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Clonal Analysis of the Tissue Specificity of Recessive Female-Sterile Mutations of *Drosophila melanogaster* Using a Dominant Female-Sterile Mutation *Fs(1)*K1237

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Using the newly isolated, germ line-dependent dominant female-sterile mutation *Fs(1)K1237*, we have characterized the germ line or somatic line dependence of 25 X-linked recessive female-sterile mutations. Since *Fs(1)K1237*/+ females fail to lay eggs, only germ line cells which lose *Fs(1)K1237* as a result of X-ray-induced mitotic recombination are capable of producing eggs. Such recombination events will render genes on the homologous chromosome homozygous. If this chromosome carries a recessive female-sterile mutation, the fertility will be restored only if the altered function is not required in the germ line. Using this test, we have classified 25 recessive female-sterile mutations: 12 affect germ line function, 12 affect somatic line function, and one gave an ambiguous result for which an explanation is proposed. For a few of the somatic line-dependent mutants, we found that some eggs derived from germ line clones showed the same phenotype as eggs laid by females homozygous for the recessive female-sterile mutation. These results are discussed in terms of a coincident production of clones in the follicle cells.

INTRODUCTION

Functions of both somatic cells (i.e., extraovarian tissue and ovarian follicle cells) and germ cells (i.e., nurse cells and oocyte) are necessary for the production of eggs in *Drosophila melanogaster*. The germ line is derived from the pole cells (Sonnenblick, 1950). Presumptive germ cells divide throughout larval development and then, at pupariation, form an oogonial stem cell line (King, 1970; Wieschaus and Szabad, 1979). These stem cells produce throughout the life of the fly clusters of 15 nurse cells and one oocyte. These clusters are surrounded by cells of mesodermal origin (Sonnenblick, 1950; Poulson, 1950) which are important for egg shape, vitellogenesis, and choriogenesis (see review by Mahowald and Kambysellis, 1980; Brennan *et al.*, 1982).

Female-sterile mutations may affect one or both of these cell lineages. There have been several extensive screens for such mutations (see review by King and Mohler, 1975; Gill, 1963; Bakken, 1973; Rice, 1973; Gans *et al.*, 1975; Mohler, 1977; Komitopoulou *et al.*, in preparation). On the basis of eggs laid, female-sterile mutants can be divided into three classes: (a) no eggs laid, (b) morphologically abnormal eggs laid, and (c) eggs laid with apparently normal morphology which undergo abnormal embryonic development.

It would be useful to know the tissue and cell type in which these mutations affect oogenesis. This problem can be approached by two complementary techniques. Reciprocal ovary transplants (Clancey and Beadle, 1937) can localize the defect to processes occurring in the ovary itself or in extraovarian tissue (e.g., fat body). The other technique is to produce a genotypically mutant germ line in a wild-type soma. If this combination of mutant germ line and wild-type soma leads to the mutant phenotype, then the function of this gene is necessary in the germ line (i.e., the mutation is germ line dependent, "GLD"), but somatic functions cannot be definitively excluded. On the other hand, if under these conditions eggs are produced which are normal in all respects, then the gene must act only in somatic tissue (i.e., the mutation is somatic line dependent, "SLD").

Such mosaics can be generated by pole cell transplantation (Illmensee, 1973) or by mitotic recombination (Wieschaus, 1978). Each technique has its own difficulties and limitations: Pole cell transplantation is technically difficult and the time of clone induction is invariable; mitotic recombination requires a means of identifying the germ line clone. To detect germ line clones, Wieschaus and Szabad (1979) first used two maternal effect germ line-dependent mutations, both recessive and X linked: *mal* (maroon-like) (Marsh and Wieschaus, 1977) and *fs(1)K10* (Wieschaus *et al.*, 1978).

Eggs laid by females homozygous for *fs(1)K10* are morphologically abnormal and are usually not fertilized; even when these eggs are fertilized, the resulting embryos die and have a "dorsal" phenotype (Wieschaus,

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1980). Using *fs(1)K10,* Wieschaus *et al.* (1981) studied the tissue specificity of some X-linked recessive female-sterile mutations placed *in cis* with *fs(1)*K10. This technique requires the observation of a large number of eggs since the few eggs derived from germ line recombinant events and identified by the *fs(1)K10* phenotype must be identified along the large number of normal eggs derived from the unaltered *fs(1)K10/+* germ line. Moreover, since *fs(1)K10* eggs do not develop, it is not possible to use this technique to study female-sterile mutations which only alter the embryonic development (class c).

The *mal* mutation affects eye color and aldehyde oxydase activity (Janning, 1976). Flies genetically homo- or hemizygous for *mal* have brownish eyes if derived from *mal/mal* mothers but the eyes are wild type if the individual is derived from a *mal/+* mother. The *mal* technique has been useful in the cell lineage study of the female germ line (Wieschaus, 1978; Wieschaus and Szabad, 1979). In this analysis, histochemical techniques were used for the detection of *mal/mal* clones in the ovary. It is easier to detect *mal/mal* clones in *mal/+* females by scoring the phenotype of the progeny when these females are crossed to *mal* males; however, the maternal effect of the wild-type gene, mal^+, is not fully penetrant and some of the progeny of a *mal/+* germ line show the mutant phenotype. This creates a background which makes it difficult to detect a small *mal/mal* germ line clone. For this reason, the *mal* marker is, in practice, difficult to use for the study of the tissue specificity of recessive female-sterile mutations placed *in cis* with it.

