

## Female Sterile Mutations on the Second Chromosome of *Drosophila melanogaster*. I. Maternal Effect Mutations

Trudi Schüpbach and Eric Wieschaus

Biology Department, Princeton University, Princeton, New Jersey 08544

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### ABSTRACT

In mutagenesis screens for recessive female sterile mutations on the second chromosome of *Drosophila melanogaster* 529 chromosomes were isolated which allow the homozygous females to survive, but cause them to be sterile. In 136 of these lines, mutant females produce morphologically normal eggs which cannot support normal embryonic development. These "maternal-effect" mutations fall into 67 complementation groups which define 23 multiply hit and 44 singly hit loci. In eggs from 14 complementation groups development is blocked before the formation of a syncytial blastoderm. In eggs from 12 complementation groups development is abnormal before cellularization, 17 complementation groups cause abnormal cellularization, 12 complementation groups cause changes in cellular morphology in early gastrulation stages, and 12 complementation groups seem to affect later embryonic development.

THE earliest events in the life cycle of *Drosophila* include fertilization, nuclear cleavage divisions, establishment of the primary body pattern and the onset of morphogenetic movements. Because of their importance for subsequent development, all these events are good candidates for a genetic dissection. Since early development relies almost exclusively on gene products which are supplied to the egg by maternal transcription during oogenesis, the relevant mutations will produce their effects when the mother, rather than the embryo itself, is mutant. In their simplest form such "maternal effect" mutations would allow homozygous females to survive, but cause them to produce abnormal progeny because vital maternal gene products are not deposited in the egg.

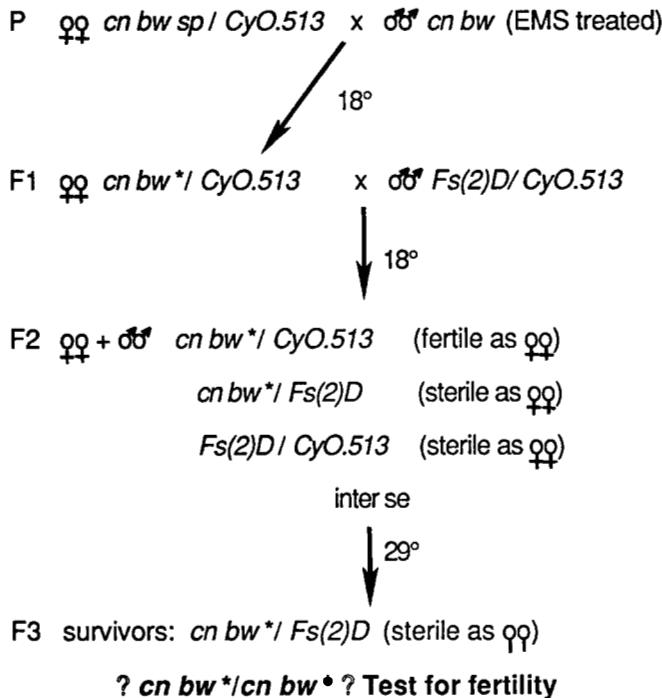
Although a genetic analysis of early embryogenesis would ideally involve mutations in each maternal component, such a detailed approach is not possible. Early development involves large numbers of common cell biological functions, and most of the genes expressed during oogenesis for use by the embryo will also be required at earlier points in the life cycle of the flies. Mutations in these genes will therefore cause homozygous females to die before reaching adult stages where their effects on subsequent embryonic development could be analyzed. On the other hand, since many of the processes which occur during early embryonic development in *Drosophila* are unique to this stage, at least some of the required gene products should be specific to embryogenesis. Mutations in such genes should allow homozygous females to survive, but would cause defects at specific stages in the embryonic development of their progeny. These muta-

tions might provide useful entry points for studying the processes ultimately in their entirety. Previous screens for female sterile mutations have indeed identified a number of such mutations (GANS, AUDIT and MASSON 1975; RICE and GAREN 1975; MOHLER 1977). We describe here the results of large scale screens for maternal effect mutations on the second chromosome, and we show that the abnormalities in such embryos range from blocks in fertilization to specific fate changes during gastrulation.

### MATERIALS AND METHODS

**Isolation of mutant lines:** Two experimental schemes were used to isolate female-sterile mutations on the second chromosome. In both schemes, males homozygous for an isogenized second chromosome were treated with EMS according to the method of LEWIS and BACHER (1968). The isogenized chromosomes were marked either with *cn bw sp* (in the W-series) or with *cn bw* (in the D-, H-, P-, Q-, R-, and S-series). All marker mutations, chromosomal rearrangements, and balancer chromosomes used in these experiments, are described and referenced in LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985); unless otherwise stated.

In the first scheme (series W and H), mutagenized second chromosomes were passed through single  $F_1$  males. In the  $F_2$  generation, heterozygous lines of such chromosomes were established at  $29^\circ$  in *trans* over the balancer *CyO* using the dominant temperature-sensitive lethal mutation *DTS91* as described in Figure 1 of NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984). In the  $F_3$  generation these stocks were examined for surviving individuals homozygous for the mutagenized second chromosome. Such individuals were easily distinguished from their heterozygous siblings by their white eye color. Homozygous females from such lines were then placed into vials or egg laying blocks and tested for fertility (for description and references of all standard procedures,



### ? cn bw\*/cn bw\*? Test for fertility

FIGURE 1.—Mutagenesis scheme. Isogenized, EMS-treated second chromosomes were propagated through three generations to produce homozygous females that could be tested for fertility. The crosses involved the use of a dominant temperature-sensitive lethal balancer chromosome, designated as *CyO.513*, and a dominant female-sterile mutation *Fs(2)D*, in order to kill or sterilize the unwanted genotypes.

unless otherwise noted, see WIESCHAUS and NÜSSLEIN-VOLHARD 1986). The first series of mutagenized chromosomes tested with this scheme (W-series) derived from the 1,547 homozygous viable lines produced by NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984) among their 5764 mutagenized lines established for isolation of zygotic embryonic lethal mutations. The second series (H-series) yielded 630 homozygous viable lines from a total of 2,176 lines established.

In this first experimental scheme, the homozygous females had to be hand-selected for the fertility test, which greatly limited the number of lines that could be screened per day. In the second experimental scheme (Figure 1) the heterozygous *F<sub>3</sub>* sibling females were either killed, or sterilized by genetic mutations, so that the only potentially fertile *F<sub>3</sub>* females were the females that were homozygous for the mutagenized second chromosome. This was achieved by using a second chromosomal balancer chromosome that carried a dominant temperature-sensitive lethal (*CyO. In(2LR)O, dpy<sup>bt</sup> Cy, I(2)DTS 513, pr cn*; STEWARD and NÜSSLEIN-VOLHARD 1986; designated in the text as *CyO.513*), and by using a dominant female-sterile mutation (*Fs(2)D*; YARGER and KING 1971). In this scheme the entire surviving *F<sub>3</sub>* generation could be directly transferred into egg laying blocks to be tested for fertility without previous hand-sorting of the various genotypes. With this experimental scheme (D- P- Q- R- S- series) 5,174 homozygous viable lines were tested. They derived from a total of 10,842 established *F<sub>2</sub>* lines. If the *F<sub>3</sub>* generation proved to be sterile, balanced stocks were established from heterozygous individuals of the *F<sub>2</sub>* generation which had been stored in vials at 18° while the *F<sub>3</sub>* generation was raised and tested.

During the fertility test in most experiments, the homo-

zygous females were kept at 29°. The egg collecting plates were changed daily and each plate was examined to determine whether or not the females had laid any eggs, whether such eggs were of normal morphology when inspected under a stereomicroscope, and whether any progeny hatched out of the eggs after a period of 24 hr at 29°. If egg morphology seemed abnormal, or if less than 10% of the progeny hatched from the egg case, the unhatched eggs were directly mounted in Hoyer's medium, and inspected with a light microscope under darkfield and phase contrast optics. This procedure allowed a preliminary classification of the putative sterile lines into lines that did not lay any eggs, lines that produced morphologically abnormal eggs, and lines that produced morphologically normal eggs, but did not give rise to viable embryos. From all putative sterile lines some individuals of the "old" *F<sub>2</sub>* generation were placed into fresh vials at 25°, and a new *F<sub>3</sub>* generation was raised and tested at 25°. Since 25° is partially permissive for the survival of *CyO.513* individuals, the homozygous females had to be hand-sorted in this retest. However, the average rate of egg fertilization is much higher at 25° than at 29° which allowed a better assessment of fertility or sterility phenotypes of the individual lines. For all lines that were found to be sterile in the retest three individual balanced lines were established from single pair matings of individuals with the genotype "mutagenized"/*CyO.513*. Homozygous progeny from these three different pair matings were again tested at 25°, and if these again proved to be sterile, one of these lines was kept and further characterized.

**Genetic mapping and complementation tests:** Genetic mapping of the mutations was performed using either a chromosome carrying the dominant markers *S Sp B1*, or an *a1 dp b pr c px sp* chromosome ("all-chromosome"), and using, in addition, the markers *cn* and *bw* present on the mutagenized chromosome. Once a female-sterile mutation had been placed between two marker mutations, a larger number of recombinants between those markers were isolated in a second recombination experiment. Usually the number of such recombinants scored was equal to, or larger than the number of genetic map units between the two flanking markers (e.g., if the mutation was found to lie between *dp* at 13.0 and *b* at 48.5 at least 35 recombinants were scored). This yields a map position with an error interval of less than ±5 map units (5% error limit). After a particular mutation had been genetically mapped, all chromosomal deficiencies within ±10 map units were tested in *trans* to the mutation. Finally, complementation tests were performed between all mutations that mapped between the same two marker mutations, in order to find all members of a given complementation group, regardless of the sterility phenotype they displayed.

