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The Effects of Zygotic Lethal Mutations on Female Germ-Line Functions in *Drosophila*

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Many genetic loci that result in lethality when mutated may also have an essential role in oogenesis. The maternal effects of EMS-induced zygotic lethal mutations at 48 loci were examined using the dominant female-sterile technique. Three categories of effects were found. In the first group (13 out of 48), no maternal effect was detected. The second set (20 out of 48) exhibited maternal effects on oogenesis, embryogenesis, or both. In 13 of this last group, only a few eggs were produced before a progressive deterioration of development occurred. It is suggested that perdurance of the wild-type gene product could produce this result. The third group (15 out of 48) produced cell lethality in germ-line clones, an effect that may be related to their role in indispensable cell functions. Three loci were found which, in germ-line clones, produced embryonic phenotypes that resemble maternal effect mutations. The implications of this study for the genetic analysis of early development are discussed.

INTRODUCTION

Genetic and molecular evidence indicates that there are approximately 5000 genes in *Drosophila* (Muller and Prokofyeva, 1935; Lefevre, 1974; Bishop *et al.*, 1975; Garcia-Bellido and Ripoll, 1978). It is of considerable interest to developmental biologists to determine the proportion of this genetic information that is essential during oogenesis for establishing the basic pattern of embryonic development. Saturation screens for female-sterile mutations (Gans *et al.*, 1975; Mohler, 1977) indicate that only 5 to 10% of all mutations produce female sterility. The possibility exists that many more genes are important contributors to oogenesis but go undetected in screens for female-sterile mutations because one of their pleiotropic effects is zygotic lethality. Saturation screens for lethality indicate that 85% of the *Drosophila* genome can be mutated to lethality (Shannon *et al.*, 1972; Hochman, 1973).

In general, mutations may produce their lethal phenotype at different times throughout development. Comparative studies of lethal phases have been made by Hadorn (1961) and Bryant and Zornetzer (1973). Although not completely comparable, the results of these studies indicate that, of the lethals examined, 35–50% die as embryos or first instar larvae, 5–10% die as second instar, 10–20% as third instar, and 20% as pupae. In addition, Bryant and Zornetzer (1973) found 10% which were polyphasic lethals. A number of previous studies have indicated that some loci that are essential for viability are also expressed during oogenesis. Ripoll (1977) and Ripoll and Garcia-Bellido (1979) observed that most cell lethals are also early lethals, leading them to suggest that perdurance of wild-type maternal information in the egg was capable of supporting embryonic, but not larval, development. This, in turn, suggests that the phenotype produced by at least some embryonic lethals may not be a simple reflection of the function of the zygotic genome. Indeed, Denell (1982) found that the degree of phenotypic expression in genetically identical embryos heterozygous for two *Polycomb* alleles was dependent on which allele was maternal. Also, Jimenez and Campos-Ortega (1982) demonstrated that the maternal contribution to the embryo is responsible for the phenotypic differences between the neurogenic mutations *Notch* and *mastermind*, and that embryos heterozygous for *Notch* do not survive when derived from homozygous *Notch* germ-line clones, but do when derived from heterozygous mothers. Finally, a temperature shift from restrictive to permissive conditions applied to female homozygous for some temperature-sensitive lethal mutations demonstrates the maternal activity of these loci (Fausto-Sterling *et al.*, 1977; Shearn *et al.*, 1978; Schneiderman *et al.*, cited in King and Mohler, 1975). None of these studies, however, has included a detailed analysis of the germ-line functions of these loci.

We have examined germ-line clones of 48 lethals distributed throughout the X chromosome in order to evaluate the proportion of diverse lethal genes which exhibit (1) specific maternal effects on development; (2) alterations of germ-line development (viability or abnormal oogenesis), and (3) the extent of perdurance. We also have examined the possibility that correlations exist between the zygotic lethal phases and the effects of mutations in homozygous germ-line clones.

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MATERIALS AND METHODS

Strains. Most of the strains with recessive lethal mutations (as well as all information relating to the cytogenetic position of the mutations) were kindly supplied by Dr. George Lefevre (see Results). All of these mutations, with the exception of *DA514* (X-ray induced), were ethylmethanesulfonate (EMS) induced. Since most recessive lethal chromosomes were maintained in combination with a duplication, the possibility that more than one lethal was present on the X chromosome was minimized. Here, all mutations are designated by their individual symbol only.

The stocks of npr^1 and npr^2 were obtained from Dr. I. Kiss; they are described in Kiss *et al.* (1978).

The stocks containing the mutations *l(1)m1*, *l(1)EN8*, and $l(1)v^{451}$ were obtained from the Bowling Green stock center. Descriptions of these mutations can be found in Lindsley and Grell (1968).

The dominant female-sterile mutation, *Fs(1)K1237* is maintained in an attached-X stock. The X chromosome containing *Fs(1)K1237* also bears $vermilion^{24}$. It was used to generate germ-line clones (Perrimon and Gans, 1983).