As discussed by Wieschaus *et al.* (1981), the use of a dominant germ line-dependent female-sterile mutation would provide a more straightforward assay for somatic or germ line tissue specificity. The best mutant would be one which laid no eggs so that all the eggs would necessarily be from a germ line clone having lost the dominant mutation. Here we describe the use of such a mutation, *Fs(1)K1237,* isolated by Komitopoulou and Gans, to test the tissue specificity of 25 X-linked recessive female-sterile mutations.

MATERIALS AND METHODS

Strains. Fs(1)K1237 was induced by K. Komitopoulou and M. Gans during an ethyl methane sulfonate mutagenesis. *Fs(1)K1237/+* females have rudimentary ovaries in which oogenesis is generally blocked before stage 4 (see King, 1970, for description of stages) and never lay eggs. The mutation was localized between *ec* (*echinus*) and *cv* (*crossveinless*) in the 4 DE region of the salivary gland chromosome and the germ line dependence was established using the *mal* marker (Busson *et al.*, 1983). The germ line dependence has been confirmed in our experiments using *fs(1)K10:* all eggs laid by *Fs(1)K1237/fs(1)K10* females X irradiated during the first larval instar were phenotypically *K10.* Throughout this paper *Fs(1)K1237* will be referred to as "*Fs(1).*"

We studied 25 recessive female sterile mutations for their tissue specificity. Some are described in Gans *et al.* (1975) and Zalokar *et al.* (1975): No. 456, 473, 571, 1242, 1246, 1501, 1502, 1561, and 1621. Those which carry a "K" were newly isolated in ethyl methane sulfonate mutagenesis by K. Komitopoulou (Komitopoulou *et al.*, in preparation). The *ty* (*tiny*) mutation was obtained from Bowling Green center and is described in King (1970); *lzlG* is a recessive female-sterile mutation which produces an almost complete atrophy of the ovaries and also effects the eyes in a manner similar to *lz* (*lozenge*); *lzlG* is in the same region as *Fs(1)K1237,* the 4 DE region of the salivary gland chromosome. It is thought that *lzlG* is a small deficiency extending from the $Fs(1)K1237^+$ gene and to a nearby gene *lzl* (*lozenge-like*) (Busson *et al.*, 1983), already described in Lindsley and Grell (1968) but since lost.

A summary of the localization of the recessive female-sterile mutations, referred to as "*fs(1)*" in this paper, is given in Fig. 1. For reasons explained later the *fs(1)* mutations were combined with morphological markers as indicated in Table 1.

Medium. Flies were grown on Gif's standard medium (Gans *et al.*, 1975) at 25°C or at 28.5°C in the case of a temperature-sensitive *fs(1).*

Induction of mitotic crossing-over. Eggs from the crosses *FM3/fs(1)* ♀ × *Fs(1)* ♂ were collected for 12-hr periods in split bottles. X irradiations were performed at the end of the first larval instar stage (40 ± 6 hr). The dose delivered was 1000 rad (X-ray machine: Siemens double dermopan; 45 kV, 25 mA, 3 mm aluminum filter).

In each experiment we studied 600 irradiated *Fs(1)/fs(1)* females and the same number of unirradiated females as a control. With this X-ray treatment, 4% of *Fs(1)/+* females show a germ line clone. The absence of egg production in 600 irradiated *Fs(1)/fs(1)* female is significant ($P < 5\%$) and such mutations were thus considered to be germ line dependent.

At emergence, *Fs(1)/fs(1)* females were collected and distributed 20 per tube and crossed with 10 *fs(1)* males. Tubes were kept for 7 days to ensure the maximum detection of clones (Wieschaus and Szabad, 1979) at 25 or 28.5°C in the base of thermosensitive *fs(1).* As soon as a clone was found, females were transferred to fresh vials, the eggs scored, and their morphology observed. If eggs developed, then the morphology of these individuals was studied.

