VIABILITY OF FEMALE GERM-LINE CELLS HOMOZYGOUS FOR ZYGOTIC LETHALS IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

We have analyzed the viability of different types of X chromosomes in homozygous clones of female germ cells. The chromosomes carried viable mutations, single-cistron zygotic-lethal and semi-lethal mutations, or small (about six chromosome band) deletions. Homozygous germ-line clones were produced by recombination in females heterozygous for an X-linked, dominant, agametic female sterile.

All the zygotic-viable mutants are also viable in germ cells. Of 16 deletions tested (uncovering a total of 93 bands) only 2 (of 4 and 5 bands) are germ-cell viable. Mutations in 15 lethal complementation groups in the zeste-white region were tested. When known, the most extreme alleles at each locus were tested. Only in five loci (33%) were the mutants viable in the germ line. Similar studies of the same deletions and point-mutant lethals in epidermal cells show that 42% of the bands and 77% of the lethal alleles are viable. Thus, germ-line cells have more stringent cell-autonomous genetic requirements than do epidermal cells.

The eggs recovered from clones of three of the germ-cell viable *zw* mutations gave embryos arrested early in embryogenesis, although genotypically identical embryos derived from heterozygous oogonia die as larvae or even hatch as adult escapers. For two genes, homozygosis of the mutations tested also caused embryonic arrest of heterozygous female embryos, and in one case, the eggs did not develop at all. Germ-line clones of one quite leaky mutation gave eggs that were indistinguishable from normal. The abundance of genes whose products are required for oogenesis, whose products are required in the oocyte, and whose activity is required during zygotic development is discussed.

AN important parameter of developmental genetics is the number of genes necessary for any particular developmental process. An estimate of this is the fraction of loci in which amorphic alleles cause cell autonomous arrest of the particular developmental process. Studies with zygotic lethals have shown

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that about 9% of lethal point mutations are also lethal in mitotic-recombination-generated clones of epidermal cells (RIPOLL and GARCIA-BELLIDO 1973; RIPOLL 1977) and a similar fraction of cell lethals was found by ARKING (1975) among temperature-sensitive lethals. These figures could be underestimates since some of the mutations classified as cell viable could be hypomorphic alleles of genes that are actually necessary for cell survival. However, a study of the cell viability of deletions, which are necessarily amorphic, gave a similar figure: about 12% of the lethal complementation groups included in the deficiencies are epidermal cell lethal (GARCIA-BELLIDO and RIPOLL 1978; RIPOLL and GARCIA-BELLIDO 1979).

The ability of germ cells homozygous for some small deletions and point-mutants to proliferate and/or differentiate into mature eggs is evaluated here. For this study we have chosen deletions and point-mutant lethals previously characterized for viability in epidermal cells.

Cells homozygous for these lethals were produced by mitotic recombination in the germ line of females that are heterozygous for a dominant, agametic, female-sterile mutation (*FS(1)K1237*) kindly given to us by M. GANS. The recombinant clones, if viable, would yield eggs derived from homozygous gonia. Should such eggs be produced, we can also study the effects on embryonic development of total absence of individual gene products in the oocyte. WIESCHAUS (1980) has already carried out an analysis of this type with some embryonic morphogenetic mutants. Studies of the viability of offspring of females heterozygous for different deletions and point mutations indicate that a large fraction of the genome must be active in the oocyte in order to support early embryonic development (GARCIA-BELLIDO and MOSCOSO DEL PRADO 1979; ROBBINS 1980; ROBBINS 1983; A. GARCIA-BELLIDO, J. MOSCOSO DEL PRADO and J. BOTAS, unpublished results). In the present study we estimate the fraction of the genome that is needed for development of the germ line itself.

MATERIALS AND METHODS

The FS(1)K1237 dominant-female-sterile mutation was found in the laboratory of M. GANS and was kindly lent to us along with some unpublished information relevant to the present work. Females heterozygous for this mutation are agametic, *i.e.*, they do not lay eggs. Oogenesis in these females is arrested at various points during early oocyte growth. The mutation maps at $4^{\pm}$ in the X chromosome based on the location of several fertile revertants that have a rough eye phenotype.

