CLONAL ANALYSIS OF DOMINANT FEMALE-STERILE, GERMLINE-DEPENDENT MUTATIONS IN *DROSOPHILA MELANOGASTER*

NORBERT PERRIMON[1]

Centre de Genetique Moleculaire du CNRS, Gif Sur Yvette, 91190 France

Manuscript received March 1, 1984
Revised copy accepted July 30, 1984

ABSTRACT

Three allelic, dominant and germline-dependent female-sterile mutations (ovo^D mutations) can be classified according to the severity of the ovarian abnormalities that they produce. The size and frequency of +/+ germline clones, induced in ovo^D/+ females, were compared with *K10/K10* germline clones induced in *K10*/+ control females. The frequency of germline clones induced by irradiation of first instar larvae is similar for the three dominant alleles and *K10*; however, the clone size increased with the strength of the allele tested, compared with *K10* clones. When clones were induced later in development, the clone frequencies decreased with the strength of the alleles. These results are discussed in the context of the antimorphic nature of these mutations and the characteristics of germline development. The use of these alleles as tools in the genetic analysis of development is discussed.

IN Drosophila, germ cells are derived from the pole cells which are the first cells to be formed, before the blastoderm stage (SONNENBLICK 1950). During larval stages, these cells proliferate and at pupariation establish a stemline population (KING 1970; WIESCHAUS and SZABAD 1979). The egg chamber is composed of three cell types: 15 nurse cells and one oocyte, derived from one germline stem cell, and follicular cells of mesodermal origin (SONNENBLICK 1950; POULSON 1950; review by KING 1970). The early segregation of the female germline precursors from the somatic tissue and the clonal lineage of germ cells has allowed development of mosaic techniques for producing females with germline clones genetically distinct from the surrounding tissues.

Two techniques exist for producing germline clones; one uses pole cell transplantation (ILLMENSEE 1973; VAN DEUSEN 1977), the other mitotic recombination. There are a number of different ways to identify induced germline clones by mitotic recombination; some use a recessive germline-dependent marker such as *K10* (WIESCHAUS, MARSH and GEHRING 1978; WIESCHAUS and SZABAD 1979); others use dominant female-sterile germline-dependent mutations (SCHUPBACH 1982; PERRIMON and GANS 1983). The problems relevant to each technique have been reviewed by PERRIMON and GANS (1983).

[1] Present address: Center for Developmental Biology, Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106.

Dominant female-sterile germline-dependent mutations (*Dfs*) provide an elegant tool with which to study the maternal effect of zygotic lethals (JIMENEZ and CAMPOS-ORTEGA 1982; KERRIDGE and DURA 1982; GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984) or to define the tissue specificity of recessive female-sterile mutations (SCHUPBACH 1982; PERRIMON and GANS 1983), since X-ray induced +/+ germline clones in *Dfs*/+ females produce normal eggs. Unfortunately, *Dfs* mutations are rare and, so far, only *Fs2D* (YARGER and KING 1971) and *Fs(1)K1237* (KOMITOPOULOU *et al.* 1983) have been used to generate germline clones. The purpose of this article is to define the characteristics of wild-type germline clones, induced at different developmental stages in females bearing *Fs(1)K1237* or one of the two other alleles *Fs(1)K1103* or *Fs(1)K155* (BUSSON *et al.* 1983). Furthermore, the present study provides some insights into the developmental features of this unusual locus.

MATERIALS AND METHODS

Strains and media: The dominant female-sterile mutations were isolated by KOMITOPOULOU and GANS in an EMS screen for *X*-linked female sterility (KOMITOPOULOU *et al.* 1983). These mutations, *Fs(1)K1237*, *Fs(1)K1103* and *Fs(1)K155*, appear to be allelic and map in region 4D-E of salivary gland chromosomes (BUSSON *et al.* 1983). These three dominant female-sterile alleles are referred to here as *K1237*, *K1103* and *K155*. The locus has been designated as *ovo*, and the dominant female-sterile mutations as a group will be referred to as ovo^D and the wild-type gene as ovo^+ (BUSSON *et al.* 1983). The new nomenclature of the dominant female-sterile alleles used by BUSSON *et al.* (1983) (*K1237* = ovo^{D1}, *K1103* = ovo^{D2} and *K155* = ovo^{D3}) has not been used in this paper because of the already large diffusion of the first symbols in the literature. They are kept in stocks as ovo^D v^{24}/Y males with attached-*X* [*C(1)DX,y f/Y*] females. All alleles were induced on an *X* chromosome marked with v^{24} (GANS, AUDIT and MASSON 1975).

The stock *fs(1)K10 w/FM3* was obtained from E. WIESCHAUS and *y v f mal* as well as *sc cv v f* from the Bowling Green stock center. Mutations and balancers are described by LINDSLEY and GRELL (1968). Flies were grown on a cornmeal-yeast medium and eggs collected on yeast-vinegar-sucrose, as described by GANS, AUDIT and MASSON (1975). Experiments were conducted at 25° ± 0.5°.