Determination of the effective lethal phase. Approximately 20 females heterozygous for each lethal mutation, after mating to wild-type males, were allowed to lay eggs for periods of up to 12 hr, after which the eggs were counted; after an additional 48 hr unhatched eggs were counted and a sample was studied in Hoyer's mounts (van der Meer, 1977). At least 50 crawling larvae were transferred to fresh medium and their development was observed daily to determine larval lethal periods. The number of pupae formed was scored as well as the number and sex of emerging adults. This procedure allowed us to accurately assign the lethal phase of each mutation to either the embryonic (E), the larval (L1, L2-L3), the pupal (P), or the adult (A) period. If mortality occurred during several stages, the primary effective lethal phase was identified as the stage during which more than 50% of the individuals died. When the majority (>50%) expired during two stages both were considered effective lethal phase. Mutations which result in mortality during more than three consecutive stages of development or during nonconsecutive stages were designated polyphasic lethal mutations. Adult lethal phases were identified when hemizygous mutant males emerged. In all cases, such males were infrequent (<5%) and abnormal, usually perished within 1 day, and never mated.

Germ-line clone induction and detection. The technique used has been described by Perrimon and Gans (1983). Briefly, the progeny of Balancer/lethal females mated to males carrying the dominant female-sterile, germ-line-dependent mutation *Fs(1)K1237* were irradiated near the end of the first larval instar stage. The dosage was 1000 rad (General Electric X-ray machine, 100 kV, 5 mA, 3 ft × 35 in., 1-mm aluminum filter). Under such conditions the frequency of germ-line clone induction using a wild-type strain, *Oregon R(P2)*, was around 8%. With such a frequency of clone induction among controls, the minimum number of females that must be examined in order to conclude that homozygous lethal germ-line clones were induced but failed to produce clones is around 200 ($P < 0.05$). In cases where the number of clones recovered was lower than expected, a larger number of flies was studied.

In each experiment, at least 100 unirradiated females were examined to ensure that the lethal gene did not alter either the frequency of occasional reversions of the dominant female-sterile gene or the frequency of spontaneous mitotic recombination (Busson *et al.*, 1983; Perrimon and Gans, 1983; Perrimon, 1984).

In cases where the lethal locus (*l*) was located more proximal to the centromere than the dominant female-sterile locus, identification of distal recombination events was made by the presence of emerging males (see Fig. 1).

At emergence, irradiated *l/Fs(1)K1237* females were distributed in lots of 10 per vial with 5-10 *Oregon R(P2)* males and checked each day for the presence of eggs. When eggs were found, the clone-containing female was then isolated and studied individually. If few or no germ-line clones were found or if the eggs were abnormal in morphology or unfertilized, a sample of females was dissected and ovaries possessing germ-line clones were then Feulgen stained. Ovarioles containing the germ-line clone (+/+) are easily distinguished from the others (+/*Fs(1)K1237*) by the presence of vitellogenic egg chambers.

In the case of fertile females with germ-line clones any unhatched embryos were studied by preparing

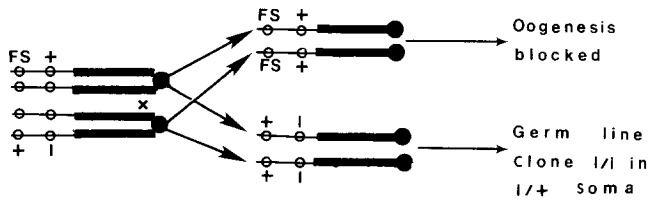

FIG. 1. Principle of the test of maternal effect of lethal mutations using the dominant female-sterile technique. Mitotic recombination events proximal to the lethal mutation tested (l), lead to a *l/l* germ-line clone in a *l/+* soma. Distal events or revertants lead to a *l/+* germ-line clone which is identified by the presence of *vermilion* male offspring. In most cases, such distal events did not occur since the lethals tested were mostly located distally or close to *Fs(1)K1237*.

Hoyer's mounts (van der Meer, 1977). The development of hatched larvae was followed daily and the morphology and fertility of any emerging adults were examined.

RESULTS

We have classified the lethals tested into three broad categories based upon their phenotypic expression observed in germ-line clones: Category I, those which did not exhibit a maternal effect, i.e., the lethal phase among male progeny derived from mothers possessing germ-line clones homozygous for the lethal was the same as among male progeny derived from *l*/+ mothers and the number, morphology, and fertility of female progeny were normal; Category II, those which showed a maternal effect or disrupted late stages of oogenesis; and Category III, those which produced germ-line lethality prior to establishment of gonial stem-cell populations in the ovaries [see King (1970) and Wieschaus and Szabad (1979) for the description of the development of the female germ line] or blocked egg chamber development prior to vitellogenesis. As indicated below, the distinction between the last two categories may have been arbitrary in some cases.

Category I: Lethals with No Maternal Effect

Among 48 lethals tested 13 exhibited no apparent maternal effect (Table 1). In 12 the male lethality, male/female ratio, morphology, and fertility among clonally derived offspring were comparable to those of controls. Although males with the polyphasic lethal mutation *VA23* occasionally survived when derived from heterozygous mothers, we found none among the progeny of homozygous germ-line clones. This might suggest a maternal effect; however, since the lethal phase among offspring of both germ-line clones and heterozygous females was mainly pupal and since very few adult males (8 out of 744 eggs) were found in the controls, we believe that background effects rather than a maternal effect were responsible for the absence of clonally derived adult males. Four additional mutations whose lethal phases included adults were studied and clonally derived adult males were found. Note that no specific lethal phase predominated among the lethals of this category. Two embryonic zygotic lethals *EA55* and *VA208* were analyzed but we could not detect any specific embryonic defect.