Viable mutants used as controls are described in LINDSLEY and GRELL (1968). The X-chromosome deletions used have a variety of origins. All had been studied in a previous work that dealt with the viability of these deletions in epidermal cells (RIPOLL and GARCIA-BELLIDO 1979). As indicated in Table 2, the deletion chromosomes also carry recessive visible mutations. The single-cistron mutations studied are listed in Table 3. They are located in the zeste-white region of the X chromosome and most have been described in SHANNON *et al.* (1972). Mutants in *zw13* have been described by LIM and SNYDER (1974). The deletion chromosomes and the zeste-white region mutations are maintained in males that carry the w^+Y chromosome which covers the lethal region. This reduces the likelihood of there being secondary lethals in these chromosomes.

Mitotic recombination was induced by X rays (Philips MG 151Be, 100 Kv, 15 mA, 2 mm Al filter, 300 r/min, total dose of 1000 r). Larvae (48 to 72 hr after egg laying), or 1-day-old imagoes were irradiated. The irradiated individuals were the offspring of a cross of mutant/*FM6* females to *FS(1)K1237*, *v* males. The irradiated mutant/*FS* females were crossed to *FM7c*, y^2 w^a *v* sn^{X2} *B* (MERRIAM and DUFFY 1972) males. Groups of 50 females and about 20 males were transferred every

2 days to new bottles for 2 weeks. Upon transfer, the bottles were examined for the presence of eggs. Egg-laying females were isolated in individual vials with new males and were allowed to lay eggs for several days. The fraction of egg-laying females among the total number of females tested was used to estimate the frequency of germ-line mitotic recombination (WIESCHAUS and SZABAD 1978). The numbers of eggs laid and hatched were also determined. When adult flies were produced, their genotypes were determined in order to confirm that a germ-line mitotic-recombination event had occurred. Sterility caused by *FS* is not fully penetrant; occasional females may contain one or several ovarioles that produce normal eggs. Since *FS* is coupled to *v*, these escapers can be readily distinguished from mitotic-recombination products. We have found only five leaky females among the several thousand females scored in this study.

In those cases in which females laid eggs but the eggs failed to hatch, the eggs were dechorionated with 7% sodium hypochlorite and were examined under paraffin oil in the light microscope.

RESULTS

Controls

The gonial cells are in the proliferation period in 48 to 72-hr-old larvae (KING 1970). Mitotic recombination occurring at that time gives rise to a clone of gonial cells that can populate one or more ovarioles (WIESCHAUS and SZABAD 1978). In fertile females heterozygous for germ-line markers, the frequency of mitotic recombination is measured by the number of females laying *some* mutant eggs. This frequency is about 10% for markers at the tip of the X chromosome in females irradiated with 1000 r as young larvae (WIESCHAUS and SZABAD 1978). In contrast, *FS(1)K1237* heterozygous females lay only eggs derived from recombinant FS^+/FS^+ gonial cells.

In our control experiments with *FS(1)K1237*, the frequency of females laying eggs varied between 2 and 6% depending on genotype (Table 1). In these control experiments the females were heterozygous for various viable marker mutations that are also present, as associated mutants, in the experimental series. In all cases, the resulting adults did not carry the *FS* chromosome. Thus, all the eggs came from recombinant germ-line clones. For 42 recombinants, the male offspring carried all the mutant markers, *i.e.*, the recombinational event must have occurred proximal to the most proximal marker. Three clones were recombinants between *ct* and *f*. Thus, mitotic recombination induced in the germ line occurs, as in epidermal cells, both in euchromatin and in heterochromatin (FRIESEN 1936; WIESCHAUS and SZABAD 1978). The existence of the FS^+ recombinant clones indicates that *FS(1)K1237* is autonomous in germ-line cells. Moreover, the low incidence of distal mitotic recombination ensures that the chromosomes recovered still bear the mutant being tested.