Induction of mitotic recombination by X-irradiation: Two sets of experiments were performed: A, in Gif, with a Siemens Dermopan X-ray machine (45 kV, 20 mA, 0.3-mm aluminum filter; the polymarked stock used was *y v f mal*) and B, in Cleveland, with a General Electric machine (100 kV, 5mA, 1-mm aluminum filter, two polymarked stocks used were *y v f mal* and *sc cv v f*). A constant dose of 1000 rads was administered, it took 45 sec in Gif and 3 min 35 sec in Cleveland. Flies were irradiated at different stages. Larvae, aged from the middle of the egg collection period (see Tables 1 and 2 for timing); white pupae (after visual selection); and young adults (immediately after emergence). Unirradiated controls were also examined.

Experimental design: Figure 1 describes the events leading to the production of germline clones.

K10 *control*: The recessive and germline-dependent female-sterile mutation *fs(1)K10*, which affects egg morphology (WIESCHAUS, MARSH and GEHRING 1978), was used as a control for germline clone frequency (WIESCHAUS and SZABAD 1979), since it does not affect ovarian organization. Progeny from a cross of *FM3,*$y^{31d}sc^8$*dm B/y v f mal* females by *fs(1)K10 w/Y* males were irradiated. Egg collections were done by transferring individual *fs(1)K10/y v f mal* females and two *y v f mal* males daily. These matings were carried out in sets of plastic tubes inverted over egg collection medium (Nüsslein-Volhard 1977). Dishes were checked every day for the presence of *K10* eggs which indicated the presence of a *K10* germline clone.

ovo^D*mutations*: Progeny from crosses of *FM3,* y^{31d} sc^8 *dm B/y v f mal* females by ovo^D v^{24}/Y males were irradiated and non-Bar females were examined for fertility and segregation of the markers in their progenies. The yellow (*y*) marker distal to the ovo^D-locus allows identification of any

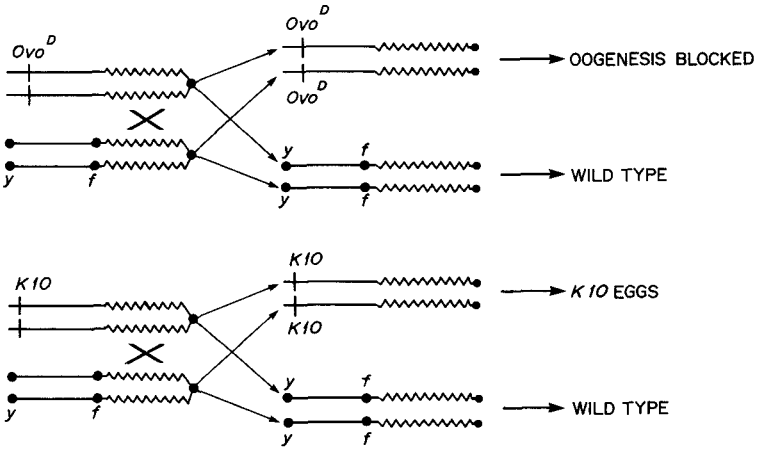


FIGURE 1.—Scheme of the experiment. When the *ovo*^{D} mutation is removed from the germline following mitotic exchange induced by X-rays, the germ cells are genetically wild type. If the germ cell can develop normally, the egg produced would have a wild-type phenotype and lead to morphologically normal progeny. The control experiment was done by substituting *fs(1)K10* (WIESCHAUS and SZABAD 1979) for *ovo*^{D}. In the control, mitotic recombination events in the female germline are detected by the presence of *K10* eggs among correctly shaped eggs (wild type).

reversions of the dominant female-sterile mutations, which would result in both *y* and *y*$^{+}$ progeny from a single clone [alternatively, the marker scute (*sc*) was used for this purpose].

K1237: In the first experiment (A), groups of 20 *K1237* v^{24}/*y v f mal* females with 10 *y v f mal* males were distributed in vials. If eggs were found in a vial, the mosaic fly was isolated by visual examination of the ovaries through the cuticle of the abdomen. This diagnosis is easy since *K1237*/+ ovaries are atrophic and +/+ germinal clones are usually large. Alternatively, mosaic females can be identified under CO_2 anesthesia in which they usually extrude an egg. When a fertile female was found and removed, the others were rechecked for the presence of a second mosaic female in the vial.

In the second experiment (B), the same method was used, but, after 7 days (in the case of larval irradiation) and 14 days (for adult irradiation), females were dissected and examined for the presence of vitellogenic egg chambers to substantiate the presence or absence of germline clones.

K1103 *and* K155: Because of differences in frequencies of clone induction, nonirradiated controls and females irradiated as larvae were distributed with males in lots of ten per vial; females irradiated as pupae, in lots of four; and adult irradiated females, in lots of three. These lot sizes also minimize the occurrence of two clone-containing females within an individual vial. These females were not dissected since heterozygotes for these alleles contain vitellogenic egg chambers.

Frequency calculations for K1237 *and* fs(1)K10: The frequencies (f) of clone induction per female were calculated from the frequencies of nonmosaic females: $f(0) = e^{-n}$ = number of females without clones divided by the total number of females studied [then (f) = 100 × n]. We did not take into account the relative positions of *fs(1)K10* and the *ovo*^{D} locus because they are only 10 map units apart and because about 80% of induced mitotic recombination events occur in the proximal heterochromatin (BECKER 1976; GARCIA-BELLIDO 1972; WIESCHAUS and SZABAD 1979).