Category II: Lethals Which Affect Embryogenesis and/or Late Oogenesis

In this category, we found only two lethal mutations which exhibited effects on embryogenesis alone; five showed defects only on oogenesis (usually producing no eggs) and the remaining 13 exhibited defects on both oogenesis and embryogenesis (Tables 2-3).

TABLE 1
THE RESULTS OF GERM-LINE CLONES OF 13 LETHAL MUTATIONS EXHIBITING NO MATERNAL EFFECTS (CATEGORY I MUTATIONS)

		Lethal phase									
Mutant	Location	E	L1	L2	L3	EP	LP	A	*N*	N_c	Male progeny
VA208	1B6-1B10	±	+						190	10	
VA23	1B10-1D4	±	±	±	±	+	+	±	280	15	
npr^1	2B					+			520	11	
npr^2	2B					+			465	9	
VE692	2C3		±	±	+	±			320	14	
EA94	3A4						+	±	165	13	+
EC287	3B3		+	+					220	13	
EA68	7B			+	+	±	±	±	270	18	+
VA156	7B		±	±	+	±	±	±	260	16	+
EA61	9F				+	±	±	±	350	13	+
EA17	10F4					+	+		74	4	
DA600	11A2	+	±	±					102	5	
EA55	11A6	±	+						212	18	

Note. The cytological location and lethal phase among progeny of control (unirradiated) heterozygous lethal females are shown. Lethal phases are: E = embryonic; L(1), L(2), L(3) = larval instars; EP and LP = early and late pupal; and A = adult, with the primary lethal period(s) indicated with "+" and other lethality times indicated by "±" (see Materials and Methods). The number of irradiated females studied (N) and the number of females containing clones (N_c) are indicated. Female offspring were recovered in all progenies. Note that in four of five cases where adult males were recovered in controls (male progeny column), males were also recovered among the offspring of germ-line clones. The lower frequencies of clone induction in *npr*^{1} and *npr*^{2} are due to the use of a different X-ray machine.

TABLE 2
RESULTS OF GERM-LINE CLONES OF 20 LETHAL MUTATIONS WHICH EXHIBIT EFFECTS ON OOGENESIS, EMBRYOGENESIS, OR BOTH (CATEGORY II MUTATIONS)

Mutant[a]	Location	Lethal phase	N	N_e	N_d	N_c	Phenotype of eggs laid
VE736	2A4-2B12	E-L1-L2	220	9	NT[b]	NT	+
VE653	2C9	L1-L2	180	5	NT	NT	+
DF958	2D4	L2-L3	763	0	763	30	−
EA75	2F6	P	220	10	NT	NT	+
DF944	3A7	P	240	5	120	20	+
EF525	3B4	P-A	305	4	305	5	+
DA670	3B5	Pol	397	3	397	8	+
VAN81	3CF	E	215	18	NT	NT	+
*l(1)m1**	10	L3	123	1	123	4	c
VA234	6D	P	350	1	100	7	c
*l(l)EN8**	13	L2-L3-P	166	9	166	15	+
VA313	7D	L3-P	262	4	262	6	+
VA40	7D	L3	450	3	100	8	c
DA583	7D-7E	E	70	1	70	3	+
VE661	8A1	L2-L3	300	5	300	16	c
DC701	9E	E-L1-L2	110	0	110	10	−
EF444	10A8	L2	155	5	155	11	+
DC705	10B13	L3	238	4	238	9	+
DC833	10C10	L2-L3	220	6	220	10	+
*l(1)v^{451}**	56.7	L2-L3	95	3	95	7	+

Note. The cytological location and lethal phase(s) (see Table 1) are indicated. "Pol" indicates a lethal mutation which demonstrated no primary lethal phase(s); i.e., hemizygotes die during all stages. The number of irradiated females (N), the number of females producing eggs (N_e), the number of females dissected (N_d), and the number of dissected females containing clones (N_c) are shown. (When N_d is less than N, N_e is included in N_c.) The phenotype of eggs produced is indicated by "+" if normal in shape, "c" if collapsed, and "−" if no eggs. Lindsley and Grell (1968) indicate that *l(l)v^{451}* is a late lethal; in our hands it exhibits larval lethality.

[a] * indicates that these mutants have not been cytologically mapped; their meiotic location is given.

[b] Not tested.

Maternal effect lethals. We found for *VAN81*, a lethal allele of *Notch*, results similar to those described by Jimenez and Campos-Ortega (1982): of 297 fertilized eggs examined, 54% exhibited normal phenotype, 43% had the *Notch* embryonic phenotype, and 3% had fused segments or large dorsal or ventral cuticular holes. Hatching larvae were never observed. *EA75* (a pupal lethal) in germ-line clones produced embryos in which structures at the posterior tips failed to develop (see Fig. 2b). This phenotype is similar to that produced by at least three female-sterile loci named *torso* or *pole hole*, *fs(2)torso* (Nüsslein-Volhard *et al.*, 1982), an allele of *fs(1)Nasrat*, and *fs(1)pole-hole* (Konrad and Mahowald, 1983). The possibility that this phenotype resulted from a small deficiency including a maternal effect lethal mutation adjacent to *EA75* was tested by examining two other alleles of this locus in germ-line clones; these also exhibited the torso phenotype. A more detailed analysis of this unusual locus will be described elsewhere.