Deletions

We have studied the viability of 16 small deletions located in the distal half of the X chromosome that had previously been studied in epidermal cells (Table 2). All these deletions are lethal in homozygous zygotes. Some of them, however, are viable in the epidermal cells of the tergites. That is, epidermal cells can proceed through seven cell divisions and differentiate normal tergite cuticular structures in the absence of the deleted genes (RIPOLL and GARCIA-BELLIDO 1979). However, homozygosis for most of these deletions is incompatible with viability of gonial cells (Table 2). We do not know in which stage the homozy-

TABLE 1

Germ-line clones in controls

X chromosome tested	N	Germ-line clones	%
Vallecas, WT	390	19	4.9
In(1)dl49, w f^{36a}	140	7	5.0
y w cv ct v g f	470	14	3.0
sta sn^{3} *m*^{D}	450	9	2.2
y Hw f^{36a}	200	12	6.0

Females heterozygous for the chromosomes listed and *FS(1)K1237* were irradiated as larvae. N is the total number of irradiated females. Each egg-laying female is scored as containing a germ-line *FS*$^{+}$ clone. All control chromosomes are themselves viable, and the eggs derived from the germ-line clones yield viable zygotes.

gous cells stall. They could be arrested during gonial proliferation or in any of the multiple steps of cell differentiation during oogenesis.

The inviability of recombinant cells probably results from germ-cell-autonomous insufficiency of a gene or genes in the deletion. It could also be caused by nonautonomous effects of the female sterile mutation, although there is no indication of such effects in controls. We tested this possibility in *FS*/deletion females that also carried a duplication that covers the deletion. *FS/Df(1)sc*^{8}*/y*$^{+}$Y and *FS/Df(1)w*$^{258\text{-}45}$*;Dp(1;4)mg/+* females were irradiated. In both cases (2% of irradiated females in the first case and 1% of irradiated females in the second case) we obtained offspring. The offspring were deletion;duplication males and deletion-heterozygous females with and without the duplication.

Females heterozygous for two of the deletions tested did lay eggs (Table 2). Both deletions are also cell viable in epidermal cells (RIPOLL and GARCIA-BELLIDO 1979). In both cases about half the eggs died as embryos and the adults recovered were females heterozygous for the deletion chromosome and *FM7c*. This was shown by the recessive markers carried by both the deletion and *FM7c* chromosomes, and was confirmed by the lethality of their male offspring. The frequency of such clone-carrying females is comparable to that found in the controls and indicates normal viability of the homozygous deletion in germ cells. Either there is no requirement for the deficient gene products in the germ cells, or cross-feeding from surrounding wild-type tissues overcomes the deficit (see DISCUSSION).

Point-mutant lethals

The inability of most deficiency-homozygous cells to yield normal eggs probably results from deletion of genes that are necessary for germ-line proliferation and/or gamete differentiation. How common are such genes? We have approached this question by studying a single chromosome region which has been saturated with zygotic lethals. The behavior of homozygous deletions may be compared with that of the most amorphic mutants in each of the lethal complementation groups in the deletion region. In this way we can ask the following specific questions: 1) What fraction of zygotically-essential genes are also needed for germ line differentiation? 2) Are there other genes needed for

TABLE 2

Germ-line clones in deletion/FS heterozygotes

Deletion	Breakpoints	No. of bands	Cell viability	N	Germ-line clones	%	Zygote viability
sc^8, *y f*	*1A1;1B1*	8	V	810	0		
sc^{19}, *y f*	*1A8;1B4-5*	4	V	70	3	4.3	V
[a]*260-1, y ct f*	*1A1;1B6-7*	14	V	280	0		
62g18	*3A1-2;3A4-6*	4–5	V	600	0		
[a]w^{rJ1}, *y sn*	*3A2-3;3C1-3*	12	L	110	0		
K95, y w	*3A3-4;3B1-2*	8	L	410	0		
64j4, y	*3A5-9;3B1-2*	2	L	480	0		
64f1	*3A9-B1;3B3-4*	3	L	410	0		
$w^{258\text{-}45}$, y^2 *f*	*3B2-3;3C2-3*	5	L	390	0		
[a]w^{-67k30}, *lz ras v*	*3C2;3C6*	5	V	450	16	3.5	V
[a]$N^{264\text{-}105}$, *f*	*3C6-7;3D2-3*	8	L	270	0		
[a]dm^{75e}, *f*	*3C12;3E4*	11	V	380	0		
[a]*c128*	*7C9-D1;7D4-5*	4	L	420	0		
[a]*KA14, f*	*7F1-2;8C6*	28	L	470	0		
[a]$v^{73\text{-}1V}$, *m f*	*10A2;10A5*	4	V	310	0		
[a]*KA10, y*	*11A1;11A7*	7	L	260	0		

Females heterozygous for the deficiency chromosomes and *FS(1)K1237* were irradiated as larvae. Germ-line clones are scored as in Table 1. Cell viability refers to viability in homozygous epidermal clones, and zygote viability refers to viability of fertilized eggs derived from deficiency homozygous clones (V = viable, L = lethal).