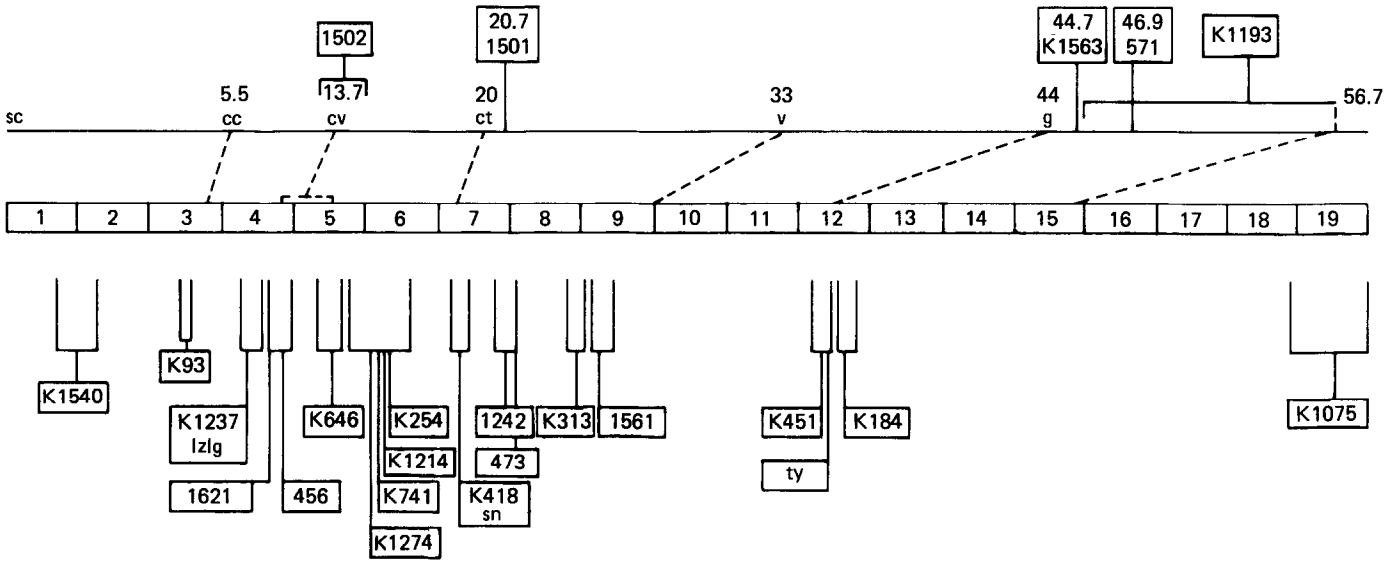

FIG. 1. Localization of the female-sterile mutations on the X chromosome. To date *1246* is not located.

RESULTS

The principle of the method we have employed is shown schematically in Fig. 2. A variety of distinguishable mitotic recombination events can occur in the germ line of a female which is doubly heterozygotes *in trans* for *Fs(1)* and the *fs(1)* mutation to be studied. Figure 2A shows the expected consequences of a recombination event occurring in the heterochromatin, in which 70–75% of mitotic recombination events occur (Garcia-Bellido, 1972; Wieschaus *et al.*, 1981). In the resulting clone ("proximal clone" or PC), the elimination of the *Fs(1)* occurs at the same time as the *fs(1)* mutation is rendered homozygous. When the effect of the *fs(1)* mutation being studied is limited to the somatic tissue, such a clone will lead to progeny bearing the *fs(1)* mutation. Mitotic recombination in the euchromatin between the two (Fig. 2B) will result in a clone ("distal clone" or DC) in which the *Fs(1)* is eliminated but the *fs(1)* remains heterozygous. Fertile females with clones of this type thus can give no information about the action of the *fs(1)* mutation in question.

There are several ways in which fertile clones of the types distal and proximal can be distinguished. The best method is to use morphological markers (Fig. 2B). If *fs(1)* is combined with a proximal marker (*forked*, *f*, was generally used), when irradiated *fs(1) f/Fs(1)* females crossed with *fs(1) f* males produce only *f* progeny, it can be unambiguously concluded that these progenies are derived from an *fs(1) f/fs(1) f* proximal clone. After five or more progeny of a clone are recovered, the absence of f^+ flies was considered to be significant. In some cases distal morphological markers located between *Fs(1)* and *fs(1)* were also used (for example *cut*, *ct*, in Fig. 2B). If an irradiated *ct fs(1)/Fs(1)* female gives progeny including both *ct* and ct^+ flies, this indicates that these progeny are derived from a distal *ct fs(1) f/+* clone.

TABLE 1
LIST OF THE *fs(1)* MUTATIONS STUDIED AND MORPHOLOGICAL MARKERS USED

fs(1)	Markers
456	.v[b]
473[a]	sc ec .g f
571	sc .ec cv v f
1242	sc ec .v f
1246[c]	v
1501	sc ec .v g f
1502	sc ec .g f
1561	.v
1621	sc .v g f
K93	.v g f
K184	sc ec cv ct. f
K254[a]	sc ec .v f
K313	sc ec cv .v g f
K418	sc ec cv .v g f
K451	sc ec v. f
K646	.v
K741[a]	sc ec .v f
K1075	sc ec cv ct g.
K1193	v.
K1214	sc ec cv. v f
K1274[a]	sc ec .v f
K1540	.ec cv ct g f
K1563	sc ec v. f
1zlG	sc. v f mal
ty	.g

[a] Thermosensible mutation.
[b] The point indicates the location of *fs(1)* with regard to the markers.
[c] To date this mutation has not been localized.