Lethals preventing egg deposition. Germ-line clones for two larval lethals (*DF958*, *DC701*) showed similar defects in oogenesis. The ovaries observed by Feulgen staining exhibited egg chambers containing different numbers of nurse cell nuclei, usually associated with later nuclear degeneration. Egg chambers containing more than 15 nurse cell nuclei were frequently observed. These chambers may represent the early stages of degeneration observed in the same ovarioles (see Fig. 3). The others, *VA234*, *VA40*, *VE661*, produced collapsed eggs (Table 3). The process which was disrupted in oogenesis has not been determined.

Lethals exhibiting variable phenotype. Thirteen lethals were found to exhibit a progressive phenotypic degeneration in germ-line clones (indicated by "+" in the variable phenotype column, Table 3). We have separated these mutations into three classes based on the terminal phenotypes observed. From early egg collections of Class AE derived from germ-line clones (Table 3), a few larvae hatched and generally produced adult females. After 2 to 3 days, embryonic lethality was observed, generally followed by abnormally shaped (fused filaments, collapsed or small egg), infertile eggs. In *VE736*, *DA583*, and *DC833*, the rapidity of the

TABLE 3
RESULTS OF PHENOTYPIC EXAMINATION OF GERM-LINE CLONES AFFECTING OOGENESIS, EARLY DEVELOPMENT OR BOTH (CATEGORY II MUTATIONS)

Mutant	Developmental stage reached				Germ-line clone phenotype[a]	Variable phenotype
	Larva	Embryonic lethal	Abnormal eggs	No eggs		
VAN81		+			E	–
EA75		+			E	–
VA234			c		NO	–
VA40			c		NO	–
VE661			c		NO	–
VE736	+	+	f, c		AE	+
DA583	+	+	s		AE	+
DC833	+	+	c		AE	+
VE653	+	+	u, f		AE	+
DF958				+	AO	–
DC701				+	AO	–
DF944	+	+	f	+	AO	+
l(1)ml			c	+	AO	+
l(1)EN8	+	+	c	+	AO	+
VA313	+			+	AO	+
DC705	+	+	c	+	AO	+
l(1)v^{451}	+		c	+	AO	+
EF525	+			+	L	+
DA670	+			+	L	+
EF444	+			+	L	+

Note. The phenotype initially found is indicated by the leftmost column containing a "+"; if the phenotype progressively degenerated with time, the changes found are indicated (+) in successive columns to the right. Intermediate phenotypes may have been missed due to their low expressivity. The variable phenotype is indicated (+) if the phenotype regressed. Usually living larvae produced heterozygous female adults, suggesting that the wild-type allele rescued the embryo. Only in a few cases was the embryonic phenotype examined; in most cases, too few were found (see text). The egg phenotypes observed are indicated: collapsed (c), small (s), short or fused filaments (f), or unfertilized (u). Five classes of germ-line clone phenotypes are indicated based on strength of phenotypic expression. Normal eggs with embryonic lethality (E), misshapen eggs (AE), no apparent oogenesis defect (NO), abnormal egg chambers (AO), and no detectable vitellogenic stages (L).

[a] For lethals which in germ-line clones exhibit a progressive phenotypic degradation this column indicates the "terminal phenotype."

transition between hatching larvae and unfertilized eggs prevented accurate characterization of a consistent embryonic phenotype. However, embryos derived from germ-line clones of *VE653*—an early larval lethal—consistently displayed posterior defects (Fig. 2c). This phenotype was confirmed in another EMS-induced allele of *VE653*, *VE849* (data not shown).

In Class AO (Table 3), those which show progressively more extreme phenotypes (*DF944*, *l(1)m1*, *l(1)EN8*, *VA313*, *DC705*, *l(1)V^{451}*) generally were similar to class AE except that the phenotype quickly regressed to that described earlier for *DF958* and *DC701*. Again, the embryonic phenotype was difficult to characterize because of the small number of embryos. The only consistent embryonic phenotype found was in germ-line clones of *DF944*. This phenotype exhibited cuticle formation but the embryos were completely shriveled and misshapen.

In Class L (*EF525*, *DA670*, *EF444*; Table 3), we generally obtained few clones and those obtained were small (two or three ovarioles) compared to clones in other classes. Early egg collections produced a few larvae but the females with clones rapidly stopped producing eggs. Later Feulgen-stained ovarian whole mounts failed to reveal any vitellogenic egg chambers. We suggest these clones represented escapers of Category III (see below). The escape may be due to a strong perdurance of the wild-type vital gene products in homozygous germ cells which may divide more slowly than others due to irradiation or other effects.