**Phenotypic descriptions:** For the mutant lines in which the homozygous females laid apparently normal eggs that did not give rise to normal embryos, embryonic development was assayed in several ways: Eggs were collected from individual lines in three hour intervals, submerged in Voltaeff oil and the eggs were then screened for the appearance of "cytoplasmic clearing," cellularization, and early gastrulation under a stereomicroscope in transmitted light. Time-lapse video films of cellularizing and gastrulating embryos were made with a Panasonic 8050 time-lapse video recorder using a compound microscope and bright-field optics. Cuticle preparations of differentiated embryos were made according to standard protocols (WIESCHAUS and NÜSSLEIN-VOLHARD 1986). All flies were raised on standard Princeton fly food (CLINE 1978); laboratories using different food receipes have sometimes reported slight differences in phe-

notypic distribution when working with some of our stocks that display variability in phenotype.

## RESULTS

To identify female-sterile and maternal-effect mutations on the second chromosome of *Drosophila melanogaster*, we treated males with EMS and established inbred lines balanced for single mutagenized chromosomes. Those lines which produced adults homozygous for the mutagenized chromosome were tested for recessive female sterility. In total 18,782 individual  $F_3$  lines were established, of which 7,351 were homozygous viable and could be tested for fertility. Using Poisson distribution the probability of a lethal hit per chromosome was therefore 0.94. Of the 7,351 viable chromosomes, 529 were sterile. The probability of obtaining a female sterile mutation per chromosome is therefore 0.075. The total target size for lethality is thus 12.5 times larger than the total target size for sterility.

In 136 of the 529 female sterile lines ( $=25\%$ ), homozygous females lay eggs of normal morphology, but the embryonic development inside those eggs is abnormal. Most of these "maternal-effect mutations" have no effects on adult viability or on the morphology of the homozygous females (see below). The effects of the mutations appear to be specific and restricted to the phenotype of the progeny.

Genetic mapping and complementation tests allowed the 136 mutations to be assigned to 67 complementation groups spread over the second chromosome (Figure 2; Tables 1–5). The distribution of alleles per locus does not follow a Poisson distribution. There are 44 loci represented by only one allele, 6 by two, 6 by three, 3 by four, and 8 by five or more alleles (deviation from Poisson significant with  $P < 0.001$ ). The major deviation is due to a surplus of loci with single mutant alleles (*i.e.*, with the mean allele frequency of 2.0 obtained in our sample, only 21 single allele loci would be expected). This surplus of single alleles probably arises because many of the single mutations represent hypomorphic alleles of genes whose expression is not restricted to oogenesis (see DISCUSSION).

Freshly laid eggs from all mutant lines were observed under oil in a dissecting microscope, and the earliest deviations from wildtype embryonic development were recorded. This procedure allowed the mutations to be classified according to the earliest time when the mutant maternal gene causes visible abnormalities in the embryo (Tables 2–5). A few loci had alleles which fell into different phenotypic classes (*e.g.*, *frh*, *bie*, *rem*, Table 2). In the final analysis, such loci were grouped according to the earliest defects shown by any of their alleles. In cases where we only identified a single allele at a given locus, the classifi-

cations may therefore not provide a reliable indication of the earliest times in development when the maternal wildtype product is required (*i.e.*, it is possible that other alleles might cause earlier defects). Nevertheless, the earliest visible defects at least indicate the latest possible stage when the maternal gene product is required for normal embryonic development.

### Class 1. Defects occurring in presyncytial stages

In eggs from females of 14 complementation groups, no visible sign of embryonic development is detected when the eggs are observed under oil (Table 2). Instead, after a period of four to six hours after egg deposition, the originally wild-type-like, uniformly dense yolk mass of these eggs starts to disintegrate into a network of darker and lighter yolk droplets, as characteristic of old, unfertilized eggs (Figure 3, a and b). The phenotype is strictly maternal and is also produced when homozygous females are crossed to wild-type males. The early developmental block might be produced by defects in maternal components necessary for fertilization, or in components necessary for very early embryonic cleavage. Three of the loci have alleles which allow a fraction of the eggs to begin embryonic development, but such eggs invariably become abnormal by the cellular blastoderm stage (Table 2; Figure 3c). This observation suggests that the corresponding genes are not specific for a single event like fertilization, but instead are required throughout early embryonic development. A hypomorphic allele which provides some wild-type product might, for example, allow development during the earliest time periods, but would result in defects at stages when larger numbers of nuclei are present in the egg.

### Class 2. Defects occurring during syncytial blastoderm formation

In eggs from mutant females of 12 complementation groups embryonic development seems to be initiated, and cytoplasmic clearing occurs in a narrow zone around the egg periphery (Table 2; Figure 3d). In normal development this process is coupled to the arrival of the embryonic nuclei at the egg periphery during cycle 9 (FOE and ALBERTS 1983). The eggs produced by the mutants seem not to develop beyond cytoplasmic clearing, pole cells are not formed and cellularization does not occur. However, about two hours after cytoplasmic clearing, the egg periphery starts to show local contractions, in what might be an attempt at gastrulation. These contractions eventually lead to eggs which typically show one or two condensed yolk balls in their center surrounded by more transparent cytoplasm.

### Class 3. Defects occur during cellularization of the blastoderm

Eggs from mutant females in 17 complementation groups allow the formation of a syncytial blastoderm,

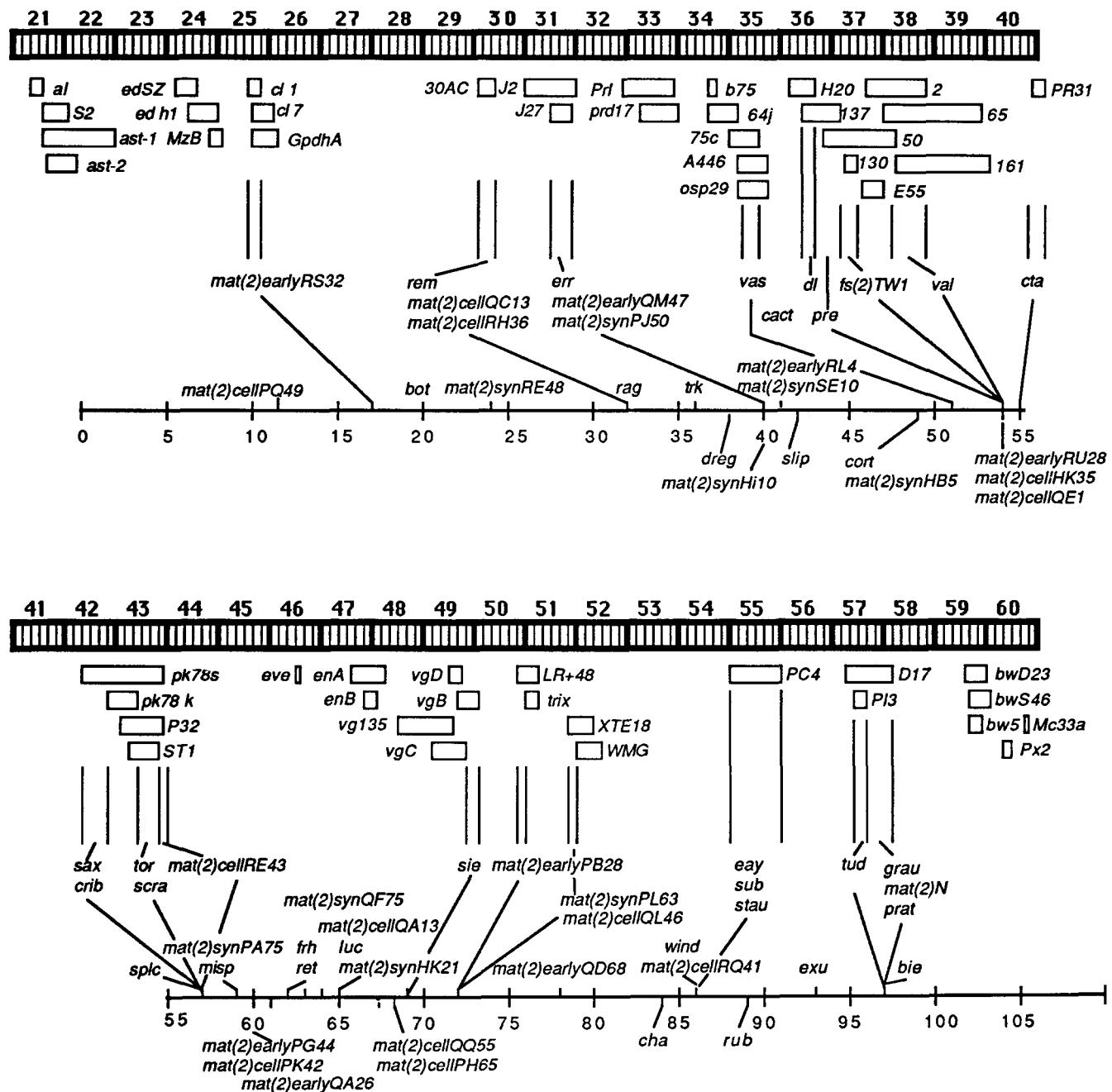


FIGURE 2.—A simplified map of the second chromosome indicating map positions and cytological localization of the 67 complementation groups. Deficiencies are represented by open bars. Cytologically undefined loci are shown directly above or below the genetic map (modified from NÜSSEIN-VOLHARD, WIESCHAUS and KLUDING 1984).

and many of these embryos begin to cellularize (Table 3). Within a given embryo, cellularization is normal in some regions, whereas in others it is irregular or does not occur at all (Figure 3f). Gastrulation takes place, but is usually very abnormal. Some of the epidermal regions which cellularize seem, however, to produce larval cuticle. Whole mounts of such eggs made at later stages contain fragmented pieces of cuticle, often with Filzkörper material, but no ventral denticles or dorsal hairs are recognizable. Mutant lines which show less extensive abnormalities at the cellular blastoderm stage, as e.g., *luc* or *mat(2)cellHK35*, dif-

ferentiate cuticle with recognizable head structures, hairs and denticles, but most of the embryos have variable holes. In addition to variable cellularization defects, eggs derived from females homozygous for *valois* lack polar granules and pole cells and show the typical maternal "knirps-like" abdominal segment deletions (SCHÜPBACH and WIESCHAUS 1986a). It is not clear how the absence of polar granules, the cellularization defects and the abdominal segment deletions relate to one another, but all four alleles of *valois* cause this particular array of embryonic defects.

