Female Sterile Mutations on the Second Chromosome of *Drosophila melanogaster*. II. Mutations Blocking Oogenesis or Altering Egg Morphology

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Manuscript received July 8, 1991
Accepted for publication August 24, 1991

ABSTRACT

In mutagenesis screens for recessive female sterile mutations on the second chromosome of *Drosophila melanogaster* 528 lines were isolated which allow the homozygous females to survive but cause sterility. In 62 of these lines early stages of oogenesis are affected, and these females usually do not lay any eggs. In 333 lines oogenesis proceeds apparently normally to stage 8 of oogenesis, but morphological defects become often apparent during later stages of oogenesis, and are visible in the defective eggs produced by these females whereas 133 lay eggs that appear morphologically normal, but do not support normal embryonic development. Of the lines 341 have been genetically characterized and define a total of 140 loci on the second chromosome. Not all the loci are specific for oogenesis. From the numbers obtained we estimate that the second chromosome of Drosophila contains about 13 loci that are relatively specific for early oogenesis, 70 loci that are specifically required in mid to late oogenesis, and around 30 maternal-effect lethals.

OOGENESIS is a highly regulated developmental process. In insects, as in many metazoans, it involves the close cooperation between the germ cells and a number of somatic cell types. Genetic and morphological studies of mutations that affect oogenesis have revealed that in *Drosophila melanogaster* a large number of genes are active during oogenesis to ensure the production of a normal egg capable of supporting the development of a normal embryo (GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984). These genes can be grouped in several classes. On the one hand, all cells involved in oogenesis require the normal complement of household genes that allow those cells to grow and divide. They also require some more specialized gene functions which are also expressed in other tissues. Because of their pleiotropic effects outside the ovary, mutations in all of these genes would usually cause lethality in homozygous individuals. A smaller, third group of genes are required more specifically for processes that only occur during oogenesis. Mutations in these genes will allow the homozygous carrier female to survive, but cause defects in oogenesis. These mutations can be used to analyze various aspects of pattern formation and differentiation processes unique to oogenesis.

Smaller, previous screens for such female-sterile mutations were focussed mostly on genes on the X chromosome. These screens identified classes of female-sterile mutations that seem to affect particular processes in oogenesis (GANS, AUDIT and MASSON 1975; MOHLER 1977; KOMITOPOULOU *et al.* 1983; PERRIMON *et al.* 1986; ORR *et al.* 1989). This present work describes the results of a large-scale mutagenesis experiment on the second chromosome of Drosophila and deals specifically with female sterile mutations affecting oogenesis. The larger number of mutations obtained in this screen allows us to compare the relative number of mutations and loci affecting various developmental processes that occur in oogenesis. These comparisons suggest that only few genes in the genome are exclusively required for the survival of germline or gonadal primordia in the stages before adulthood. Another small group of genes appear to be required specifically for the initial establishment and maintenance of the 15 + 1 nurse cell-oocyte cluster. On the other hand, a larger number of genes appear to be specifically involved in follicle cell functions such as secretion of the egg shell, or the migration patterns of the follicle cells within the developing egg chamber.

MATERIALS AND METHODS

experiments are described and referenced in LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985, 1986, 1987, 1990), unless otherwise indicated. Genetic mapping of the mutations was performed using an *al dp b pr c px sp* chromosome ("*all*-chromosome") or in some cases a chromosome carrying the dominant markers *S Sp Bl*, and using, in addition, the markers *cn* and *bw* which are present on all mutagenized chromosomes. Once a female sterile mutation had been placed between two marker mutations, a larger set of additional recombinants between the two markers was isolated and tested in a second recombination experiment. This yielded a genetic map position with an error interval of less than 5 map units ($P = 5\%$). After a particular mutation had been mapped, all chromosomal deficiencies within 10 map units on either side were tested in *trans* to the mutation (Table 1). Finally, all mutations mapping between the same two markers were complemented against each other in order to find all members of a complementation group, regardless of the female sterile phenotype. In addition, a set of 14 deficiencies (indicated with an asterisk in Table 1) were crossed to all 528 sterile lines, in order to find all the mutations in the collection that are uncovered by these deficiencies. This analysis revealed that the gene *vasa* was actually represented by six rather than two alleles as previously reported (SCHÜPBACH and WIESCHAUS 1989), and, given the phenotype of the additional alleles, the gene had to be placed into the group of female sterile mutations affecting mid to late oogenesis. In addition, the gene previously designated as *mat(2)cellHK35* was found to be allelic to the gene *squash* which also affects mid to late oogenesis. The mutation designated as *spliced*^{RL3} was found to be allelic to *torso* by KLINGLER *et al.* (1988), and by STRECKER *et al.* (1989). As compared to the previous report, the class of maternal effect lethals has therefore been reduced to 133 lines (64 loci) from the previously reported 136 lines (67 loci).

Phenotypic descriptions: Eggs were collected, fixed, and mounted in Hoyer's medium for inspection with a compound microscope, as described in WIESCHAUS and NÜSSLEIN-VOLHARD (1986). For mutant lines that did not lay any eggs, ovaries of several females were dissected, fixed for 5–10 min in 2% glutaraldehyde, transferred to phosphate-buffered saline (PBS) and after further dissection of ovarioles and egg chambers, the ovaries were inspected with phase contrast optics, or DIC optics. The ovaries of selected mutations were stained with Hoechst stain for 5 min (1 µg/ml in PBS, after fixation with glutaraldehyde) and inspected with a fluorescence microscope. Enhancer trap lines (*i.e.*, lines carrying insertions of engineered *P* elements with lac-Z inserts (BIER *et al.* 1989) which show expression patterns in ovarian cell types were crossed to some of the mutant lines, and the resulting lines were stained for β-galactosidase expression as described by FASANO and KERRIDGE (1988)]. The enhancer trap lines were obtained in our laboratory (T. SCHÜPBACH, L. J. MANSEAU and E. SHADDIX, unpublished results) after mobilization of a *P* element insert containing the lac-Z gene on the *CyO* chromosome, which had been provided to us by the laboratories of L. Y. JAN and N. Y. JAN.

RESULTS

In screens for female-sterile mutations on the second chromosome of *D. melanogaster* 528 sterile lines were isolated from a total of 18,782 individual mutated chromosomes (SCHÜPBACH and WIESCHAUS

1989). In all these sterile lines the homozygous females survive but fail to produce viable offspring. The female-sterile lines were grouped into three broad phenotypic categories, similar to the phenotypic groups defined for female-sterile lines on the X chromosome (KING and MOHLER 1975; GANS, AUDIT and MASSON 1975; PERRIMON *et al.* 1986). In 133 lines the defects appeared to be restricted to embryogenesis. These maternal-effect loci have been described earlier (SCHÜPBACH and WIESCHAUS 1989). The remaining 395 lines caused defects already visible at the time the egg is laid. They could be grouped into two general categories. In the first group of 62 mutant lines, early stages of oogenesis are often abnormal, and these females usually do not lay any eggs. In the second group of 333 lines, oogenesis apparently proceeds relatively normally through stage 8 [for stages of oogenesis see KING (1970) and MAHOWALD and KAMBYSELLIS (1980)]. Morphological defects become detectable at later stages of oogenesis and may involve a lack of transport of the nurse cell content into the oocyte, abnormal egg shape, abnormal migration of follicle cells, or abnormal secretion of the egg shell. The females in these lines will usually lay morphologically defective eggs.

Within each group phenotypic subgroups were defined, and several lines from each subgroup were selected as representatives. The mutations in these representative lines were genetically mapped and crossed to all the remaining lines within the phenotypic subgroup. This allowed us to define several loci, often with multiple alleles, within each subgroup. Subsequently, complementation tests were performed between alleles of loci which mapped to similar positions on the chromosome regardless of their phenotype, and the genetically characterized mutants were also tested over chromosomal deficiencies (Table 1). These tests revealed that a number of loci in the early defective class have alleles that had originally been put into the late defective class. Similarly, late defective loci sometimes had alleles in the maternal-effect class. These complementation tests allowed us to define 140 loci on the second chromosome which are represented by a total of 341 lines. In the following descriptions, loci have been placed into the categories defined by the allele that produced the earliest visible defect, given that such alleles were found to be closer to the amorphic state of the gene in all cases where alleles could be tested over chromosomal deficiencies. On the other hand, the classification of loci with only one mutant allele may sometimes be inaccurate.