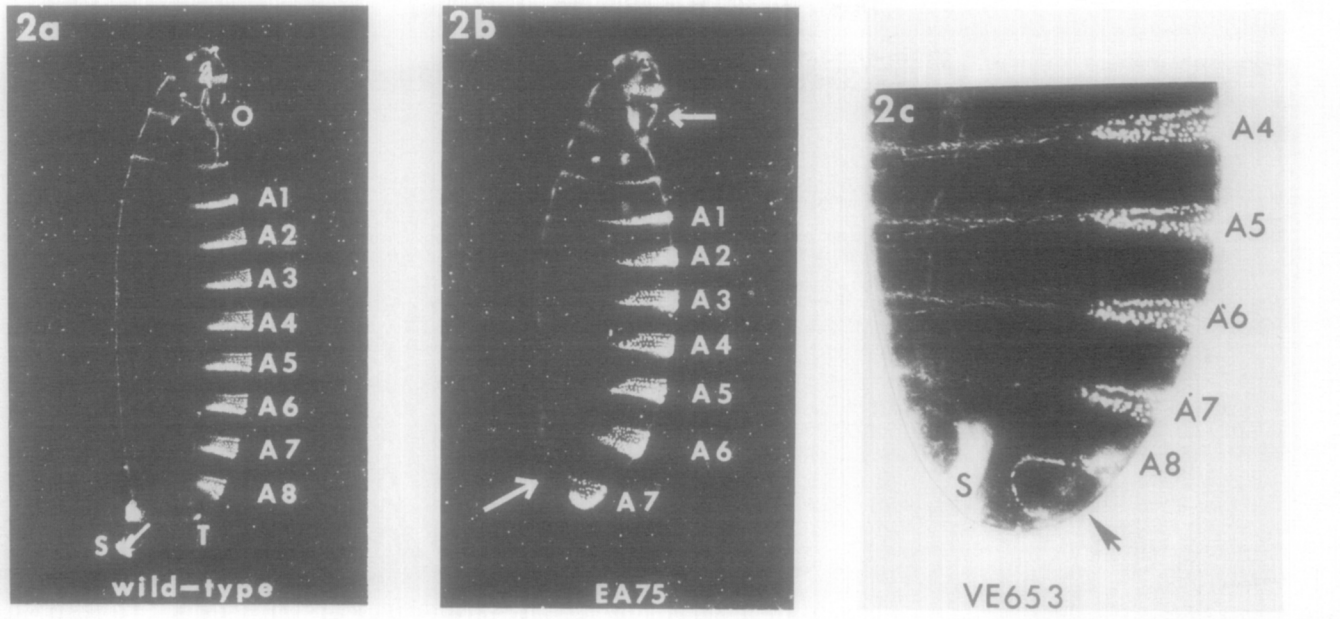

FIG. 2. Lateral dark-field photographs of normal (a), germ-line clonally derived *EA75* (b) and *VE653* (c) embryos. The absence (lower arrow) of posterior spiracles (S) and tuft (T) and abdominal segment A8 is the consistent phenotype of *EA75*. The anterior arrow in b points to the external oral apparatus (O) which appears slightly abnormal. The anterior end, abdominal segments, and spiracles of germ-line clonally derived embryos of *VE653* (c) appear normal but the embryos consistently possessed a cuticular hole posterior to A8 (arrow). A1 through A8 indicated the ventral abdominal denticle belts. (a, b) 85×, (c) 225×.

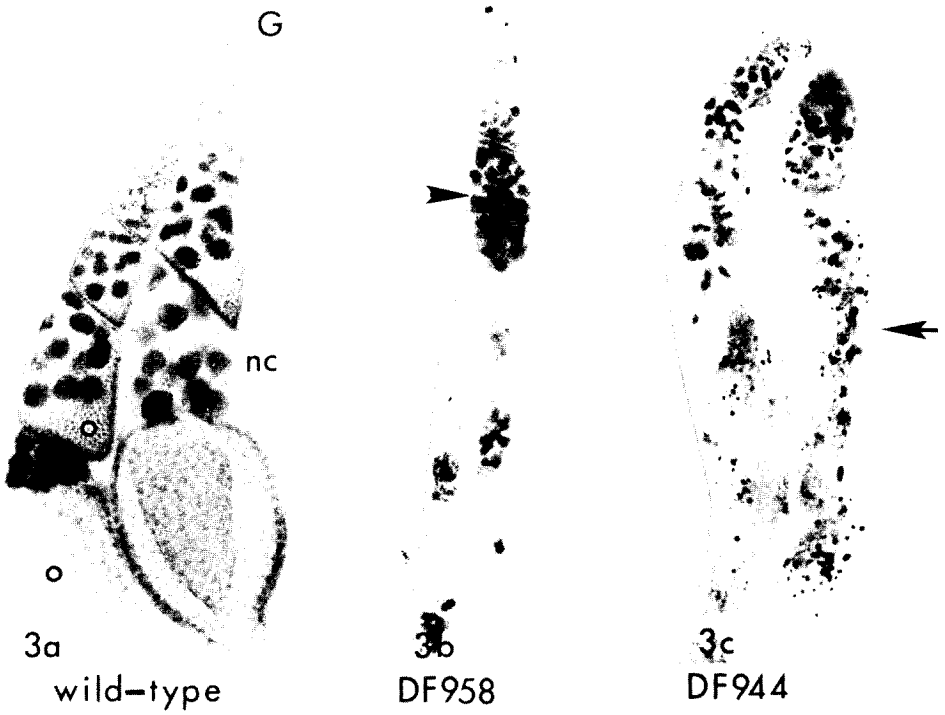

FIG. 3. Whole mounts of Feulgen-stained ovarioles of wild-type (a) and germ-line clones of *DF958* (b) and *DF944* (c). A tumorous egg chamber (many nurse cells) is indicated (arrow) in b and degenerating egg chamber (arrow) in c. G, germarium; o, oocyte; nc, nurse cell. Approximately 125×.