Frequency calculations for K1103 *and* K155: Since it was impossible to visually identify females possessing germline clones, the frequencies were calculated as follows. The frequency (f) of mosaic induction per female = 100 × $n1$, where $f(0) = e^{-n}$ = number of vials with no fertile females divided by the total number of vials, and $n1 = n$ divided by the number of flies per vial.

RESULTS

The morphological phenotypes of females bearing the dominant female-sterile mutations have been described by BUSSON *et al.* (1983). *K1237*/+ fe-

males have atrophic ovaries in which oogenesis is blocked before vitellogenesis and eggs are never produced. Females heterozygous for the other two dominant female-sterile mutations contain vitellogenic egg chambers. A few *K1103/+* females lay some eggs that are always flaccid; during oogenesis many vitellogenic egg chambers are degenerating. Females heterozygous for *K155* lay eggs at a rate of approximately 40% that of wild-type females; such eggs exhibit phenotypes ranging from normal to collapsed, with a large fraction possessing one large fused dorsal filament.

Unirradiated control

Nonirradiated ovo^D/+ flies can lay fertile eggs only if a spontaneous mitotic recombination event occurs in the germline or a spontaneous reversion of the ovo^D mutation occurs. To simplify the presentation of the results, revertants have been omitted from the table. Revertants of three ovo^D mutations were indeed found in both control (C) and irradiated females (I). The frequency (f) of such events per female was for *K1237*: f(C) = 1.6%, f(I) = 0.9%; for *K1103*: f(C) = 0.5%, f(I) = 1.4%; for *K155*: f(C) = 2.1%, f(I) = 1.2%. These frequencies indicate that reversions occurred at about the same frequency in all three alleles and that X-ray irradiation did not influence such events. Spontaneous mitotic recombination events occurred with low frequencies (see Tables 1 and 2), and no significant differences were observed between ovo^D alleles and the *K10* control.

Frequency of germline clones

I shall first compare results of experiment A (in Gif), because experiment B (in Cleveland) was designed specifically to detect germline clones in adult flies heterozygous for *K1237*.

Following first instar larval irradiations, the frequency of clones for *K1237* was significantly lower than for *K1103*, *K155* and *K10* (Table 1 and 2). The differences in frequencies among the latter mutations were not significant. Comparison of the frequency of germline clone induction for first instar irradiation can be arranged: *K1237* < *K1103* = *K155* = *K10*. Second (L2) and third-instar (L3) irradiations of *K1237/ y v f mal* flies were performed. Larvae were irradiated at 55 ± 5 hr for L2 irradiations. Six germline clones were found among 200 flies [f = 3% (1.8–4.2%)]. In case of L3 irradiations, larvae were irradiated at 76 ± 5 hr, and three germline clones were obtained among 320 flies [f = 1% (0.5–1.5%)]. Therefore, second instar irradiations of *K1237/+* females show that the frequency of germline clones is about the same as for first instar irradiations. However, a decrease in this frequency is observed for third instar irradiation compared with first and second instar irradiations. Irradiations of *K1103* and *K155* were not performed on second or third instar larvae.

Pupal irradiations produced a significant difference in clone frequency between *K1103 vs. K155* and *K10* (Table 1). The latter two showed similar frequencies. Thus, at this stage the alleles can be arranged: *K1103* < *K155* = *K10*. Pupal irradiations were not performed on *K1237/+* females.

TABLE 1

Clonal analysis of K10 *and* ovo^{D} *mutations (in Gif)*

	Control			XR L1 (40 ± 8 hr)			XR WP (120 ± 1 hr)			XR YA (192 ± 1 hr)		
	N	*N*Cl	*f* (%)	*N*	*N*Cl	*f* (%)	*N*	*N*Cl	*f* (%)	*N*	*N*Cl	*f* (%)
fs(1)K10	150	2	1.3 (0.4–2.2)	175	15	8.9 (6.8–11)	110	22	22 (19–25)	76	23	36 (30.5–41.5)
Fs(1)K155	500	4	0.8 (0.4–1.2)	400	20	6.9 (5.6–8.2)	100	11	14.5 (11–18)	84	12	18.6 (14.4–22.8)
Fs(1)K1103	500	5	1.1 (0.7–1.5)	420	20	6.5 (5.3–7.7)	65	4	6.7 (3.6–9.8)	100	7	7.9 (5.2–10.6)
Fs(1)K1237	600	3	0.5 (0.2–0.8)	640	21	3.3 (2.6–4)		NT		440	2	0.5 (0.2–10.8)

ovo^{D}/y v f mal and *K10/y v f mal* flies were irradiated. The controls were unirradiated. The number of flies studied (*N*), the stage of irradiation and the number of clones found (*N*Cl) are given. The frequency of germline clone induction per female is given with the 95% confidence interval. Note that when compared with *K10*, the values of *f* decrease at later irradiation times for *ovo^{D}* mutations and the two alleles decrease by differing amounts. Nomenclature: first instar larval stage, L1; white pupae, WP; young adult, YA. XR, X-ray. NT, not tested.