To carry out a complete complementation analysis on a subset of loci, without relying on the phenotypic groups, a set of 14 chromosomal deficiencies were chosen and all 528 mutant lines were crossed to each of these deficiencies. (These deficiencies are indicated

TABLE 1

Deficiencies on the second chromosome

Deficiency	Deficiency breakpoints	Loci uncovered	Reference
Df(2L)al	21B8-C1; 21C8-D1		a
Df(2L)S²	21C6-D1; 22A6-B1		a
Df(2L)ast-1	21C7-8; 23A1-2		a
Df(2L)ast-2	21D1-2; 22B2-3		a
*Df(2L)ed^SZ	24A3-4; 24D3-4	capu, fs(2)ltoQE45	b
Df(2L)ed-dph1	24C1,2-3; 25A1-4	capu, fd(2)ltoQE45	b
Df(2L)M-zB	24E2-F1; 24F6-7		a
Df(2L)cl 1	25D7-E1; 25E6-F3	mat(2)earlyRS32	a
Df(2L)cl 7	25D7-E1; 26A7-8	fs(2)ltoQ J42	a
Df(2L)GpdhA	25D7-E1; 26A8-9		a
Df(2L)TE62X2	27E5-F1; 28D3-4		i
Df(2L)30A;C	30A; 30C	rem, mat(2)cellQC13, mat(2)cellRH36	c
Df(2L)J-der2	31A; 32A1,2	trk, mat(2)earlyQM47, mat(2)synPJ50, err fs(2)ltoDG25, fs(2)ltoP123 fs(2)ltoRU26	d
*Df(2L)J-der27	31D; 31F3	mat(2)earlyQM47, mat(2)synPJ50, err fs(2)ltoDG25, fs(2)ltoP123 fs(2)ltoRU26	d
*Df(2L)Prl	32F1-3; 33F1-2	aret, zuc	a
Df(2L)prd1.7	33B3-7; 34A1-2	aret, zuc	a
Df(2L)b75	34D4-6; 34E5-6		a
*Df(2L)64j	34D1-2; 35B9-C1	fs(2)ltoQL53	a
Df(2L)75c	35A1,2; 35D4-7	vas, fs(2)ltoRJ36	a
Df(2L)A446	35B1-3; 35E6-F2	vas, Bic-C, fs(2)ltoRJ36	a
*Df(2L)osp29	35B2-3; 35E6	vas, Bic-C fs(2)ltoRJ36	a
*Df(2L)r10	35D1,2; 36A6,7	Bic-C, cact, chif, mat(2)synHB5, fs(2)ltoRN48	j
*Df(2L)H20	36A8-9; 36E1,2	dl, kel, qua, BicD fs(2)ltoHC44, mdy	a
Df(2L)TW137	36C2-4; 37B9-C1	dl, pre, kel	a
*Df(2L)TW50	36E4-F1; 38A6-7	fs(2)TW1, pre, fs(2)ltoPM43, fs(2)ltoHD43 fs(2)ltoRE57, fs(2)ltoRV64 fs(2)ltoPP43	a
Df(2L)TW130	37B9-C1; 37D1-2	fs(2)TW1, fs(2)ltoPM43	a
Df(2L)E55	37D2-E1; 37F5-38A1	fs(2)ltoRE57, fs(2)ltoRV64	a
Df(2L)TW2	37D2-E1; 38E6-9	val, del, spir, fs(2)ltoRE57, fs(2)ltoHD43 fs(2)ltoRV64, fs(2)PP43	a
*Df(2L)TW65	37F5-38A1; 39E2-F1	val, del, spir, fs(2)ltoHD43, fs(2)PP43	a
Df(2L)TW161	38A6-B1; 40A4-B1	val, del, spir	a
Df(2L)PR31 (=C')	2L heterochromatin	cta	a
*Df(2R)pk78s	42C1-7; 43F5-8	tor, crib, scra sax, mat(2)cellRE43 sat, fs(2)ltoRN73	a
Df(2R)pk78k	42E3; 43C3		a
Df(2R)P32	43A3; 43F6	tor, scra, sat, mat(2)cellRE43, fs(2)ltoRN73	e
Df(2R)ST1	43B3-5; 43E1-8	tor, scra, sat, fs(2)ltoRN73	a
Df(2R)eve1.27	46C3-4; 46C9-11		a
Df(2R)enA	47D3; 48B2-5		a
Df(2R)enB	47E3-6; 48A4		a
Df(2R)vg135	48D-E; 49D-E	stil	f
Df(2R)vgC	49B2-3; 49E7-F1		a
Df(2R)vg^D	49C1-2; 49E2-6		a
*Df(2R)vg^B	49D3-4; 50A2-5	sie	a
*Df(2R)L^R+4R	50F-51A1; 51B	mat(2)earlyPB28	g
Df(2R)trix	51A1,2; 51B6		g
Df(2R)XTE 18	51E3; 52C9-D1	mat(2)synPL63 mat(2)cellQL46 fs(2)ltoPA77	g
Df(2R)WMG	52A; 52D		g
*Df(2R)PC4	55A; 55F	eay, sub, stau hal, fs(2)ltoDF6	a
*Df(2R)D17	57B4; 58B	grau, tud, mat(2)N, prat, top	h
Df(2R)Pl3	57B13-14; 57D8-9	tud	h
Df(2R)bw-D23	59D4-5; 60A1-2	egl, qui, shu, pep, mi, retn, fs(2)ltoAHK35 fs(2)ltoAPV63, fs(2)ltoDC37 fs(2)ltoHM11, fs(2)ltoRM7	a
*Df(2R)bw-S46	59D8-11; 60A7	egl, qui, shu, pep, mi, retn, fs(2)ltoAHK35 fs(2)ltoAPV63, fs(2)ltoDC37 fs(2)ltoHM11, fs(2)ltoRM7	a
Df(2R)bw5	59D10-E1; 59E4-F1		a
Df(2R)Px²	60C5-6; 60D9-10		a
Df(2R)M-c33a	60E2-3; 60E11-12		a

Cytology according to: a, LINDSLEY and ZIMM (1987); b, REUTER and SZIDONYA (1983); c, T. LAVERTY (personal communication); d, D. SINCLAIR (personal communication); e, P. SIMPSON (personal communication); f, E. GOLDSTEIN (personal communication); g, R. MACINTYRE (personal communication); h, J. O'DONNELL (personal communication); i, WUSTMANN et al. (1989); j, ASHBURNER et al. (1990). Deficiencies designated by an asterix (*) have been crossed to all female sterile lines in the collection.
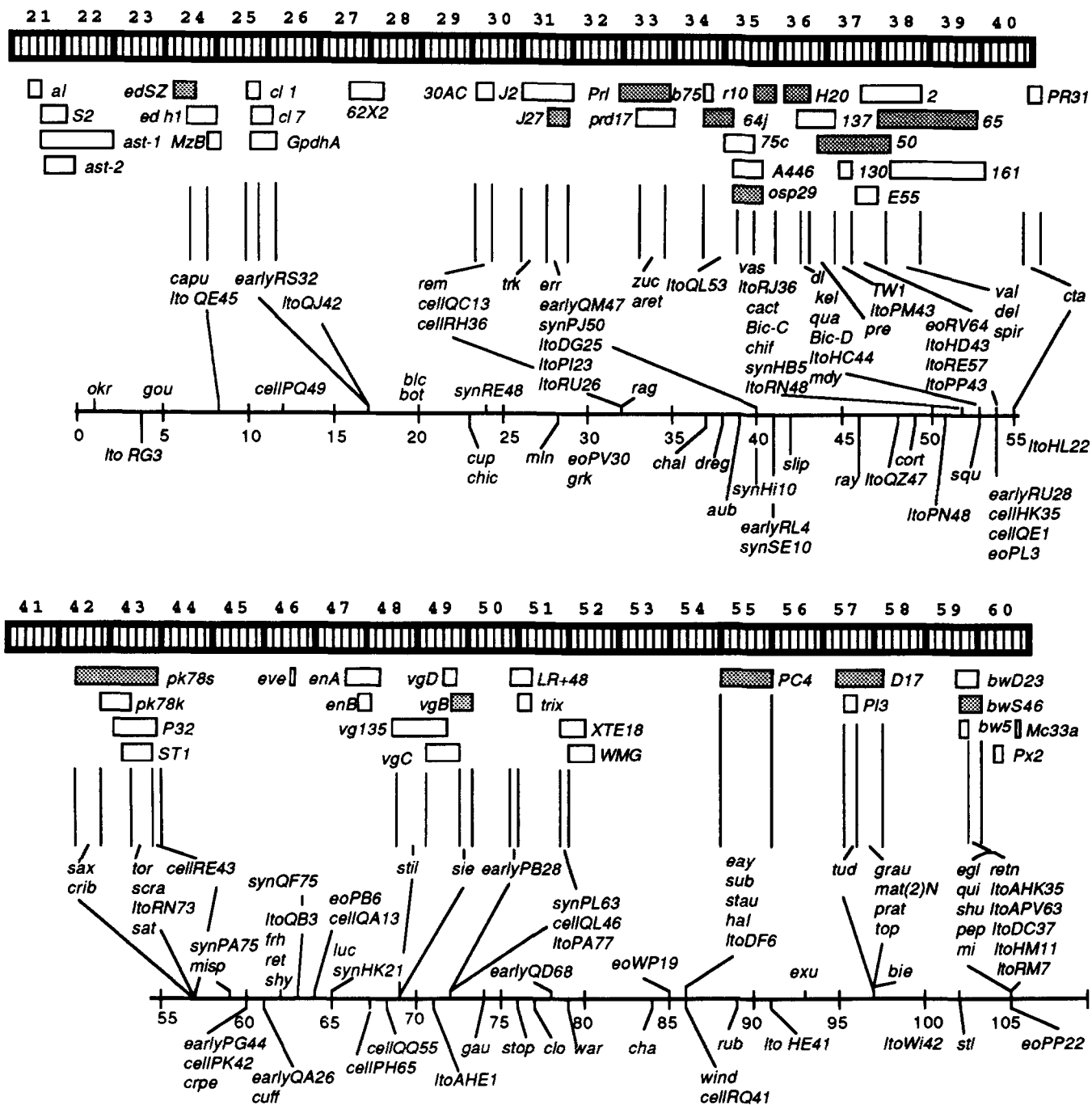
FIGURE 1.—A simplified map of the second chromosome indicating map positions and cytological localization of the 140 mapped loci. Deficiencies are represented by bars. The crosshatched bars indicate deficiencies that have been complementation tested against all 528 mutant lines. Cytologically defined loci are shown directly below the corresponding deficiencies, cytologically undefined loci are shown directly above or below the genetic map (modified from NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984).

with an asterix on Table 1, and a cross-hatched bar in Figure 1.) In total these deficiencies uncover approximately 532 bands on the second chromosome, corresponding to approximately 27.4% of the banded regions on that chromosome. Of the mutant lines, 163 (31% of all lines, including the maternal-effect lines) failed to complement one of these deficiencies. These 163 mutant lines define a total of 62 loci yielding an average allele frequency of 2.6 per locus.

