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Maternal-effect loci involved in *Drosophila* oogenesis and embryogenesis: P element-induced mutations on the third chromosome

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ABSTRACT A collection of 1609 recessive P-lethal mutations on the third chromosome was tested in germline clones for effects on egg differentiation and embryonic development. In 164 lines, normal differentiation of the egg chamber is prevented and in 841 lines, embryos develop abnormally. This latter group of maternal-effect mutations was subdivided into 23 classes based on the cuticular phenotypes. Our collection comprises new alleles of previously characterized genes (e.g. *kayak, punt, string, tramtrack).* For some of the genes identified in this screen, a maternal contribution to embryonic development has not been described previously (e.g. *extramacrochaete, Trithorax-like, single minded, couch potato, canoe).* The genes classified in our study with a dual function during oogenesis and embryogenesis not only substantially extends the existing collection of maternal-effect genes but will also aid further understanding of how patterning of the *Drosophila* embryo is controlled by the maternal genome.

KEY WORDS: *oogenesis, germline clones, germband etension, segmentation, dorsal closure*

Introduction

Early patterning of the Drosophila embryo is governed by two classes of genes, maternal-effect genes and zygotic genes. During oogenesis maternal-effect gene products are synthesized in the nurse cells and deposited into the developing egg where some become confined to specific regions in the egg. At these locations they provide positional information for the main body axes and regulate the activity of zygotic genes which in return will refine the patterns along these axes (St Johnston & Nuesslein-Volhard, 1992). Among maternal-effect genes two classes are recognized, namely those with an exclusive maternal participation in patterning of the embryo and those which perform additional essential functions during zygotic development. Many of the genes belonging to latter class are thought to have escaped detection in previous large-scale saturation screens. These screens were primarily based on testing mutant animals either for female sterility to identify maternal effect genes (Schupbach & Wieschaus, 1989) or embryonic lethality to identify zygotically required genes (Jürgens *et al.,* 1984; Wieschaus *et al.*, 1984; Nüsslein *et al.,* 1984). These classical screens did not permit the detection of genes whose products are required both maternally and zygotically. In particular, it has been difficult to assess whether zygotic loci also have a maternal contribution to development. This can only be achieved by selective removal of gene function during oogenesis, an approach that relies on a method that can generate mutant germ cells in an otherwise wild-type soma.

The efficient generation of germ line mosaics became possible with the introduction of FLP-DFS technique (Chou an Perrimon, 1996). Using this technique it became evident that many genes previously known to be zygotically required also provide maternal information essential for patterning of the embryo. For example, a maternal contribution of the *dpp* receptors, *punt* and *thickveins*, in the establishment of the D/V axis was only uncovered after removal of maternally provided products (Letsou *et al.,* 1995). In the same way, the function of the transcriptional repressor *groucho* and the coactivator, CTD, in embryonic patterning was identified only in embryos specifically devoid of maternal product (Paroush *et al.,* 1997). Screens for zygotic lethal loci with a maternal contribution were conducted on the X chromosome (Perrimon *et al.,* 1989) and on the second and the third chromosome (Perrimon *et al.,* 1996). In this paper, we report the results of a systematic screen for maternal-effect phenotypes in a collection of recessive lethal P element insertions on the third chromosome (Deak *et al.,* 1997).

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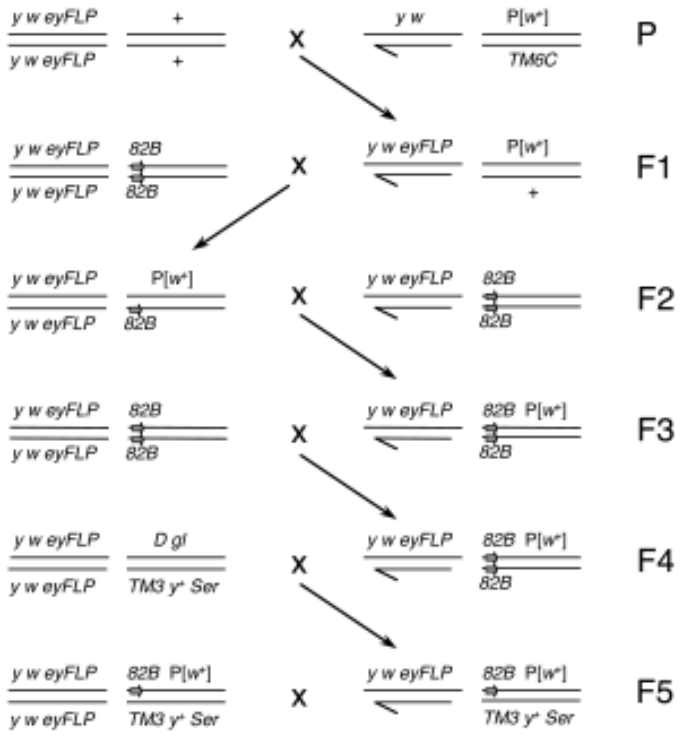

Fig. 1. Strategy for recombining P-induced mutations onto *FRT* bearing chromosomes. *Mutations are marked with the* mini-white *gene* (P[w+]). *In the first cross the* y w *chromosome of the original stock was replaced by a* y w eyFLP *chromosome. F1 male* w+ *progeny were mated to both* FRT *stocks separately. Five to seven F2* w+ *females were backcrossed to males of the corresponding homozygous* FRT-*stock. The resulting F3 progeny were then screened for males having mosaic eyes. When such mosaic males were found in one of the two* FRT *crosses, a single male was tested by mating it to females of the corresponding* FRT *stock. When no mosaic flies were found in any of the two crosses,* w+ *females (or recombinant females) and* w- *males from each cross were set up in a bottle each. This cross was a repetition of the third cross but with larger numbers to increase the chance to obtain a recombined chromosome.*

We generated and analyzed germline clones of 1609 lines. Of these we recovered 1005 lines that show abnormal egg development or embryonic development when homozygous mutant in the female germ line. In addition to the discovery of a substantial number of previously uncharacterized maternal-effect genes, our study led to the identification of hitherto unknown maternal contribution of well-characterized zygotic genes.

Results

Perrimon and colleagues (Perrimon *et al.,* 1996) have previously screened a collection of P-lethal insertions on the second and third chromosome for maternal-effect phenotypes. We chose a similar strategy to screen for maternal-effect phenotypes in an independent collection of lethal P element insertions on the third chromosome generated by one of our groups (Deak *et al.,* 1997). To this end, each P-insertion of the collection was recombined onto a chromosome arm containing *FRT* sites. Recombination was performed according to the scheme shown in Fig. 1. Since it was not known on which arm of the third chromosome the insertions were located each insertion line was crossed with the *FRT80B* and *FRT82B* lines containing *FRT* site at the base of the left and the right arm of the third chromosome, respectively (Xu and Rubin, 1994). The *eyFLP*-system (Newsome *et al.,* 2000) was used to detect recombinant chromosomes carrying both the *FRT* and the *P(w+)* insertion: Recombinants carrying the *P(w+)* insertion and the *FRT* sequence in *cis* generate mosaic eyes when placed over the corresponding *FRT* bearing chromosome in a *w eyFLP* background. From the original 2419 P insertion lines we recovered 1968 recombinant lines (81.4 percent). In addition, we recovered 73 lines with insertions on both *FRT80B* and *FRT82B*-chromosomes, and in 27 cases recombinants from the same line showed different levels of *white* expression. The total number of recombinant *FRT P(w+)* lines was therefore 2068. Of these 1737 still carried a lethal mutation and were kept as stocks. The remaining 331 lines proved to be homozygous viable. In these cases, lethality observed in the original stock most likely arose from a mutation not associated with the P insertion and that thus could be removed by recombination.