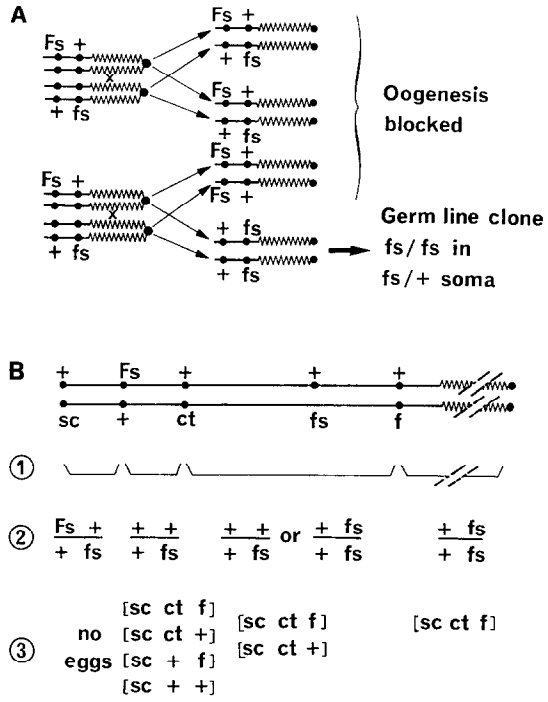

FIG. 2. Principle of the test of tissue specificity of recessive female-sterile mutations using *Fs(1)K1237*. (A) Mitotic recombination in heterochromatin (proximal to *fs and Fs*) leads to an *fs(1)/fs(1)* germ line clone in an *fs(1)/+* soma. If the *fs(1)* affects only a function required in the soma, females with this proximal clone should be fertiles. Otherwise, if the *fs(1)* affects a germ line function, the clone should have the same characteristic as the *fs(1)* mutant itself. (B) Use of morphological markers for distinguishing proximal (*fs(1)/fs(1)*) and distal (*fs(1)/+*) clones. (1) Region of the recombination event; (2) genotype of the resulting germ line clone; (3) phenotypes of the progeny when the irradiated *Fs(1)/sc ct fs(1) f* females are crossed with *sc ct fs(1) f* males. Only when clones producing five flies or more, all *sc ct f*, are found, can the *fs(1)* mutation be designated as somatic line dependent.

sc, has been used to identify possible cases of reversion of *Fs(1)K1237*, an event which occurs occasionally (Busson *et al.*, 1983). Following such an event, the progeny would include a mixture of *sc* and sc^+ flies.

Even when an *fs(1)* mutation is not combined with proximal morphological markers, it is still possible to conclude that the fertile clones obtained are proximal if the observed frequency of the fertile clones is significantly higher than the frequency of clones expected from mitotic recombination events between *Fs(1)* and *fs(1)*. To determine the expected frequencies of proximal and distal clones, it is necessary to take into account the location of the *fs(1)* mutation and the known distribution of mitotic recombination events along the chromosome: 25 to 30% of mitotic recombination events take place in euchromatin at random sites (Garcia-Bellido, 1972; Wieschaus *et al.*, 1981; Becker, 1976). Another possible means of distinguishing between proximal and distal clones is to test individually the flies from a fertile clone to determine whether or not they carry the *fs(1)* mutation. Progeny of five or more flies all carrying *fs(1)* may be considered as the derivatives of a proximal clone; if any $fs(1)^+$ flies are found, the progeny must be derived from a distal recombination event.

In our experiments, the frequency of females laying eggs varied between 2 and 3% (Table 3). As each vial contains 20 females (see Materials and Methods), the probability that a vial contained more than one mosaic female is low but cannot be neglected ($P(0) = 0.67$, $P(1) = 0.27$, $P(>2) = 0.06$). This ambiguity was removed by dissecting at the end of the experiment all the flies derived from a vial where eggs were found (mosaic flies contain ovarioles with postvitellogenic stages). Alternatively, as most of these X-ray-induced clones have a very large size, such events were easily detectable based on the large number of eggs found and immediately flies of the vials were checked and separated (the diagnosis of fertile flies was done by examination of the abdomen).

Spontaneous mitotic recombination events and reversion of *Fs(1)K1237* were found in the nonirradiated lot with respective frequencies of 0.75 and 0.05% (males sc^+v derived from these putative reversions were generally checked for the absence of *Fs(1)K1237*. A similar rate of reversions was found during clonal analysis of *fs(1)*.

Female-Sterile Mutations Affecting a Germ Line Function

A summary of clone data for germ line dependence of 12 mutations is given in Table 2. Except for *fs(1)1621*, no fertile proximal clones were found. Using the technique with *fs(1)K10* associated *in cis* with *fs(1)1621*, Wieschaus *et al.* (1981) obtained an ambiguous result. In our experiments we found one clone which produced eight progeny *sc f* indicating that the mitotic recombination event occurred proximal to *f* and that the genotype of the clone was *fs(1)1621/fs(1)1621*. However, we do not interpret this result as evidence for the somatic line dependence of *fs(1)1621*. While ovaries of females homozygous for *fs(1)1621* are atrophied and tumorous egg chambers are observed it is known that this mutation is not fully penetrant and some homozygous females lay normal eggs which develop into adults (Gollin and King, 1981). The female *Fs(1)/fs(1)1621* with the proximal fertile clone was isolated and tumorous egg chambers were observed in the functional ovary following Feulgen staining. These data and the very low number of fertile clones lead us to conclude that *fs(1)1621* is a germ line-dependent mutation.