Mutations affecting early oogenesis

Class 1A. Production of few, defective germ cells: Females homozygous for strong mutations at eight loci contain small, underdeveloped ovaries which contain few, if any, egg chambers (Table 2). The rare egg chambers usually become abnormal at early stages of oogenesis and degenerate. Occasionally, single egg chambers can develop further, and an abnormal cho-

rion with reduced or fused dorsal appendages may be formed. The only locus that consistently caused complete absence of germ cells from all homozygous females was *aret*. This phenotype would be expected of a locus specifically required for germline survival during embryonic, larval or pupal stages. Weaker alleles of *aret*, however, cause a range of different phenotypes, not easily explained if *aret* were only required for survival of germ cells in preadult stages. Zygotically acting mutations that cause complete absence of germ cells from the adult gonads, therefore, appear to be rare. This may imply that most germline survival functions are not exclusively used by germ cells alone. Alternatively, more loci might be specific for germline survival, but may encode partially redundant functions.

For most of the mutations in this class the small, empty ovaries were subdivided into ovarioles, as are normal ovaries (Figure 2b). But in *fs(2)eoPL3* and *fs(2)eoPP22* the ovaries were misformed and no subdivision into ovarioles was observed. The absence of developing germ cells in these females may therefore be a secondary consequence of a defect in the differentiation of the somatic portions of the ovary.

In several loci in this group, males homozygous or *trans*-heterozygous for different alleles were also found to be sterile (Table 2). This is in contrast to groups of female-sterile loci which produce later phenotypes and usually affect only female fertility (data not shown). The higher incidence of male/female sterility in the early class may reflect the similarities observed in the the early stages of development in male and female germ cells.

Class 1B. Production of egg chambers with abnormal numbers of nurse cells and ovarian tumors: In mutations at seven loci, egg chambers are formed that do not contain the normal complement of 15 nurse cells and 1 oocyte (Table 2). During normal oogenesis a stem cell division at the tip of the ovariole will produce one differentiating daughter cell, which undergoes four more mitotic divisions giving rise to a cluster of 16 cells. Normally one of these cells becomes the oocyte, the other 15 assume a nurse cell fate [for a review of oogenesis, see *e.g.*, KING (1970) and MAHOWALD and KAMBYSELLIS (1980)]. Mutations that produce egg chambers with abnormal numbers of nurse cells may interfere with these initial events. With the exception of *egalitarian* and *Bicaudal-D*, the actual number of nurse cells (or pseudo-nurse cells, KING and RILEY 1982) in all these lines appears variable. Usually the abnormal egg chambers degenerate. In many of the lines some egg chambers are found that contain normal numbers of nurse cells and may develop further and form abnormal eggs. The high degree of variability in these lines would suggest that these loci function in a mechanism that is not abso-

lutely necessary for germline divisions themselves, but are involved in a process that confers regularity to these divisions.

In two of the loci (*stall* and *pep*) ovarian tumors are formed. These ovaries show ovarioles which contain many small, undifferentiated cells. A similar phenotype has been described in detail for the locus *otu*, a female sterile locus on the X chromosome (KING and RILEY 1982; STORTO and KING 1988). Like *otu*, the alleles of the locus *stall* show a range of egg chamber phenotypes ranging from tumorous egg chambers to egg chambers with abnormal numbers of nurse cells and egg chambers that will develop into variably abnormal eggs. The allele *stall*^{PH} often forms egg chambers in which two cells can be observed to take up yolk (Figure 2d). The allele *pep*^{QS} of *peppercorn* forms egg chambers which contain many small cells with large nuclei, somewhat reminiscent of the phenotype produced by germ cells homozygous for mutations at the locus *Sex-lethal* (SCHÜPBACH 1985; Figure 2c). These egg chambers are surrounded by a monolayer of follicle cells which will eventually secrete a tiny round chorion around the cluster of undifferentiated cells.

In females homozygous for mutations at *egalitarian* or homozygous for the recessive loss-of-function allele *Bicaudal-D*^{PA66} all egg chambers contain 16 nurse cells and no oocyte (Figure 2g). These two loci therefore do not seem to be involved in controlling the correct number of the four mitotic germline divisions, but instead seem to be required for the establishment or maintenance of the oocyte (see also MOHLER and WIESCHAUS 1986; SUTER, ROMBERG and STEWARD 1989; WHARTON and STRUHL 1989).

Class 1C. Early degeneration: In mutations at four loci, and in 16 genetically uncharacterized lines, egg chambers are formed, but the cells in these egg chambers appear morphologically abnormal, and usually degenerate before yolk uptake begins. Already at early stages the cells in the egg chambers are often abnormally shaped and appear very refractile in phase contrast optics, indicating that they are beginning to degenerate. Since usually both the follicle cells as well as the nurse cells and oocyte appear abnormal, it is not easy to determine whether the primary defect in these lines results from germline or follicle cell malfunction.

Mutations affecting mid- and late stages of oogenesis

Class 2A. Oogenesis is arrested at stage 8/9: In one locus and eight genetically uncharacterized lines, early stages of oogenesis appear to develop normally, but egg chambers arrest around stage 8 or 9 and begin to degenerate. At this stage the follicle cell epithelium that surrounds the elongated egg chamber is of uniform thickness, and the oocyte has taken up

TABLE 2

Female sterile loci that cause defects early in oogenesis (class 1)

Locus	Map position/ cytology	No. of alleles	Allele names	Special remarks
Class 1A: No or few egg chambers in ovaries				
arrest (aret)	2-48 33B3-7; 33F1-2	8	WH53 WQ47 PA62 PD41 PE27 QB72 PC42 RM47	$aret^{WH}$, $aret^{WQ}$, $aret^{PE}$ show absence of differentiating egg chambers (Figure 2b). $aret^{QB}$ $aret^{PC}$ usually have a number of small egg chambers in which the follicle cells secrete a tiny round chorion around the germline (Figure 2e). $aret^{PA}$, $aret^{PD}$, $aret^{RM}$, have almost normal numbers of egg chambers which degenerate early. Males $aret^{WH}/aret^{WQ}$, or $aret^{QB}/aret^{PC}$ are sterile, and have a reduced number of sperm bundles in testis
cut off (cuff)	2-61	3	WM25 WL52 QQ37	$cuff^{WM}$ and $cuff^{WL}$ often cause absence of egg chambers, or degeneration of early egg chambers. Males $cuff^{WM}/cuff^{W}$ are only weakly fertile, contain a small number of sperm bundles as well as degenerating material in testis
deadlock (del)	2-54 38A6-B1; 38E6-9	4	WH22 WK36 PS23 HN56	del^{WH} and del^{WK} usually have no egg chambers. del^{PS} and del^{HN} have a normal number of egg chambers and produce eggs with fused dorsal appendages or without dorsal appendages. Males del^{WH}/del^{WK} are fertile
halted (hal)	2-86 55A; 55C	3	DB48 PI56 PK13	hal^{DB} and hal^{PK} usually have no egg chambers. hal^{PI} has a normal number of egg chambers and produces normal unfertilized eggs. Males hal^{DB}/hal^{PK} are fertile.
stopped (stop)	2-76	2	WS59 WS61	Reduced viability of homozygotes and transheterozygotes. Males fertile
fs(2)eoPL3	2-54	1	PL3	Somatic gonadal structures not clearly subdivided into ovarioles. Males sterile with small testis, no sperm bundles
fs(2)eoPP22	2-105	1	PP22	Somatic gonadal structures not subdivided into ovarioles. Males sterile with small testis, no sperm bundles
fs(2)eoPV30	2-30	1	PV30	Homozygotes have reduced viability. Males sterile with small number of sperm bundles in testis.
Class 1B: Abnormal number of nurse cells and tumorous egg chambers				
Bicaudal-D (Bic-D)	2-53 36C8-11	1	PA66	$Bic\text{-}D^{PA66}$ is a recessive allele at the locus and produces egg chambers with 16 nurse cells and no oocyte. Egg chambers increase in size to stage 6. No chorion is formed. Males fertile. (see also MOHLER and WIESCHAUS 1986; SUTER, ROMBERG and STEWARD 1989; WHARTON and STRUHL 1989)
egalitarian (egl)	2-105 59D8-11; 60A1-2	5	WU50 RC12 PB23 PV27 PR29	Egg chambers contain 16 nurse cells and no oocyte. egl^{PB}, egl^{PV}, egl^{PR} also contain many degenerating egg chambers. Males egl^{WU}/egl^{RC} fertile
shut down (shu)	2-105 59D8-11; 60A1-2	3	WM40 WQ41 PB70	Few egg chambers, sometimes with abnormal numbers of nurse cells, usually early degeneration, few small abnormal eggs. Males shu^{WM}/shu^{WQ} are sterile, testis contain sperm bundles and degenerating material
stand still (stil)	2-63 48D-E; 49B2-3	2	WE42 PS34	$stil^{WE}$ produces no egg chambers, $stil^{PS}$ forms many egg chambers, some with abnormal numbers of nurse cells, many degenerate, some form eggs with abnormal chorions. Males $stil^{WE}/stil^{PS}$ are fertile
stall(stl)	2-102	4	WU40 AWK26 PA49 PH57	stl^{WU} forms ovarian tumors, or egg chambers with variable numbers of nurse cells, stl^{PH} often shows yolk containing cells at various location within the egg chambers (Figure 2d), also many degenerating egg chambers, few collapsing eggs. Males are fertile
peppercorn (pep)	2-105 59D8-11; 60A1-2	2	QS2 QW34	Ovarian tumors, ovaries contain many egg chambers filled with small cells (Figure 2c). Follicle cells secrete a tiny round egg shell around the egg chambers. Males $pep^{QW}/Df(2R)bwS46$ or pep^{QS2}/pep^{QS2} are sterile, testis contain germ cells, but no sperm bundles.
fs(2)eoWP19	2-85	1	WP19	Ovarian tumors, some egg chambers with abnormal numbers of nurse cells, usually one oocyte at posterior end. Degeneration of many chambers, some eggs with very irregular chorions are formed