**TABLE 1**  
**Deficiencies on the second chromosome**

	Deficiency breakpoints	Loci uncovered	Reference
<i>Df(2L)al</i>	21B8-C1; 21C8-D1		a
<i>Df(2L)S<sup>2</sup></i>	21C6-D1; 22A6-B1		a
<i>Df(2L)ast-1</i>	21C7-8; 23A1-2		a
<i>Df(2L)ast-2</i>	21D1-2; 22B2-3		a
<i>Df(2L)ed<sup>sz</sup></i>	24A3-4; 24D3-4		b
<i>Df(2L)ed-dph1</i>	24C1,2-3; 25A1-4		b
<i>Df(2L)M-zB</i>	24E2-F1; 24F6-7		a
<i>Df(2L)cl 1</i>	25D7-E1; 25E6-F3	<i>mat(2)earlyRS32</i>	a
<i>Df(2L)cl 7</i>	25D7-E1; 26A7-8		a
<i>Df(2L)GpdhA</i>	25D7-E1; 26A8-9		a
<i>Df(2L)30A;C</i>	30A; 30C	<i>rem, mat(2)cellQC13, mat(2)cellRH36</i>	c
<i>Df(2L)J-der2</i>	31A; 32A1,2	<i>trk</i>	d
<i>Df(2L)J-der 27</i>	31D; 31F3	<i>mat(2)earlyQM47, mat(2)synPJ50, err</i>	d
<i>Df(2L)Prl</i>	32F1-3; 33F1-2		a
<i>Df(2L)prd1.7</i>	33B3-7; 34A1-2		a
<i>Df(2L)b75</i>	34D4-6; 34E5-6		a
<i>Df(2L)64j</i>	34D1-2; 35B9-C1		a
<i>Df(2L)75c</i>	35A1,2; 35D4-7	<i>vas</i>	a
<i>Df(2L)A446</i>	35B1-3; 35E6-F2	<i>vas</i>	a
<i>Df(2L)osp29</i>	35B2-3; 35E6	<i>vas</i>	a
<i>Df(2L)H20</i>	36A8-9; 36E1,2	<i>dl</i>	a
<i>Df(2L)TW137</i>	36C2-4; 37B9-C1	<i>dl, pre</i>	a
<i>Df(2L)TW50</i>	36E4-F1; 38A6-7	<i>fs(2)TW1, pre</i>	a
<i>Df(2L)TW130</i>	37B9-C1; 37D1-2	<i>fs(2)TW1</i>	a
<i>Df(2L)E55</i>	37D2-E1; 37F5-38A1		a
<i>Df(2L)TW2</i>	37D2-E1; 38E6-9	<i>val</i>	a
<i>Df(2L)TW65</i>	37F5-38A1; 39E2-F1	<i>val</i>	a
<i>Df(2L)TW161</i>	38A6-B1; 40A4-B1	<i>val</i>	a
<i>Df(2L)PR31 (=C')</i>	2L heterochromatin	<i>cta</i>	a
<i>Df(2R)pk78s</i>	42C1-7; 43F5-8	<i>tor, crib, scra, sax, mat(2)cellRE43</i>	a
<i>Df(2R)pk78k</i>	42E3; 43C3		a
<i>Df(2R)P32</i>	43A3; 43F6	<i>tor, scra, mat(2)cellRE43</i>	e
<i>Df(2R)ST1</i>	43B3-5; 43E1-8	<i>tor, scra</i>	a
<i>Df(2R)eve1.27</i>	46C3-4; 46C9-11		a
<i>Df(2R)enA</i>	47D3; 48B2-5		a
<i>Df(2R)enB</i>	47E3-6; 48A4		a
<i>Df(2R)ug135</i>	48D-3; 49D-E		f
<i>Df(2R)ugC</i>	49B2-3; 49E7-F1		a
<i>Df(2R)ug<sup>D</sup></i>	49C1-2; 49E2-6		a
<i>Df(2R)ug<sup>B</sup></i>	49D3-4; 50A2-5	<i>sie</i>	a
<i>Df(2R)L<sup>R+48</sup></i>	50F-51A1; 51B	<i>mat(2)earlyPB28</i>	g
<i>Df(2R)trix</i>	51A1,2; 51B6		g
<i>Df(2R)XTE 18</i>	51E3; 52C9-D1	<i>mat(2)synPL63, mat(2)cellQL46</i>	g
<i>Df(2R)WMG</i>	52A; 52D		g
<i>Df(2R)PC4</i>	55A; 55F	<i>eay, sub, stau</i>	a
<i>Df(2R)D17</i>	57B4; 58B	<i>grau, tud, mat(2)N, prat</i>	h
<i>Df(2R)Pl13</i>	57B13-14; 57D8-9	<i>tud</i>	h
<i>Df(2R)bw-D23</i>	59D4-5; 60A1-2		a
<i>Df(2R)bw-S46</i>	59D8-11; 60A7		a
<i>Df(2R)bw5</i>	59D10-E1; 59E4-F1		a
<i>Df(2R)Px<sup>2</sup></i>	60C5-6; 60D9-10		a
<i>Df(2R)M-c33a</i>	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).

#### Class 4. Abnormalities visible during early stages of gastrulation

Eggs produced by homozygous females from 12 complementation groups develop into blastoderm em-

bryos which seem to cellularize normally, but become abnormal at gastrulation (Table 4). At final differentiation, embryos from all these complementation groups form some differentiated cuticle. These cuticles show characteristic defects which can often be

**TABLE 2**  
**Maternal effect loci that cause defects before cellularization of the blastoderm embryo**

Locus	Map position/ cytology	No. of alleles	Special remarks
<b>Class 1: Presyncytial arrest, eggs show no visible signs of development</b>			
<i>early (eay)</i>	2-86 55a; 55f	7	
<i>fruh (frh)</i>	2-62	6	Embryos from <i>frh<sup>PH</sup></i> , <i>frh<sup>PM</sup></i> and <i>frh<sup>PL</sup></i> form sometimes irregular cellular blastoderms and fragmented cuticle
<i>bientot (bie)</i>	2-97	3	Embryos from <i>bie<sup>PV</sup></i> form sometimes irregular cellular blastoderms and fragmented cuticle. Homozygous adults have tergite defects
<i>subito (sub)</i>	2-86 55A; 55F	2	Embryos from <i>sub<sup>HM</sup></i> form rare syncytial blastoderms
<i>remnants (rem)</i>	2-32 30A; 30C	2	<i>rem<sup>HG</sup></i> shows embryonic development, embryos form fragmented cuticle
<i>fs(2)TW1<sup>a</sup></i>	2-54 37B9-C1; 37D1-2	4	
<i>mat(2)earlyPB28</i>	2-72 50F; 51A1,2	1	
<i>mat(2)earlyPG44</i>	2-60	1	
<i>mat(2)earlyQA26</i>	2-61	1	
<i>mat(2)earlyQD68</i>	2-78	1	
<i>mat(2)earlyQM47</i>	2-40 31D; 31F3	1	
<i>mat(2)earlyRL4</i>	2-41	1	
<i>mat(2)earlyRS32</i>	2-17 25D7-25E1	1	
<i>mat(2)earlyRU28</i>	2-54	1	
<b>Class 2: Syncytial blastoderm arrest, narrow halo of clear cytoplasm appears at egg periphery</b>			
<i>grauzone (grau)</i>	2-97 either 57B4; 57B13-14, or 57D8-9; 58B	5	
<i>cortex (cort)</i>	2-49	2	
<i>presto (pre)</i>	2-54 36E4-F1; 37B9-C1	2	
<i>mat(2)synHB5</i>	2-49	1	Cytoplasmic clearing appears irregular
<i>mat(2)synHi10</i>	2-40	1	
<i>mat(2)synHK21</i>	2-65	1	
<i>mat(2)synPA75</i>	2-59	1	
<i>mat(2)synPJ50</i>	2-40 31D; 31F	1	Narrow halo of clear cytoplasm in 80% of embryos, remainder show variable cellularization
<i>mat(2)synPL63</i>	2-72 51E3; 52A	1	
<i>mat(2)synQF75</i>	2-63	1	Narrow halo of clear cytoplasm in 50% of embryos, remainder show variable cellularization
<i>mat(2)synRE48</i>	2-24	1	Narrow halo of clear cytoplasm in 80% of embryos, remainder show variable cellularization
<i>mat(2)synSE10</i>	2-41	1	Narrow halo of clear cytoplasm in 50% of embryos, remainder show variable cellularization

correlated to a particular aberrant pattern of cell movements and cell shape changes during gastrulation. This indicates that these maternal gene products are important either for the correct spatial instruction of the blastoderm cells, or for the adequate response of the embryonic cells at the onset of gastrulation.