[a] Largest non-overlapping deficiencies.

germ-line differentiation besides those genes known to have lethal phenotypes? 3) If lethals do not prevent the formation of eggs, what fraction of them cause embryonic arrest unrescuable by the genes of the paternal gamete?

We have chosen the zeste-white region (1 to 1.5) for our analysis. This region has been genetically studied in detail and has been shown to contain lethal and semilethal alleles belonging to 15 complementation groups (*zw1* to *zw13*, *gt* and *tko*) in the 3A2-3C1 segment of the X chromosome (JUDD, SHEN and KAUFMAN 1972). Lethal phase in hemizygous embryos and viability in epidermal cells (gynandromorphs) have been studied for several alleles in 13 of the 15 complementation groups (SHANNON *et al.* 1972). Based on those data, we have selected alleles that show the most extreme phenotypes—earliest lethal phase and cell-autonomous lethality in mosaics—as the most amorphic available. We studied two *zw3* alleles, two *zw6* alleles and a single mutation in each of the other loci.

The effect of homozygosis of these lethals on proliferation and/or differentiation of the female germ line can be deduced from the data in Table 3. Two-thirds of the genotypes studied did not lay any eggs. The absence of germ-line clones is, in each case, highly significant by a contingency X^2 comparison with the pooled, homogeneous, controls. This result is especially surprising in those cases in which the mutant studied is viable in large spots in gynandromorphs (V in Table 3) and in the few cases in which even hemizygous males occasionally survive (E in Table 3).

We have found egg-laying females of six genotypes corresponding to mutants

TABLE 3

Germ-line behavior of zeste-white region mutants

Deficiency region	Locus	Allele	Cell viability	N	Germ-line clones	%	Zygote viability
:	*gt*	*123, y*	?(E)	453	14	3.1	V
:							
62g18	*tko*	*k11*	V[a]	704	0		
:							
: :	*zw1*	*d13*[b]	V	384	8	2.1	L
: :							
:	*zw8*	*g10, y*	L	580	0		
:							
:	*zw4*	*k16*	V	460	0		
:							
K95	*zw10*	*i20, y w f*	V(E)	550	2	0.4	L
:							
:	*zw13*	*13*	?	550	0		
:							
:	*zw2*	*c21*	V	750	0		
:							
: :	*zw3*	*k22*	V	550	6	1.1	L
: :		*h22*	V	200	6	3.0	L
:							
64f1	*zw6*	*a25, y*	V	790	10	1.2	V
:		*e5*	V	250	0		
:							
: :	*zw12*	*k1*	V	650	0		
:							
:	*zw7*	*e3, y sn*	L	700	0		
:							
w258-45	*zw5*	*g27*	V(E)	500	0		
:	*zw11*	*a5*	L	520	0		
:							
:	*zw9*	*k18, sn*	V(E)	520	0		

Females heterozygous for the mutations indicated and *FS(1)K1237* were irradiated as larvae. Germ-line clones are scored as in Table 1. Cell viability refers to viability in gynandromorphs (SHANNON *et al.* 1972), and zygote viability refers to viability of fertilized eggs derived from mutation-homozygous clones (V = viable, L = lethal). (E) indicates recovery of hemizygous mutant escapers.

[a] Male tissue seen only in the abdomen.