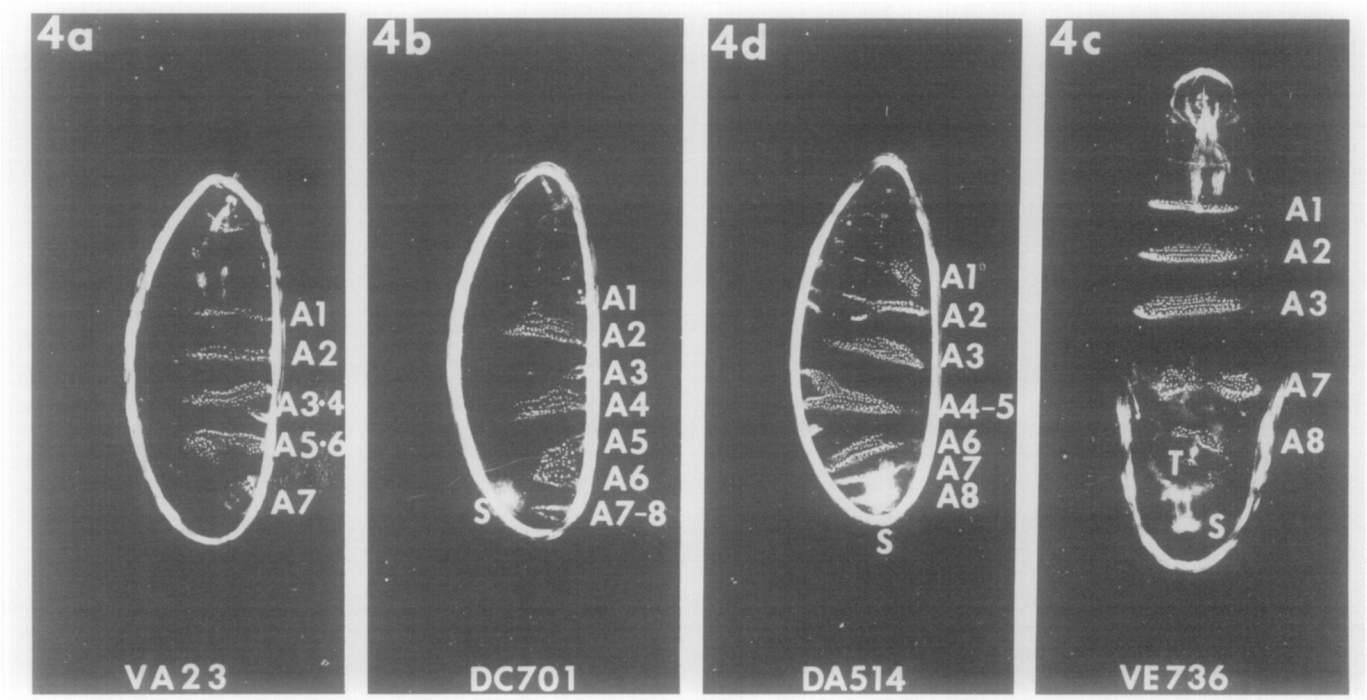

FIG. 4. Dark-field photographs of embryonic zygotic lethal embryos of *VA23* (a), *DC701* (b), *VE736* (c), and *DA514* (d). Note fusion of segments in a, b, and d. The reduction of denticle belts especially A1 and A3 in b and the apparent abdominal segment gap in c which we interpret to be missing A4 through A6. 60×.

It is clear that the rate of division of clonally marked cells is often slower than normal division rates (Wieschaus and Szabad, 1979).

General considerations. In Category II, larval lethal phases appeared enriched relative to Category I. Of 20 zygotic lethals, 13 were embryonic or larval lethals, 5 were clearly pupal lethals, and 2 lethals died at the larval-pupal transition. Two embryonic zygotic lethals exhibited interesting phenotypes. Some segments are often absent in *VE736* embryos (Fig. 4c), and *DC701* (Fig. 4b) exhibited fusion of segments. Only two pure embryonic lethals were found, *Notch* (*VAN81*) whose phenotype was the same as that described by Jimenez and Campos-Ortega (1982) and *DA583* whose embryonic phenotype appeared wild type.

Category III: Lethals Which Affect Germ Cell Viability or Produce Early Arrest of Oogenesis

Fifteen lethals of this category were found (Table 4). The hypothesis proposed in the case of *EF525*, *DA670*, and *EF444* concerning the subviability of germ cells (see Table 3) is supported by results obtained in the study of *DA514* and its allele *L12*. *DA514* was found to be lethal in germ-line clones whereas its allele *L12* disrupted oogenesis. In the case of *L12*, among 139 flies

TABLE 4

RESULTS OF GERM-LINE CLONES OF 15 LETHAL MUTATIONS WHICH EXHIBIT GERM-CELL LETHALITY OR EARLY OOGENESIS ARREST (CATEGORY III MUTATIONS)

Mutant	Location	Lethal phase	N	N_e	N_d	N_c
VE737	1E1-1E3	L3	220	1	112	1
VE795	1E3-2A2	L1-L2	300	0	150	0
VE676	2A3-2A4	L2-L3	650	0	186	0
VA130	2B2-2B17	L2-L3	360	0	150	0
DF967	2D1	L2	420	0	190	0
EC205	2E2	P	359	0	100	0
EA96	3A9	L1-L2	330	1	100	1
VA276	7C	L3	600	0	100	0
VA75	7D	L2-L3	450	1	105	1
VA293	7E	EP	350	2	150	2
VA195	7E-7F	L3-P	600	0	95	0
DA514	10A4	Pol	111	1	111	1
EC230	10B4	E-L1	400	1	185	1
DF912	10C5	E	280	0	102	0
DF939	10E6	L2	198	1	198	1

