, 14, 15, 31	B1
pentagon	7E10-8A5	0	*M71*(1)	1	NT	1, 6	B2
cor 36	7F1	*K79*(1)	*C2*(1)	1	SD	19, 27, 34, 37	A
goggle	7F10	0	0	1	NT	1, 6	B2
lozenge	8D	NT	*M69*(2)	3	NT	1, 14, 15	B1
K313	8E-9B1	*K313*(2)	0	0	SD	30, 31	C
raspberry	9E3	0	0	1	GLD	6, 37	B2
disheveled	10B5	0	0	1	GLD	6, 37	C
180	36*	*180*(1)	0	0	SD	30	B2
K451	12A6-D3	*K451*(1)	NT	0	—[a]	20, 31	B2
K1563	44.7*	*K1563*(1)	NT	0	SD	31	C
120	47*	*120*(6)	0	0	SD	31	C
mei 41	12B13-D2	NT	*M37*(5)	1	NT	16	B2
yolkless	12E	*148*(11)	*M25*(8)	10	GLD	7, 37	A
K499	v-f	*K499*(2)	*M23*(2)	0	GLD	37	A
K1075	18E1-20A	*K1075*(2)	*M114*(1)	0	SD	31	A
125	56*	*125*(1)	NT	0	GLD	6, 37	B2
refringent	67.4*	0	0	1	NT	1	B2
Embryogenesis							
cinnamon	*0**	*0*	*M50*(1)	1	GLD	1, 21	B2
deep orange	*2A2-5*	*0*	0	2	GLD	1, 35	B2
pecanex	*2E2-3*	*387*(2)	*M10*(8)	1	GLD	8, 32	B1
k10	*2E3*	0	*M9*(3)	1	GLD	8, 9, 30, 32	A
paralog	*3B3*	*par*(1)	NT	0	GLD	26, 32	C
Y2	*3B4-6*	*73*(2)	*M12*(3)	1	GLD	4, 5, 37	A
107	*4A-F*	*107*(1)	0	0	GLD	6, 37	B2

TABLE 3—Continued

	Location	GANS	MOHLER	Others	Spec.	References	NC
swallow	14*	*1502*(2)	*M44*(2)	1	GLD	5, 10, 31	A
fs(1)h	7D1-6	*1456*(1)	*M16*(1)	2	GLD	22, 37	B2
platinum	7E11	0	*M47*(1)	1	NT	1, 6	B2
1242	7F	*1242*(1)	NT	0	GLD	5, 31	B2
almondex	8D	0	0	3	GLD	1, 17, 37	B1
gd	11A2-5	*573*(1)	*M18*(6)	4	GLD	5, 11, 37	A
457	49*	*457*(4)	*M22*(1)	0	GLD	5, 6, 37	A
M53	Near v	NT	*M53*(1)	1	GLD	6, 37	B1
151	53*	NT	*M29*(6)	1	GLD	12, 37	B1
rudimentary	15A1	Many	*M34*(16)	6	GLD	1, 24, 37	B2
fused	17D-E	0	0	6	GLD	1, 6, 23, 37	B2
1074	NT	*1074*(1)	NT	0	GLD	37	B2

Female steriles are grouped according to their effects on oogenesis and embryogenesis. In each category, a rough phenotypic classification is given, the cytological location or, alternatively, the meiotic position (*) is shown. The allelic series numbers in GANS and MOHLER are provided with the respective number of alleles (in parentheses); finally, numbers of mutations available from other sources are given. The tissue specificity of each fs locus is indicated by GLD (germline dependence) or SD (somatic dependence). For each fs locus, references for the map location, tissue specificity and mutant description are given. NT, not tested; NC, new classification. GANS and collaborators have identified two semidominant mutations: *fs(1)180* (GANS, AUDIT and MASSON 1975) and *fs(1)K1563* (KOMITOPOULOU *et al.*, 1983), but other characteristics of these loci have not been determined. Finally, an interesting environmental effect is observed in the case of the *fs(1)120* locus; although mutations are fs when flies are reared on standard Drosophila media, they are fertile on the media used at the University of Iowa.