Several of the irradiated females produced eggs (Table

TABLE 2
SUMMARY OF CLONE DATA FOR GERM LINE-DEPENDENT (GLD) *fs(1)* MUTATION

Mutation	Number of tubes with eggs	Number of tubes with progeny		Tubes with eggs but no progeny	
		PC[a]	DC[b]	Number of tubes[c]	Number of eggs in each tube
lzl^{G}	0				
K741^{TS}	0				
K1274^{TS}	1	0	1		
1621	1	1			
1246	3	0		3	1,2,3
ty	2	0		2	2,3
K418	5	0	1	4	6,18,28,30
K646	6	0		6	3,5,8,9,12,18
K1193^{TS}	5	0		5	2,6,8,9,12
K1540	9	0		9	1,4,5,6,10,15,18,25,32
1242	10	0		10	1,2,5,15,20,23,38,45,62,68
1502	12	0		12	2,4,4,9,13,13,15,45,49,58,60,70

Note. For each *fs(1)*, 30 tubes with 20 irradiated *Fs(1)/fs(1)* females crossed with *fs(1)* males (i.e., 600 females) were studied. For the markers associated with *fs(1)* see Table 1.

[a] "Proximal clone" *fs(1)/fs(1)* arising from recombination events proximal to *fs(1)*.

[b] Number of "distal clones" *fs(1)/+* arising from recombination events distal to *fs(1)*.

[c] In all cases the morphology of the eggs found was similar to the morphology of the eggs laid by the *fs(1)/fs(1)* mutant females.

2). All of the homozygous clones of the mutations listed in Table 2 produced eggs with the phenotype characteristic of eggs produced by homozygous *fs(1)/fs(1)* mothers, suggesting that they arose from a proximal clone. In our clonal analysis of *fs(1)1246* and *ty*, eggs were found rarely and only in a few tubes; in the case of *Fs(1)/ty*, the morphology of these eggs was abnormal. It is probable that additional *Fs(1)/fs(1)1246* and *Fs(1)/ty* females with germ line clones were present but were not detected because they failed to produce eggs. Females homozygous for *fs(1)K418* (allelic to *singed*) produce small eggs and those homozygous for *fs(1)K646*, *fs(1)K1193*, and *fs(1)K1540* produce flaccid eggs; these same characteristics were observed in the eggs produced by the irradiated *Fs(1)/fs(1)* females. Using the *K10* technique, Wieschaus *et al.* (1981) studied *fs(1)371* which is allelic to *fs(1)K1540*, but could not conclude with certainty that *fs(1)371* was germ line dependent. In our clonal analysis of *fs(1)K1540*, the facts that no clone gave progeny and that the eggs laid showed the mutant morphology strongly suggest that the mutation is germ line dependent. Females homozygous for *lzlG* and for *fs(1)K741* and *fs(1)K1274*, when raised at the nonpermissive temperature, lay no eggs; none of the 600 irradiated females heterozygous for *Fs(1)* and one of these three mutations laid any eggs except for one *Fs(1)/fs(1)K1274* female which proved to have a distal clone.

Only two of the present group of mutations which produce morphologically normal eggs that are blocked in subsequent embryonic development have been studied: *fs(1)1242*, which affects cleavage nuclei (Zalokar *et al.*, 1975) and *fs(1)1502*, which affects cephalogenesis (Komorowska, 1980). Although no histological study has been made of the eggs produced by irradiated *Fs(1)/fs(1)* females, in both cases development resembled that of eggs laid by the homozygous *fs(1)* females.

Female-Sterile Mutations Affecting a Somatic Line Function

A summary of clone data for somatic line dependence of 12 mutations is given in Table 3. For 11, the fertility of clones obtained from mitotic recombination proximal to the *fs(1)* demonstrated the somatic line dependence of these mutations without ambiguity. The last mutation, *fs(1)K1075*, is near the heterochromatin and, in this study, it was not combined with a proximal marker. Females homozygous for *fs(1)K1075* produce eggs with appendages of abnormal size and shape which sometimes (±5%) develop and give adults. In this experiment, among eight fertile clones, three arose from mitotic recombination distal to *fs(1)K1075* and one could not be

TABLE 3
SUMMARY OF CLONE DATA FOR SOMATIC LINE-DEPENDENT (SLD) *fs(1)* MUTATIONS

Mutation	Number of tubes with eggs	Tubes with progeny			Tubes with eggs but no progeny[d]	
		PC[a]	DC[b]	PC[c] or DC	Number of tubes	Number and morphology of eggs
K93	14	9	0	5	0	
K184[e]	10	5	0	3	2	+ (6,7)
K254	9	2	0	3	4	+ (4,5,10,30)
K313	9	3	0	4	2	+ (2,9)
K1214	11	6	0	1	4	+ (1,3,4,6)
K1563	11	4	0	5	2	+ (2,7)
456	5	3	0	2	0	
473^{TS}	5	4	0	0	1	m (30)
571	15	10	0	3	2	+ (4,7)
1501	14	9	0	3	2	+ (1,1)
1561	11	7	0	1	3	+ (6),1 Mosaic (10m,11+)
K1075	10	0	3	5	2	2 Mosaics (6m,8+),(8m,4+)

[a] See legend to Table 2.