Note. The cytological location and lethal phase(s) (see Table 1; Pol = polyphasic lethal) are indicated. The number of irradiated females (N), the number of females producing eggs (N_e), the number of females dissected (N_d), and the number of dissected females containing vitellogenic stages of oogenesis (N_c) are indicated. (All N_c individuals were identical to the N_e individuals and genetic tests of the offspring of each of these individuals proved that all such "clones" either were revertants of *Fs(1)K1237* or were due to recombination between *Fs(1)K1237* and the lethal locus.)

studied, one laid collapsed eggs; after dissection of all the females, Feulgen staining revealed two additional clones both exhibiting abnormal oogenesis. This probably indicates that *L12* is a weaker allele than *DA514* and that the low frequency of clones obtained in the study of *L12* may indicate subviability of germ cells. Note that the lethal phases of the mutations in this class are mainly (11 of 15) larval. *EC230* embryos were found to die at hatching and *DA514* embryos exhibited a fused segment pattern (see Fig. 4d).

DISCUSSION

The analysis presented here indicates that many zygotic lethal mutations exhibit maternal effects on development. In some instances, the resulting embryonic phenotypes resemble phenotypes produced by maternal effect female-sterile or early zygotic lethal mutations.

We found that 73% of the lethals examined did affect germ-line functions in homozygous germ-line clones. In a similar study of lethals in the *zeste-white* region, Garcia-Bellido and Robbins (1983) found that 13 of 15 (86%) loci exhibited germ-line effects; 10 produced no eggs; and 3 were maternal effect lethal. We suspect that our figure of 73% is an underestimate. Careful examination of the zygotic lethal phase of the mutations analyzed indicated that 69% of the mutations with no maternal effects (Category I) are polyphasic or late lethals. Since we have examined only one allele per locus, it is not clear whether that allele represents the amorphic state of the gene or not. If the alleles are amorphic, then we can conclude that the function of such genes is restricted to somatic tissues. Our data, however, suggest that the quality of a particular allele must be carefully considered during such evaluations. For example, we obtained differing results with alleles *L12* and *DA514*. Furthermore, in a recent study of alleles at a locus encoding a subunit of RNA polymerase II, two were germ-line lethal and one was germ-line viable and affected egg morphology (Mortin and Perrimon, in preparation). Similar observations have been made by Garcia-Bellido and Robbins (1983) who examined two alleles of the *zw6* locus. Homozygous germ-line clones of one of them ($zw6^{a25}$) exhibited a maternal effect embryonic lethal phenotype, whereas homozygous clones of the other allele ($zw6^{e5}$) failed to produce eggs. Robbins (1983), however, presented evidence that the $zw6^{e5}$ mutation was associated with another mutation located outside the zeste-white area so this apparent allele-dependent difference might be due to the effects of the second lethal mutation. Different alleles of the same locus may exhibit different phenotypes in germ-line clones. For example, it is possible that some of the 13 zygotic lethal mutations shown here to exhibit no germ-line function would display maternal effects or germ-line lethality if we examined other alleles.

One might expect a lower proportion of late lethal phase mutations to exhibit maternal effects since a different subset of gene activities may be specifically required for pupariation. This proved to be the case for two nonpupariation mutants (npr^1 and npr^2) which are known to affect the 2B chromosome puff (Kiss *et al.*, 1978). Furthermore, we observed an enrichment of early lethal phase mutations among those affecting germ-line functions (Categories II and III in Table 5). The genes involved probably represent general metabolic functions, the maternal component being sufficient to support early, but not later, development. This point was previously suggested by the results of Ripoll (1977) and Ripoll and Garcia-Bellido (1979) who found that the majority of zygotic lethals which are cell lethals in mitotic recombination clones of somatic tissue also exhibit early zygotic lethality. Similar interpretations have been suggested by Wood *et al.* (1980) for *Caenorhabditis elegans*. In fact, Isnenghi *et al.* (1983) found that over 50% of thermosensitive embryonic lethals in this nematode require maternal expression.

The approach used here may be compared to other methods of studying the maternal effects of lethal mutations. As described in the Introduction such effects can be analyzed using thermosensitive (ts) lethal mutations in which the maternal effect is analyzed after shifting homozygous lethal adult females to the restrictive temperature. Although many ts lethal mutations have been isolated, most of them have not been tested for their effects on germ-line functions. Rice (1973) described the isolation of 16 such mutations. Females homozygous for five of these mutations did not lay any eggs at the permissive temperature while the others laid eggs showing embryonic defects mainly

TABLE 5
CORRELATION BETWEEN ZYGOTIC LETHAL PHASE(S) AND GERM-LINE CLONE PHENOTYPE

Category	*N*	EL	P	Pol
I	13	31%	31%	38%
II	20	65%	30%	5%
III	15	73%	20%	7%

Note. Descriptions of the categories are found under Results. *N* equals the number of lethals in each group. Embryonic and larval zygotic lethals (EL) have been combined and compared to the pupal (P) and to polyphasic (Pol) zygotic lethals. Note *early* to *late* lethal phases correlate with *more* to *less* extreme phenotypes in germ-line clones.