In the following brief descriptions we have grouped the loci according to the cuticular aberrations they cause:

**i. Defects in the dorsoventral axis:** *dorsal* and *windbeutel*: Two loci were identified which cause a dorsalization of embryos derived from homozygous mothers. The *dorsal* locus has already been well characterized (NÜSSLEIN-VOLHARD *et al.* 1980; STEWARD and NÜSSLEIN-VOLHARD 1986). Six new alleles of *dorsal* were isolated in these screens. They all result in an incompletely dorsalized embryonic phenotype, resembling the phenotype produced by the allele *dl<sup>2</sup>*. The embryos usually form some Filzkörper material and a

small piece of head skeleton, rather than producing only the dorsal most cuticle elements as seen in embryos derived from *dl<sup>1</sup>*. Two of these new alleles are temperature-sensitive. At 18° they produce embryos which are much less dorsalized and form ventral setae, whereas at 29° they show the *dl<sup>2</sup>* phenotype.

Mutations in *windbeutel* also produce the dorsalized embryonic phenotype. Only one mutant allele of *wind* was isolated in this screen, it causes an embryonic phenotype similar to that produced by *dl<sup>2</sup>*. Homozygous *wind<sup>RP</sup>* flies show a reduction in viability. Since no deficiency is presently available for this locus it is not clear whether the null-phenotype at the locus would cause zygotic as well as maternal-effect lethality.

**cactus:** Embryos derived from females homozygous for *cactus* appear ventralized. These embryos lack dorsal cuticle as well as dorsally derived cuticle elements such as Filzkörper and spiracles. Instead, they are enveloped by a cuticle carrying ventral setae belts

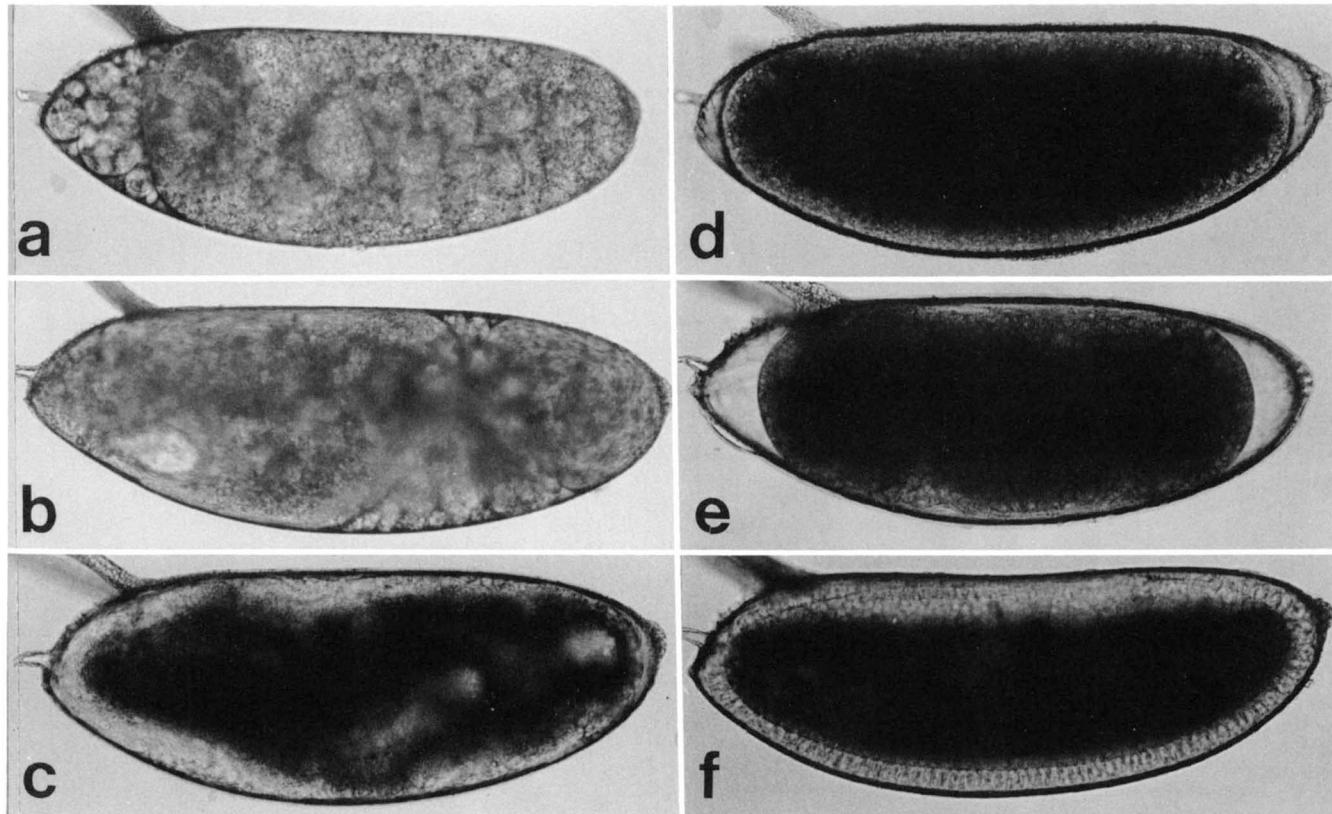


FIGURE 3.—Mutant phenotypes of the class 1, 2 and 3 early acting loci. **a**, wild-type, old, unfertilized egg; **b**, egg from *frh*<sup>PM</sup>, resembling an unfertilized egg; **c**, egg from *frh*<sup>PM</sup>, undergoing partial cellularization. **d**, egg from *grau*<sup>QQ</sup>, showing a narrow zone of cytoplasmic clearing around the egg circumference. **e**, egg from *grau*<sup>QQ</sup> contracting away from the vitelline membrane. **f**, egg from *mat(2)cellQC13*. Note irregular cellularization along the dorsal side.

which extend further dorsally than the ones found in normal larvae, often occupying most of the larval circumference. Most of the head structures are absent (Figure 5c). This phenotype resembles the cuticle phenotype produced by females that carry a dominant allele of the female-sterile mutation *Toll* (ANDERSON, JÜRGENS and NÜSSLEIN-VOLHARD 1985; ANDERSON, BOKLA and NÜSSLEIN-VOLHARD 1985) as well as embryos homozygous for certain alleles of *dpp* (IRISH and GELBART 1987). In films of gastrulating *cactus* derived embryos, several mutant features are evident. The cephalic furrow is very deep on the dorsal side, as it would be on the lateral side of wild-type embryos. In many cases the germband does not extend at all, or moves only a short distance around the posterior pole onto the dorsal side of the egg. Consequently, the posterior midgut invagination always occurs close to the posterior pole. The dorsal folds do not form and no amnion-serosa becomes apparent at later stages of gastrulation (Figure 4). These changes are consistent with a ventralization of the embryo before the onset of gastrulation, and similar defects at gastrulation have been described for other ventralizing mutations such as *Toll*, *dpp*, *zen*, *gurken* and *torpedo* (WAKIMOTO, TURNER and KAUFMAN 1984; ANDERSON, JÜRGENS and NÜSSLEIN-VOLHARD 1985; ANDERSON, BOKLA and

NÜSSLEIN-VOLHARD 1985; IRISH and GELBART 1987; RUSHLOW *et al.* 1987; SCHÜPBACH 1987). One of the three alleles of *cactus* is temperature-sensitive.

*saxophone*: Embryos produced by females homozygous for *sax* form a cephalic furrow which is abnormally deep at its dorsal side. During gastrulation, the germband does not extend its full length along the dorsal side, but buckles in under the dorsal epithelium when it is one third the distance from the posterior end (Figure 4). This phenotype resembles that of several zygotic embryonic lethal mutations such as *twisted-gastrulation* (*tsg*), *zerknüllt* (*zen*), and weak alleles of *dpp*, all of which are thought to cause a “ventralization” of the embryo. On the other hand, the cuticle of *sax* embryos does not show any obvious ventralized phenotype, *i.e.*, the ventral denticle belts are normal in width and the dorsal hypoderm is clearly present. Since the initial defects are limited to the dorsal side of the embryo, the abnormalities may simply result from an inability of the dorsal cells to respond to dorsal cues with the appropriate cell shape changes, thus failing to get out of the way of the advancing germband.