[b] The $zw1^{d13}$ chromosome used had been freed of all second-site mutations by recombination (ROBBINS 1983).

in five complementation groups. The behavior of the germ-line viable mutations is detailed in the following paragraphs.

gt: gt^{123} is a sub-vital mutation that often survives to adulthood. For example, a cross of y gt/+ females by +/Y males yielded 283 daughters, 110 + sons, and 53 *gt* sons. Some of the *gt* males are quite large, confirming the presence of the mutation, but the penetrance is only about 10%. This mutation is germ-line viable, and the eggs show no evidence of a maternal deficit. In one sample of 172 eggs, only 6 failed to hatch and 4 of those had apparently normal segmentation. Eighty-nine daughters and 34 *gt* sons made it to adulthood. Since

twice the females is comparable to the total number of eggs, all lethality is accounted for as postembryonic lethality of *gt* males. Furthermore, the viability of the *gt* embryos in the sample of eggs from homozygous germ-line clones is indistinguishable from the viability in the mating of *gt*/+ females (34/89 = 0.38; (2 × 53)/283 = 0.37). Whether a less leaky mutation at the *gt* locus would have a germ-line or maternal effect is moot.

zw1: The mutation tested gave control numbers of germ-line clones, but none of the eggs were viable. Microscopic examination revealed no signs of development. In the absence of any evidence of development, it is impossible to state whether eggs from *zw1* homozygous clones cannot be fertilized, or whether they have a deficiency in a maternally packaged gene product so severe that development is precluded even when a normal allele is present in the zygote.

zw3: Two alleles in the *zw3* complementation group (*k22* and *h22*) gave fertile females at a frequency comparable to that of control females. However, none of the eggs laid by these females hatched. Microscopic examination of their embryos showed some developmental progress, but morphogenesis was arrested before cuticular differentiation. For these mutants, zygotic gene activity does not overcome the defect in oocytes derived from mutant-homozygous clones. It is possible that the single gene dose brought in by the sperm is insufficient to rescue these eggs, but that additional copies could act early enough and at high enough level to overcome the deficit. To test this, we remated the egg-laying *zw3/FS* females after about 10 days of oviposition to males that carried a duplication (*Dp(1;4)mg*) in the fourth chromosome in addition to the wild-type genes carried in the X chromosome. The eggs produced after the second mating were examined, and were also found to contain embryos arrested in early development.

zw6: Of the two *zw6* alleles studied, only one (*a25*) yields eggs from homozygous germ-line clones. Ten egg-laying females were found. These gave rise to both inviable (arrested in early embryogenesis) and viable eggs. The latter developed into adult females carrying the *y a25* mutant chromosome of the mother and the balancer *FM7c* chromosome of the male. The lethality of the *a25* chromosome was confirmed in the progeny of these females. Females carrying the other *zw6* allele (*e5*) were sterile. Based on its lethality phase, this allele is weaker than *a25*. However, it has been shown in other experiments (ROBBINS 1983) that the *e5* chromosome also carries a closely linked deleterious mutation that is covered by w^+Y but not by *Dp(1;4)mg*. Since recombinant clones would be homozygous for this extraneous mutant as well, it seems likely that its presence accounts for the failure of irradiated *FS/e5* females to yield eggs.

zw10: Two *zw10* females (both heterozygous for the *i20* allele) laid more than 100 eggs over several days, but none of those eggs hatched. Upon examination under the microscope, we found the embryos to be arrested in early development. These eggs probably came from recombinant clones because leaky *FS* females lay viable eggs. However, since the eggs die, no genetic test of their recombinant origin could be made. In any case, the fraction of fertile females (0.4%) is well below the control frequencies indicating poor germ-line viability of the recombinant cells.

The failure of egg production in cells homozygous for most point lethals could result from gene product insufficiency during gonial proliferation, when the clones were induced, or later, in any of the several steps leading to oocyte differentiation. In adult females, ovariole formation and germarium organization has already occurred (KING 1970). Mitotic-recombination clones induced in adults will, therefore, only result from recombination in the gonial stem cells. Lethals that only affect the germ line before the cambial divisions should permit egg production in clones generated in the adult. Some of the lethals have been re-examined by irradiating adult *FS*/mutant females.

The number of gonial stem cells in the adult is larger than the number of gonial cells found during the proliferation period of the larva. Data on mitotic-recombination target size in adults confirms this (WISCHAUS and SZABAD 1978). To provide wild-type controls we studied irradiated *FS(1)K1237*/*y* females. Recombinant eggs are not expected before 8 days after irradiation—the time between the cambial division and oviposition in normal fertile females (KING 1970). Eggs were regularly recovered from irradiated *FS*/*y* females after 4 or 5 days. This finding suggests that competition between ovarioles may slow the rate of oogenesis in normal fertile females. The frequency of egg-laying females was lower (11/500 = 2.2%) than expected (25%, WIESCHAUS and SZABAD 1978).