References concerning the mapping and phenotypes: The following references GANS, AUDIT and MASSON (1975); KOMITOPOULOU *et al.* (1983); MOHLER (1977); and MOHLER and CARROLL (1984) have been omitted in the tables. (1) LINDSLEY and GRELL (1968); (2) KING *et al.* (1981); KING and RILEY (1982); R. C. KING *et al.* (unpublished results); (3) COUNCE and EDE (1957); KERN (1979); (4) YOUNG and JUDD (1978); (5) ZALOKAR, AUDIT and ERK (1975); (6) N. PERRIMON *et al.* (unpublished observations); (7) WARING, DIORIO and HENNEN (1983); P. J. DIMARIO and N. PERRIMON (unpublished observations); (8) PERRIMON, ENGSTROM and MAHOWALD (1984b); (9) WIESCHAUS, MARSH and GEHRING (1978); (10) KOMOROKSWA (1980); E. STEPHENSON and A. P. MAHOWALD (unpublished observation); MAHOWALD (1983); (11) K. D. KONRAD, T. GORALSKI and A. P. MAHOWALD (unpublished observations); (12) L. ENGSTROM and A. P. MAHOWALD (unpublished observations); (13) BYERS, DAVIS and KIGER (1981); (14) KING (1970); (15) KING and MOHLER (1975); (16) BAKER and CARPENTER (1972); MASON *et al.* (1981); (17) SHANNON (1972, 1973); (18) GOLLIN and KING (1981); (19) SPRADLING and MAHOWALD (1981); (20) ORR, KOMITOPOULOU and KAFATOS (1984); (21) BAKER (1973); (22) FORQUIGNON (1981); DIGAN *et al.* 1985; (23) COUNCE 1956; (24) NORBY (1970); JARRY (1979); SEGRAVES *et al.* (1983); (25) BUSSON *et al.* (1983); PERRIMON 1984; (26) THIERRY-MIEG (1982); (27) A. CARROLL and J. D. MOHLER (unpublished results).

References concerning the tissue specificity: (30) WIESCHAUS, AUDIT and MASSON (1981); (31) PERRIMON and GANS (1983); (32) PERRIMON, ENGSTROM and MAHOWALD (1984b); (33) DIMARIO and HENNEN (1982); (34) UNDERWOOD and MAHOWALD (1980); (35) MARSH *et al.* (1977); (36) THIERRY-MIEG (1982); (37) this study; (38) SCHUPBACH (1985); (39) E. M. UNDERWOOD, J. D. MOHLER and A. P. MAHOWALD (unpublished results).

[a] Ambiguous results were obtained in PERRIMON and GANS (1983).

k10 may have a more global effect than *gd* because the egg chambers as well as the embryos appear dorsalized (WIESCHAUS, MARSH and GEHRING 1978). Embryos derived from females homozygous for *fs(1)457* exhibit a twisted phenotype (Figure 3D; N. PERRIMON, unpublished results), and those from homozygous *fs(1)swallow* females exhibit clypeolabrum defects (Figure 3E) (KOMOROWSKA 1980; *cf.* figure 3 in MAHOWALD 1983). Mutations at the *paralog* locus