Adult irradiations produced significantly fewer clones in *K155* than in *K10* and fewer in *K1103* than in *K155* (Table 1). The frequency of germline clones found in *K1237* (Table 1) was not significantly different from the control. The frequencies of clone induction in the mutations at this stage can be arranged: *K1237* < *K1103* < *K155* < *K10*.

These results indicate that the decrease in frequency of germline clone induction observed for the ovo^D mutations is a time-dependent phenomenon and that these decreases are different for the different ovo^D alleles. This allelic dependence is correlated with the severity of the ovarian defect; *i.e.*, the less drastic the ovarian defect, the less dramatic is the decline in frequency of germline clone induction.

Special interest in K1237: Whether it would be possible to induce some germline clones in *K1237* adults under different X-ray conditions was tested. Varying the X-ray dose failed to increase the frequency of germline clones (results not shown). However, it was possible to induce some germline clones in late irradiations using a different X-ray machine (compare Table 2 with Table 1). In experiment B (Table 2), irradiations at all stages resulted in elevated frequencies of germline clone induction when compared with those in experiment A (Table 1). In experiment B, the frequency of germline clone induction was significantly higher when irradiations were performed at L2 than at L1. (In experiment A no difference between these stages was found.) The decrease in frequency observed in experiment A when irradiations were performed after L2 was also found in the second experiment. Although almost no fertile clones were recovered for adult irradiations in the first experiment, a few clones were found in experiment B. Dissection of the females in experiment B revealed that, in every case, some females possessing germline clones never laid eggs (fewer females were dissected than originally studied because of the death of some flies). Such clones were generally large in size following larval irradiations, and it is likely that egg retention in such clones was due to mechanical problems caused by the presence of the second agametic ovary. The observation of "egg retention" after late irradiations was more surprising since ovaries of females irradiated late generally possessed only one or two mature eggs. It is possible that egg retention was due to an inability of the ovarian musculature to extrude the egg. This might suggest that a normal number of egg chambers within an ovariole is necessary for normal egg extrusion.

Clone size

When a germline clone of genotype +/+ was induced at an early larval stage in a *K1237*/+ female, it was frequently of very large size (Figure 2). Such germline clones generally filled an entire ovary. In contrast, the expected size (as in *K10*) was generally less than five ovarioles (WIESCHAUS and SZABAD 1979). Because large germline clones may be a general feature of such clones when induced in ovo^D/+ females, it was important to carefully quantitate this for all alleles. Although clone sizes in *K1237* females can be determined by simply scoring the number of ovarioles possessing vitellogenic oocytes, this is

TABLE 2

Clonal analysis of K1237 *(in Cleveland)*

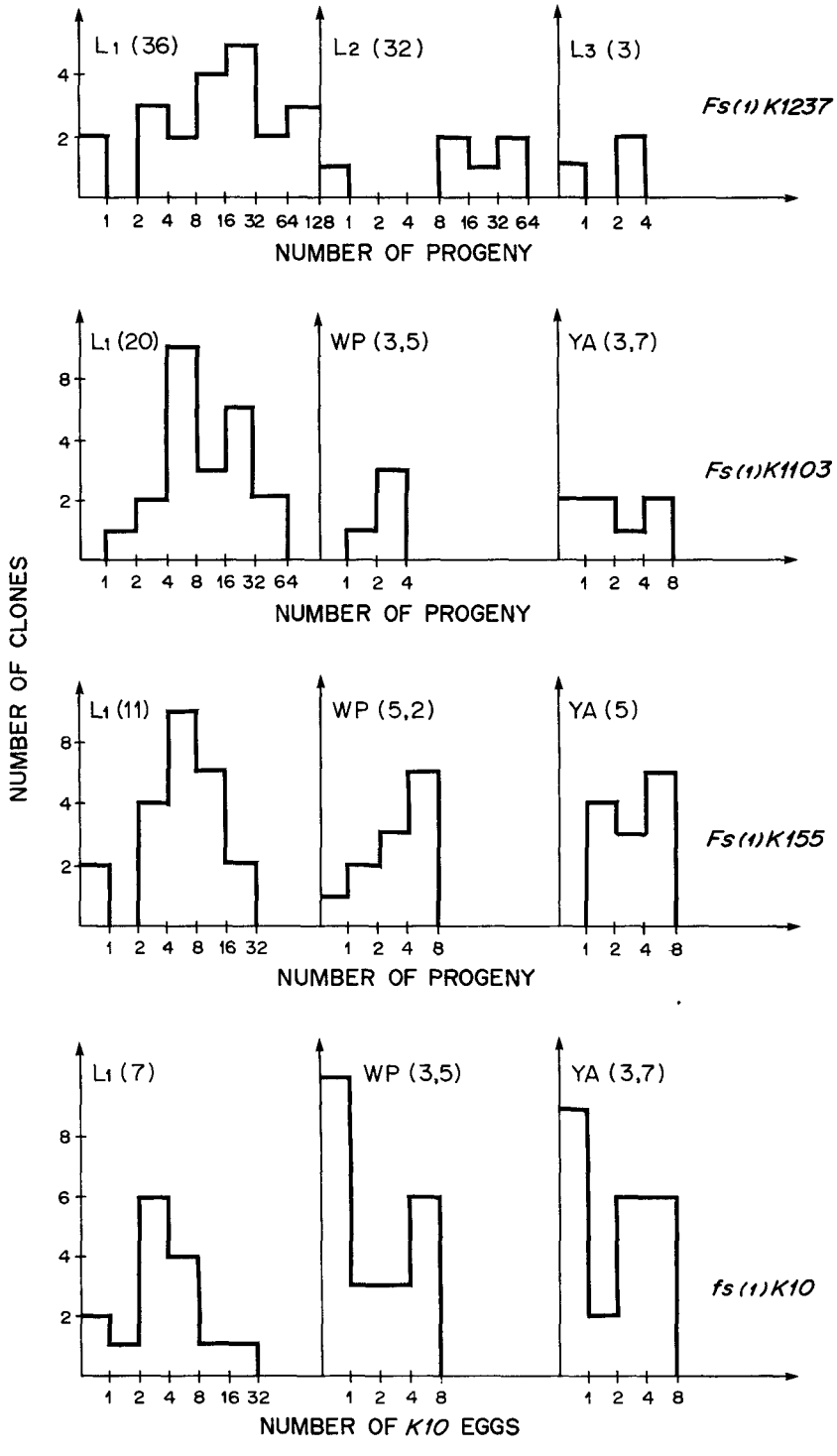
	Time of irradiation	*N*	*Nd*	*NCl*		f(1) %	f(2) %
				Fertile	Dissected		
Control		300	265	1	1	0.3 (0–0.6)	0.3 (0–0.6)
XR L1	40 ± 8 hr	400	305	22	28	5.6 (4.5–6.7)	9.3 (8–10.7)
XR L2	60 ± 8 hr	120	110	15	19	13.3 (10.3–16.3)	17.2 (13.8–20.6)
XR L3	90 ± 10 hr	54	45	6	8	11.7 (7.4–16)	16 (11.1–20.9)
XR YA	192 ± 1 hr	274	242	7	14	2.6 (1.6–3.6)	5.8 (4.3–7.3)