[b] See legend to Table 2.

[c] It was not possible to distinguish between DC and PC, generally because of the small number of progeny.

[d] In some cases eggs but not progeny were found; the number of eggs was scored and their phenotypes were observed: eggs with normal morphology and eggs with morphology of the *fs(1)* mutant are designated by (+) and (m), respectively.

[e] See text.

evaluated due to the small number of flies; the four other clones with 15, 18, 21, and 26 flies, arose from mitotic recombination proximal to *g* (*garnet:* 1.44) marker 4 *cM* to the left of *fs(1)K1075.* It is unlikely that all of these four clones arose from recombination events between *g* and *fs(1)K1075.* As these clones produced a large number of progeny, they cannot be explained simply by the partial fertility of the mutant. Therefore we think that *fs(1)K1075* is somatic line dependent.

In the study of these somatic line-dependent mutations, tubes were observed with eggs but no progeny. The failure of morphologically normal eggs to develop can be explained in a number of ways. It could be due to a lack of fertilization (death of the multiply marked males defective female genitalia resulting from the X-ray treatment) or a low viability of embryos homo- or hemizygous for multiply marked *fs(1)* X-chromosome. In the tubes where progeny were found, the percentage of eggs giving adults was often low (results not shown), so it is not surprising that some clones did not produce any adult. In the studies of *fs(1)456* and *fs(1)K93* we did not use marker genes because the mutations were located near or to the left of *Fs(1)* and subsequently all the tubes with eggs produced viable progenies.

From irradiated females carrying some of the mutations which were classified somatic line dependent by the above criteria, we surprisingly obtained eggs which showed the same morphological anomalies as eggs laid by females homozygous for the mutations themselves. Such eggs were found in the studies of four mutants: *fs(1)473, fs*(1)*1561, fs*(1)*K184,* and *fs(1)K1075.* In the case of *fs(1)473,* numerous abnormal eggs were found in a tube. When the female laying these eggs was isolated and dissected, she proved to have one ovary with six functional ovarioles. The somatic line dependence of this mutation had been unequivocally demonstrated by the fertility of four proximal clones. As there is evidence that this mutation affects follicle cells (it alters the chorion structure dramatically (Margaritis, 1980) and reduces the production of the two major chorion proteins s36 and s38 (Petri, personal communication), a likely explanation for the surprising production of mutant eggs by this irradiated *Fs(1)/fs(1)473* female is the coincident induction of homozygous *fs(1)* clones in both follicle and germ line cells. Wieschaus *et al.* (1981) obtained a similar result during the study of another somatic line-dependent mutation, *fs(1)384,* for which there also was evidence for a functional defect in the follicle cells.

In the case observed during the analysis of *fs(1)473,* all the eggs were phenotypically mutant. This requires, in the context of the proposed explanation, that the germ line clone was entirely encompassed by the mutant follicular cell clone. If this were not the case, then one would expect a mixture of eggs to be produced: mutant eggs from that part of the germ line clone associated with the homozygous follicle cell clone, normal eggs from that part associated with the heterozygous follicle cells of the unaltered background. Just such a mixture of mutant and normal eggs was observed during the analysis of *fs(1)1561* and *fs(1)K1075* (Table 3). The identification of a mixed population of mutant and normal eggs requires a very careful examination of the eggs and it is possible that some have escaped our notice. Such partial phenotypes may be the result of mosaicism in other cell types contributing to oogenesis. Our knowledge of the function of other tissues is actually limited to the fat body (Mahowald and Kambysellis, 1980), and in the context of the hypothesis proposed there is no evidence that a fat body mosaic for yolk protein mutants could lead to a mixture of mutant and wild-type eggs. Different culture conditions have recently been observed to "rescue" the sterility of females homozygous for alleles of *fs(1)K184* (D. Mohler, personal communication). It is probable that a similar phenomenon occurs with clones of this mutation. One tube with viable progeny was observed to contain a mixed population of mutant and normal eggs (not indicated in Table 3). Since less than 10 progeny were obtained for each of the five PC clones of this mutant, it is likely that each clone produced a similar mixed population. These eggs would not have been detected because larvae disturbed the media.