We have tested the mutants that are viable in somatic cells (complementation groups 2, 3, 4, 5, 6 and 12) for egg production after irradiation of adult *FS*/mutant females. Between 350 and 500 females of each genotype were scored for egg production. All the genotypes were sterile. Although the absence of late clones may be a function of homozygosity for the mutations, the paucity of clones in the control suggests an alternative explanation. It is possible that the defect caused by *FS*, either at the morphological level or because of perdurance of its products, inhibits oogenesis of even wild-type gonia. Although *FS* is autonomous among ovarioles, it may be nonautonomous within an ovariole. Even a marginal defect in *zw* mutant-homozygous clones could then result in total sterility.

DISCUSSION

The present study of genetic requirements in the development of female germ cells is based on recovery of eggs from sterile (agametic) females. This sterility is caused by a dominant factor (*FS*) in the X chromosome that can be removed by mitotic recombination induced in the germ line of FS/FS^+ females. The resulting FS^+/FS^+ gonial cells proliferate and then differentiate into FS^+ mature oocytes. The FS^+ nature of these oocytes can be demonstrated in the F_1 adults derived from the fertilized eggs.

We have studied viability throughout gonial proliferation and oogenesis of homozygous FS^+ clones that are: wild-type, homozygous for viable mutations, homozygous for deletions, or homozygous for single-cistron lethals. All of the viable chromosomes tested allow oogenesis to proceed normally. This is no surprise since females homozygous for these mutants are fertile. The only exception to this is *Hw*; homozygous *Hw* females are agametic sterile. The viability and fertility of *Hw/Hw* clones found in this test supports the finding

of HOLZWORTH *et al.* (1974). They showed that the *Hw* sterile effect (in flies homozygous for another allele—Hw^{49c}) is not caused by cell-autonomous germ-line defects. The frequency of clones in various *FS*/viable-mutant females is similar to that occurring in *FS*/wild-type females, suggesting the absence of significant deleterious effects of these mutants during oogenesis.

The viability of germ cells homozygous for 16 small deletions in the distal end of the X chromosome was studied. The nonoverlapping deletions (with asterisk in Table 2) uncover 93 bands, possibly corresponding to about as many lethal complementation groups (LEFEVRE 1974). Within these deletions there are two regions, defined by $Df(1)sc^{19}$ and $Df(1)w^{-67k30}$, which are viable in germ-line cells. Together, these two regions account for nine bands. In the earlier study of epidermal-cell viability of the same deletions, these two and others adding up to 39 bands were found to be viable (Table 2). Thus, although all deletions viable in the germ line are also viable in epidermal cells, the genetic requirements of the germ cells are more stringent than those of epidermal cells.

Lethality of the small deletions can result from inclusion within them of genes which are germ-line specific and/or of genes whose functions are required for both zygotic viability and germ-line development. In order to distinguish between these, we have studied single-cistron lethals. We have chosen the most extreme alleles known, based on effective lethal phase and viability of somatic cells, of the complementation groups included in the zeste-white region (SHANNON *et al.* 1972).

Of the 15 lethal complementation groups tested, 5 have alleles that permit completion of oogenesis. Germ-line clones of the leaky *gt* allele tested gave eggs indistinguishable from those produced by *gt* heterozygotes. One *zw6* allele (*a25*) of the two tested produced viable eggs, half of which developed into heterozygous females. The other *zw6* allele (*e5*), although itself less severe than *a25*, is closely linked to a semi-lethal (ROBBINS 1983) that most likely accounts for the germ-line lethality of *e5/e5* clones. The two *zw3* alleles and the single *zw10* allele tested produced eggs. However, none of these eggs developed past early stages of embryogenesis. We do not know whether less leaky alleles of the apparently germ-line viable loci would have been germ-cell inviable. Nor do we know whether some of the mutants in the other *zw* groups gave a germ-line lethal result because of the presence of closely linked lethals. This is unlikely, however, since the same experiments that exposed the closely linked mutation in $zw6^{e5}$ do not reveal any second-site mutations in the other chromosomes. Any lethals or semi-lethals outside the region of overlap of *Dp(1;4)mg* and $w^{+}Y$ would have been detected.