All females were dissected at the end of the experiment. The number of dissected flies (*Nd*) and the number of clones found is shown. Clones found upon dissection include the number of fertile clones. Two frequencies are given: f(1) refers to the number of fertile flies and f(2) to the total number of clones in dissected flies. See Table 1 for other abbreviations. Note that, in contrast to experiments in Gif (Table 1), higher frequencies of clones are found for all irradiations and clones are obtained after adult irradiations. Results represent the data pooled from two experiments in which two different stocks, *y v f mal* and *sc cv v f*, were used (see MATERIALS AND METHODS). Since frequencies were similar in both, they were combined.

impossible in females bearing the other alleles. An alternative method is to score the number of germline clone progeny. This was done for the three ovo^D alleles (Figure 2).

Larval instar irradiation: First instar irradiations were done for the three ovo^D alleles and for *K10*. The mean number of *K10* eggs per fly was seven (range 1–29). The mean number of progeny, corrected for the presence of more than one mosaic female per vial, was 36 (range 1–123) for *K1237*, 20 (2–63) for *K1103* and 11 (1–30) for *K155*. These results indicate that clone size decreased as the severity of the allele decreased (*K1237* < *K1103* < *K155* < *K10*). Second and third instar irradiations were done for *K1237*. Although very few clones were recovered, results for second instar irradiations indicate that clones were still large (mean 32, range 1–61). The three clones recovered following third instar irradiation were small (mean 3).

Pupal and adult irradiations: The germline clone frequency for *K1237*/+ females was very low at these irradiation times; not enough clones were recovered to draw any conclusions about clone sizes (results not shown). Such germline clones were, however, found for *K1103* and *K155*. Comparisons of clone size means did not reveal any clear difference among germline clones induced in these two alleles and *K10*. Nevertheless, the "one progeny" size class of germline clones following pupal and adult irradiations appeared dra-


L1 (36)
L2 (32)
L3 (3)
Fs(1)K1237
1 2 4 8 16 32 64 128 1 2 4 8 16 32 64 1 2 4
NUMBER OF PROGENY
L1 (20)
WP (3,5)
YA (3,7)
Fs(1)K1103
1 2 4 8 16 32 64 1 2 4 1 2 4 8
NUMBER OF PROGENY
L1 (11)
WP (5,2)
YA (5)
Fs(1)K155
1 2 4 8 16 32 1 2 4 8 1 2 4 8
NUMBER OF PROGENY
NUMBER OF CLONES
L1 (7)
WP (3,5)
YA (3,7)
fs(1)K10
1 2 4 8 16 32 1 2 4 8 1 2 4 8
NUMBER OF K10 EGGS

matically reduced in the two *ovo^D* mutations when compared with the "one *K10* egg" size class in *K10*. This class probably represents the loss of the germline clone with the first sister cell (WIESCHAUS and SZABAD 1979).