Our objective was to produce germline clones of these lines in females using the FLP-DFS technique (Chou *et al.,* 1996) and examine the contribution of loci affected by these insertions to egg differentiation and embryonic development. Clones homozygous for the insertion were generated by expression of FLP at the late larval stage (for details see Materials and Methods). In total, 1609 lines were tested in germline clones and sorted into three major groups (Table 1).

Group 1: P-Lethal Lines with No Germline or Maternal-Effect Phenotype

Group 1 represents all lines of which germline clones produce normal eggs giving rise to fertile progeny. It thus appears that the 604 zygotic lethal loci belonging to this group (37.5 percent of the total number tested) are not required for normal egg differentiation

TABLE 1

CLASSIFICATION OF P INSERTION LINES

Group 1			Group 2			Group 3			Total		
	e	56		*e*	45		*e*	151		*e*	252
	e	56		*e*	45		*e*	151	**3L**	*l*	120
153	*p*	45	102	*p*	16	433	*p*	124		*p*	185
	a	15		*a*	7		*a*	68	**688**	*a*	90
	n.d	6		*n.d*	10		*n.d*	25		*n.d*	41
	e	180		*e*	34		*e*	168		*e*	382
	l	55		*l*	10		*l*	52	**3R**	*l*	117
451	*p*	104	62	*p*	8	408	*p*	94		*p*	206
	a	63		*a*	3		*a*	44	**921**	*a*	110
	n.d	49		*n.d*	7		*n.d*	50		*n.d*	106
	e	236		*e*	79		*e*	329		*e*	634
	l	86		*l*	34		*l*	117		*l*	237
604	*p*	149	**164**	*p*	24	**841**	*p*	218	**1609**	*p*	391
	a	78		*a*	10		*a*	112		*a*	200
	n.d	55		*n.d*	17		*n.d*	75		*n.d*	147

Group 1 summarizes P lines with no germline requirement or maternal effect. Group 2 represents P lines that are defective in oogenesis and group 3 represents P lines that when deprived from the maternal component to development lay embryos that are abnormal or fail to hatch. Each group was subdivided according to which chromosomal arm the respective insertion was located (3R or 3L), and to the lethality stage of the zygotic phenotype: e (embryonic lethal), l (larval lethal), p (pupal lethal), a (pharate-adult lethal), n.d. (not determined). See also Deak *et al.,* 1997.

and do not provide essential maternal contributions to embryonic development.

Group 2: P-Lethal Lines Affecting Germline Differentiation

We recovered 154 lines (about 10 percent) that produced few or no eggs upon clonal induction. These lines were of interest as they may identify genes that perform an essential function at some stage in the assembly of a functional oocyte. We used the nuclear dye DAPI to examine the cytology and organisation of mutant germ cells. Of the 154 lines, 17 lines contained normal looking ovarioles that exhibit no conspicuous morphological aberrations at any of the stages of egg differentiation (wild type-like [*wtl*] in Table 2; Fig. 2). The other 137 lines revealed defects at different stages of ovarian development and were grouped into 9 classes by virtue of their most prominent phenotype.

The earliest aberration detected in group 2 is described by the *agametic* class (*aga* in Table 2, Fig. 2). Mosaic ovaries of these lines contain a number of germaria with no germ cells. Due to the absence of germ cells, the somatically derived follicle cells collapse and appear as string-like structures (Fig. 2). This phenotype suggests that mutant germ cells are lost in pre-adult stages presumably because of the loss of an essential function for cell survival. The second class comprises lines (26) that produce cysts with large numbers of small undifferentiated germ cells. In some of these lines the number of germ cells per cyst is exceedingly high, a phenotype similar to that generated by mutations of the ovarian tumor type. We referred this class as ovarian tumors (*tum* in Table 2, Fig. 2).

All of the remaining lines produced cysts with polyploid nuclei indicating that mutant germ cells were able to proceed into post-germarial stages. Among these we found a range of phenotypes affecting different aspects of egg chamber differentiation. For instance, we observed lines producing mutant cysts with deviations from the normal nurse cell number per cyst. Some lines contain cysts with too few (< 15) nurse cells (*rnn* in Table 2, Fig. 2). In some of these cases the nuclear size of the few enveloped nurse cell nuclei were abnormally enlarged. Other lines produced cysts containing supernumerary (> 15) nurse cells (*snn*).

Another class, referred to as abnormal cyst (*abc*, Fig. 2), comprises lines that are capable to form cysts with a normal number of polyploid nurse cells (15), but show abnormalities in ensuing cyst development. Some of these lines failed to form a discernible oocyte or mispositioned the oocyte to regions other than the posterior pole where the oocyte normally locates. In some lines the polyploid nurse cell nuclei display an aberrant nuclear morphology and start to degenerate in late previtellogenic stage (*deg,* Fig. 2).

The last class we obtained in our screen displays a late phenotype resembling the previously described *dumpless* phenotype. Accordingly we referred to these lines as dumpless (*dmp* in Table 2, Fig. 2). A general feature observed in this class is the failure of nurse cells to deposit their contents into the oocyte at stage 10. As a result, eggs are formed that are only about half the size of a normal egg. These small eggs are rarely laid and therefore these lines were placed into the no or few eggs class. The class referred to as small egg (*sma*, Fig. 2) also produces eggs which are smaller than wildtype eggs. However, the reduced size of these eggs appear not to be a consequence of a dumping defect at stage 10.