Locus	Map position/ cytology	No. of alleles	Allele names	Special remarks
Class 1C Early degeneration				
blocked (blc)	2-20	1	WE13	Males are sterile
close down (clo)	2-77	3	WG17 WK50 WU5	Males clo^{WG}/clo^{WK} fertile
fs(2)eoPB6	2-64	1	PB6	Males are sterile
in addition: 16 genetically undefined lines (WN32, WS10, PE32, PK33, PM41, PN50, PQ16, PT37, PW21, PY64, QG27, QW63, RD13, RG62, RL36, RS53)				

some yolk. It is not obvious what developmental process is primarily affected by these mutations; possibly the posterior migration of the follicle cells cannot be initiated, or alternatively, yolk synthesis or yolk uptake may be abnormal. The arrested egg chambers eventually degenerate.

Class 2B. Mutations that interfere with normal follicle cell migration patterns: During mid- and late stages of oogenesis the follicle cells undergo a series of cell shape changes and migrations which result in the complex pattern of the mature egg shell (for descriptions see *e.g.*, KING 1970, MAHOWALD and KAMBYSELLIS 1980; MARGARITIS 1985). A large number of female sterile lines can cause abnormalities in follicle cell migration patterns resulting in abnormally patterned egg shells. This indicates that many different genes are required, either directly or indirectly, to produce the correct pattern of follicle cell migration.

Open-ended chorion phenotype: Mutations at eight loci on the second chromosome cause an open-ended chorion phenotype in homozygous mutant females (Table 3). In these egg chambers the centripetal migration of the follicle cells, which should lead to the closing over of the anterior end of the egg shell, does not occur. In a normal egg chamber the follicle cells initially surround the nurse cell-oocyte complex in a uniform monolayer. During stage 8 and 9 of oogenesis the follicle cells at the posterior end of the chamber begin to change their shape. They become columnar and more densely packed over the oocyte. As more follicle cells come to overlie the oocyte, only few follicle cells are left to cover the nurse cells. These follicle cells assume a very flattened morphology. At the end of this process there is a clear boundary in the follicle cell epithelium distinguishing the columnar follicle cells from the flattened cells. In normal egg chambers this boundary coincides precisely with the boundary between the oocyte and the nurse cells. In the loci which cause the open ended chorion phenotype this boundary in the follicle cell epithelium is frequently shifted anteriorly and lies over the nurse

cells. In *cup*, *chal* and *Bic-C* the first tier of nurse cells is usually enclosed by columnar follicle cells (Figure 3). In *kel* and *quit* the follicle cell boundary is more variable and is often in the proper position. In strong mutant alleles at any of the five loci there is no "centripetal" migration of follicle cells in between the oocyte and nurse cells. The follicle cells remain on the outside of the nurse cell-oocyte complex. They continue to differentiate and secrete a structured, multilayered chorion, but in the absence of the centripetal follicle cell migrations these egg shells remain open-ended ("cup-shaped"). In cup-shaped eggs dorsal appendages are not formed. Instead, a patch of dorsal appendage material is often found within the surface of the egg shell (Figure 3), indicating that a number of follicle cells were apparently determined to secrete dorsal appendage material, but did not migrate properly out of the epithelium. In contrast, migration of border cells which occurs during stage 9 (KING 1970) is initiated normally in these mutations. However, the border cells will only migrate as far posterior as the plane defined by the boundary of flat *vs.* columnar follicle cells, *i.e.*, in these mutants, the border cells usually do not reach the anterior end of the oocyte. This indicates that the anterior-posterior pattern of the entire follicle cell population is affected in the mutants.

Mutations at the *Bicaudal-C* (*Bic-C*) locus also have a dominant haplo-insufficient phenotype and cause a low frequency of bicaudal embryos to form inside the morphologically normal looking eggs produced by heterozygous females (MOHLER and WIESCHAUS 1986). No bicaudal production was ever observed in heterozygous females of the other loci. Whereas strong mutations at the loci *cup*, *chal*, *kel*, *Bic-C* and *quit* consistently produce open ended chorions, weaker alleles at *cup*, *chal* and *Bic-C* lead to the production of more normal looking eggs which very frequently have abnormal anterior ends and fused and reduced dorsal appendages. Weaker alleles at the locus *kelch*, in contrast, will produce very small eggs with short, stubby appendages, a phenotype that is

TABLE 3

Female sterile loci that cause defects in midoogenesis

Locus	Map position/cytology	No. of alleles	Allele names	Special remarks
Class 2A: Female sterile loci that cause arrest and degeneration of egg chambers in midoogenesis				
midway (mdy)	2-53 36A8-9; 36E1,2	2	QX25 RF48	
in addition: 8 genetically undefined lines (WG18, WG44, PB7, PK9, PS66, QR7, RC24, RS37)				
Class 2B: Female sterile loci that cause abnormalities in follicle cell migration				
i. Open ended chorion chalice *(chal)*	2-37	3	WP46 QC23 HC49	$chal^{WP}$ shows strong phenotype. $chal^{QC}$ more variable, produces some eggs with fused dorsal appendages. $chal^{HC}$ produces normal, unfertilized eggs
cup (cup)	2-23	37	—[a]	Alleles vary from cup^{WQ52}, cup^{R177} with strong phenotype to cup^{PB53}, cup^{PB9} which produce eggs with fused dorsal appendages, to cup^{RM12} with mostly normal eggs and 10% normal embryos
[a] WQ52, PB1, PB53, PF21, PF63, PH69, PL11, PN14, PS73, PV36, PW3, PY76, QD57, Q J9, Q J36, Q J65, QK4, QK12, QK24, QL54, QM66, QR55, QR56, QV7, QV50, QW45, RC11, RG6, RH13, RH24, RI77, RM12, RN61, RS2, RS50, RU45, RV4.				
Bicaudal-C (Bic-C)	2-53 35D1,2; 35E6	5	WC45 PE37 PX1 QL53 RU35	$Bic\text{-}C^{WC}$ all egg chambers are open ended, heterozygous females produce low frequency of bicaudal embryos. Other alleles may produce eggs with fused dorsal appendages
kelch (kel)	2-53 36C2-4; 36E1,2	7	Wi20 WB6 DE1 HL39 PL25 QD64 RF41	
quit (qui)	2-105 59D8-11; 60A1-2	3	QL24 PX61 APE36	
fs(2)ltoHE41	2-91	1	HE41	
fs(2)ltoQZ47	2-48	1	QZ47	
fs(2)ltoRG3	2-4	1	RG3	
ii. Abnormalities in dorso-ventral pattern of follicle cells				
cappuccino (capu)	2-8 24C1,2-3; 24D3-4	3	RK12 HK3 HK38	Variable expansion (dorsalization) of dorsal appendages, at expense of main body egg shell. Embryos frequently dorsalized. In addition, in embryos absence of polar granules, pole cells and abdominal segments (MANSEAU and SCHÜPBACH 1989)
spire (spir)	2-54 38A6-B1; 38E6-9	4	RP48 HP10 PJ56 QF70	Same phenotype as *capu*
gurken (grk)	2-30	7	WG41 DC29 HF48 HG21 HK36 HL23 QI66	Reduction or absence of dorsal appendages (ventralization) with increase in main body egg shell. Frequently micropyles at both ends of eggs. Embryos ventralized (SCHÜPBACH 1987)
torpedo (top)	2-100	1	QY1	top^{QY1} produces phenotype similar to *gurken*, but less extreme. Amorphic alleles at the locus are lethal (SCHÜPBACH 1987; CLIFFORD and SCHÜPBACH 1989)