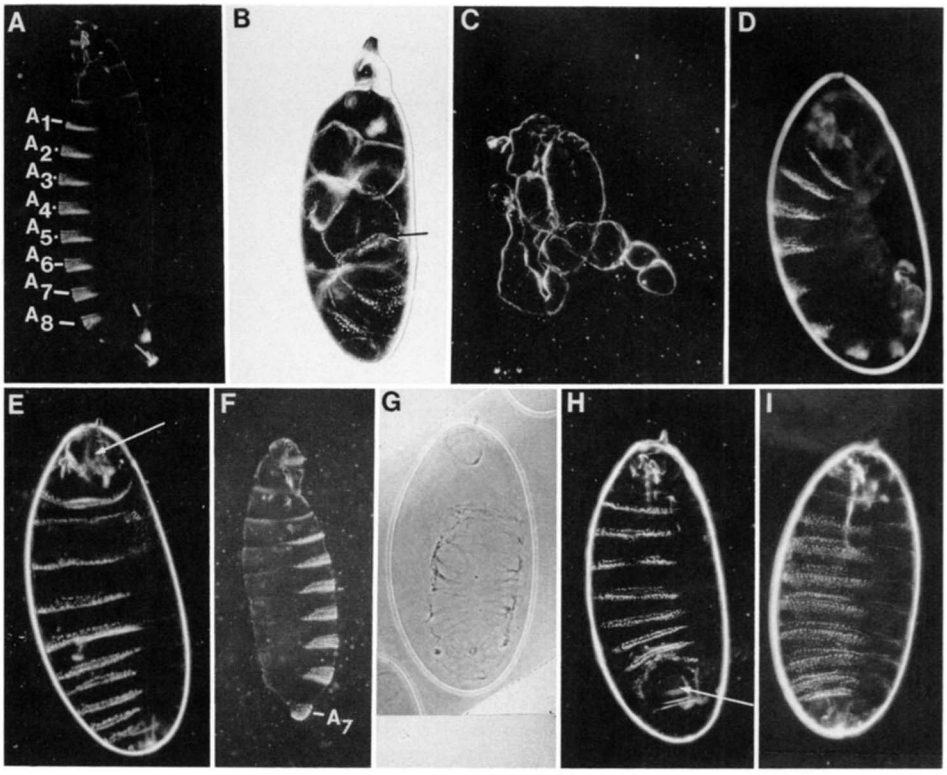

FIGURE 3.—Embryonic phenotypes of *X*-linked female-sterile loci. A, The pattern of a wild-type larva is shown; eight abdominal segments are clearly distinguishable. Maternal-effect female-sterile phenotypes can be paternally influenced (class 2) or not (class 1). In class 1, three types of phenotypes are found. The dorsalized phenotype is exhibited by embryos derived from females homozygous for *fs(1)k10* (B) and *fs(1)gastrulation-defective* (C); note, in case of *fs(1)k10*, the dorsalized egg shape. Embryos derived from homozygous *fs(1)457* females have a twisted phenotype (D) and those derived from females homozygous for *fs(1)swallow* exhibit a clypeolabrum defect (arrow in E). Embryos which exhibit the torso-like phenotype (F) are lacking structures posterior to abdominal 7; this phenotype is exhibited by only one allele at two fs loci: *fs(1)Nasrat*^{211} and *fs(1)pole hole*^{1901}. In class 2, three phenotypes are found. The neurogenic phenotype (G), which consist in an hypertrophy of the nervous system, is exhibited by *fs(1)pecanex*, *fs(1)107*, *fs(1)almondex*, *fs(1)M53* and *fs(1)1074*. Posterior defect are observed in case of *fs(1)151* (*fs(1)posterior midgut defect*) (H). Finally, embryos with a reverse polarity phenotype are found at the *fs(1)fused* locus (I).

produce pleiotropic effects and exhibit antimorphic behavior (THIERRY-MIEG 1982).