**ii. Defects in the anteroposterior axis:** The loci *torso*, *trunk*, *exuperantia*, *vasa*, *staufen* and *tudor* have already been described in detail (SCHÜPBACH and

**TABLE 3**  
**Maternal effect loci that cause defects during cellularization of the blastoderm embryo**

Locus	Map position/ cytology	No. of alleles	Special remarks
<b>Class 3: Irregular cellularization at blastoderm stage, some embryos form pieces of cuticle</b>			
<i>cribble (crib)</i>	2-57 42C1-7; 42E3, or: 43F6-8	3	
<i>scraps (scra)</i>	2-57 43C3; 43E1-8	6	Cellularization seems to begin normally, but is often not completed; embryos form pieces of cuticle. <i>scra</i> <sup>1</sup> <i>scra</i> <sup>PQ</sup> , <i>scra</i> <sup>RS</sup> are lethal over the deficiency
<i>luckenhaft (luc)</i>	2-65	3	Cellular blastoderms with local defects, embryos form cuticles with big holes, variable head defects and segment fusions
<i>sieve (sie)</i>	2-68 49E7-F1; 50A2-5	3	Variable cellularization, embryos form only pieces of cuticle. <i>sie</i> <sup>III</sup> has semidominant effects, causing considerable embryonic lethality when mother heterozygous
<i>valois (val)</i>	2-53 38A6-7; 38E6-9	4	"Grandchildless-knirps" phenotype (see text), in addition 80-90% of the embryos fail to cellularize
<i>mat(2)cellHK35</i>	2-54	1	Variable cellularization defects; embryos form cuticles with holes, variable head defects, and segment fusions
<i>mat(2)cellPH65</i>	2-67	1	
<i>mat(2)cellPK42</i>	2-60	1	
<i>mat(2)cellPQ49</i>	2-12	1	
<i>mat(2)cellQA13</i>	2-64	1	
<i>mat(2)cellQC13</i>	2-35 30A; 30C	1	
<i>mat(2)cellQE1</i>	2-54	1	
<i>mat(2)cellQL46</i>	2-72 51E3; 52A	1	
<i>mat(2)cellQQ55</i>	2-68	1	Phenotype is ameliorated by paternal wild-type allele
<i>mat(2)cellRE43</i>	2-57 43E1-8; 43F6	1	
<i>mat(2)cellRH36</i>	2-31 30A; 30C	1	Phenotype very variable, 30% of embryos hatch
<i>mat(2)cellRQ41</i>	2-86	1	Paternal wild-type allele can rescue some of the embryos to adulthood

WIESCHAUS 1986a, b) and therefore only a brief summary of these phenotypes will be presented:

*torso* and *trunk*: The embryos produced by mutant females lack those structures normally formed by cells at the anterior and posterior ends of the blastoderm (*i.e.*, the labral segment and telson). Instead, they form structures characteristic of less extreme positions of the fate map, as though more central regions of the fate map had been stretched out over the entire egg length.

*vasa*, *staufen* and *tudor*: Mutations in these loci cause two defects in embryos produced by homozygous mothers. All of such embryos lack polar granules and pole cells (BOSWELL and MAHOWALD 1985; SCHÜPBACH and WIESCHAUS 1986a). In addition to this germline defect, the embryos show deletions of abdominal segments similar to those associated with the zygotically active *knirps* locus. The same array of mutant features is also produced by the locus *valois* (see above), and this particular phenotype is sometimes designated as "grandchildless-knirps phenotype."

In addition to the germline deletion and the abdominal segment deletion, embryos derived from *staufen* also show deletions of anterior head structures.

*exuperantia*: In the differentiated cuticle of embryos from homozygous *exu* mothers the anterior-most head structures are missing. At gastrulation the anterior most cells in *exu* embryos form a posterior midgut, proctodeal region and often Malpighian tubules.

*spliced*: Only one allele of *spliced* was isolated in our screen and it is temperature-sensitive. Embryos developing at 29° show an extreme mutant phenotype and form only head and telson structures, whereas cuticle structures derived from the thorax and anterior abdomen are missing. When embryos are raised at 18° they develop an almost normal cuticular pattern with the exception of small deletions and segmental fusions centered over the fourth abdominal segment (Figure 5). At 22° an intermediate phenotype is produced, where larger deletions of posterior head structures, thoracic segments and abdominal segments occur, leaving only irregular patches of intervening segments

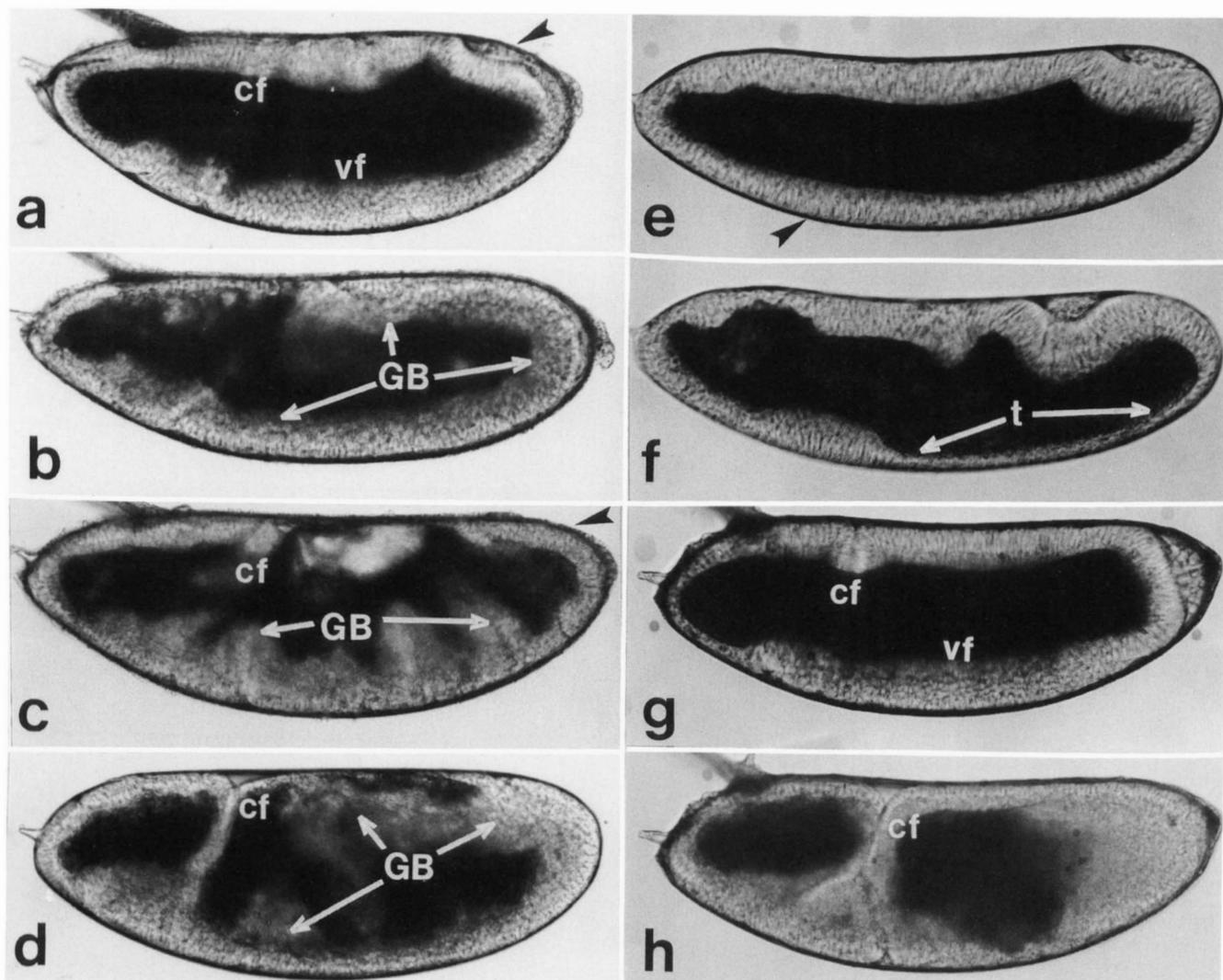
TABLE 4  
Maternal effect loci that cause abnormalities visible at early stages of gastrulation

Locus	Map position cytology	No. of alleles	Special remarks
<b>Class 4: Abnormal cell behavior at onset of gastrulation</b>			
<i>cactus (cact)</i>	2-52 35E6; 36A8-9	3	Embryos are ventralized; in cuticle, ventral denticle belts expanded laterally, dorsal cuticle elements missing. <i>cact<sup>HE</sup></i> is temperature-sensitive
<i>dorsal (dl)</i>	2-53 36C8-11	6	Embryos are dorsalized; in cuticle, tube of dorsal cuticle is formed; <i>dl<sup>PZ</sup></i> and <i>dl<sup>QD</sup></i> are temperature-sensitive and form ventral denticles at 18° but no denticles at 29°
<i>windbeutel (wind)</i>	2-86	1	Embryos are dorsalized, same phenotype as dorsal
<i>exuperantia (exu)</i>	2-93	4	At gastrulation: inverted posterior end forms at anterior end, cephalic furrow is shifted forward; cuticle: anterior head structures missing
<i>torso (tor)</i>	2-57 41C1-7; 43F5-8	9	At gastrulation: cephalic furrow is shifted forward, posterior midgut missing, germband forms to the posterior end of embryo. Cuticle: anterior head structures are missing, as well as structures posterior to A7
<i>trunk (trk)</i>	2-36 31A; 31D	6	(Same phenotype as <i>torso</i> )
<i>concertina (cta)</i>	2-54.8 2L heterochr	5	At gastrulation: posterior midgut missing, no advancement of germband around posterior pole, folding of germband. Cuticle: holes at anterior and posterior end
<i>saxophone (sax)</i>	2-57 42C1-7; 42E3, or: 43F6-8	2	At gastrulation: germband extension abnormal, posterior midgut invagination occurs at abnormal dorsal position. Cuticle: abnormalities at anterior and posterior end
<i>staufen (stau)</i>	2-86 55A; 55F	1	"Grandchildless-knirps" phenotype (see text), in addition at gastrulation cephalic furrow shifted anterior, in cuticle: torso-like deletion of anterior head
<i>tudor (tud)</i>	2-97 57B13-14; 57D8-9	1	"Grandchildless-knirps" phenotype (see text), around 30% of embryos survive and grow up into sterile adults
<i>vasa (vas)</i>	2-51 35B9-C1; 35D4-7	1	"Grandchildless-knirps" phenotype (see text)
<i>spliced (splc)</i>	2-57	1	<i>splc<sup>RL</sup></i> is temperature-sensitive, at 18° small segment defects, at 29° posterior head segments, thoracic segments, and abdominal segments A1-A8 are absent

between the anterior head and posterior abdominal segments. The phenotype caused by *spliced* is therefore the opposite of the *torso* phenotype, in that *spliced* removes the middle elements of the embryonic pattern, but leaves intact the terminal structures affected by *torso*.