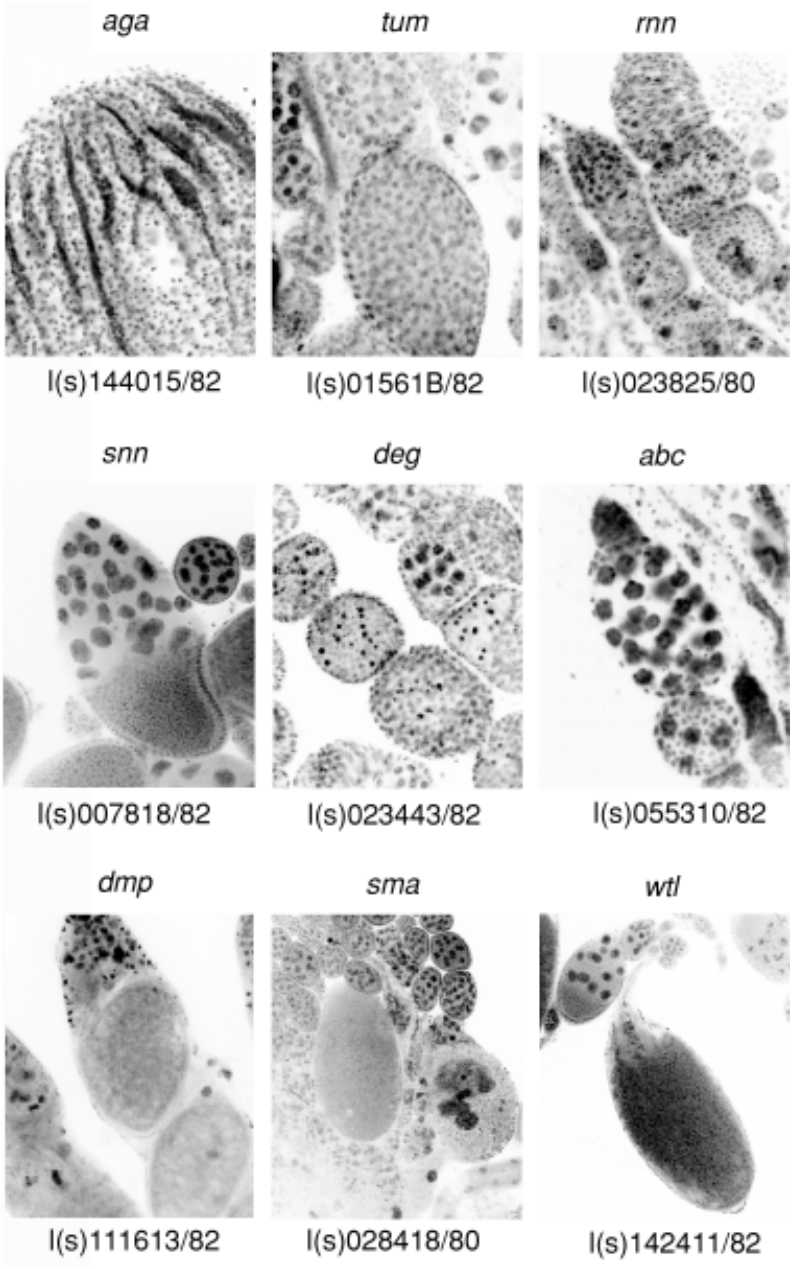

Fig. 2. Ovarian phenotypes of germline clones homozygous for P-induced mutations on the third chromosomes. *Each panel shows the nuclear cytology of a representative line of the phenotypic classes listed in Table 2. A detailed description of the phenotype is given in the text.*

Group 3: P-Lethal Lines Affecting Embryonic Development

Group 3 contains the largest fraction of lines tested in our screen for maternal effects (841 lines). Lines belonging to this category produce homozygous mutant eggs that do not develop normally. We subdivided this group into 3 main phenotypic classes (Table 3).

Subgroup A. This subgroup composed of 153 lines is characterized by the lack of a morphologically visible defect in mutant embryos and, therefore, we referred to them as amorphous phenotype lines. Lethality caused by these mutations is not accompanied with detectable changes in cuticular morphology. We thus could not apply a scheme to classify these lines by virtue of cuticle phenotypes. Nondescript lethal phenotypes are expected to arise from mutations in genes involved in the development of internal structures such as the nervous system, the tracheal system, the gut, the musculature and the circulatory system. For instance, among the 153 lines of this subgroup we found an allele of the *bonus* gene (l(3)s024108/82) which encodes a transcriptional co-activator required for neuronal development (Salzberg *et al.,* 1997).

Subgroup B. Typical for this subgroup (370 lines) is that they lay only few eggs (less than 20 eggs per 20 mated females during a 24 h period of egg-laying) indicating that egg differentiation is impaired. In some lines, the few mutant embryos recovered displayed specific cuticular phenotypes which permitted us to conduct complementation tests with other mutations that cause similar phenotypes. In these tests, we discovered new alleles of loci for which a maternal contribution to zygotic development had already been described. These loci include *string* [l(3)s022406/80] (Alphey at al., 1992)*, rotated abdomen* (*roa*) [l(3)s059011/80] (Martin-Blanco & Garcia-Bellido, 1996; Salzberg *et al.,* 1997; Cooley *et al.,* 1988), *tartan* [l(3)s093413/80] (Chang *et al.,* 1993; Morimoto *et al.,* 1996; Salzberg *et al.,* 1997), *pavarotti* [l(3)s093413/80] (Salzberg *et al.,* 1997; Adams *et al.,* 1998), *tramtrack* [l(3)s132515/82, l(3)s137108/82] and *single minded* [l(3)s111004/82](Golembo *et al.,* 1996; Salzberg *et al.,* 1997).

Subgroup C. This subgroup of maternal-effect lines consists of the remaining 318 lines. Different from subgroup B, mosaic females of these lines produced normal amounts of mutant eggs indicating that oocyte differentiation is not significantly impaired by homozygosity for the insertion. All embryos that derive from homozygous eggs die, however, and display specific pattern defects. We applied a phenotypic classification scheme similar to that used in previous screens for embryonic pattern defects (Jürgens *et al.,* 1984; Wieschaus *et al.,* 1984; Nüsslein-Volhard *et al.,* 1984). According to this scheme, subgroup C was catalogued into 23 phenotypic classes (Table 4) that we present in an alphabetical listing. For some of the classes, we performed complementation analyses with P-insertions that belong to the same phenotypic class.

TABLE 2

CLASSIFICATION OF OVARIAN PHENOTYPES

Phenotypic class	Abbreviation	Number of lines
agamatic	*aga*	18
undifferentiated or tumorous cyst	*tum*	26
reduced nurse cell number (<15)	*rnn*	10
supernumerary nurse cell number (> 15)	*snn*	19
degenerating nurse cell nuclei	*deg*	12
abnormal cyst	*abc*	14
dumpless	*dmp*	4
small egg	*sma*	15
ovo^{D1} -like	*ovo*	19
wildtype-like	*wtl*	17
Total		**154**

TABLE 3

CLASSIFICATION OF GROUP 3 P LINES

Subgroup A			Subgroup B			Subgroup C			Total		
	e	19		*e*	92		*e*	40		*e*	151
	l	6		*l*	48		*l*	11	**3L**	*l*	65
46	*p*	11	274	*p*	80	113	*p*	33		*p*	124
	a	7		*a*	40		*a*	21		*a*	68
	n.d	3		*n.d*	14		*n.d*	8	**433**	*n.d*	25
	e	46		*e*	40		*e*	82		*e*	168
	l	10		*l*	16		*l*	26	**3R**	*l*	52
107	*p*	27		*p*	16		*p*	51		*p*	94
	a	10		*a*	9		*a*	25	**408**	*a*	44
	n.d	14		*n.d*	15		*n.d*	21		*n.d*	50
	e	65		*e*	132		*e*	122		*e*	329
	l	16		*l*	64		*l*	37		*l*	117
153	*p*	38	**370**	*p*	96	**318**	*p*	84	**841**	*p*	218
	a	17		*a*	49		*a*	46		*a*	112
	n.d	17		*n.d*	29		*n.d*	29		*n.d*	75

Subgroup A represents P lines that when homozygous in the female germ line give rise to a reduced amount of eggs. Subgroup B represents P lines with an essential maternal contribution to embryonic development but no obvious cuticular phenotype. Subgroup C represents P lines with a maternal effect and an aberrant cuticular phenotype. Each subgroup was subdivided according to the chromosomal position of the respective insertion (left of right arm), and according to the lethality stage of the zygotic phenotype: e (embryonic lethal), l (larval lethal), p (pupal lethal), a (pharate-adult lethal), n.d. (not determined). See also Deak *et al.,* 1997.

