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# DEVELOPMENTAL GENETICS OF THE 2E-F REGION OF THE DROSOPHILA *X* CHROMOSOME: A REGION RICH IN "DEVELOPMENTALLY IMPORTANT" GENES

NORBERT PERRIMON, LEE ENGSTROM AND ANTHONY P. MAHOWALD

*Department of Developmental Genetics and Anatomy, Developmental Biology Center, Case Western Reserve University, Cleveland, Ohio 44106*

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ABSTRACT

We have analyzed the 2E1-3A1 area of the *X* chromosome with special attention to loci related to embryogenesis. Published maps indicate that this chromosomal segment contains ten bands. Our genetic analysis has identified 11 complementation groups: one recessive visible (prune), two female steriles and eight lethals. One of the female sterile loci is *fs(1)k10* for which homozygous females produce both egg chambers and embryos with a dorsalized morphology. The second female sterile is the paternally rescuable *fs(1)*pecanex in which unrescued embryos have a hypertrophic nervous system. Of the eight lethal complementation groups two are recessive embryonic lethals: hemizygous giant (*gt*) embryos possess segmental defects, and hemizygous crooked neck (*crn*) embryos exhibit a twisted phenotype. Analysis of these mutations in the female germ line indicates that *gt* does not show a maternal effect, whereas normal activity of *crn* is required for germ cell viability. Analysis of the maternal effect in germ line clones of the remaining six recessive lethal complementation groups indicates that four are required for germ cell viability and one produces ambiguous results for survival of the germ cells. The remaining, *l(1)*pole hole, is a recessive early pupal lethal in which embryos derived from germ line clones and lacking wild-type gene activity exhibit the "torso" or "pole hole" phenotype.

DETAILED genetic analysis of selected regions of the Drosophlia genome is an excellent approach to gaining insight into both the types of loci that are closely linked and the relationships between adjacent genes (JUDD, SHEN and KAUFMAN 1972; LEWIS 1978; LEWIS *et al.* 1980a,b). We have been concerned with the region of the *X* chromosome distal to zeste-white in which a number of interesting female sterile loci are found: *fs(1)k10* (WIESCHAUS, MARSH and GEHRING 1978), *fs(1)*pecanex (formerly *mel(1)R1)* (ROMANS, HODGETTS and NASH 1976) and *l(1)*pole hole (PERRIMON, ENGSTROM and MAHOWALD 1984; N. PERRIMON, L. ENGSTROM and A. P. MAHOWALD, unpublished data). BRIDGES (1938) counted nine bands in the 2E-F region of the chromosome, three in 2E and six in 2F. Electron microscopy of salivary gland chromosomes is in accordance with these numbers (SORSA and SAURA 1980). Because of the extensive studies of the adjacent zeste-white interval (JUDD, SHEN and KAUFMAN 1972; SHANNON *et al.* 1972; KAUFMAN *et al.* 1975), many defi-

TABLE 1

*List of deficiencies and duplications in the 2E-F area*

| Rearrangement | Cytology | References |
|---|---|---|
| *Df(1)Pgd kz* | *Df(1)2D3;2F5* | GERASIMOVA AND ANANJEV (1972) |
| *Df(1)Pgd*$^{35}$ | *Df(1)2C2-4;2E2-F1* | D. NERO, personal communication[a] |
| *Df(1)64c18* | *Df(1)2E1-2;3C2* | CRAYMER and ROY (1980) |
| *Df(1)pn*$^{7a}$ | *Df(1)2E1;3A4* | G. LEFEVRE, personal communication |
| *Df(1)TEM* 304 | *Df(1)2E2-F1;3A4-6* | J. LIM, personal communication |
| *Df(1)278.4B.1a* | *Df(1)2E3-F3;3A5-B4* | T. WU, personal communication |
| *Df(1)2F1-3A4* | *Df(1)2F1;3A4* | GREEN, personal communication |
| *Df(1)JC19* | *Df(1)2F3;3C5* | CRAYMER and ROY (1980) |
| *Df(1)X12* | *Df(1)2F6-3A1;3B5* | KAUFMAN *et al.* (1975) |
| *Df(1)62g18* | *Df(1)3A1-2;3A4* | KAUFMAN *et al.* (1975) |
| *Df(1)TEM 75* | *Df(1)2F5-3A1;3C2-4* | J. LIM, personal communication |
| *Dp(1;Y)w*$^{+303}$ | *Dp(1;Y)2D1-2;3D3-4* | G. LEFEVRE, personal communication |
| *Dp(1;3)w*$^{vco}$ | *Dp(1;3)2B17-C1;3C4-5;77D3-5;81* | LINDSLEY and GRELL (1968) |

Breakpoints and reference for each rearrangement are noted.

[a] CRAYMER and ROY (1980) list the cytology of *Df(1)Pgd*$^{35}$ as *Df(1)2D3-2F5*; we believe that the deficiency they studied was *Df(1)Pgd kz*.

ciencies are available which extend into the 2E-F region. LEFEVRE (1981; and G. LEFEVRE, personal communication) identified seven lethal complementation groups within the nine-band region of 2E-F. Overlapping deficiencies were used to further delimit the location of these loci. Furthermore, germ line clonal analysis of each lethal was used to determine the presence and extent of any maternal effect on embryonic development exhibited by each mutation.