This view is consistent with the changes in cell behavior seen in *spliced* embryos at gastrulation (Figure 4, e and f). At room temperature (23-25°) no ventral furrow is formed along the length of the embryo. Instead, at the time when the anlage of the posterior midgut is moving dorsally, the ventral cells from 2-50% egg length become very thin. This is very characteristic of the proctodeal precursor cells in the wild-type embryo which normally occupy a position between 2-10% egg length. The posterior midgut anlage itself initially assumes a normal shape, but

it advances only to about 30% egg length and never closes off properly. A cephalic furrow and anterior midgut are not visible. In the anterior head region only one of the two "dorsal cell clusters" is formed. It therefore appears that in *spliced* embryos at room temperature only the cells at the very anterior and posterior end assume their normal fates, whereas cell behaviors characteristic for the middle region of the wild-type embryo (ventral furrow formation, cephalic furrow formation) are eliminated. Moreover, at least part of the terminal regions (*i.e.*, the proctodeum) is formed over a much larger portion of the embryo than normal. Based on the phenotype in cuticle preparations and at gastrulation, it appears therefore that normal activity at the *spliced* locus is required for pattern specification in the middle region of the embryo. However, since the single allele of *spliced* is



**FIGURE 4.**—Gastrulation phenotypes of class 4 mutations, living embryos photographed under oil. **a**, wild type at early gastrulation stage. Cephalic furrow (cf) and ventral furrow (vf) visible; arrow indicates formation of posterior midgut. **b**, wildtype at the end of gastrulation. The germband (GB) has extended to the dorsal side, cephalic furrow mainly visible at the ventral side, posterior midgut has invaginated. **c**, *cta*<sup>WU</sup> late in gastrulation, arrow indicates the absence of a posterior midgut; the germband has not advanced to the dorsal side, but has been thrown into folds along the ventral side, cephalic furrow (cf) visible. **d**, *sax*<sup>WO</sup> late in gastrulation. The cephalic furrow (cf) is deep on the dorsal side; the germband (GB) and posterior midgut have internalized and are visible dorsally underneath the dorsal cells which have not formed a typical, flexible, amnion-serosal sheet. **e**, *splc*<sup>RL</sup> at room-temperature early in gastrulation. No cephalic furrow is formed, arrow indicates the absence of a ventral furrow. **f**, *splc*<sup>RL</sup> at room-temperature later in gastrulation. The ventral cells from 0–50% egg length have thinned out (t) and do not form mesoderm. **g**, *cact*<sup>PD</sup> early in gastrulation. The cephalic furrow (cf) is already abnormally deep and the ventral furrow (vf) is visible, the posterior midgut does not assume a completely normal shape. **h**, *cact*<sup>PD</sup> late in gastrulation. Cephalic furrow (cf) is deep on the dorsal side, the germband has not advanced to the dorsal side and the posterior midgut has invaginated at a posterior location.

temperature-sensitive and therefore probably not amorphic, the “null-phenotype” of the locus (and thus the wild-type role of the gene) may be rather different.

*concertina*: At the beginning stages of gastrulation, embryos produced by females homozygous for *cta* fail to form a posterior midgut. The germband extends but does not advance to the dorsal side of the embryo, and is thrown into extensive folds along the ventral side of the egg. The anterior midgut appears to form normally (Figure 4). This phenotype resembles that of the zygotic embryonic lethal mutation *folded gastrulation* (ZUSMAN and WIESCHAUS 1985). At final differentiation, the embryos from *cta* have a fairly normal

array of thoracic and abdominal segments with morphologically normal denticle belts, which suggests that in spite of the abnormal folding of the germband, morphogenesis of these segments proceeded rather normally. The most frequent cuticle abnormalities are observed in the head skeleton of such larvae.

#### Class 5. Abnormalities visible only late in development

*i. maternal(2)Notchlike*: One of the maternal-effect loci identified in our screen results in a characteristic cuticle phenotype in which the embryos form only a piece of dorsal cuticle (Figure 5d). This phenotype

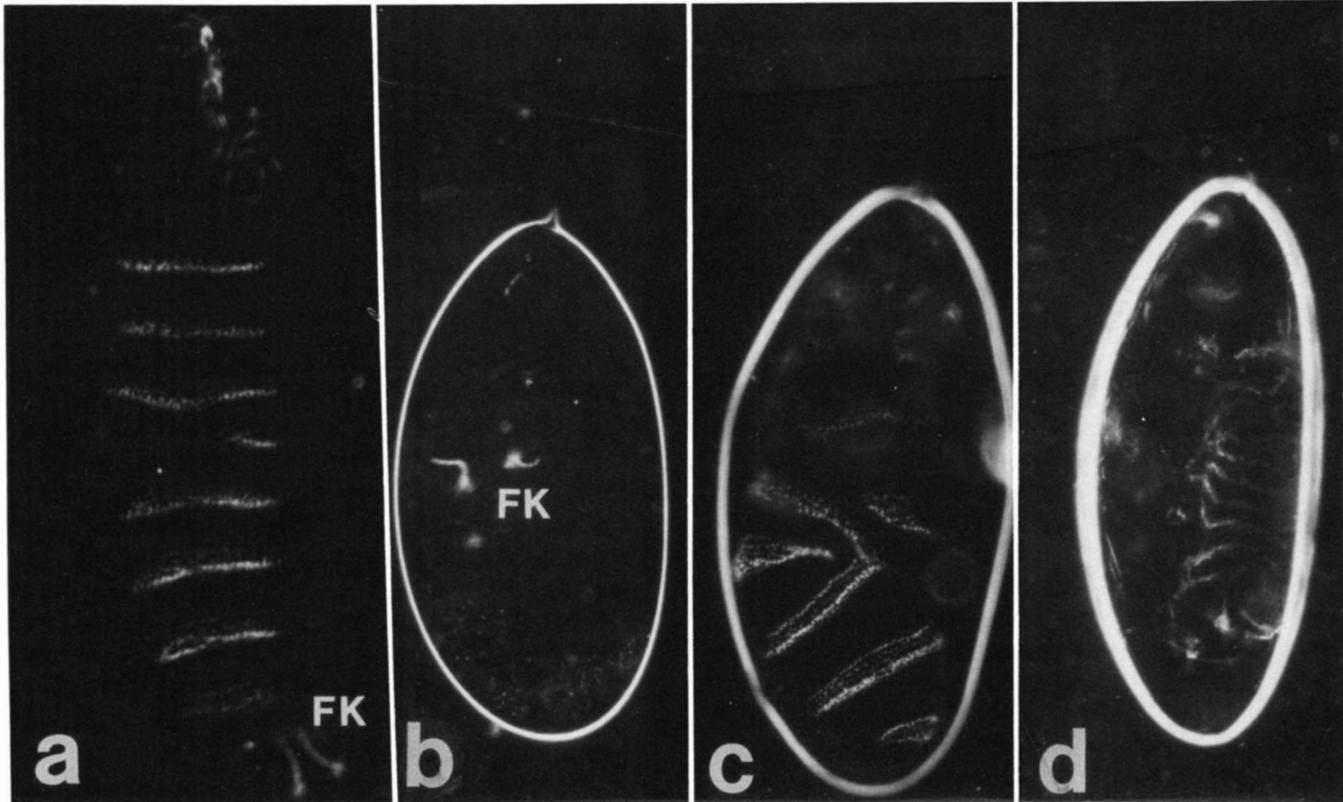


FIGURE 5.—Cuticle phenotypes of class 4 and 5 mutations. **a**, *splc<sup>RL</sup>* at 18°. Cuticle is almost normal except for a defect in abdominal segment A4. Filzkörper (FK) visible at posterior end. **b**, *splc<sup>RL</sup>* at 29°. The only recognizable cuticle structures are the Filzkörper (FK). **c**, *cact<sup>PD</sup>*. The denticle belts cover a wider area of the circumference than in wildtype, they are usually also abnormally arranged. Filzkörper are missing and the head is very abnormal. **d**, *mat(2)N<sup>QJ</sup>*. Only a piece of dorsal cuticle is formed at the dorsal side of the embryo.

resembles the cuticle phenotype produced by the zygotic lethal mutation *Notch* (POULSON 1937, 1940; LEHMANN *et al.* 1981; HOPPE and GREENSPAN 1986). In *Notch*/Y embryos, an abnormally large fraction of the cells in the neurogenic ectoderm enter the neuronal pathway, leaving the embryo with only a patch of dorsal epidermal cells that will form cuticle at final differentiation. We have not tested whether any of the cells in the neurogenic region of the *mat(2)N* embryos develop into neuroblasts. However, all six zygotic embryonic lethal loci with this particular cuticle phenotype (JÜRGENS *et al.* 1984; NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984) have been shown to overproduce neuroblasts at the expense of epidermal cells (LEHMANN *et al.* 1981) and *mat(2)N* most likely has a similar mutant effect. We have isolated three alleles of *mat(2)N*. The homozygous females in those lines survive and produce the embryonic phenotype, however, flies transheterozygous for either of the three alleles and the *Df(2R)D17* show reduced viability and are very shortlived. This suggests that the gene has a zygotic as well as a maternal function. The analysis of embryos derived from homozygous germline clones demonstrated that the mutant phenotype is germline-dependent, *i.e.*, the gene has to

be transcribed in the germline of the mother in order to ensure normal embryonic development (data not shown).