Anterior Patterning Defects. 37 lines affecting the anterior portion of the embryo were classified into 4 distinct groups: anterior defects (and) (6 lines), head defects (hed) (21 lines), cli-like (cli) (where cli stands for clift, as described by Nüsslein-Vollard *et al.,* 1984) (2 lines) and anterior open (aop) (8 lines) (see Table 4). "and" lines have general defects which disrupt most of the anterior portion of the embryos, while in the hed group defects are confined to the anterior-most region, the pseudocephalon, and/or the mouth hooks. Among these lines an allele of *tramtrack* was characterized: *l(3)s120910/82* (see Salzberg *et al.,* 1997). No complementation groups with more than one member were found among the "and" and aop lines tested. On the other hand, among the hed lines, several combinations were found that produced less than 20 percent survivors. These combinations were l(3)s096108/82 and l(3)s041303/82, l(3)s096108/82 and l(3)s083902/82, l(3)s083902/82 and l(3)s086405/82, l(3)s044738/82 and l(3)s019002/82, l(3)s147406/82 and l(3)s028218/82, l(3)s102312/82 and l(3)s096108/82, l(3)s142216/82 and l(3)s096108/82. No complementation was found between lines l(3)s120710/82 and l(3)s096108/82 suggesting that these insertions affect the same locus. The cuticles of embryos derived form lines belonging to the aop group display an anterior hole, indicating a possible involvement of the corresponding loci in the process of dorsal closure as previously described for the gene *yan* (also known as *anterior open:* Riesgo-Escovar *et al.,* 1997b).

Posterior Patterning Defects. 3 lines showed a mutant phenotype similar to mutations in *nanos*. The *nanos* locus (Wang & Lehmann, 1991) encodes an RNA binding protein, which specifies posterior cell fates. Proper nanos mRNA localization to the posterior end requires the gene products of the *cappucino*, *spire*, *staufen*, *tudor*, *vasa*, *valois* and *oskar* loci. 3 lines in this group represent new alleles of *Tropomyosin II* (Miklos Erdelyi, personal communication) which was shown to be required for the localization of *oskar* mRNA to the posterior end (Erdelyi *et al.,* 1995).

Terminal Defects. 28 lines showed absence or defective termini at both poles, a phenotype which resembles that caused by loss-of-function *torso* (*tor*) alleles (Sprenger and Nüsslein-Volhard, 1993). We characterized this class by complementation analysis and we found few semi-lethal heteroallelic combinations with less than 20 percent survival rate, namely l(3)s099808/82 and l(3)s027821/82, l(3)s104415/82 and l(3)s027821/82, l(3)s104415/82 and l(3)s080104/82, l(3)s135216/82 and l(3)s067401/82, l(3)s140612/82 and l(3)s023426/82. Another 19 lines in this class displayed a range of morphological aberrations of the posterior spiracles such as loss, incomplete formation, and splitting of the spiracles along the A/P axis. Similar phenotypes have been described for mutations in the Abdominal- A cascade (Hu & Castelli-Gair, 1999).

Dorso-Ventral Patterning Defects. In this class we found 47 lines with patterning defects along the dorso-ventral axis. Typical defects observed among these lines are: internalization of the Filzkörper, mismatching of the externally visible cephalo-pharyngeal skeleton, the fusion of cuticular patterns along the ventral midline and changes in the width of ventral denticle belts (Arora & Nüsslein-Volhard, 1992). Two lines were classified as dorsalized (drs): *l(3)s116206/82* and *l(3)s072409/82*. The insertion *l(3)s123713/82* is a new allele of the *punt* gene (see Fig. 3), which was shown to encode a type II receptors that mediates signaling by Dpp (Letsou *et al.,* 1995) during the establishment of the dorso-ventral polarity in the embryo: strong *punt* hypomorphs display a ventralization of the cuticle.

Defects in Epidermal Development and Cuticle Differentiation. 50 insertion lines showed aberrations in epidermal development and cuticle differentiation. These were subdivided into 3 classes: body section compressed embryos (bsc) (6 lines), collapsed (cps) (14 lines) and cuticle defects (ctd) (30 lines). A novel phenotype is described by the bsc lines: while the overall organization of the embryonic body looks normal, the segments along the A/P axis are compressed like an accordion. In the cps group the cuticle surface is preserved but the structure of the embryos has partially collapsed towards the inside of the body. This phenotype could also be due to mechanical stress during the cuticle preparation procedure, indicating a somehow fragile cuticle structure. The ctd group refers to lines producing embryos with abnormal cuticular appearance. For instance, in some lines, mutant embryos were recovered with naked cuticles. In other lines, only parts of the embryonic cuticle is present as exemplified by line *l(3)s10400982* (Fig. 2).

TABLE 4

PHENOTYPIC CLASSES IN SUBGROUP C

Phenotypic class	Abbreviation	Number of lines
Anterior defects	and	6
Anterior open	aop	8
Body section compressed embryos	bsc	6
cli-like	cli	4
Collapsed	cps	14
Cuticle defects	ctd	28
Curled tail	cut	3
Embryos twisted with reduced ventral denticle belts	etr	11
Dorsal closure defective	dcd	7
Dorsal holes	dho	2
Dorsalized	drs	2
Dorso-ventral defects	dvd	45
Filzkörper defective	fzd	19
Head defects	hed	21
Latero-ventral holes	lvl	6
Multiple holes	muh	14
Nanos-like	nan	3
Segment polarity defects	spd	2
Segmentation defects	std	58
Terminal defects	ted	29
Ushaped-like	ush	15
Ventral holes	veh	4
Ventral midline defects	vmd	11
Total		**318**

P-lines displaying similar cuticular phenotypes were grouped into the same phenotypic class. The definition of each class listed above represents a condensed description of the phenotype. For a more detailed description see text.

Defects in Dorsal Closure. 9 lines present a dorsal closure defective (dcd) phenotype. The process of dorsal closure is a morphogenetic event that is initiated at mid-embryogenesis. During this process, the two lateral epithelia stretch toward the dorsal midline, the suture line of the two leading edges. Cell migration during dorsal closure relies on cell shape changes in the leading edge cells and is controlled by the activity of the JNK and the Dpp pathway (Noselli, 1998; Riesgo-Escovar & Hafen 1997b). The lines *l(3)s0200/04A/82, l(3)s020004B/82 and l(3)s135103/82* are new alleles of *kayak*, that was previously shown to be required for dorsal closure (Riesgo-Escovar & Hafen, 1997a; Zeitlinger *et al.,* 1997).