From this detailed study of the 2E-F region, we have identified an amazing variety of loci affecting early embryogenesis. Two female sterile mutations were found affecting the early embryo, one producing a dorsalized maternal effect and the other a neuralizing effect that is partially rescued by paternally provided gene activity. Two of the lethals in the region affect the pattern of determination at the blastoderm. Finally, a pupal lethal in germ line clones shows a maternal effect on early development. Thus, this region is unusually rich in both the number and diversity of loci affecting oogenesis and early development.

## MATERIALS AND METHODS

*Stocks*: The lethal stocks, with the exception of the hybrid dysgenesis (MR)-induced lethals, were obtained from G. LEFEVRE, who also provided their approximate cytological location. Of this group, those carrying the initial letter E, V or D are ethyl methanesulfonate (EMS) induced; the others are x-ray induced. These lethal mutations were kept either as *FM7* balanced stocks or as attached-*X* (C(1)DX/Y) stocks bearing a duplication of the entire 2E-F area on the *Y* chromosome, *D(1;Y)w*$^{+303}$, or on the third chromosome, *Dp(1;3)w*$^{vco}$ (see Table 1 for the extent of the duplications).

The MR-induced lethals, *D62* and *D72* and *l(1)24*, were obtained from F. SOBELS; *L154* and *L271* were obtained from M. GANS.

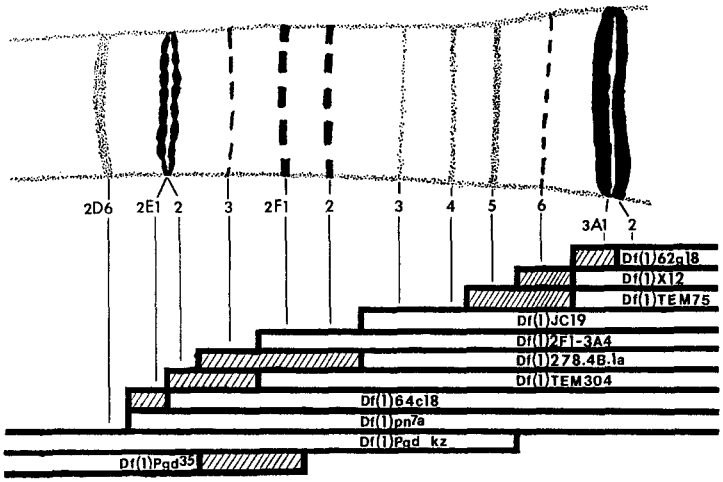


FIGURE 1.—Anatomy of the 2E-F area and extent of deficiencies. Deficiencies are represented by open bars and are shown with respect to the missing chromomeres. Shaded area indicates the uncertainty of the breakpoints (see Table 1).

The deficiencies used in this study (see Table 1) were kindly provided by M. M. GREEN, J. LIM, D. NERO, L. ROBBINS, P. SANTAMARIA and T. WU and by the Pasadena stock center.

*Germ line clones*: Germ line clones were induced using the dominant female sterile technique as previously described by PERRIMON and GANS (1983) and PERRIMON, ENGSTROM and MAHOWALD (1984). The dominant female sterile mutation, *K1237*, is maintained in an attached-*X* stock. A constant dose of 1000 rads was administered at the end of the first instar larval stage (General Electric, 100 kV, 5 mA, 3 min 35 sec, 1-mm aluminum filter). Hoyer's mounts of unhatched embryos were prepared according to the technique of VAN DER MEER (1977). A recessive lethal mutation analyzed in a germ line clone was determined to produce germ line lethality when no fertile clones were found among at least 200 females (PERRIMON, ENGSTROM and MAHOWALD 1984).

*Genetic analysis*: The possibility that the recessive zygotic lethals provided by G. LEFEVRE contained multiple mutations was tested by placing each mutation with one of the duplications to reveal other lethals on the chromosome outside the region covered by the duplication. Each lethal complementation group was mapped relative to the deficiencies covering the area. Individual alleles of each lethal complementation group were checked for complementation with at least two alleles (when they existed) of the adjacent flanking lethal loci. In all cases, in which two mutations or a mutation and a deficiency complemented, *trans*-heterozygous progenies were kept and their morphology and fertility were examined.

Flies were grown on standard Drosophila medium at 25 ± 0.5°.

## RESULTS

### *Genetic analysis*

Utilizing the information already available (see Introduction, Table 1 and Figure 1) and by performing complementation tests as indicated in MATERIALS AND METHODS we were able to construct a general map of the area (see Figure 2). We characterized five MR-induced lethals *l(1)24*, *D62*, *D72* (F. SOBELS, unpublished results) and *L154*, *L271* (M. GANS, unpublished results). *D62* and *D72* were found to be allelic with the lethal complementation group *EC226*, and both complemented lethal groups *VA172*, *HF330* and *l(1)*pole hole (*l(1)ph*).