Locus	Map position/cytology	No. of alleles	Allele names	Special remarks
iii. Variable abnormalities in dorsal appendage formation				
aubergine (aub)	2-39	5	QC42 HM23 HN2 AHN56 AWE13	Most extreme eggs are spindle shaped without dorsal appendages. Other eggs have fused dorsal appendages, or are of normal morphology and remain unfertilized
gourd (gou)	2-5	2	RI33 QD67	Similar to *aub*
okra (okr)	2-1	2	RU47 WS1	Similar to *aub*. Eggs of okr^{WS} often have a second micropyle at posterior end
zucchini (zuc)	2-45 33B307; 33F1-2	3	RS49 HM27 SG63	Similar to *aub*
squash (squ)	2-53	3	PP32 HE47 HK35	Similar to *aub*
vasa (vas)	2-51 35B2-3; 35D4-7	6	PD23 HE1 PW72 QS17 RG53 AQB3	vas^{PW72}, vas^{QS17}, vas^{AQB3}, vas^{RG53} produce eggs with fused dorsal appendages. Eggs sometimes small. vas^{PD23} and vas^{HE1} produce normal eggs. In all alleles, embryos lack polar granules, pole cells and abdominal segments (see also SCHÜPBACH and WIESCHAUS 1986; LASKO and ASHBURNER 1988; HAY, JAN and JAN 1988)
fs(2)ltoRE57	2-54 37D2-E1; 37F5-38A1	1	RE57	Similar to *aub*
fs(2)ltoWi42	2-100	2	Wi42 Wi46	Eggs of variable size, sometimes fused dorsal appendages
fs(2,ltoRJ36	2-50 35B2-3; 35D4-7	1	RJ36	
fs(2)ltoRN48	2-51 35E6; 36A6,7	1	RN48	
fs(2)ltoRV64	3-54 37D2-E1; 37F5-38A1	1	RV64	

in addition: 33 genetically uncharacterized lines (Wi9, WN49, AWD18, HE59, Hi36, DD23, PW41, QG56, QI6, QJ34, QM57, QM63, QS10, RH33, RI44, RI67, RJ27, SD22, Wi36,HF27, HL51, DB22, DC41, DD15, PR56, PU13, QG75, QQ64, RC7, RE42, RF14, RS12, RU44)

usually observed in mutations where the nurse cell content is not transported into the oocyte (see below). In spite of the phenotypic similarities seen in the egg shells produced by strong alleles at the four loci, the differences observed in the weaker, or heterozygous phenotypes therefore suggests that the five loci may affect different processes in oogenesis.

Mutations that cause alterations in the dorso-ventral pattern of the egg shell: Mutations at the loci *gurken* (*grk*) and *torpedo* (*top*) cause a ventralization of the follicle cell epithelium and cause reduction or absence of the dorsal follicle cell populations that would normally form operculum and dorsal appendages. Embryos which develop inside these eggs are also ventralized (SCHÜPBACH 1987). All alleles of *gurken* consistently produce this ventralized egg shell phenotype, whereas amorphic alleles at the locus *torpedo* are lethal (CLIFFORD and SCHÜPBACH 1989). Mutations at the

loci *spire* (*spir*) and *cappuccino* (*capu*) produce a variable egg shell phenotype with a fraction of the eggs showing a dorsalized egg shell where follicle cells from the entire circumference have secreted dorsal appendage material. Other eggs have one broadened dorsal appendage on the dorsal midline, some eggs show a reduced, fused dorsal appendage, and a fraction of the eggs are always of normal morphology (MANSEAU and SCHÜPBACH 1989). Embryos developing inside *spir* or *capu* derived eggs lack polar granules and pole cells, show deletions in their abdominal segmentation pattern and are frequently dorsalized. Mutations at *spir* and *capu* therefore consistently block the formation of the posterior pole plasm which should harbor the determinants for the germline as well as the determinants for abdominal segmentation, whereas the effect of the mutations on dorso-ventral pattern for-
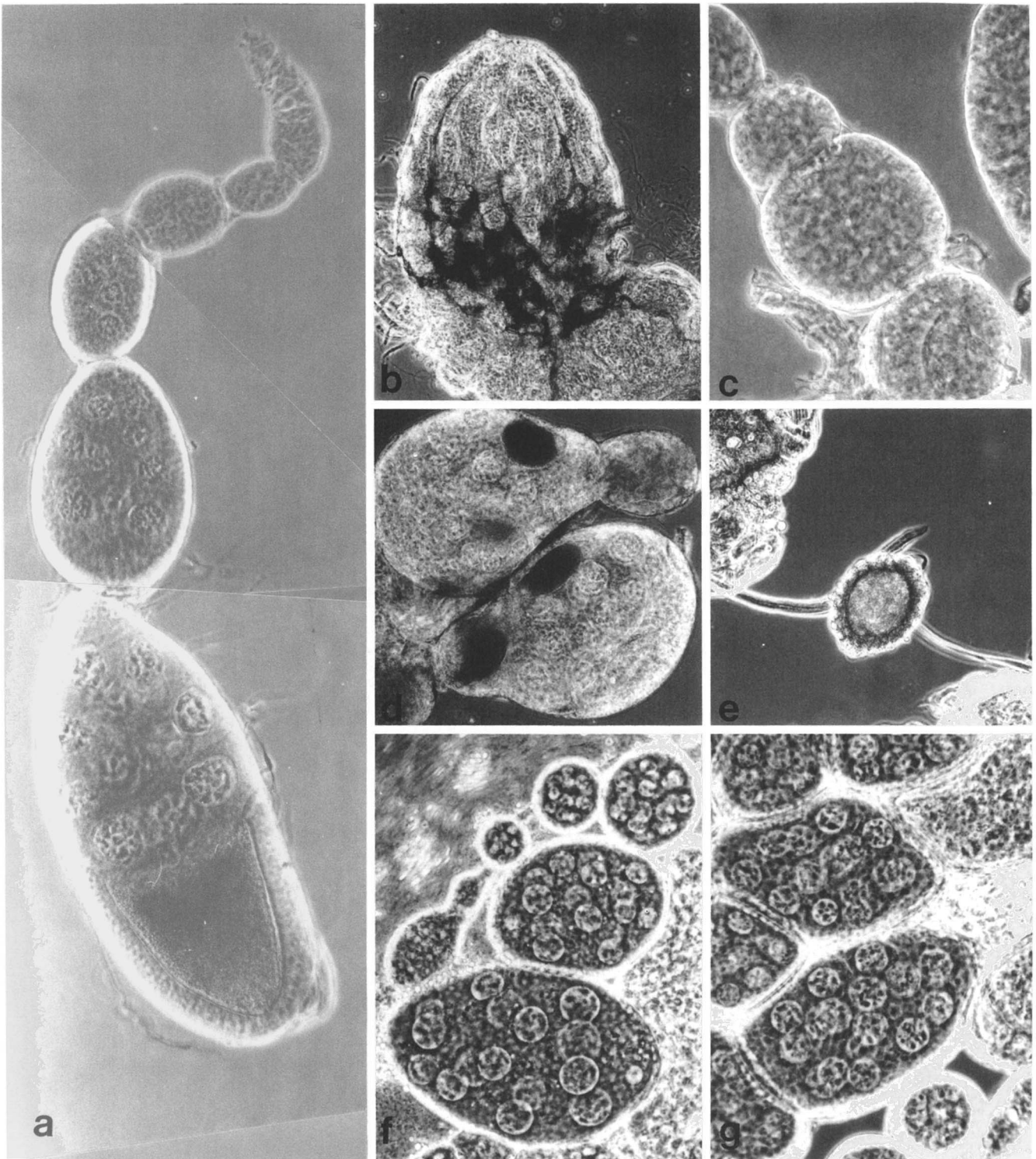
FIGURE 2.—Wild-type and mutant egg chambers, photographed under phase contrast optics. **a**, Wild-type ovariole containing germarium at the top, and egg chambers of approximately stages 2, 3, 5, 7 and 10 in descending order. **b**, Ovary without germ cells of $aret^{WH}$. The dark structures are trachea which contact the empty ovarioles, as in wild-type ovaries. **c**, Egg chambers of pep^{QS} containing many small, undifferentiated cells. **d**, Egg chambers of stl^{PH} in which two cells accumulate yolk. **e**, Small egg chamber of $aret^{QB}/aret^{PC}$ where the follicle cells have secreted a tiny round chorion around the abnormal cell cluster. The same phenotype is also frequently produced by the alleles of *pep*. **f**, Artificially flattened wild-type egg chambers, showing 15 nurse cell nuclei and 1 oocyte nucleus at the posterior end. **g**, Flattened egg chambers of *egl*, showing 16 nurse cell nuclei and no oocyte.
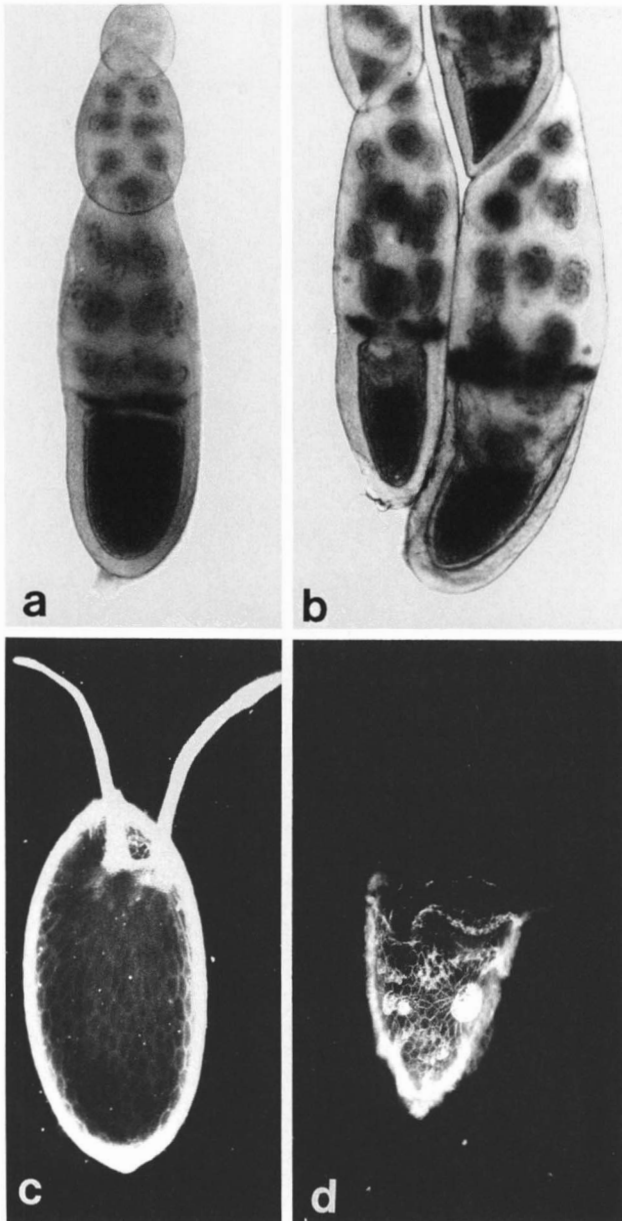
FIGURE 3.—Egg chambers and chorions from wild type and from mutations that produce an open-ended chorion phenotype. **a** and **b**, egg chambers from females carrying the transposant BB127 which contains the lac-Z gene, stained with X-gal. The transposant BB127 expresses β-galactosidase in nurse cells, and beginning at stage 10 of oogenesis, in a ring of follicle cells at the boundary between columnar and flattened follicle cells. **a**, In wild-type egg chambers this ring of follicle cells is exactly aligned with the boundary between oocyte and nurse cells. **b**, In $Bic-C^{WC}$ homozygous egg chambers this ring of staining follicle cells encircles the nurse cells. **c**, Wild-type chorion with two dorsal appendages, **d**, open ended chorion from kel^{Wi}, only a knob of dorsal appendage material is formed.