Ambiguous Data for fs(1)K451

This mutation has been shown to cause a decrease in the amounts of all the chorion proteins (Komitopoulou, personal communication); the homozygous females have reduced viability and produce eggs with very abnormal chorions. We obtained 10 females with germ line clones: four gave progeny but in all four cases the clones resulted from mitotic recombination distal to *f.* It was not determined whether the recombination events occurred distal to *fs(1),* but as the distance between *fs(1)K451* and *Fs(1)* is three times greater than that between *fs(1)* and *f,* this is likely to have been the case. Since proximal fertile clones should be the most common type in the case of an *fs(1)* mutation not affecting germ line functions, their apparent absence is a strong indication of the germ line dependence of *fs(1)K451.* If *fs(1)K451* is germ line dependent, then proximal clones should yield mutant eggs. We found six irradiated females which produced eggs which did not develop (number of eggs found in each case: 3, 7, 11, 12, 25, 27) but in every case all of the eggs appeared perfectly wild type. These results can be understood if the gene involved is required in both germ line and somatic cells. Another interpretation

is that the tested chromosome includes two mutations, one of them, somatic line dependent, affecting the chorion structure, and the other, germ line dependent, affecting embryonic viability; these two mutations would have to be close together since both are included within *Df(1)HA92*.

DISCUSSION

In these experiments, the germ line or somatic line dependence of 25 recessive female sterile mutations has been analyzed. The main object of this study was to test the efficacy of the *Fs(1)K1237* mutation for the identification of mitotic recombination in the germ line. In addition, the results have provided some insights into the role of the somatic and germ line cells during oogenesis.

It is well known that certain somatic tissues play important roles in the processes of vitellogenesis and choriogenesis (see review by Mahowald and Kambysellis, 1980 and Postlethwait and Shirk, 1981). Thus, it is not surprising to find that many somatic line-dependent *fs(1)* mutations interfere with these processes. Direct evidence now indicates that synthesis of chorion proteins is reduced in the SLD mutants: *fs(1)473*, *fs(1)K254*, *fs(1)K1214*, and *fs(1)K1563* (Lombard and Petri, personal communication; Komitopoulou *et al.*, in preparation). The SLD mutation *fs(1)1501*, though not causing chorion anomalies by itself, has been shown to interact with a closely linked gene, *fs(1)384*, which does affect dramatically the chorion structure (Komitopoulou *et al.*, in preparation). Two other mutations may be involved in vitellogenesis. The SLD mutation *fs(1)K313* is located in the same region of the X chromosome as the genes coding for the YP_1 and YP_2 proteins and is probably allelic to the partial dominant mutation *fs(1)1163* which reduces the quantity of YP_1 in the hemolymph (Bownes and Hames, 1978; Bownes and Hodson, 1980). In the eggs produced by females homozygous for the *fs(1)K184* mutation, the quantity of all the three yolk proteins (YP_1, YP_2, and YP_3) appears to be reduced (Komitopoulou *et al.*, in preparation). Germ line clones of a stronger allele under different culture conditions established the GLD of this gene (Perrimon, unpublished); therefore the differing results obtained are probably due to the culture conditions or allele used.

In the sample of *fs(1)* mutations studied here, all of the SLD mutations affect the morphology of the eggs. But some GLD mutations (*fs(1)K418*, *fs(1)K646*, *fs(1)K1193*, and *ty*) also show this characteristic. The germ line dependence of *fs(1)K418* (allelic to *singed, sn*) is not surprising since mutations at the *sn* locus are known to decrease the level of polyploidization in the nurse cells (King, 1970); the nurse cells degenerate prematurely and their cytoplasm is not injected into the oocyte, with a consequent reduction in the size of the eggs. In the case of *ty*, the earliest observed abnormality of oogenesis concerns the follicle cells (Falk and King, 1964) and it seems that the primary defect is a precocious transition of the follicle cells from a migratory to a secretory phase (King, 1970). The germ line dependence of this mutation demonstrated here suggests that normal behavior of the follicle cells depends upon an interaction with the germ cell component of the egg chamber since the gene involved acts in a cell type other than the one in which the abnormalities are observed. Dimario and Hellen (1982) showed that the mutation *ty* was GLD and has no apparent effect on the follicle cells which is in accordance with our results.

A similar type of interaction was found for the mutant *fs(1)K10* which affects the morphology of the chorion appendages but is germ line dependent (Wieschaus *et al.*, 1978). The two other GLD mutations, *fs(1)K646* and *fs(1)K1193*, affect the turgidity of the eggs but they have not been analyzed in detail and the primary detect causing flaccid eggs is not known.

Among the other GLD mutations, there are four which disturb early oogenesis. *lzlG* stops oogenesis before egg chamber formation. Three mutations, *fs(1)1621* (Gollin and King, 1981) and two newly isolated thermosensitive mutations, *fs(1)K741* and *fs(1)K1274*, produce ovarin tumors. Another mutation causing ovarian tumors, *fs(1)116* (King *et al.*, 1978), has been found to be germ line dependent (Wieschaus *et al.*, 1981). King *et al.* (1981) observed giant polytene chromosomes in nuclei of the nurse cells which sometimes form. These observations demonstrate the important role of germ line functions in the initial steps of oogenesis, the formation of egg chambers, and the differentiation of the nurse cells.