#### **ii. Variable defects in the head and final cuticle:**

Mutations at four maternal loci resulted in embryos which show segment deletions or segment fusions in the thorax and abdomen, as well as occasional head defects (Table 5). The segmental deletions do not follow a pattern, but occur at random in the various embryonic segments. No defects were detected in these embryos at earlier stages, yet given the size of the cuticular defects (1–2 segments in width), it is well possible that corresponding irregularities at earlier stages might have gone unnoticed.

Mutations in five additional loci, each represented by only one allele, cause more variable cuticular defects in the resulting larvae. Usually these larvae have large holes in various locations, they may form only fragmented cuticles and often develop very abnormal heads. These phenotypes resemble the larvae formed by weaker alleles of the loci which cause defects during cellularization (class 3). In the five mutations we observed no defects during cellularization and the phenotype may therefore arise by cell death during later stages of embryonic development. It is also possible, that smaller irregularities during cellularization in these mutant lines went unnoticed.

**TABLE 5**  
**Maternal effect loci that cause visible abnormalities only late in development**

Locus	Map position cytology	No. of alleles	Phenotype of embryos from homozygous females
<b>Class 5: Abnormalities visible in cuticle of embryos</b>			
<i>maternal Notchlike</i> ( <i>mat(2)N</i> )	2-97 57D8-9; 58B	3	Embryos form small piece of dorsal cuticle, most embryonic cells do not form cuticle; all three alleles are semilethal over Df(2R)D17
<i>botch</i> ( <i>bot</i> )	2-20	1	Irregular segmentation, variable segment fusions and holes in cuticle, phenotype is ameliorated by paternal wild-type allele
<i>misstep</i> ( <i>misp</i> )	2-59	1	Irregular segmentation, variable segment fusions
<i>pratfall</i> ( <i>prat</i> )	2-98 either in 57B4; 57B13-14, or in 57D8-9; 58B	1	Irregular segmentation, variable segment fusions
<i>slipshod</i> ( <i>slip</i> )	2-42	1	Irregular segmentation, variable segment fusions
<i>chaff</i> ( <i>cha</i> )	2-84	1	Variable head defects and holes
<i>dregs</i> ( <i>dreg</i> )	2-38	1	Variable head defects and holes
<i>erratic</i> ( <i>err</i> )	2-41 31D; 31F3	1	Variable head defects and holes; lethal over Df(2L)Jder27
<i>rags</i> ( <i>rag</i> )	2-32	1	Large ventral holes and head defects
<i>rubbish</i> ( <i>rub</i> )	2-89	1	Variable head defects and holes
<b>Class 6: No visible abnormalities, apparently normal larvae that do not hatch</b>			
<i>reticent</i> ( <i>ret</i> )	2-62	1	
<i>shy</i> ( <i>shy</i> )	2-62	1	

### Class 6. Normal cuticular phenotype

Mutations in only two loci (*reticent* and *shy*), each represented by a single allele, resulted in apparently normal larvae which did not hatch out of the egg case. The cause of their death was not further investigated. Originally a third mutant line was placed into this class, but it was subsequently found to be allelic to *luc*<sup>1</sup> which causes defects at cellularization. Since no chromosomal deficiencies are available for either *ret* or *shy*, the nature and strength of these mutations is at present not known.

### Activity of paternal alleles during embryogenesis

In most tests for fertility, homozygous females had mated to their heterozygous brothers. In those crosses 31 of the 136 mutant lines showed variability in their phenotype. In some cases this variability allowed a small fraction of larvae to hatch and even to survive to adult stages. In one case (*mat(2)cell RQ41*), all the surviving escaper adults were heterozygous, suggesting that their survival depended on their having received a wild type allele from their fathers. In all other lines which showed some survival of progeny to adult stages, both heterozygous as well as homozygous progeny were found among the escaper adults, indicating that survival was not dependent on the presence of a paternal wildtype gene.

We also tested one mutation at each of the variable loci to determine whether the paternal wild-type gene could influence (ameliorate) the mutant phenotype. In this test homozygous females were collected and divided into two groups. The first group was mated to homozygous mutant males, the second to wild-type males. In addition to *mat(2)cell RQ41*, there were two more cases (*mat(2)cell QQ55* and *botch*) where the phenotypic distribution of the embryos was shifted when the eggs were fertilized by wildtype sperm as compared to mutant sperm. In these three mutants, the amelioration consisted in an increase of more than 20% in the class of embryos with no or small cuticular defects and a corresponding decrease in the percentage of embryos with large cuticular defects and holes. In all these tests, the absolute percentages of embryos with small or large defects varied, however, from one test to the next, indicating that environmental factors like possibly food, temperature and age of females usually play a role in the expression of most of the maternal-effect mutations with variable phenotypes. A very minor paternal (*i.e.*, zygotic) contribution might therefore have been missed. With this reservation in mind there are only 3 loci in the collection, each represented by only a single allele, in which the paternal wildtype allele seems to be sufficiently active during embryogenesis to make a (small) contribution to the embryonic phenotype.

### Are the maternal effect mutations specific for oogenesis?

In the course of the mapping we found that 31 of the female sterile loci were located in regions of the second chromosome covered by cytologically defined deficiencies. Mutations at three of these loci were found to be almost lethal in trans over the corresponding deficiency (*err*, *scraps*, *mat(2)N*). These three loci, therefore, encode functions that are necessary for viability of the carrier as well as the progeny. One locus (*bie*) also has a zygotic phenotype, in that all homozygous adult individuals show tergite defects. This is true for all three alleles at the locus. Therefore, not all mutations isolated in this screen are true maternal effect mutations specific for oogenesis. The fact that in the case of the (semi-) lethal loci the homozygous mutant females survive indicates that the mutations do not eliminate all wild type product. It is, however, remarkable that three of these zygotically active loci (*scraps*, *mat(2)N*, *bie*) are represented with more than one allele. Certain zygotically active loci appear to have a relatively higher probability to produce maternal effect alleles than others.

### DISCUSSION

Insect eggs contain large quantities of maternal gene products. Most of those molecules are required at various other stages of the life cycle, and as the maternal supply diminishes, the developing zygote will eventually synthesize them from its own genome (WRIGHT 1970; GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984). A small number of genes seem to be required only during embryonic development and to be expressed exclusively during female oogenesis. Such maternal-effect genes have been identified in all previous screens for female-sterile mutations in *Drosophila*, as well as in other genetically well studied organisms (GANS, AUDIT and MASSON 1975; RICE and GAREN 1975; MOHLER 1977; ISNENGH et al. 1983; KEMPHUES et al. 1986). If the term maternal-effect mutation is restricted to those female-sterile mutations that do not alter the morphology of the egg itself, but affect only the formation and development of the zygote, such mutations comprise roughly one quarter of the female-sterile lines in a given screen [59 out of 245 mutant lines on the X chromosome (PERRIMON et al. 1986); 136 lines out of 529 in our screen on the second chromosome].

The 136 maternal-effect lines recovered in our screen fall into 67 complementation groups. We are relatively confident that the majority of the 23 loci represented by more than one allele represent true maternal effect genes whose expression is only required during oogenesis. The problem lies in interpreting the large group of 44 mutations which com-

plement all others. In some cases these mutations may be single alleles which define maternal effect loci hit only once in our screens. Such single hits are expected by chance and may be fairly common if some genes are not as easily affected by EMS as others. On the other hand, an unknown fraction of the single hits do not identify "true" maternal-effect genes but represent hypomorphic (or possibly neomorphic, etc.) mutations of genes that in amorphic condition would have much more severe consequences on the homozygote (PERRIMON et al. 1986). The existence of such mutations is demonstrated by our observation that of the 31 loci in our collection for which a chromosomal deficiency is available, three are lethal or semilethal in trans over that deficiency. This estimate sets a lower limit for the fraction of our mutations which are actually hits in lethal loci, since not all hypomorphic alleles that allow viability in homozygotes would be predicted to be lethal over a deficiency.

These considerations suggest that the distribution of alleles per complementation group is actually two distributions superimposed on each other: a distribution of hypomorphic alleles of various vital loci (mostly single hits per locus) superimposed on the allele frequency distribution for true maternal effects, the latter being approximately Poisson distributed around the average hit per locus, with most loci represented by multiple hits. Although it is not straightforward to separate the lethal from the "true" maternal-effect loci, the two distributions might be sorted out if we knew the average number of alleles induced in true maternal effect loci (which would then allow calculating the predicted frequency of the various classes in a Poisson distribution). Judging from the loci we have detected, this value must lie between 2.0 and 4.0. The value 2.0 must be the minimum since 67 complementation groups were identified for the 136 mutations, and 4.0 must be the maximum if only the 92 mutations representing 23 multiple hit loci are used. Based on the distribution of loci with multiple hits, the best empirical fit with our actual distribution of alleles is obtained with an average value of 3.0 hits per locus (Figure 6). On this assumption, the 23 loci with multiple hits would represent 80% of the maternal-effect loci on the second chromosome, four of the 44 single hits in the collection would still represent true maternal effect loci, and 1-2 such loci would have been undetected. The total number of maternal-effect genes on the second chromosome would then add up to 28-29. Of the 136 mutant lines in the collection 40 would therefore represent unusual alleles of lethal loci and 96 would represent mutations in 27 maternal effect genes.