mation is more variable (MANSEAU and SCHUPBACH 1989).

Mutations which cause variable abnormalities in dorsal appendage formation: Mutations at eleven loci and 33 genetically uncharacterized lines produce eggs with variable dorsal appendage abnormalities. Eggs pro-

duced by females homozygous for these mutations often have only one fused dorsal appendage which may be reduced in size. A fraction of the eggs may lack dorsal appendages altogether. Such eggs are usually spindle shaped, a phenotype which is similar to the eggs produced by the ventralizing mutations *torpedo* or *gurken*. In addition, eggs derived from *okra* (*okr*) homozygous females often show an anterior and a posterior micropyle, similar to eggs from strong *gurken* alleles (SCHÜPBACH 1987). Despite the similarities to *gurken* and *torpedo* it is however not clear whether this variable fusion of dorsal appendages or incompletely penetrant spindle-shaped egg phenotype represents a ventralization of the follicle cell epithelium. The ventralized eggs of *gurken* and *torpedo* are larger than wild-type eggs, because more follicle cells contribute to the main body of the egg shell at the expense of the two populations of migrating appendage producing follicle cells. In contrast, the eggs produced by many of the mutations in this variable egg shell class are frequently smaller than wild-type eggs. Fusion or absence of dorsal appendages in eggs from these lines may therefore not necessarily reflect a ventralization of the egg shell, but may be caused by a variety of defects in germline and follicle cell development. In addition, this variable fused dorsal appendage phenotype is also frequently produced by weak alleles of loci that block oogenesis at early stages (Table 2). It is possible that the correct establishment and differentiation of the migrating dorsal appendage producing cell populations is a very sensitive process. Slight disturbances in various processes of oogenesis may lead to reductions in the number of cells committed to this fate, or alternatively, may interfere with survival, migration, or differentiation of these particular cell populations.

Based on our previous analysis of the two alleles vas^P and vas^{HE} the locus *vasa* was originally placed into the category of maternal-effect mutations that have no visible effect on oogenesis or egg shell patterning. Using the deficiency $Df(2L)osp29$ to screen all mutant lines we found, however, four more alleles of *vasa* which cause variable defects in oogenesis and lead to the production of eggs with fused and reduced dorsal appendages. The eggs that are laid are also usually somewhat smaller than wild-type eggs. Similar defects in oogenesis were also observed for strong *vasa* alleles by LASKO and ASHBURNER (1990) and LEHMANN and NÜSSLEIN-VOLHARD (1991).

Class 2C. Mutations which cause the egg shell to be structurally abnormal: *Thin, fragile chorion phenotype:* The eggs produced by females at 9 loci and in 14 genetically uncharacterized lines usually have a chorion that appears unstructured and fragile (Table 4). Often the chorions rupture at the time of egg deposition by the female and such eggs appear to be

TABLE 4

Female sterile loci that affect the structure and integrity of the egg shell (class 2C)

Locus	Map position/cytology	No. of alleles	Allele names	Special remarks
i. Thin, fragile chorion				
chiffon (chif)	2-53 35E6; 36A6,7	6	WD18 QW16 QY42 WF24 PS55 DB23	
gauze (gau)	2-74	7	PT61 HF19 PB55 PY16 QN68 RJ31 RP65	
rayon (ray)	2-46	1	QN54	
satin (sat)	2-57 43C3; 43E1-8	1	SC46	
fs(2)ltoAHE1	2-71	1	AHE1	
fs(2)ltoHL22	2-55	1	HL22	
fs(2)ltoPA77	2-72 51E3; 52A	1	PA77	lethal over Deficiency (see also UNDER- WOOD *et al.* 1990)
fs(2)ltoPN48	2-51	1	PN48	
fs(2)ltoPP43	2-54 37F5-38A1; 38A6-7	1	PP43	lethal over Deficiency

in addition 14 genetically uncharacterized lines: HN12 PU34, PZ61, QE55, QL57, QM25, QU35, RB37, RE74, RM33, RO31, RP65, SP28, ASB62

ii. Collapsed eggs				
crepe (crpe)	2-59	6	PZ3 HM38 DB3 PL4 QY25 RI35	
muslin (mln)	2-28	2	PP41 Hi3	
fs(2)ltoHD43	2-54 37F5-38A1; 38A6-7	1	HD43	
fs(2)ltoHM11	2-105 59D8-11; 60A1-2	1	HM11	
fs(2)ltoQJ42	2-17 25E6-F3; 26A7-8	1	QJ42	SAVANT and WARING (1989)
fs(2)ltoRN73	2-57 43C3; 43E1-8	1	RN73	
fs(2)ltoRU26	2-41 31D; 31F3	1	RU26	

in addition 37 genetically uncharacterized lines: HB6, HE2, HH15, HH32, HK29, HL48, HM22, HN43, DE17, DG18, PE70, PG42, PG69, PK75, PR67, PT44, PU48, QE31, QF3, QF57, QH1, QJ5, QI56, QK35, QK36, QK52, QL73, QT48, QT68, RA37, RD30, RD77, RQ63, RS54, RU3, SG10, SG71

surrounded only by a vitelline membrane. Upon inspection of the chorion, the follicle cell imprints seem less distinct than in wild type, the dorsal appendages are thin and reduced in size, or they may be altogether absent (*e.g.*, in eggs from *chiffon* and *gauze*). The various layers of the normal egg shell are secreted by the follicle cells during middle and late stages of oogenesis, a process which requires a well controlled