Twelve loci exhibit rescuable MEL phenotypes. Interestingly, five of them (*almondex*, *pecanex*, *fs(1)M53*, *fs(1)107* and *fs(1)1074*) produce neurogenic phenotypes (Figure 3G). Embryos derived from homozygous *fused* females exhibit a reversed polarity phenotype (Figure 3I) (COUNCE 1956; NÜSSLEIN-VOLHARD and WEISCHAUS 1980). *fs(1)posterior midgut defect* (formerly *fs(1)151*; MAHOWALD 1983; ENGSTROM *et al.*, unpublished results) appears to affect posterior midgut invagination (Figure 3H). The embryonic phenotypes associated with three other rescuable MEL do not produce consistent phenotypes, because

TABLE 4

Clonal analysis of female-sterile mutations

fs locus	No. of alleles tested	N GLC	Conclusion
K575	1	6	GLD
dec 1	2	10, 11	SD
cor 36	2	6, 12	SD
yolkless	3	6, 4, 7	GLD
K499	1	7	GLD
Y2	1	6	GLD
gd	2	13, 8	GLD
457	2	5, 9	GLD
dunce	1	6	GLD
M53	1	10	GLD
151	1	14	GLD
almondex	2	12, 8	GLD
diminutive	1	0	GLD
Sxl	1	4	GLD
raspberry	2	11, 8	GLD
disheveled	1	9	GLD
125	1	7	GLD
107	1	13	GLD
fs(1)h	3	5, 8, 7	GLD
fused	2	12, 7	GLD
rudimentary	2	10, 7	GLD
1074	1	11	GLD

The number of females possessing germline clones (N GLC) for each allele is shown. Nomenclature: germline dependent (GLD) and somatic dependent (SD).

lethality occurs at various embryonic stages (*cinnamon*, *deep orange*, *platinum* and *rudimentary*). *Cinnamon* and *deep orange* are involved in the synthesis of eye pigments and probably some essential pathways, and *rudimentary* is known to be responsible for three enzymes of the pyrimidine biosynthetic pathway. Although detailed information on the homeotic effects of *fs(1)h* have been published (FORQUIGNON 1981; DIGAN *et al.* 1986), no detailed analysis of the MEL phenotype produced by the *fs(1)h* locus has yet been reported.

It should be pointed out that all mutant alleles at one fs locus do not necessarily cause identical phenotypes. Striking examples of different phenotypes produced by different alleles are the *otu*, *fs(1)Nasrat* and *fs(1)pole hole* loci. Most alleles at the latter two loci result in collapsed egg phenotypes; however, *fs(1)Nasrat*^{211} (DEGELMANN *et al.*, unpublished results) and *fs(1)pole hole*^{1901} both cause torso-like (NÜSSLEIN-VOLHARD, WIESCHAUS and JURGENS 1982) embryonic MEL phenotypes (Figure 3F). This outcome indicates that different fs phenotypes can be generated by different mutations of one locus.

Germline clonal analysis of fs: The tissue specificity of mutations at 47 of the 57 loci listed in Table 3 was tested. In the present paper we analyze 22 of these 47 loci (Table 4) using the dominant female-sterile technique (PER-

RIMON and GANS 1983). Usually, more than one allele was analyzed when available (Table 4). Among the 22 new loci tested, 20 were found to be germline-dependent and only two somatic-dependent. There were no ambiguous results. In total, 36 of the 47 fs loci listed in Table 3 disrupt a germline function. It is known only in the case of two somatic line-dependent fs (*dec 1* and *cor 36*) that the defect is associated with ovarian (*i.e.*, follicle cells) and not extraovarian cell type. It is interesting to notice that all fs loci which exhibit MEL phenotype are germline-dependent.

DISCUSSION

As part of our long-term study of the genetic control of oogenesis, we have analyzed the female sterility mutations on the *X* chromosome identified in a number of laboratories. Because of the number of chromosomes (8000) analyzed, previous workers (*e.g.*, KING and MOHLER 1975) have suggested that the chromosome was nearly saturated for all of the complementation groups affecting female fertility. A number of observations argue against this proposal. Thus, we have found that most of the loci are represented by only one allele, even when we tested mutations from different screens. We have also discovered that lethal alleles exist for 12 of these female-sterile mutations. In these cases it is clear that the locus is involved in some essential process during the life cycle and that the fs mutation does not affect the vital function of the locus. It appears possible that these loci with only one allele are actually essential genes and that the female-sterile allele is a rare mutation that does not affect viability.