Epidermal Holes. 24 mutant lines displayed holes in the cuticle and were subdivided into 3 classes: latero-ventral holes (lvh) (6 lines), multiple holes (muh) (14 lines) and ventral holes (veh) (4 lines). Known loci displaying such a phenotype comprise *crumbs* and *stardust* (Grawe *et al.,* 1996), which are required for the formation of epithelia. Ventral holes embryos display a hole on the ventral side of the body as reported for Ultrabithorax-like (Mortin and Kaufman, 1984).

Segmentation Defects. We identified 56 lines with defects in their segmentation pattern. Of these, 4 lines showed aberrations typically found in segment polarity mutants. Segment polarity genes subdivide anterior and posterior compartments in Drosophila segments. They are responsible for the positioning of parasegment boundaries and for the establishment of the final segment boundaries of the larval epidermis. Each larval segment has a well defined antero-posterior polarity, which is easily seen in the ventral epidermis of the abdomen. The formation of the segment polarity pattern relies on the secretion (by the anterior cells of the embryonic segments) of the morphogen Hedgehog, which is received by cells in the posterior compartment of the adjacent segment. Hedgehog targets genes are regulated by the transcription factor Cubitus interruptus and include *decapentaplegic*, *patched* and *wingless* (Aza-Blanc and Kornberg, 1999). In this class we identified a new component of the Hedgehog signaling pathway, *dispatched* (*disp*) [*l(3)s037707/80*], that was shown to encode a novel sterol-sensing domain protein (Burke *et al.,* 1999).

Ventral Midline Defects. In 11 lines, the width of ventral denticle belts is narrowed, the setae are misplaced or fused, denticle belts are interrupted by naked cuticle or other aberrations along the ventral midline. As described in previous sections, those phenotypes could also be interpreted as mild aberrations of genes belonging to the D/V patterning class or the terminal class of genes.

Germband Retraction Defective Lines. We isolated 29 lines, which showed defective germ-band retraction. These were divided into 3 classes: curled tail (cut) (3 lines), U-shaped-like (ush) (15 lines) and embryo twisted with reduced ventral denticle (*i*) (11 lines). We distinguish curled tail from the U-shaped like class because the tail of embryos in the former class is only slightly bent up dorsally. Among the ush class we recovered new alleles of *canoe* [*l(3)s009105A/82*], previously identified as a *dosal open* mutant (Jürgens *et al.,* 1984; Takahashi *et al.,* 1998), *kayak* [*l(3)s064007/82*] and *Trithorax-like* [*l(3)029105/80, l(3)002401/80, l(3)053018/80 and l(3)114513/80*] (Farkas *et al.,* 1994; Ohtsuki and Levine 1998). The fact that the *l(3)s064007/82* insertion in *kay* causes an arrest in germband retraction while three other insertions produce a dorsal open phenotype (see above) suggests that Dfos is also required for this earlier process and that the different insertions impair the maternal contribution of *kay* function to a different degree.

In the class, named *embryo twisted with reduced ventral denticle belts*, like *Egfr* (*faint little fall*) mutant embryos (Schüpbach & Wieschaus, 1989), are twisted along the A/P axis, the ventral denticle belts are reduced in their length (along the L/R axis) and their tail is (slightly) bent up dorsally. In this class we recovered new alleles of *extramacrochaete* [*l(3)s058004/80*] (Van Doren *et al.,* 1992), and *couch potato* [*l(3)s032412/82* and *l(3)s145301/82*] (Bellen *et al.,* 1992).

Discussion

In this study we describe the results of a large-scale screen for maternal-effect mutations in a collection of recessive lethal insertions on the third chromosome (Deak *et al.,* 1997). We tested 1609 lethal P-insertions in germline clones using the FLP-DFS method. Of these, 604 lines produced normal and fertile progeny, 164 lines did not produce eggs and 841 lines produced eggs that developed abnormally after fertilization. Thus, 38 percent of the lethal loci showed no maternal effect. The product of these genes are not required during oogenesis or during early embryonic development before zygotic transcription starts. About 10 percent of the loci are essential for oogenesis and 52 percent of the loci show an essential maternal contribution to development. The percentage of lines with a maternal effect is slightly lower than estimates made in previous screens (García-Bellido & Robbins 1983; Perrimon *et al.,* 1989). On the other hand, our number is within the range of the one reported by Perrimon *et al.* (1996). These authors analyzed 496 independent P element induced lethals on the second and third chromosomes for maternal effect phenotypes. They found that 60 percent of 284 tested lines on the third chromosome produced a maternal-effect in the embryo. Besides the size of the collection tested, a notable difference between the work reported here and the study of Perrimon *et al.* (1996) was the percentage of lines which was classified to belong to the defective egg formation group. In our screen 10 percent of the lines were assigned to this group, while Perrimon *et al.* (1996) reported that a significantly higher number of lines tested, namely 27 percent, affected egg formation. This difference may be explained simply by the application of different criteria for assigning a line to a specific group. For instance, we also included a number of lines that produced only very few eggs to the maternal-effect group. These may have not met the same criteria for this type of classification by Perrimon *et al.* (1996).

The stringent selection of lines affecting oogenesis permitted us to carry out a detailed phenotypic analysis of this class. The