The two maternal effect embryonic lethal mutations tested here (*fs(1)1242* and *fs(1)1502*) were found to be GLD. Several other mutations of this type have also been shown to be GLD by the transplantation of the pole cells: *dor* (Marsh *et al.*, 1977), *mat-3* (Regenass and Bernard, 1978), *par* (Proust and Thierry-Mieg, personal communication; Thierry-Mieg, 1982). In the case of *fs(1)1242* and *fs(1)1502*, there were no indications that the genes act in somatic line: the morphology and viability of homo- or hemizygous females is not rescued by a wild-type allele supplied by the sperm; the properties of *fs(1)/fs(1)* germ line clones in an *fs(1)/+* soma appears similar to those of the germ line of nonmosaic homozygous females. Therefore it seems that these two mutations affect some maternal functions specific to the germ line involved in the first steps of embryonic development. It is likely that many genes acting in both

soma and germ line (housekeeping genes such as *rudimentary*) have maternal functions which are important in embryonic development. If mutations in them lead to a zygotic lethality, a maternal effect can be detected only by germ line mosaic techniques since homozygous females are lethal.

The study of female sterile mutations is a useful approach to dissecting the complex process of oogenesis. As outlined by Wieschaus (1978), clonal analysis can provide information on the tissue specificity of female-sterile mutations and thus form an important part of these studies. Since there are several hundred loci known which, when mutated, can lead to female sterility, a technique allowing the rapid and effective study of these numerous mutants is essential. The technique outlined here using the dominant female-sterile mutation *Fs(1)K1237* has several advantages over the technique of clonal analysis using *fs(1)K10:* (1) no eggs are produced by the unaltered germ line of the irradiated *Fs(1)/fs(1)* females; (2) the size of germinal clones in which Fs(1) is lost is greater than that of *fs(1)K10* homozygous clones induced in *fs(1)K10 fs*(1)/+ females at the same stage of development (Perrimon and Gans, in preparation); (3) with the *Fs(1)* technique, the ability of the eggs produced by the clone to develop depends only on the genotype at the *fs(1)* locus and on the properties of the *fs(1)* mutation itself. This last feature allows the study of female-sterile mutations which cause abnormal development of the eggs. The technique also allows the analysis of the function of zygotic lethal mutations in the germ line and the study of possible maternal effects of lethal mutations which are viable in the germ line (Kerridge and Dura, 1982; Jimenez and Campos-Ortega, 1982).

The first two features allow a quick diagnosis of the germ line-dependent and somatic line-dependent mutations based simply on the absence or presence of fertile proximal clones following irradiation. This study has shown that it can also be informative to observe the morphology of the eggs produced. When a mutant proves to be SLD, it remains to be determined whether the mutation affects ovarian or extraovarian somatic tissues. This determination is obviously possible by the transplantation of ovaries. But, if the hypothesis which we have previously proposed is correct, then the production of a mixture of normal and mutant eggs by irradiated *Fs(1)/fs(1)* females is suggestive of a functional defect of the mutant follicle cells. On the other hand, when a functional defect in the mutant germ line is demonstrated by the absence of fertile proximal clones, the possibility of a functional defect in other tissues cannot be excluded. The production by irradiated *Fs(1)/fs(1)* females of eggs which differ morphologically from eggs laid by homozygous *fs(1)* females is a good indication that all functions of the gene required in oogenesis are not restricted to the germ line. The *fs(1)K451* mutation is probably an example of such a gene with several sites of action. In the case of genes whose functions are required in germ line as well as in somatic tissue it may be necessary to test various alleles in germ line clones, since a weak allele may lead to the conclusion of a SLD of the gene when a more extreme mutation would establish the GLD of the gene.

A major drawback of the *Fs(1)K1237* technique is that its application is restricted to X-linked mutations. To date only two autosomal dominant female sterile mutations have been isolated and used: *Fs(2)D* on the right arm of chromosome 2 (Lindsley and Grell, 1968; Yarger and King, 1971; Wieschaus, 1978; Schupbach, 1981) and *Toll* on the right arm of chromosome 3 (Wieschaus and Nusslein-Volhard, personal communication; Perrimon, in preparation). However, it is possible to extend the use of *Fs(1)* for the study of autosomal mutations in different ways. First, a combination of an X-linked dominant female-sterile mutation with an X duplication of the wild-type allele of the autosomal mutation to be studied has recently allowed the analysis of the maternal effect of lethal mutation of the bithorax complex (Kerridge and Dura, 1982). Other possibilities for extending the *Fs(1)* technique for the study of autosomal genes include translocations linking the *Fs(1)*+ allele and the recessive mutation to be studied or duplications of the *Fs(1)* mutant allele on the autosome arm where the wild-type allele of the gene to be studied is located. For the successful application of the first two methods, it is necessary that the *Fs(1)* mutation employed not be dominant over two wild-type alleles. Females of the genotype *Fs(1)K1237*/+/+ are sterile but two other dominant female-sterile mutations, (*Fs(1)K1103* and *Fs(1)K155*), allelic to *Fs(1)K1237*, are fertile over two doses of the wild-type alleles and can be used in this fashion.

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