Such a pleiotropic character for these female-sterile loci for which there is only one allele is supported by previous studies. For example, most female-sterile mutations listed in LINDSLEY and GRELL (1968) exhibit morphological phenotypes. Thus, among 46 *X*-linked fs loci, 45 are associated with pleiotropic effects: 14 with eye defects (*e.g.*, *almondex*, *goggle*, *lozenge*); 13 with bristle defects (*e.g.*, *dishevelled*, *Hairy-wing*, *singed*); 11 with small wing or body size (*e.g.*, *rudimentary*, *tiny*); three with eye color phenotypes (*e.g.*, *cinnamon*, *deep orange*); and four with antennal defects. In fact, LINDSLEY and GRELL (1968) list only one sex-linked fs mutation (*fs(1)Nasrat*) which exhibits no pleiotropic effects. Many of these loci show a lethal phenotype with strong alleles (*e.g.*, *rudimentary*, *deep orange*).

A number of lines of evidence support the suggestion that hypomorphic allele of lethal loci can exhibit female sterility. SCHNEIDERMAN *et al.* (cited in KING and MOHLER 1975) list 13 temperature-sensitive lethal mutations that were found to be female sterile at the permissive temperature. Another line of evidence comes from germline clonal analyses of recessive lethal mutations (PERRIMON, ENGSTROM and MAHOWALD 1984a; see review in PERRIMON and MAHOWALD 1986) that demonstrated the types of maternal effects on oogenesis of essential genes. Hence, we suggest that most of the fs loci, identified as female sterile because of only one mutation, are actually essential genes and that the fs allele represents a hypomorphic allele.

It is useful for a genetic analysis of oogenesis to distinguish loci that are

uniquely active during oogenesis because they perform some ovary-specific function (*i.e.*, luxury genes) and those loci that are needed both for oogenesis and for other functions. To exemplify this distinction, we propose the following reclassification of female sterility loci.

Class A: Ovary-specific functions: If transcriptional activity of a gene is required only for the process(es) of oogenesis, amorphic and some hypomorphic mutations of it would result in female sterility. We would also expect saturation screens for fs mutations to recover mutations of such loci again and again. Therefore, fs loci will be placed in this class when they possess many mutant alleles and exhibit no pleiotrophic effects on viability or morphology.

Class B: Female sterile with pleiotropic somatic effects: We have subdivided this group into two classes: (1) B1, fs which show somatic expression with no effect on viability and (2) B2, fs with effects on viability.

Class B1: Female sterile with morphological pleiotropy: If transcriptional activity of a gene is required zygotically for a nonvital function in addition to its need during oogenesis, amorphic and some hypomorphic mutations of it may result in female sterility and in a second phenotype in hemizygous or homozygous flies. Because of its nonvital character, we would expect to recover many alleles of such genes during mutagenesis screens for female steriles. Rescuable maternal-effect mutations are included in this class since expression of the wild-type gene in the embryo is sufficient to overcome a maternal deficiency.

Class B2: Female-sterile hypomorphic alleles of zygotic lethals: If transcriptional activity of a gene is required as in Class B1 but is necessary for viability, mutations of it generally result in lethality. Flies hemizygous or homozygous for some hypomorphic mutations of such a gene could be viable. However, females homozygous for such a gene might be sterile if oogenesis is sensitive to its reduced activity (BISCHOFF and LUCCHESI 1971). We would rarely expect to recover alleles of such genes during fs mutagenesis screens. Such fs mutations would be allelic to zygotic lethals.

Class C: Unusual female-sterile mutations: In some instances, the mutation shows a complex pattern suggesting that it is neomorphic, hypermorphic or antimorphic (*cf.* MULLER 1932). We would expect these female-sterile mutations to be rare.

We have classified the loci listed in Table 3 according to these criteria (Table 3, right column). Class A is represented by 15 loci, class B1 by 7, class B2 by 29 and class C by 6 (summary in Table 5).