TABLE 5

LIST OF P-LINES BELONGING TO SUBGROUP C

Subgroup C	lines
and	l(3)s148016/80,l(3)s146004/80, l(3)s138102/82, l(3)s120307/80, l(3)s120910/82 (ttk), l(3)s144113/82
aop	l(3)s145005/82, l(3)s023549/80, l(3)s028002/82, l(3)s067108/82, l(3)s066909/82, l(3)s097406/80, l(3)s079604/80, l(3)s0700016A/80
bsc	l(3)s107707/82, l(3)s 050501/82, l(3)s 096115/82, l(3)s 073906/82, l(3)s124310/82, l(3)s060006/82
cli	l(3)144113/82, l(3)097016/82
cps	l(3)s000624/82, l(3)s006013/82, l(3)s018809/82, l(3)s022309/82, l(3)s042834/82, l(3)s047526/80, l(3)s066310B/82, l(3)s101702/82, l(3)s129607/82, l(3)s134217/80, l(3)s136403/80, l(3)s143302/82, l(3)s145609/80, l(3)s145610/80.
ctd	l(3)s003010/80, l(3)s008418/80, l(3)s010409/82, l(3)s028011/80, l(3)s028411/80, l(3)s029718/82, l(3)s030701/82, l(3)s038307/82, l(3)s044631/80, l(3)s050016/80, l(3)s05124B/80, l(3)s051614/80, l(3)s059205/80, l(3)s059419/82, l(3)s059510/80, l(3)s060015B/82, l(3)s063813/80, l(3)s066121/80, l(3)s071806/80, l(3)s080503/80, l(3)s081705/82, l(3)s084210/82, l(3)s085301/82, l(3)s089707/82, l(3)s102412/82, l(3)s116013/82, l(3)s123304/82, l(3)s130813/82, l(3)s143909/82, l(3)s145609/80
cut	l(3)s040201/82, l(3)s066719/82, l(3)s066813/82.
etr	l(3)s015012B/80, l(3)s032301/82, l(3)s032412/82 (cpo), l(3)s043029/82, l(3)s052804/80, l(3)s056015/80, l(3)s058004/80 (emc), l(3)s100209/80, l(3)s122211/80, l(3)s125605/80, l(3)s145301/82 (cpo)
dcd	l(3)s0200/04A/82 (kay), l(3)s020004B/82 (kay), l(3)s135103/82 (kay), l(3)s040406/80, l(3)s066122/80, l(3)s127416/82, l(3)s145016/82, l(3)s147207/80,
dho	l(3)s027013/80, l(3)s137402/82
drs	l(3)s116206/82, l(3)s072409/82
dvd	l(3)s005302/80, l(3)s008729/82, (3)s009413/82, l(3)s028310/82, l(3)s040402/80, l(3)s042610/80, l(3)s050116/82, (3)s051407/82, l(3)s052516/82, l(3)s057806/82, l(3)s060402/80, l(3)s061708/82, l(3)s066677/80, l(3)s068002A/82, l(3)s069605/82, l(3)s073414/82, l(3)s075315/80, l(3)s079309/82, l(3)s080407/82, l(3)s095814/80, l(3)s096414/82, l(3)s097703/82, l(3)s103408/80, l(3)s103505/82, l(3)s108113/80, l(3)s110511/82, l(3)s115101/80, l(3)s115915/82, l(3)s116013/82, l(3)s122511/80, l(3)s123713/82 (pnt), l(3)s126015/80, l(3)s126201/82, l(3)s128202/80, l(3)s130315/82, l(3)s130815/80, l(3)s132601/82, l(3)s132607/82, l(3)s135305/82, l(3)s135710/80, l(3)s136403/82, l(3)s136414/80, l(3)s140410/80, l(3)s143517/82, l(3)s147313/82.
fzd	l(3)s016603/82, l(3)s018611/80, l(3)s023911/82, l(3)s024409A/82, l(3)s027114/80, l(3)s031805/82, l(3)s033913/82, l(3)s039603/82, l(3)s043420/82, l(3)s0492107B/80, l(3)s060801/80, l(3)s061903/82, l(3)s064717/82, l(3)s077009/82, l(3)s093008/82, l(3)s106006/82, l(3)s141203/82, l(3)s146006/80, l(3)s148906/82
hed	l(3)s000710/82, l(3)s0102312/82, l(3)s0116512/82, l(3)s0138107/82, l(3)s014917/82, l(3)s017117/82, l(3)s019002/82, l(3)s019203/82, l(3)s028218/82, l(3)s029309/80, l(3)s040608/82, l(3)s041303/82, l(3)s044738/82, l(3)s064009/82, l(3)s078105/80, l(3)s083902/82, l(3)s086405/82, l(3)s096108/82, l(3)s120710/82, l(3)s142216/82, l(3)s147406/82
lvl	l(3)s004019/80, l(3)s004021/80, l(3)s034107/80, l(3)s078402/82, l(3)s093806/80, l(3)s117408/80
muh	l(3)s007412/80, l(3)s011420/82, l(3)s047003/80, l(3)s047212/82, l(3)s048103/82, l(3)s054618/82, l(3)s057401/82, l(3)s066317/82, l(3)s087602/80, l(3)s101206/82, l(3)s119003/80, l(3)s126215/80, l(3)s144002/80, l(3)s147506/82
nan	l(3)s005515/82, l(3)s085005/82, l(3)s091005/82
spd	l(3)s037707/80 (disp), l(3)s134803/80
std	l(3)s006517/82, l(3)s008614/82, l(3)s009826/80, l(3)s010013/82, l(3)s010407/80, l(3)s010415/82, l(3)s023713/82, l(3)s025637/80, l(3)s027402/80, l(3)s030009/82, l(3)s032513/82, l(3)s034505/80, l(3)s037901/82, l(3)s041509/82, l(3)s044408/82, l(3)s044632/82, l(3)s046604/82, l(3)s050002/80, l(3)s050810/82, l(3)s052001/80, l(3)s058712/82, l(3)s061308/80, l(3)s062914/80, l(3)s063207/82, l(3)s063319/82, l(3)s063614/82, l(3)s065303A/80, l(3)s070701/82, l(3)s083407/82, l(3)s088501/82, l(3)s090001/80, l(3)s095304/82, l(3)s097301/82, l(3)s098105/82, l(3)s106101/82, l(3)s107717/82, l(3)s108306/80, l(3)s108408/82, l(3)s108602/82, l(3)s111611/82, l(3)s113403/82, l(3)s113503/80, l(3)s114105/82, l(3)s119608/82, l(3)s121414/80, l(3)s124802/82, l(3)s125011/82, l(3)s126202/82, l(3)s126607/82, l(3)s127816/82, l(3)s127914/82, l(3)s131207/82, l(3)s132304/82, l(3)s140512/82, l(3)s146508/80 l(3)s148318/80
ted	l(3)s023426/82, l(3)s023634/82, l(3)s025247/80, l(3)s027313/80, l(3)s027821/82, l(3)s042825/82, l(3)s04703/82, l(3)s059904/82, l(3)s067401/82, l(3)s067611/82, l(3)s080104/82, l(3)s081401/80, l(3)s092011/80, l(3)s097002/82, l(3)s099808/82, l(3)s104415/82, l(3)s129213/82, l(3)s129803/82, l(3)s129805/82, l(3)s132901/80, l(3)s135216/82, l(3)s136910/82, l(3)s138603/80, l(3)s140102/82, l(3)s140612/82, l(3)s140713/82, l(3)s147804/82, l(3)s148815/82, l(3)028510/82
ush	l(3)s002401/80, l(3)s008320/80, l(3)s009105A/82 (cno), l(3)s024421/80, l(3)s025807/82, l(3)s028113/80, l(3)s029105/80, l(3)s053018/80, l(3)s064007/82 (kay), l(3)s100201/82, l(3)s106707/82, l(3)s136808/82, l(3)s139506/82, l(3)s146501/82
veh	l(3)s015102/82, l(3)s049105/82, l(3)s067712/82, l(3)s130502/82
vmd	l(3)s001407/82, l(3)s023844/80, l(3)s033805/80, l(3)s044823/80, l(3)s051204A/80, l(3)s051513/80, l(3)s071501/82, l(3)s090613/80, l(3)s111814/80, l(3)s144306/80, l(3)s148307/80

P-lines numbering referred to the original publication by Déak *et al.* (1997).