Special feature of K1103: During the clonal analysis of *K1103*, females, irradiated at a late stage (white pupae or adult), were found that laid three classes of eggs; (1) collapsed, (2) normal morphology with arrest in early development and (3) normal morphology that hatch and lead to adult progeny. The first class of eggs are probably derived from *K1103*/+ germ cells, whereas the two others are from +/+ germ cells, since unirradiated *K1103*/+ females never lay class 2 and 3 eggs. Examination of these females indicated that the class 2 eggs were generally produced prior to class 3 eggs (results not shown). However, other small clones were found that produced only class 1 and 2 eggs. Analysis of these clones is difficult because the numbers of eggs laid by germline clone females irradiated at these stages were very small. These results do, however, suggest that not all germline clone-derived eggs of genotype +/+ can produce viable progeny in the *K1103* background.

DISCUSSION

It is now well established that dominant female-sterile germline-dependent mutations are powerful tools for the study of the germline dependence of female-sterile mutations (SCHUPBACH 1982; PERRIMON and GANS 1983 and to study the maternal effect of zygotic lethals (JIMENEZ and CAMPOS-ORTEGA 1982; KERRIDGE and DURA 1982; GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984). This paper describes the characteristics, and conditions for use, of three dominant female-sterile alleles at the *ovo^D* locus.

K1237 is the most useful allele, since heterozygote flies do not lay eggs. The present analysis demonstrates that the highest clone frequency in heterozygous *K1237* females is observed when irradiation is carried out during the second instar stage. This may be a problem, since one would like to be able to induce germline clones later in development, in order to determine the time of action of genes in the germline and the perdurance of the wild-type allele. Although it may be possible to induce some late clones in *K1237*/+ females depending on the X-ray conditions used (see RESULTS), the use of *K1103* or *K155* may be easier. *K1103* would appear preferable to *K155* since very few (and always abnormal) eggs are laid by heterozygous females, but the presence of occasional

FIGURE 2.—Clone sizes. Clone sizes induced at different times in the different mutations studied (experiment A, see text). The abscissas represent the absolute sizes of clones (number of *K10* eggs for *K10* and number of progeny for *ovo^D* mutations). The ordinates indicate the number of mosaic females for *K10* and *K1237*; for *K1103* and *K155* the ordinates indicate the number of vials with clones. Egg production was studied during 7 days for L1 irradiation, 10 days for L2 and L3 and 16 days for white pupal (WP) and young adult (YA). Mean numbers are shown in parentheses. They were corrected, in the case of *ovo^D* mutations, for the presence of more than one mosaic female per vial. Note the large size of germline clones for larval irradiation (L1) in *K1237* and *K1103* mutations and the reduced frequency or absence of class 1 clones (single offspring) after late irradiations of *K1103*/+ and *K155*/+ females. Larval stages-L1, L2, L3.

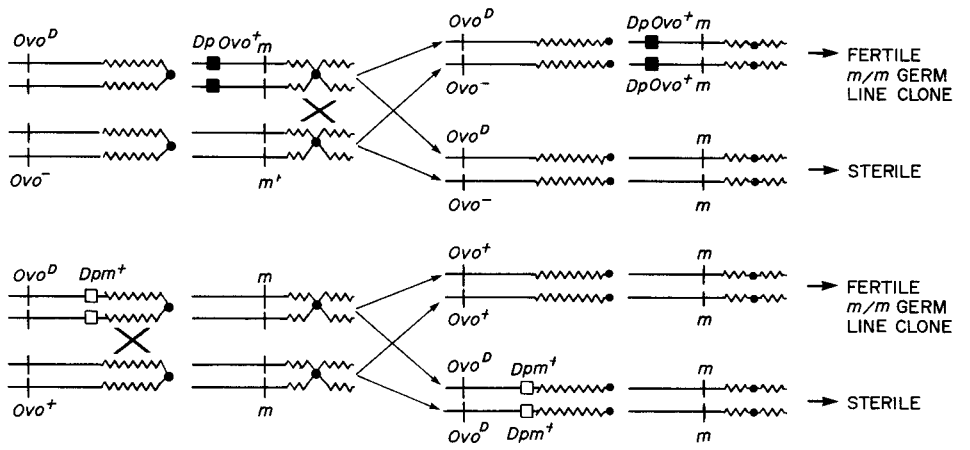


FIGURE 3.—Extension of the dominant female-sterile technique. Two methods for induction of germline clones of autosomal mutations (*m*) using the *X*-linked dominant female-sterile mutations *K155* and *K1103*. a, In the first method (top), females of genotype ovo^D/ovo$^-$; *Dp* ovo^+ *m*/+ are irradiated. Any mitotic recombination event occurring on the autosome would result in segregant germ cells homozygous for *Dp* ovo^+m and generate a *m*/*m* germline clone. For ovo^-, either a deficiency or a recessive allele of *ovo* can be used. b, In the second method (bottom), irradiated females of genotype ovo^D *Dp* m^+/+; *m*/*m* are fertile when a mitotic recombination event occurs on the *X* chromosome because +/+; *m*/*m* germ cells are produced. These methods can only be applied using *K155* and *K1103* because *K155*/+/+ and *K1103*/+/+ females are fertile; whereas, *K1237*/+/+ individuals are sterile.

abnormal (+/+) germline clone-derived eggs may frustrate accurate interpretation of the results (see RESULTS). The use of *K155* for the detection of germline clones appears tedious since *K155*/+ females lay many eggs, some of normal morphology. Therefore, an appropriate choice must be made depending on the mutation being clonally analyzed and the questions being asked.