TABLE 5

Female sterile loci that produce abnormally shaped eggs, or fail to lay eggs

Locus	Map position/cytology	No. of alleles	Allele names	Special remarks
Class 2D: Abnormal egg size				
i. Tiny eggs (failure in transport)				
chickadee (*chic*)	2-23	3	WC57 WF57 WK26	Molecularly characterized by L. COOLEY (personal communication)
quail (*qua*)	2-53 36C8-11	6	WP20 HK25 HM14 PX42 QE24 QT21	(see also STEWARD and NÜS-SLEIN-VOLHARD 1986)
warbler (*war*)	2-79	1	RU10	
minus (*mi*)	2-105 59D8-11; 60A1-2	2	PT43 QX54	(Locus originally discovered by BIDDLE, see LINDSLEY and ZIMM 1990)
fs(2)ltoAHK35	2-105 59D8-11; 60A1-2	1	AHK35	
fs(2)ltoDC37	2-105 59D8-11; 60A1-2	1	DC37	
fs(2)ltoDG25	2-41 31D; 31F3	1	DG25	
fs(2)ltoQB3	2-62	1	QB3	
fs(2)ltoQT50	2-54 37D2-E1; 37F5-38A1	1	QT50	lethal over Deficiency, allelic to *l(2)TW37Bc*. (T. WRIGHT, personal communication)
fs(2)ltoRM7	2-105 59D8-11; 60A1-2	1	RM7	

in addition 11 genetically uncharacterized lines: HL58, HP11, PE29, PI42, PV1, QH9, RC1, RC25, RD54, RF76, RO3

ii. Short and variable egg size

Locus	Map position/cytology	No. of alleles	Allele names	Special remarks
fs(2)ltoAPV63	2-105 59D8-11; 60A1-2	1	APV63	
fs(2)ltoP123	2-41 31D; 31F3	1	PI23	
fs(2)ltoQE45	2-8 24C1,2-3; 24D3-4	1	QE45	

in addition 41 genetically uncharacterized lines: HA21, HD45, HF43, HP16, HP26, DB37, PA15, PE69, PF29, PJ47, PK16, PP52, PU5, PU6, PU36, QC43, QD1, QD21, QE12, QE44, QF9, QG48, QI76, QS61, QU33, QX48, QX63, RH53, RJ33, RK35, RM29, RP64, RR14, RS74, RU12, RV15, RV31, RV53, RV62, SD36, SG09

Locus	Map position/cytology	No. of alleles	Allele names	Special remarks
Class 2E: Egg retention				
retained (*retn*)	2-105 59D8-11; 60A1-2	2	RO44 RU50	
fs(2)ltoHC44	2-53 36A8-9; 36E1,2	1	HC44	
fs(2)ltoPM43	2-54 37B9-C1; 37D1,2	1	PM43	

in addition 27 genetically uncharacterized lines: WQ40, HB3, HC22, HD51, Hi6, HL56, PC46, PG49, PK50, PL21, PL22, PW34, QB4, QB62, QD44, QD76, QO7, QU70, RB30, RC32, RF15, RH61, RO54, RQ69, RS35, RS44, RV57

and coordinated pattern of gene expression in these cells (MARGARITIS, KAFATOS and PETRI 1980; MARGARITIS 1985; PARKS and SPRADLING 1987; ROMANO et al. 1988). Most likely the mutations with thin and fragile egg shells cause abnormalities in the secretion of one or more layers of the egg shell. In fact, the egg shell of fs(2)ltoPA77 has been analyzed by UNDERWOOD et al. (1990) and was found to show abnormalities in chorion gene expression. chif was found to be defective in chorion gene amplification (J. TOWER and A. SPRADLING, personal communication and cited in SPRADLING 1991).

Collapsed, flaccid egg shells: In mutations at seven loci and in 37 genetically uncharacterized lines the mutant females lay eggs that appear collapsed (Table 4). In normal eggs the vitelline membrane and the waxy layer seal the egg from its surrounding and prevent the eggs from dehydration. Abnormalities in the deposition of the vitelline membrane or the waxy layer, or alternatively, failure to completely cover the anterior end of the oocyte via the late migrations of the anterior follicle cells might cause eggs to lose water and show the collapsed phenotype. fs(2)ltoQJ42 has been analyzed by SAVANT and WARING (1989) and was found to cause absence of a major vitelline membrane protein. On the other hand, mutations in the yolk protein YP2 locus can also cause a collapsed egg phenotype (WILLIAMS et al. 1987). Alterations of various steps in yolk production or yolk uptake might therefore also account for this phenotype.

Class 2D. Abnormalities in egg size and proportion: *Tiny eggs: failure to transport nurse cell content into the oocyte:* In normal eggs the nurse cells attain their largest size at stage 10B (KING 1970). Subsequently the nurse cell content is transported into the oocyte via the system of interconnected ring canals. In the egg chambers from mutant females at 10 loci and an additional 11 uncharacterized lines, the nurse cells remain large (Table 5). The oocyte arrests growth at around stage 10. The follicle cells proceed with their regular migration movements to enclose the anterior end of the oocyte and form dorsal appendages. Since the nurse cell content largely remains excluded from the oocyte, tiny eggs are produced. The follicle cell imprints on these eggs appear thickened, presumably because the follicle cells do not stretch to cover the growing oocyte, but instead, retained their columnar shape. The dorsal appendages are usually short and stubby, possibly due to a topological barrier for the migrating follicle cells posed by the large nurse cells.

Short eggs: The mutant females in 44 lines lay eggs which are shorter and usually fatter than wild-type eggs. In contrast to the tiny eggs described above, these short eggs are frequently fertilized and often give rise to normal embryos, or to embryos that show irregular reductions and fusions in their anterior-posterior segmentation pattern. Upon dissection of ovaries from such lines usually no consistent abnormalities are visible and the nurse cells seem to regress normally at later stages. This short egg phenotype is very similar to the phenotype of the X chromosomal locus *short egg* (*seg*) which is caused by an abnormal follicle cell function (WIESCHAUS, AUDIT and MASSON 1981). The second chromosomal lines which cause this short egg phenotype were not further analyzed.

Class 2E. Mutations that cause retention of morphologically normal eggs in the ovary: The mutant females in three loci and 27 genetically uncharacterized lines very rarely lay any eggs (Table 5). Their ovaries contain, however, a large number of apparently mature eggs, similar to ovaries of females that, for lack of suitable egg laying medium, retain their eggs. It is possible that the oviducts of these females are not functional, or alternatively, their egg laying behavior may be abnormal.

DISCUSSION

Number of genes: In the mutagenesis screens described in this paper we recovered 528 female sterile lines from a total of 7351 lines tested. These 7,351 lines derived from 18,782 lines, 60% of which were lethal. A traditional way to estimate the number of female sterile loci is based on comparing the frequency of female sterile mutations to the frequency of lethals in a given screen (GANS, AUDIT and MASSON 1975; MOHLER 1977). Using this method we can calculate that the target size for lethality on the second chromosome is 12.5 times larger than the target size for female sterility (see also SCHUPBACH and WIESCHAUS 1989). Using an estimate of 1700 lethals on the second chromosome (NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984), this result would suggest that 140 loci on the second chromosome can mutate to female-sterility in a standard EMS mutagenesis. This uncorrected number compares well with similar estimates derived from screens for female sterile mutations on the X chromosome (KING and MOHLER 1975; GANS, AUDIT and MASSON 1975). These calculations are, however, subject to major reservations. Similar to PERRIMON et al. (1986) we have previously argued that the distribution of alleles in such female-sterile screens represents the combination of alleles at two sets of loci: the more specific female-sterile loci with an allele distribution approximately following a Poisson distribution, and a set of nonamorphic alleles at loci with functions in various tissues. Indeed, in our collection, there is a large excess of loci represented by only one allele, and a smaller group of loci with multiple alleles which are approximately Poisson distributed (see also SCHÜPBACH and WIESCHAUS 1989). In order to obtain an estimate of the number of loci

that are more specific for oogenesis we can use the loci in our collection for which all alleles have been identified, *i.e.*, the 62 loci uncovered by the 14 deficiencies which were complemented against all 528 sterile lines. The allele frequency of the loci uncovered by the 14 deficiencies deviates significantly from a Poisson distribution (30 loci are represented by one allele, 8 by two alleles, 8 by three alleles, 4 by four alleles, 3 by five alleles, and 9 by six or more alleles). The average allele frequency for true female-sterile loci in the collection should therefore be somewhere between 2.6 (if all 62 loci represented by the 163 alleles within the deficiencies were true *fs* loci) and 4.1 [if, similar to PERRIMON *et al.* (1986), only the 32 loci with more than one allele are counted as true female-sterile loci]. Our best empirical fit for either the results from the deficiency complementation, or from the previously published set of maternal-effect mutations suggests an average allele frequency of approximately 3.0 for the more specific female sterile mutations in the collection (SCHÜPBACH and WIES-CHAUS 1989). We would thus estimate that the 163 mutations uncovered by the 14 deficiencies (corresponding to approximately 27% of the euchromatin of the second chromosome) define 30–32 female-sterile loci with multiple hits, 4 female sterile loci with single hits, and include an additional 26–28 mutations at loci that are not specific to oogenesis and mostly represented by single alleles. Extrapolating from these 163 lines (31% of the total) to the entire collection and thus to the entire chromosome, we would estimate that the second chromosome contains a total of around 110 female-sterile loci, *i.e.*, loci that will mutate to female sterility (including maternal-effect sterility) at a rate similar to that with which the average vital gene mutates to lethality. Of these loci, 95% would be represented by at least one allele in our collection, accounting for 67% of the lines recovered in the screen. The remaining 33% of the lines in the collection would be thus be alleles of loci that are also required in other tissues, and are mostly represented by single alleles. Therefore, although a majority of the lines (67%) recovered in the screen represent alleles at more specific loci, when simply counting the number of loci identified, the majority of the loci defined in the screen are not true female sterile loci (178 *vs.* 110). Similar calculations based on the 133 maternal-effect lines yields very similar estimates for numbers of loci (SCHÜPBACH and WIESCHAUS 1989). Of the 110 predicted female sterile loci, 88 (= 80%) should be represented by more than one allele. However, there are presently only 57 loci in the collection that have more than one allele, which may largely be due to the fact that 187 of the 528 lines have not been genetically characterized.