Except for *gt* which had not been tested, all the germ-line viable alleles had been shown to also be viable in male epidermal tissue in gynandromorphs (SHANNON *et al.* 1972). However, most of the germ-line inviable alleles are cell viable in epidermal cells. Moreover, some of them ($zw10^{i20}$, $zw5^{g27}$, $zw9^{k18}$, and *tko*) are sometimes even viable as hemizygous flies (SHANNON *et al.* 1972, corroborated in our crosses). Taken at face value, these results indicate more stringent gene-function requirements in germ cells than in epidermal cells. Based on these results, around 67% (10/15) of those genes needed for viability

are also required for oogenesis. In contrast, the fraction of the same genes that is required for epidermal cell viability is 23% (3/13). The zeste-white region probably represents a random, albeit small, sample of the genome and these results are indicative of the fraction of all genes required for oogenesis. This inference is supported by the germ-cell inviability of deletions extending some distance outside the zeste-white region, and by some recent observations of F. MIÑANA made in our laboratory. He has studied a sample of newly induced zygotic lethals in the X chromosome and found, with the same assay, that 28/40 = 70% are germ-line lethal. Thus, whereas about 80% of zygotic lethals are viable in epidermal cells, only around 30% of them are viable in female germ-line cells.

These differences in genetic requirements in the two systems could merely reflect different cell proliferation dynamics and/or different levels of (or responses to) diffusible metabolites. Epidermal cell differentiation in tergites occurs about seven divisions after irradiation of the progenitor cells (GARCIA-BELLIDO and MERRIAM 1971; GARCIA-BELLIDO 1973). Thus, dilution ensures that differentiation does not merely use gene products generated in the ancestral heterozygous cell. Competition between homozygous mutant cells and heterozygous cells (MORATA and RIPOLL 1975) could account for the differences between germ-line and epidermal cell behavior. In tergites, such competition is very low but it could be more extreme in gonial cells, as it is in imaginal discs. The higher viability of epidermal cells compared to gonial cells could also be caused by freer diffusion among epidermal cells than among cells in the gonads. Whatever the underlying mechanism, it remains the case that the genetic requirements of the germ cells include most of the genome.

We do not know in which stages of germ-line development the homozygous lethal cells are arrested. The developmental arrest could occur during proliferation of the gonial cells, in the gonial stem cells or during differentiation of the oocyte. An attempt to study the viability of gonial cells after the proliferation period in irradiated adults has failed. The viability of even FS^+ control chromosomes in late clones is very low and the effects of *FS* on the mature ovary probably prevent normal development of the gonia.

One of the aims of the present study was estimation of the fraction of genes that are specific for and autonomous to germ-line development. Sterile mutations that are autonomous in transplanted germ cells and have no morphological effects obviously belong to this class (GEHRING 1976). Even in the most specific cases, however, the possibility remains that the alleles studied are hypomorphic mutations and that less active alleles would have detectable zygotic effects. We expected to detect genes of this class by finding inviability of deletions in which all the included lethal complementation groups are germ-line viable. Unfortunately, among the deletions in the zeste-white region, even the smallest contains zygotic lethals that affect oogenesis. Thus, the abundance of oogenesis-specific genes remains unknown.

Two deletions, and point mutants in five complementation groups, have been shown to be viable in the germ line. This indicates that their wild-type gene products either are non-cell-autonomous or are not required in oogenesis, or

that the particular mutation tested was leaky. We know that $Df(1)w^{-67k30}$ includes several genes in a 5-chromosome-band segment and that mutations in all but one of them are viable in hemizygous zygotes (LEFEVRE and GREEN 1972). The present result implies that there are also no germ-line lethal genes in this interval. $Df(1)sc^{19}$ is homozygous lethal, but it is thought to contain only the zygotic gene yellow and the zygotic gene(s) of the achaete-scute complex (GARCIA-BELLIDO 1979). Developmental analysis of this complex indicates that these genes are involved in differentiation of the central and peripheral nervous system. Moreover, genetic-mosaic analysis has shown that these genes are cell autonomous in epidermal cells (GARCIA-BELLIDO and SANTAMARIA 1978; JIMENEZ and CAMPOS ORTEGA 1979). The finding that $Df(1)sc^{19}$, which lacks four chromosome bands, is viable in germ-line clones (confirmed by F. JIMENEZ and J. CAMPOS-ORTEGA, personal communication) is interesting in several respects. It reinforces the earlier conclusions that: a) this deletion may not contain genes other than those already detected; at the least it does not contain a germ-line viability locus; and b) these genes are specifically involved in developmental operations not expected to affect oogenesis. Otherwise, the specific phenotypes of their mutants would have to be explained as secondary effects of failures in a more general cell function. Interestingly, the mutation *Hw* which is a derepression mutant of the achaete gene is, as seen above, also viable in germ cells. It is also of interest to note that small deletions for the bithorax complex are also, in a similar test, viable in germ line cells (S. KERRIDGE, personal communication).