Although dominant female-sterile mutations have not yet been characterized on the autosome arms, it is still possible to induce germline clones of autosomal mutations by using the ovo^D mutations. One such technique would use a duplication carrying the ovo^+ gene on an autosome arm (Figure 3a). To date, the only such duplication available is *Dp(1;2)*rb^{+71g} (CRAYMER and ROY 1980). Using *Dp(1;2)*rb^{+71g} and *K1103*, I have produced germline clones at a frequency of about 2% when second instar larvae are irradiated (results not shown). A second technique is to use a duplication on the *X* chromosome of the wild-type allele of the mutation studied (Figure 3b) (see also KERRIDGE and DURA 1982). With both techniques, only *K155* and *K1103* can be used because such analyses require that the dominant female-sterile allele be fertile in the presence of two wild-type doses. This is not true for *K1237* (BUSSON *et al.* 1983).

The present analysis demonstrates a correlation between the strength of the ovo^D allele used and the size of the induced germline clone: the more extensive the ovarian abnormalities, the larger the clones induced by early irradiation. The frequency of induction of germline clones appears to be dependent on the time of irradiation as well as upon the allele tested. The frequency of germline clone induction decreases as clones are induced later in development

for all alleles, and the stronger alleles show a relatively more pronounced decrease in clone number with time.

The fact that the frequencies of germline clones induced at the end of first instar are about the same for all dominant alleles tested (although a slight difference may exist in *K1237*) indicates that the number of germ cells present in the early larval ovary is about the same in females possessing any of the ovo^D alleles or *K10*. The large size of +/+ germline clones observed in mosaic ovo^D/+ females irradiated as early larvae suggests that induced +/+ germ cells undergo more larval divisions than ovo^D/+ germ cells. The decreased frequency of the germline clones found following successively later irradiations may reflect abnormal proliferation of ovo^D/+ germ cells during larval stages (some may even degenerate). This possibility is supported by the observed phenotype of recessive (amorphic) mutations at the *ovo* locus (BUSSON *et al.* 1983). Females homozygous for recessive alleles are fully viable, but agametic, whereas males are fertile. Therefore, the effect of ovo^D appears detrimental only to female germ cell division, and the large size of early +/+ clones may be a direct consequence of germ cell regulation.

In temperature-sensitive or incompletely penetrant grandchildless mutations, only one full ovary (not two partial ovaries) per female is frequently found, often following a shift from permissive to restrictive temperature (THIERRY MIEG 1976; MARIOL 1981; NIKI and OKADA 1981; ENGSTROM *et al.* 1982). This puzzling observation can be explained either by a common migration of all existing pole cells into one embryonic gonad or by the migration or survival of only one pole cell within one ovary. If the second hypothesis is correct, then the regeneration of one normal ovary from one pole cell would support the regulative process that we observe in ovo^D/+ females possessing +/+ germline clones.

After late irradiations of *K1103* and *K155*, the one-progeny size classes are found with very low (for *K1103*) or zero (for *K155*) frequencies. In the *K10* control, this class corresponds to the loss of one segregant with the first sister cell derived from the recombinant stem cell. This probably indicates that, within ovo^D/+ females, the first germline clone-derived eggs cannot produce viable progeny. With the assumption that the first germline clone-derived egg chambers do not produce progeny, it is possible to correct the frequency of adult germline clones. When this is done, the frequencies of germline clones in *K155* (39%) and *K10* (36%) become identical, whereas a large difference is still observed in the case of *K1103* (16%).

Since the dominant female-sterile mutations at the *ovo* locus behave as antimorphs (BUSSON *et al.* 1983), it is possible that recovery of unhatched, correctly shaped eggs derived from germline clone events in *K1103*/+ females, as well as the lack of single progeny clones in the case of *K155* and their decreased frequency for *K1103*, reflects a perdurance effect of the ovo^D product. [Perdurance has been defined by GARCIA-BELLIDO and MARRIAM (1971) as the minimum time required for a cell to express the mutant phenotype after loss of the wild-type gene.] The elimination of the ovo^D product would then require a few stemline divisions. The presence of numerous germline clones that do

not produce eggs in late irradiated *K1237*/+ females may also be due to perdurance. This also correlates with the ovarian phenotype of *K1103*/+ females (*i.e.*, few abnormal vitellogenic stages).

Since the ovo^D mutations behave as antimorphs, it is possible that they disrupt division of germ cells during larval proliferation and during oogenesis. This would explain the observations that, when a +/+ germline clone is induced in an ovo^D/+ female, an apparent developmental regulation for germ cell number is observed and that, following late irradiations, the development of such clones appears to be affected by perdurance of the ovo^D product. The germline clone frequencies observed in the background of ovo^D mutations would thus depend on the number of cells in the germline at the time of irradiation and on the development of +/+ clones in cells showing the perdurance effect.