"/80" and "/82" describe lines recombined on the FRT80B and the FRT82-chromosome, respectively.

classification of 164 lines into 10 phenotypic classes ranging from agametic ovaries to ovaries with no morphological defects provides valuable information for the further molecular charactization of these loci and the processes involved in egg maturation.

A general problem with P element lethal collections is that the lethality is frequently not associated with the dominantly marked P element insertion. Indeed by recombining the P element insertions on FRT bearing chromosomes we found that in 16 percent of the cases (331 of 2'068 lines tested) the P element insertion was homozygous viable. Thus the recombination event led to the removal of second site lethal mutations. Salzberg *et al.* (1997) analyzed the same collection of lethal P element insertion on the third chromosome for zygotic defects during the development of the peripheral nervous system. They determined, by remobilizing the P element, that in 22 percent (22/101 lines) of the cases that P element was not responsible for the phenotype. From this is can be estimated that after recombination on FRT chromosomes, the P element insertion is responsible for the mutant phenotype in more than 90 percent of the cases.

One of the main goals of this large-scale maternal-effect screen was to identify those genes whose function during embryogenesis has escaped detection by analyzing phenotypes in the absence of zygotic gene function because of the maternally contributed gene products. Indeed, we identified a new segment polarity gene *dispatched* which is required for the production of the *hedgehog* protein (Burke *et al.,* 1999). In addition, we identified new alleles of genes previously known for their zygotic requirement during later stages of embryonic development. Amongst these, we identified new alleles of the Drosophila *fos* gene (*Dfos, kayak*) and *canoe* (*cno*). The phenotypes recovered for different insertion alleles in the two genes imply that they are not only required for dorsal closure but also for germband retraction. These newly identified *Dfos* alleles exemplify an additional strength of the P element induced mutations. Since the P elements frequently insert into the 5' end or into intronic sequences of genes or they often do not cause a complete disruption of gene function. Together with chemically induced complete loss of function mutations it is thus possible to obtain an allelic series of different phenotypes. Such an allelic series was important to uncover different functions of *Dfos* during oogenesis and embryonic develompent. Complete removal of *Dfos* function during oogenesis blocks egg production. Removal of *Dfos* function zygotically, however, arrests embryogenesis at the dorsal closure stage, presumably because earlier Dfos function is provided by maternal product. Indeed, the new P element induced alleles in *Dfos* indicate that embryos with reduced maternal Dfos products arrest already at the germ band retraction stage, thus suggesting an involvement of *Dfos* in this earlier process. The often hypomorphic nature of the P element induced mutations may thus provide important insights into the early embryonic requirement of genes whose function is essential during oogenesis.

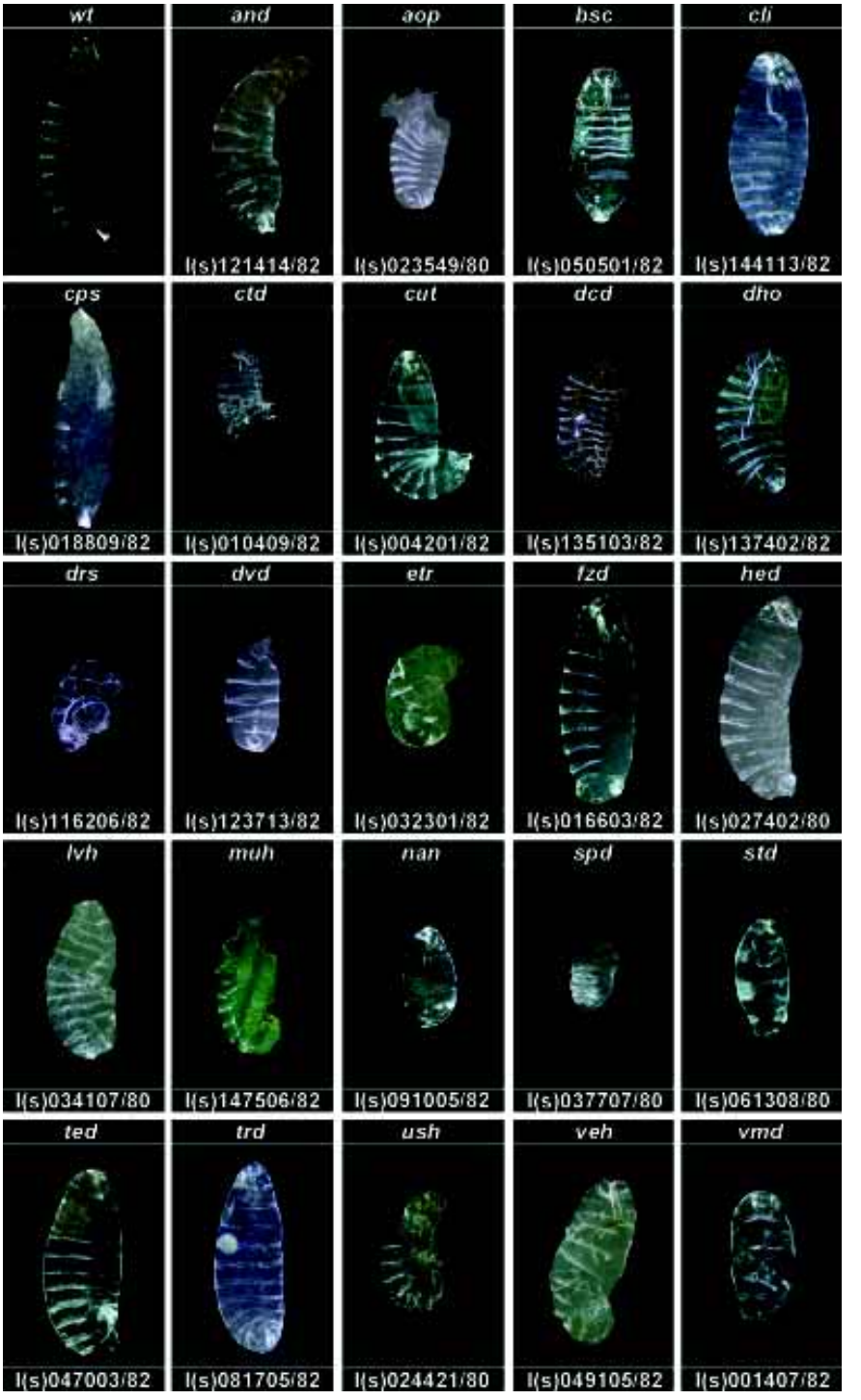

Fig. 3. Cuticular phenotypes of germline clones homozygous for P-induced mutations on the third chromosomes. *Each class (see Tables 4 and 5) is represented by one P-line displaying the typical phenotype. For a detailed description, see text.*