The oocytes resulting from recombinant clones of these two deletions can support the development of zygotes produced by fertilization with wild-type sperm. That is, half the eggs laid by $Df(1)w^{-67k30}/FS$ females gave rise to adult females. There are two classes of embryos in the case of $Df(1)sc^{19}$: females heterozygous for the deletion that develops normally, and hemizygous males that die as embryos. F. JIMENEZ and J. CAMPOS-ORTEGA (personal communication) have found that these males have the same lethal phase and phenotype as males derived from heterozygous females. There is, therefore, no maternal effect of the achaete-scute-complex deletion. A similar situation holds for bithorax-complex deletions (S. KERRIDGE, personal communication).

Of the single-cistron lethals that allowed germ-line development, the $zw6^{a25}$ homozygous-clone-derived oocytes supported complete development of heterozygous female zygotes, and eggs from gt^{123} clones are indistinguishable from eggs produced by *gt/+* females. However, all eggs produced by *zw1/FS*, *zw3/FS* and *zw10/FS* females failed to hatch. Eggs derived from *zw3* and *zw10* clones were morphologically normal and had been fertilized; partial development of the embryos was visible upon microscopical examination. Thus, maternal homozygosity for these mutations causes an oocyte defect that prevents embryonic development. Eggs from *zw1* clones showed no evidence of development, and may be so defective that they cannot be fertilized.

That germ-line mutant homozygosity yields inadequate levels of a maternally-packaged gene product rather than an irremedial structural defect is indicated by the fact that partial maternal insufficiency can be overcome by sufficient zygotic gene activity (ROBBINS 1980; ROBBINS 1983). The importance of maternal

gene activity for early development is even more striking in light of the observation that hemizygous embryos produced by females heterozygous for these mutants survive until advanced larval stages (*zw3*) or are semilethal (*zw10*) (SHANNON *et al.* 1972), whereas heterozygous embryos suffering from these maternal defects fail to proceed past the earliest embryonic stages. F. MIÑANA'S ongoing study of newly induced zygotic lethals is yielding a similar result. Although it is not known whether these mutations are amorphs or hypomorphs, 2 of the 12 germ-cell viable lethals produce eggs that all die early in embryogenesis. The zeste-white region does not appear to be unique.

Genes like these are important because they contribute in a fundamental way to early development. Their function and role in development can only be fully understood by studying embryos derived from mutant gonia; oocytes derived from heterozygous germ cells still contain wild-type products that can support development of homozygous mutant zygotes to advanced stages and even to adulthood. The abundance of maternally and zygotically acting genes may be very high. Many may be included among those that are inviable in germ cells and whose embryonic effects can not be studied with this procedure. There is, however, other evidence that suggests that they are common. Maternal heterozygosity for any one of many deletions throughout the genome yields significant early embryonic lethality (GARCIA-BELLIDO and MOSCOSO DEL PRADO 1979; A. GARCIA-BELLIDO, J. MOSCOSO DEL PRADO and J. BOTAS 1982). Moreover, studies in which zygotic gene expression is manipulated by using position-effect variegation permit evaluation of the developmental effects of different degrees of maternal and zygotic insufficiency (ROBBINS 1980; ROBBINS 1983). Both methods indicate that a majority of genes have both maternal and zygotic effects. The results presented here indicate that most gene products are not only needed in the oocyte, but that most of them are also essential for oogenesis. Although the germ-line-clone technique alone will permit developmental analysis of only a fraction of them, their analysis by this and other methods will be important to our understanding of the dynamics of early development.

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