I thank M. GANS and L. ENGSTROM for useful discussions and comments on the manuscript and P. HARDY for critical reading of the manuscript. This work was aided by the Centre National de la Recherche Scientifique.

LITERATURE CITED

BECKER, H. J., 1976 Mitotic recombination. pp. 1020–1090. In: *The Genetic and Biology and Drosophila*, Vol. 1c, Edited by M. ASHBURNER and E. NOVITSKY. Academic Press, London.

BUSSON, D., M. GANS, K. KOMITOPOULOU and M. MASSON, 1983 Genetic analysis of three dominant female-sterile mutations located on the *X* chromosome of *Drosophila melanogaster*. Genetics **105:** 309–325.

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

ENGSTROM, L., J. H. CAULTON, E. M. UNDERWOOD and A. P. MAHOWALD, 1982 Developmental lesions in the agametic mutant of *Drosophila melanogaster*. Dev. Biol. **91:** 163–170.

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

GARCIA-BELLIDO, A., 1972 Some parameters of mitotic recombination in *Drosophila melanogaster*. Mol. Gen. Genet. **115:** 54–72.

GARCIA-BELLIDO, A. and J. R. MERRIAM, 1971 Genetic analysis of cell heredity in imaginal discs of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **68:** 2222–2226.

GARCIA-BELLIDO, A. and L. G. ROBBINS, 1983 Viability of female germline cells homozygous for zygotic lethals in *Drosophila melanogaster*. Genetics **103:** 235–247.

ILLMENSEE, K., 1973 The potentialities of transplanted early gastrula nuclei of *Drosophila melanogaster*: production of their image descendants by germline transplanation. Wilhelm Roux' Arch. **171:** 331–343.

JIMENEZ, F. G. and J. A. CAMPOS-ORTEGA, 1982 Maternal effects of zygotic mutants affecting early neurogenesis in *Drosophila*. Wilhelm Roux' Arch. **191:** 191–201.

KERRIDGE, S. and J. M. DURA, 1982 Lethal *bithorax complex* mutations of *Drosophila melanogaster* show no germline maternal effects. Dev. Genet. **3:** 207–214.

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

KOMITOPOULOU, K., M. GANS, L. M. MARGARITIS, F. C. KAFATOS and M. MASSON, 1983 Isolation and characterization of sex-linked female-sterile mutations in *Drosophila melanogaster* with special attention to eggshell mutants. Genetics **105:** 897–920.

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

MARIOL, M. C., 1981 Genetic and developmental studies of a new grandchildless mutant of *Drosophila melanogaster*. Mol. Gen. Genet. **181:** 505–511.

NIKI, Y. and M. OKADA, 1981 Isolation and characterization of grandchildless like mutants in *Drosophila melanogaster*. Wilhelm Roux' Arch. **190:** 1–10.

NÜSSLEIN-VOLHARD, C., 1977 A rapid method for screening eggs from single *Drosophila* females. Drosophila Inform. Serv. **52:** 166.

PERRIMON, N., L. ENGSTROM and A. P. MAHOWALD, 1984 Analysis of the effects of zygotic lethal mutations on germline functions in *Drosophila*. Dev. Biol. In press.

PERRIMON, N. and M. GANS, 1983 Clonal analysis of the tissue specificity of recessive female sterile mutations of *Drosophila melanogaster* using a dominant female sterile mutation *Fs(1)K1237*. Dev. Biol. **100:** 365–373.

POULSON, D. F., 1950 Histogenesis, organogenesis and differentiation in the embryo of *Drosophila melanogaster* Meigen. pp. 168–274. In: *Biology of Drosophila*, Edited by M. DEMEREC. John Wiley and Sons, New York.

SCHUPBACH, T., 1982 Autosomal mutations that interfere with sex determination in somatic cells of *Drosophila* have no direct effect on the germline. Dev. Biol. **89:** 117–127.

SONNENBLICK, B. P., 1950 The early embryology of *Drosophila melanogaster*. pp. 62–67. In *Biology of Drosophila*, Edited by M. DEMEREC. John Wiley and Sons, New York.

THIERRY MIEG, D., 1976 Study of a temperature sensitive mutant grandchildless like in *Drosophila melanogaster*. J. Microsc. Biol. **25:** 1–6.

VAN DEUSEN, E. B., 1977 Sex determination in germline chimeras in Drosophila melanogaster. J. Embryol. Exp. Morphol. **37:** 173–185.

WIESCHAUS, E., J. L. MARSH and W. J. GEHRING, 1978 *fs(1)K10*, a germline dependent female sterile mutation causing abnormal chorion morphology in *Drosophila melanogaster*. Wilhelm Roux' Arch. **184:** 75–82.

WIESCHAUS, E. and J. SZABAD, 1979 The developmental and function of the female germline in *Drosophila melanogaster*: a cell lineage study. Dev. Biol. **68:** 29–46.

YARGER, R. J. and R. C. KING, 1971 The phenogenetics of a temperature sensitive autosomal dominant, female sterile germ in Drosophila melanogaster. Dev. Biol. **24:** 166–177.

Corresponding editor: T. C. KAUFMAN