Although this is our best estimate, it is subject to several reservations: even among true maternal effect loci, the distribution of alleles deviates from a simple

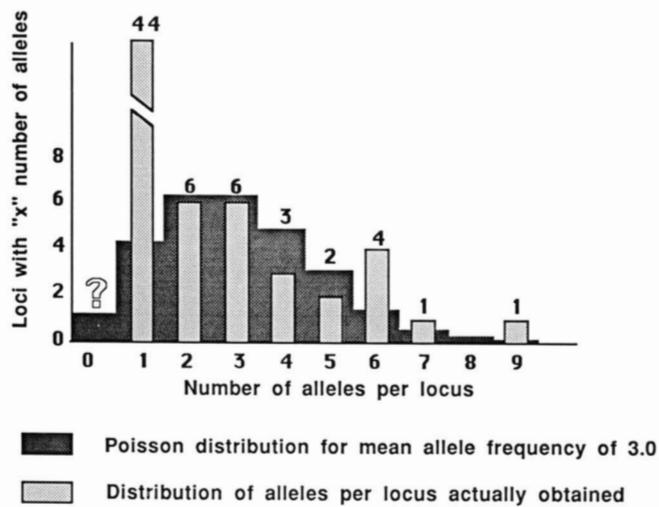


FIGURE 6.—Predicted and actually observed distribution of allele frequencies. The predicted distribution was calculated assuming that the allele frequency per “true” maternal effect locus is Poisson distributed with a mean of 3.0 (see text for explanation).

Poisson distribution. The Poisson distribution assumes that all loci are equimutable, yet hypomorphic alleles of certain loci might be easier to detect than others. [In our screen, mutations were usually only retained if less than 10% of the embryos hatched out of their egg case, which is, e.g., the case for all known hypomorphic alleles of *dorsal* (STEWARD and NÜSSLEIN-VOLHARD 1986), but not for the hypomorphic alleles of *tudor* (BOSWELL and MAHOWALD 1985).] Loci which are relatively insensitive to EMS may not have been detected at all in our screens. On the other hand, certain zygotically active loci have a high probability to produce maternal effect alleles and are not always easy to distinguish from “true” maternal effect loci. Therefore, the phrase “maternal effect locus” has a practical usefulness but is not an absolute distinction. It designates loci which frequently mutate to a maternally dependent embryonic lethality without killing the homozygous carrier, although in some instances either an unusual allele, or a complete deficiency of the locus may also cause reduction in zygotic viability (see e.g., *Toll*; GERTULA, JIN and ANDERSON 1988).

**Maternal effect mutants predominantly affect processes occurring early during embryogenesis:** Among the 23 loci with multiple hits, nine (39%) encode functions that are required before the onset of cellularization. Another five (22%) are required before the completion of cellularization and eight (35%) showed abnormalities in early gastrulation stages. In one case (*mat(2)N*) abnormalities were detected only after the first 4 hr of development. The early defects associated with these maternal effect mutations suggest that most maternal products that are specific for embryogenesis are utilized during the first 3 hr of embryonic development. This contrasts with products of a more general nature (e.g., ribo-

somal RNA, acid phosphatase-1) where maternal products tend to be utilized throughout embryonic development and zygotic transcription can be eliminated with no effect on embryonic development (SAWICKI and MACINTYRE 1978; GARCIA-BELLIDO and MOSCOSO DEL PRADO 1979; ZUSMAN and WIESCHAUS 1987). A small number of zygotic loci are known which are required for early embryonic development (JÜRGENS *et al.* 1984; NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984). Most of these are probably specific for embryogenesis (WIESCHAUS and NOELL 1986). The stage when they affect embryogenesis follows that of most of the maternal effect loci described here. This suggests that zygotically active genes take over the specific control of development processes at gastrulation.

**Maternal effect mutations which change cellular fates can be grouped to define a small number of processes which control embryonic patterning:** There are at least twelve maternal effect loci on the second chromosome which cause cells at certain positions in the embryo to assume fates normally characteristic of cells in other positions. Such genes could be involved in the synthesis, distribution, or maintenance of maternal determinants which establish the initial spatial pattern of the embryo. Alternatively, the genes might be involved in the interpretation mechanisms by which such maternal signals are transduced to the zygotic genome. The twelve loci on the second chromosome share characteristic mutant phenotypes with loci identified in similar screens on the X chromosome (GANS, AUDIT and MASSON 1975; MOHLER 1977; PERRIMON *et al.* 1986) and on the third chromosome (C. NÜSSLEIN-VOLHARD, G. JÜRGENS, K. V. ANDERSON and R. LEHMANN, unpublished data). The “dorsalized” embryonic phenotype is produced by mutations in two loci on the second chromosome (*dl*; NÜSSLEIN-VOLHARD *et al.* 1980; *wind*) as well as by eight genes on the third chromosome and one gene on the X chromosome (ANDERSON and NÜSSLEIN-VOLHARD 1984; KONRAD, GORALSKI and MAHOWALD 1988a, b). A maternal neuralized phenotype is produced by mutations in *mat(2)N*, as well as by two maternal-effect genes on the X chromosome (*pecanex*, *almondex*) (PERRIMON *et al.* 1986). The “torso-phenotype” is produced by the maternal-effect genes *tor* and *trk* on the second chromosome, by *torso-like* on the third chromosome and by certain alleles of *fs(1)Nasrat* and *fs(1)pole hole* on the X chromosome (DEGELMANN *et al.* 1986; PERRIMON *et al.* 1986; NÜSSLEIN-VOLHARD, FROHNHÖFER and LEHMANN 1987). Seven loci are known in the genome which produce the “maternal knirps” phenotype in the abdomen and most of these have an effect on pole cells (*vas*, *val*, *stau*, *tud*, *osc*, *pum*, *nos*) (BOSWELL and MAHOWALD 1985; LEHMANN

and NÜSSLEIN-VOLHARD 1986, 1987; NÜSSLEIN-VOLHARD, FROHNHÖFER and LEHMANN 1987). It is also possible to place *exu* into a phenotypic group with the gene *bicoid* on the third chromosome and with the gene *swallow* on the X chromosome (formerly *fs(1)1502*) (GANS, AUDIT and MASSON 1975; STEPHENSON and MAHOWALD 1987), if one allows for a difference in the extent of the pattern deletion and pattern duplications caused by these mutations (FROHNHÖFER and NÜSSLEIN-VOLHARD, 1986; NÜSSLEIN-VOLHARD, FROHNHÖFER and LEHMANN 1987).

The limited spectrum of maternal phenotypes is perhaps their most striking feature. Most phenotypes are produced by more than one locus, suggesting that the various loci participate in a common process. This view is confirmed by the analysis of double mutants within each phenotypic group (ANDERSON, JÜRGENS and NÜSSLEIN-VOLHARD 1985; SCHÜPBACH and WIESCHAUS 1986a, b). These analyses suggest that the genes within a given group may be responsible for different steps of a particular pathway where the "active end product" rather than each of the single gene products itself, would be used to define the embryonic pattern.

In such a model, the two genes with truly unique phenotypes (*cactus* and *spliced*) could also be members of the basic groups, given that they cause a phenotype opposite to that of particular groups (*cact* ventralizes the embryos, and the mutant phenotype of *splic* causes the opposite of the *torso* phenotype). Such genes might act as negative "regulators" in the pathway, and when mutated cause an "overproduction" of the necessary endproduct. This would produce a complementary phenotype, if correct embryonic patterning depended on the proper level of the endproducts of the *dorsal* group or the *torso* group, rather than on the simple presence of those products. The maternal-effect genes, therefore, identify at least five maternal-dependent pathways for embryonic patterning: the *dorsal-Toll*, the neuralized, the *torso*, the *tudor-oscar* ("grandchildless-knirps"), and the *bicoid-exu* pathway. Not all the gene products involved in these pathways are necessarily supplied by simple maternal-effect genes. For example certain members of the *torso* and of the neuralized pathway are required zygotically as well as maternally (PERRIMON, ENGSTROM and MAHOWALD 1984, 1985). Such loci would not be detected in simple female sterile screens. Given that the screens for maternal effect mutations have demanded that the homozygous carrier females themselves survive, it is possible that there are additional maternally dependent patterning pathways in the embryo which cannot be easily identified because all contributing genes are also required for the survival of the zygote. Examples of such genes and pathways would be *fused*, *esc*, *exd* and *caudal* (COUNCE 1956; NÜSSLEIN-VOLHARD and

WIESCHAUS 1980; STRUHL 1983; MACDONALD and STRUHL 1986; WIESCHAUS and NOELL 1986). On the other hand, in analyses of germline effects of random lethals, very few novel maternal phenotypes have been discovered (PERRIMON, ENGSTROM and MAHOWALD 1984). This suggests that the number of such additional, yet undiscovered patterning pathways may not be very large.

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**Note added in proof:** KLINGLER *et al.* (1988) have recently shown that *spliced* is in fact a hypermorphic allele of *torso*.

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