during early nuclear divisions. King and Mohler (1975) cited the characterization by Schneiderman *et al.* of 22 X-linked ts lethals representing 16 loci. One of them was a maternal effect lethal which prevented embryonic development while the others disrupted different stages of oogenesis. Careful analyses of the maternal effects of ts lethals have been performed so far on only three loci: *l(1)ts1* analyzed by Fausto-Sterling *et al.* (1977) is described as affecting midgut formation and dorsal closure, two alleles of the small disk mutation *l(3)1902* characterized by Shearn *et al.* (1978) affect early embryonic development, and *fs(1)h* shows two separate temperature-sensitive periods, the first during oogenesis extending into early embryogenesis and the second during the pupal stage (Forquignon, 1981). Potentially, the characterization of ts lethals would appear to be a convenient method for investigation of germ-line effects of lethal mutations in *Drosophila.* Since apparently 12% of EMS-induced lethals are ts (Suzuki, 1970), such an approach is feasible, but it is not clear that at the restrictive temperature the true amorphic state of a ts lethal gene is expressed. That residual activity of ts lethal mutations exists at restrictive temperatures has been indicated for *mutagen sensitive-101*^{ts1} (Gatti *et al.*, 1983) and for *Ubl*^{ts}, a temperature-sensitive RNA polymerase II mutation (Mortin and Kaufman, 1982). Partial gene activity at the restrictive temperature would render the examination of maternal effects difficult. This problem can be overcome by using the dominant-female-sterile technique, if known amorphic alleles of each lethal mutation are tested. Alternatively, pole cell transplantations (Van Deusen, 1976) could also be used to examine the germ-line effects of lethal mutations.

The study of the maternal effects of embryonic zygotic lethals in germ-line clones may also be the method of choice to distinguish between genes with functions specifically related to embryonic development and those with more general functions. A few of the recessive embryonic lethal mutations which have been characterized so far in germ-line clones do not exhibit maternal effects: they include *Krüppel* (Wieschaus, 1980), *Ultrabithorax* mutations (Kerridge and Dura, 1982), and embryonic lethal of *giant*, *gt*^{X11} (Perrimon and Engstrom, unpublished). Examples of weak influences of wild-type products stored in the eggs on embryonic zygotic phenotypes were shown in the cases of *Notch* and *mastermind* (Jimenez and Campos-Ortega, 1982). It is possible that lethal mutations which affect metabolic processes exhibit germ-line defects preventing egg production, whereas those which disrupt correct embryonic pattern determination exhibit either weak or no maternal effects. If this statement were true, then the polyphasic lethals exhibiting embryonic phenotypes (Fig. 4) which are comparable to *pair rule* or *gap* mutations (Nüsslein and Wieschaus, 1980) may reflect reduced levels of gene activities responsible for more general cellular functions and not genes specifically related to events in embryonic development. Their germ-line clone phenotypes (except in the case of *VA23*) indicate that each of the genes involved is required for germ-line development.

The observation of progressive phenotypic deterioration among lethal clones was unexpected, since the induction of a homozygous clone is followed by a number of cell divisions (Wieschaus and Szabad, 1979) and the germ cells undergo extensive growth and metabolic activity during oogenesis. However, this phenomenon may reflect a perdurance (term defined by Garcia-Bellido and Merriam, 1971) of the wild-type gene product of "housekeeping" genes present in germ cells prior to the mitotic recombination event. It may be that the alleles tested possessed partial activity and that the perdurance accompanied by this residual activity was capable of supporting oogenesis for a period. It may be possible to investigate this perdurance phenomenon in the female germ line more thoroughly by induction of germ-line clones at various developmental stages using other dominant female-sterile mutations (Perrimon, 1984) and by examining cell viability of somatic clones homozygous for these mutations.

The possibility that the extent of perdurance is in some cases limited may explain the behavior of those lethals where only a few small homozygous clones were found. The degenerating ovarian phenotype observed (Fig. 3) is similar to the phenotype produced by several female-sterile loci. On the X chromosome, for example, four loci are known to produce ovarian tumors. For one of them, *ovarian tumor*, many alleles are known (Gans *et al.*, 1975; King and Riley, 1982). However, the other three, *fs(1)K741*^{ts}, *fs(1)K1274*^{ts}, and *fs(1)1621*, are represented by single alleles (Komitopoulou *et al.*, 1983). This may suggest that the latter three represent leaky mutations at vital loci, their expression being limited to ovaries because of the high metabolic activity of oogenesis (Bischoff and Lucchesi, 1971).

The observation that a lethal mutation can produce a *torso* or *pole hole*-like phenotype (Nüsslein-Volhard *et al.*, 1982; Konrad and Mahowald, 1983) indicates that zygotic lethals studied in germ-line clones can exhibit phenotypes similar to those produced by female-sterile mutations. Among 48 zygotic lethals we found 3 (*VE653, EA75, VA81N*) which exhibited a maternal effect on cell determination (6%). A comparison of this percentage to an estimate of the number of maternal effect

female-sterile mutations affecting cell determination (0.5 to 1%) (see review Konrad and Mahowald, 1983) suggests that saturation screens for this type of female-sterile mutation may detect only a fraction of the maternally active loci needed for embryonic development.

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