The number of fs loci has previously been estimated utilizing the Poisson distribution (RICE 1973; GANS, AUDIT and MASSON 1975; MOHLER 1977; KING and MOHLER 1975). The parameter of the distribution is calculated as twice the number of cistrons with two mutations divided by the number of cistrons with one mutation. In order to correctly calculate the number of fs loci using the Poisson distribution, two assumptions are made: (1) all loci exhibit the same mutability and (2) all mutational events in the same cistron lead to female sterility. Examination of mutation frequencies at different loci clearly illustrate that not all loci are equally mutable; some loci because of their large size (*e.g.*,

TABLE 5

Germline and somatic line dependence of *X*-linked fs loci

	No. of loci	No. tested	N GLD	N SD
Class A	15	14	11	3
Class B1	7	6	6	0
Class B2	29	21	17	4
Class C	6	6	2	4
Total	57	47	36	11

The number of fs loci disrupting a germline function (N GLD) and somatic (N SD) is indicated according to the four classes of fs mutation described in Table 2.

rudimentary; SEGRAVES *et al.* 1983), or for other reasons, are more mutable than others. Probably the most significant problem with such calculations is that many, if not most, fs loci represented by a single allele are probably hypomorphic mutations of essential genes; null mutations of these loci would not be detected in fs mutagenesis screens.

Therefore, since two of the assumptions made in the use of the Poisson distribution for calculating the number of fs loci are not met, such calculations overestimate the number of such loci. The new categories of fs mutations proposed by us eliminates some of the problems inherent in previous estimations of the number of fs loci. If we assume that mutations in class A represent ovary-specific fs loci (*i.e.*, they are not lethal loci, semilethal loci, or exhibit morphological defects and are not paternally rescuable), then we can estimate the number of loci in this class. Class A contains a total of 15 fs loci, each with many alleles. If we assume that the *X* chromosome represents one-fifth of the total Drosophila genome, and that the Drosophila genome contains 5000 mutable genes (GARCIA-BELLIDO and RIPOLL 1978), then perhaps only about 75 "true fs" or "ovary-specific" loci are present in the genome.

These conclusions are interesting because they suggest that very few Drosophila genes are used exclusively during oogenesis. Some of these genes may be responsible for important developmental decisions during germline development (*e.g.*, *fs(1)k10* may control dorso-ventral polarity of the egg chamber and embryo). Other such genes may be responsible for production of specialized products (*e.g.*, chorion proteins, *dec*^{1} and *cor 36*, or sequestration of yolk protein, *yolkless*).

Most of the fs mutations listed in Table 3 have been analyzed using the dominant female-sterile technique (PERRIMON and GANS 1983). In Class A, 11 of 14 loci are required specifically for germline functions; the others are necessary for somatic functions (see summary in Table 5). This indicates that approximately 80% of all true female-sterile mutations represent loci producing germline-dependent phenotypes. In a previous analysis of germline function, PERRIMON and GANS (1983) found 12 germline-dependent and 12 somatic cell-dependent mutations among 25 fs mutations. However, these 25 were not a representative sample of fs loci, because they were enriched for mutations causing collapsed egg phenotypes. Interestingly, all *X*-linked MEL loci (Figure

3) are germline-dependent. This suggests that the germline derivatives themselves are responsible for establishing the maternal information necessary for the formation of a normal embryo.

It should be emphasized that, as yet, with the exception of *ocelliless* (SPRADLING and MAHOWALD 1981), *fs(1)h* (DIGAN *et al.* 1985), *fs(1)k10* (HAENLIN *et al.* 1985) and *rudimentary* (SEGRAVES *et al.* 1983), none of the fs mutations listed in Table 2 have been characterized molecularly. Molecular analysis of mutant alleles and developmental transcription profiles must be available before we can unambiguously state that "luxury" genes specific to oogenesis exist in Drosophila.

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