The prediction that roughly two-thirds of the re-covered lines in the collection represent alleles of female-sterile loci more specific for oogenesis may not hold when other mutagens are employed. In particular, *P* element insertions, which may cause a higher frequency of non amorphic alleles, and also show clear preferences for particular loci, may produce a different ratio of mutations at pleiotropic *vs.* more specific loci (SPRADLING 1991)

Of the total number of lines, 62 showed defects in early oogenesis, 333 had defects in mid- to late oogenesis, and 133 produced morphologically normal eggs that do not support normal embryonic development (maternal-effect lethals). If the arguments above apply to all the classes, we can estimate that in each class about 33% of the lines represent mutations in pleiotropic loci, and that the remaining 66% represent the more specific loci on the second chromosome. With an average allele frequency of approximately 3.0, about 13 second chromosomal loci would be specific for early oogenesis, about 70 loci would affect mid- to late oogenesis, and about 30 would specifically cause maternal-effect lethality.

Phenotypic classes: The various mutant classes can be subdivided and used as a basis for an analysis of specific events in gonad development and oogenesis. The gonad primordium is established during stages 11–14 of embryogenesis when the migrating germ cells become enveloped by groups of somatic cells of mesodermal origin (SONNENBLICK 1941; CAMPOS-ORTEGA and HARTENSTEIN 1985). During larval stages the female germ cells and the precursors of the somatic follicle cells divide continuously and begin differentiation only in pupal stages (KING 1970; WIESCHAUS and SZABAD 1979). None of the mutations in our collection causes the complete absence of a gonad, a phenotype that would be expected if the somatic components of the gonad were completely absent. Only two genes when mutated caused abnormal gonadal differentiation. Consequently, genes that are specifically (or exclusively) required to establish the somatic gonad primordia, or genes that specifically control survival and overall differentiation of the gonad are rare. With respect to early germ cell functions we found six genes that had mutant alleles which could cause the absence of differentiating germ cells in the ovaries of homozygous mutant females. It is presently not known at which stages of germ cell development these genes are required. In only two of these loci were the mutant males also sterile, but in both cases the testis still contained some germ cells. There is therefore presently no clear candidate for a gene required for early survival of both male and female germ cells in the collection.

The stem cells in the adult ovary divide continuously during adult life (WIESCHAUS and SZABAD 1979). The differentiating daughters of the stem cell divi-

sions will undergo four incomplete mitotic divisions ("cystocyte divisions") giving rise to a cluster of sixteen cells connected to each other by ring canals, the remnants of the mitotic division. One of the two cells in the cluster that have four ring canals will become the oocyte and will move from its initial central position within the cluster to the posterior end. The remaining fifteen cells will differentiate into nurse cells. Seven of the genes in our collection interfere at some level with the normal establishment of the 15 + 1 nurse cell-oocyte cluster. Three of these loci can be counted into the "ovarian tumor" class giving rise to egg chambers that contain many small, undifferentiated cells, similar to the phenotypes produced by mutations at the X chromosomal locus *otu*, or the third chromosomal locus *bag-of-marbles* (KING and RILEY 1982; McKEARIN and SPRADLING 1990). Clearly the control of the cystocyte divisions is affected in these mutants at some level. In one allele of *stl* egg chambers with variable numbers of nurse cells are formed which will often contain more than one cell that accumulates yolk, strongly suggesting that more than one of the cells in the cluster has acquired the characteristics of an oocyte. Given that normally the cell lineage of the cystocyte divisions is fixed, and only one of two particular daughters within the cluster can become the future oocyte (see *e.g.*, KING 1970), it is perhaps not unexpected that a mutation that disrupts the normal control of cell division could lead to egg chambers with more than one oocyte.

Mutations at the two loci *egalitarian* and *Bic-D* can give rise to egg chambers that contain 16 nurse cells and no oocyte. In these mutants the distinction between oocyte and nurse cells is either never established or fails to be maintained. *Bic-D* has been molecularly cloned and its protein has been shown to show sequence similarity to the tail portion of the myosin heavy chain. The RNA and the protein have also been shown to accumulate in the oocyte of wild-type egg chambers (SUTER, ROMBERG and STEWARD 1989; WHARTON and STRUHL 1989), but the gene products fail to accumulate in *egl* egg chambers (SUTTER and STEWARD 1991). Given the additional observation that *egl* mutations will suppress the dominant gain-of-function phenotype of *Bic-D* alleles (MOHLER and WIESCHAUS 1986), it is possible that *egl* function is necessary for *Bic-D* localization, and that localization of *Bic-D* in turn is necessary to maintain the oocyte in its determined state.

After the initial cystoblast divisions the somatically derived follicle cells envelop the 16 cell cluster as it enters the lower portion (region 2B) of the germarium. Further development of the egg chamber will depend on the close cooperation between the three cell types—oocyte, nurse cells and follicle cells. The follicle cells contribute yolk to the developing oocyte,

and in mid-oogenesis they undergo a series of complex migrations and cell shape changes before beginning with the secretion of the various layers of the egg shell (see *e.g.*, MARGARITIS 1985). A large fraction of the mutations recovered in this screen affects follicle cell behavior. Mutations at 23 loci and in 33 uncharacterized lines will cause abnormalities in follicle cell migration patterns, as reflected in the mature secreted egg shell, and mutations in 16 loci and 51 uncharacterized lines lead to the formation of a structurally abnormal egg shell. This demonstrates that a relatively large number of genes are involved in the development and the differentiation processes that take place in the follicle cell epithelium. This finding corresponds well with the results obtained from recent screens involving random insertion of lac-Z containing P elements into the genome ("enhancer-trap" screens) performed in various laboratories. The lines isolated in these screens have revealed a high complexity of transcriptional activity in the follicle cells during oogenesis (FASANO and KERRIDGE 1988; GROSSNIKLAUS *et al.* 1989; SPRADLING 1992; T. SCHÜPBACH, L. J. MANSEAU and E. SHADDIX, unpublished results). The majority of the mutant lines affecting follicle cell behavior that were obtained in the present screen have very variable phenotypes. This variability may in part arise because many of the mutations may not be null alleles at the corresponding loci. In fact, genetic mapping and complementation tests revealed that several of the lines that showed variable effects on follicle cell migration leading to fusion and reduction of dorsal appendages, represented alleles at loci where strong alleles blocked oogenesis at earlier stages. However, there also exist several loci represented by multiple alleles in the collection, for which even the strong alleles when tested over a deficiency still produce variable egg shell phenotypes. Possibly there exists a certain amount of redundancy among the genes involved in late follicle cell functions such that single mutations will not completely block these later differentiation processes. Studies of genetically mosaic egg chambers have shown that the dorso-ventral development of the follicle cell epithelium depends on signals from the underlying germ line (WIESCHAUS, MARSH and GEHRING 1978; SCHÜPBACH 1987). Since the movements of the follicle cells occur in relation to the underlying oocyte and nurse cells, it is conceivable that some of the mutations affecting follicle cell migration would act in the germline whereas others might be expected to block follicle cell intrinsic processes.

Mutations in seven loci cause a block of the centripetal migrations of the follicle cells that normally occur after stage 10 of oogenesis and lead to the formation of the anterior specialized regions of the eggshell (operculum, micropyle, ventral collar). The resulting

egg shell consequently remains open-ended ("cup-shaped"). Morphological examination of the phenotype during oogenesis and use of "enhancer-trap" lines that express lac-Z in particular subsets of follicle cells have shown that as an initial defect in these lines the posterior migration of the follicle cells at stages 8 and 9 of oogenesis is arrested before the population of columnar follicle cells is properly aligned with the oocyte surface. Border cell migration in these lines is similarly arrested before the border cells reach the anterior end of the oocyte. It is therefore possible that in these lines the populations of columnar *vs.* flat follicle cells mis-position themselves in the anterior-posterior axis with respect to the underlying germline. Alternatively, it is possible that oocyte growth is arrested or slowed down in these mutants such that although the follicle cells position themselves correctly within the egg chamber, the oocyte remains too small and thus causes the relative misalignment. In either case, the mutations reveal that follicle cells do not necessarily have to be in contact with the oocyte itself to make a clear distinction between the population attaining the columnar *vs.* flattened cell shape, and likewise, that the border cells can arrest their posterior migration without having made contact with the anterior end of the oocyte itself. This observation argues against a strict chemotactic model for border cell migration with the oocyte providing the source of the signal. In the future, the morphological and mosaic study of such mutations in combination with available molecular techniques should allow a more precise understanding of the various signals and patterning processes that govern the development and differentiation of the somatic and germline components of the ovary of Drosophila.

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