TABLE 6

LIST OF P-LINES WITH OVARIAN PHENOTYPES

aga	l(3)s144015/82 ,l(3)s047501/82, l(3)s051311/82, l(3)s052713/82, l(3)s143907/82, l(3)s022348/82, l(3)s023844/80, l(3)s044203/80, l(3)s041315/80, l(3)s093411/80, l(3)s095808/80, l(3)s095914/80, l(3)s061113/80, l(3)s044215/80, l(3)s057310/82, l(3)s092006/80, l(3)s130405/82, l(3)s121211/82
tum	l(3)s01561B/82, l(3)s097301/82, l(3)s103705/82, l(3)s124110/82, l(3)s126908/82, l(3)s003220/82, l(3)s118306/82, l(3)s011130/80, l(3)s045801/80, l(3)s052201/80, l(3)s043934/80, l(3)s058106/80, l(3)s067206/80, l(3)s075307/80, l(3)s094603/80, l(3)s134217/80, l(3)s028323/80, l(3)s058911/80, l(3)s059614/80, l(3)s073804/80, l(3)s095501/80, l(3)s122404/80, l(3)s139114/82, l(3)s117109/82, l(3)s130405/82, l(3)s139908/82
rnn	l(3)s010127/82, l(3)s072603/80, l(3)s092902/82, l(3)s124311/82, l(3)s125015/82, l(3)s059504/80, l(3)s105406/80, l(3)s107905/80, l(3)s023825/80, l(3)s139908/82
snn	l(3)s007818/82, l(3)s090906/82, l(3)s101310/10, l(3)s045002/82, l(3)s008825/80, l(3)s030305/80, l(3)s042806/80, l(3)s100413/80, l(3)s108709/80, l(3)s112416/80, l(3)s144606/80, l(3)s089302/80, l(3)s145202/80, l(3)s043926/80, l(3)s146205/80, l(3)s071513/80, l(3)s119304/82, l(3)s143103/80, l(3)s043926/80
deg	l(3)s020514/82, l(3)s023443/82, l(3)s084402/82, l(3)s086102/82, l(3)s087113/82, l(3)s037106/82, l(3)s051311/82, l(3)s007329/80, l(3)s026222/80, l(3)s099716/80, l(3)s092712/80, l(3)s145202/80
abc	l(3)s026020/82, l(3)s039003/82, l(3)s040419/80, l(3)s092712/12, l(3)s038611/82, l(3)s058004/80, l(3)s055310/82, l(3)s029110/80, l(3)s027714/80, l(3)s042931/80, l(3)s147423/80, l(3)s113416/82, l(3)s106810/80, l(3)s044626/80
dmp	l(3)s111613/82, l(3)s111704/82, l(3)s085407/80, l(3)s003612/80
sma	l(3)s035313/82, l(3)s064301/82, l(3)s070701/80, l(3)s012815/80, l(3)s028418/80, l(3)s041506/80, l(3)s051204/80, l(3)s060310/80, l(3)s067309/80, l(3)s059511/80, l(3)s143103/80, l(3)s146411/80, l(3)s011101/80, l(3)s058316/80, l(3)s038611/82
ovo	l(3)s023635/82, l(3)s036909/82, l(3)s074206/82, l(3)s090114/82, l(3)s126504/82, l(3)s132413/82, l(3)s144812/80, l(3)s117708/82, l(3)s004805/80, l(3)s023826/80, l(3)s047501/82, l(3)s106503/80, l(3)s116919/80, l(3)s021204/80, l(3)s057310/82, l(3)s062912/82, l(3)s071802/80, l(3)s071513/80, l(3)s010715/82
wtl	l(3)s059701/80, l(3)s073714/82, l(3)s074407/82, l(3)s130415/82, l(3)s142411/82, l(3)s058313/82, l(3)s026908/80, l(3)s090101/80, l(3)s110108/82, l(3)s115915/80, l(3)s119601/80, l(3)s141904/80, l(3)s146001/80, l(3)s049115/80, l(3)s139114/82, l(3)s143301/80, l(3)s043816/80

P-lines numbering referred to the original publication by Déak et al. (1997).
"/80" and "/82" describe lines recombined on the FRT80B and the FRT82-chromosome, respectively.

Materials and Methods

Recombination of P-element Insertions on FRT Chromosomes

The stocks used for recombining the P element insertions onto the FRT80B and FRT82B chromosomes are described in detail Newsome *et al.* (2000). The following genotypes were used: *y w; P(w$^+$)1 / TM6C, y w P(ry$^+$; eyFLP)1;+/+, y w P(ry$^+$; eyFLP)1; P(ry$^+$; hs-neo, FRT) 80B / P(ry$^+$; hs-neo, FRT)80B, y w P(ry$^+$; eyFLP)1; P(ry$^+$; hs-neo, FRT) 82B / P(ry$^+$; hs-neo, FRT)82B, y w P(ry$^+$; eyFLP)1; D,gl / TM3, P(y$^+$), Ser.*

The P element insertion strains that remained recessive lethal after recombination have been deposited in the Szeged P element stock center.

To generate P[*ovo^{D1}*] on the FRT80B and FRT82B chromosomes, the P[*ovo^{D1}*] insertions on 3L and 3R described in Cho and Perrimon (1996) were recombined onto the FRT containing chromosomes using X-ray induced male recombination as described by Cho and Perrimon (1996).

Production of Germline Clones with the Autosomal FLP-DFS Technique

10 females of the genotype *y w*; *FRT m** / *TM3*, *Ser, y$^+$* were crossed to 10 males of genotype *hs70-FLP / Y; FRT P[ovo^{D1}] / TM3*, *Sb*. After a prelay at 25°C for one day, laid eggs were collected over a period of 24 h and incubated for another 36 h at 25°C. First instar larvae were then heat shocked for 1 hr at 37°C and put back to 25°C to complete development. 15-20 eclosed females of genotype *y w* / *y w FLP; FRT m** / *FRT P [ovo^{D1}]* were mated with *y w / Y; FRT m* / TM3, Ser, y$^+$* males. Due to presence of the dominant female sterile mutation *ovo^{D1}* in the female germline, only germline clones homozygous for the mutation *m** can form eggs. *FRT* represents either *FRT80B* for the left arm or *FRT82B* for the right arm.

Examination of Ovaries and Embryo Cuticles

Ovaries of *y w* / *y w FLP; FRT m** / *FRT P [ovo^{D1}]* females which did not produce eggs were prepared for examination. They were dissected in Ringer's solution and fixed for 30 min in 3.6 percent formaldehyde. Fixed tissue was stained for 5 min with DAPI (0.2 µg/ml) and cytologically examined by fluorescence microscopy (Zeiss, Axiophot).

From egg-laying females, eggs were collected and cuticles were prepared according to the protocol described by Van Meer (1977). These preparations were examined by dark-field microscopy. Progeny of the cross *y w* / *y w FLP; FRT m** / FRT *P [ovo^{D1}]* females with *y w* / *Y; FRT m* / TM3*, *Ser, y$^+$* males consist of two classes of embryos. One inheriting the paternal mutant chromosome and thus lacking maternal as well as zygotic activity, and the other class which inherits the wild-type allele from the father. The two classes could be distinguished based on the presence or absence of the *y$^+$* marker that is only inherited with the paternal wild-type chromosome. In cuticle preparations, the denticle belts of *y$^+$* marked embryos appear darker than in *y* embryos.

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