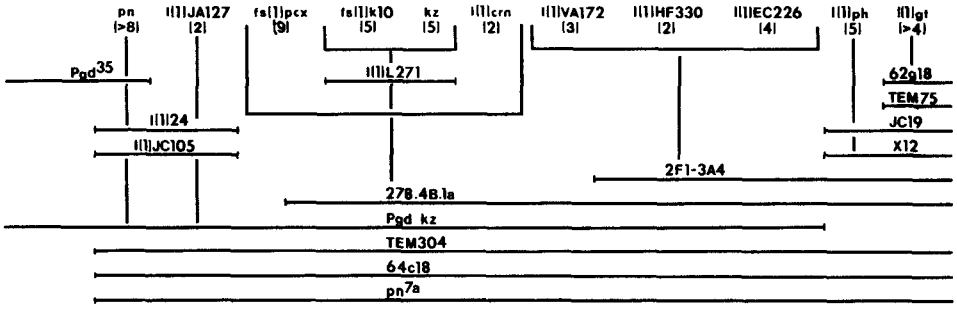


FIGURE 2.—Complementation map of the 2E-F area. Deficiencies are shown with respect to the complementation groups that they fail to cover and are indicated by their numbers only. Numbers below the name of the complementation groups indicate the number of alleles at each loci. The relative order of genes grouped above a bar is unknown. *Df(1)Pgd kz/Df(1)X12* is viable and fertile. *JC105*, *l(1)24* and *L271* are depicted as deficiencies.

*L154* was found to be allelic to *l(1)*pole hole and *L271* to the lethal complementation group kurz (*kz*). *L154* complemented lethal groups *EC226*, *HF330* and *VA172* and $gt^{X11}$ as did *L271* for lethal groups *JA127* and *crn*. However, when we tested *L271* for complementation with mutations of the two other complementation groups delimited by the distal breaks of *Df(1)278·4B·1a* and *Df(1)2F1-3A4* (*i.e.*, *fs(1)k10*, *fs(1)pcx*) we found that *L271* did not complement *fs(1)k10*, thus locating *fs(1)k10* close (right or the left) to the lethal complementation group in *kz*. Two lethal alleles of the *JA127* locus, *JC105* and the MR-induced *l(1)24*, uncovered the recessive visible mutation prune (*pn*). Also, *JC105* did not complement $Df(1)Pgd^{35}$ and probably uncovered a distal lethal complementation group not yet identified.

*Cytological location*

In many cases the deficiencies' breakpoints have been identified within ±1–3 bands (Table 1). However, it is possible to estimate the approximate location of the 2E-F complementation groups by connecting the results shown in Figure 2 with the extent of the deficiencies shown in Figure 1. The *pn* locus is probably located in 2E1-E2. In the interval 2E2-2F1, delimited by *Df(1)278·4B·1a* and *Df(1)2F1-3A4* are the complementation groups *JA127*, *pcx*, *k10*, *kz* and *crn*. Because of the extent of *Df(1)2F1-3A4* and *Df(1)JC19*, the complementation groups *VA172*, *HF330* and *EC226* are probably in 2F1-F2. In 2F6 delimited by *Df(1)Pgd kz*, *Df(1)X12* and *Df(1)62g18* is *l(1)ph* and, finally, the *gt* locus is located in 3A1 (KAUFMAN *et al.* 1975). If this correlation is correct, it would suggest that no loci have yet been identified in the area 2F3-F5.

*Phenotype of deficiency embryos*

The 2D6-3A1 region contains two embryonic lethals: *crn* and *gt*. All progeny hemizygous for any deficiencies of the area are embryonic lethals. Embryos hemizygous for *Df(1)TEM75*, *Df(1)62g18*, *Df(1)X12*, *Df(1)JC19* and *Df(1)2F1-3A4* showed the *gt* embryonic phenotype. Also, embryos hemizygous for *Df(1)278·4B·1a* showed an additive embryonic phenotype: *crn* and *gt*. Those for *Df(1)64c18* also showed the *crn* plus *gt* phenotype, but additional nonspecific

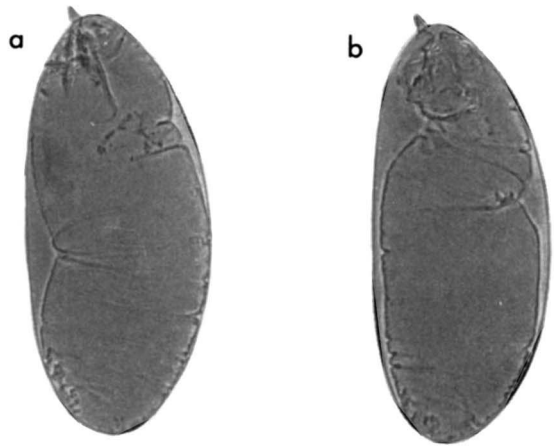


FIGURE 3.—Embryonic phenotype of crooked neck (*crn*). Phase-contrast micrographs of two 24-hr hemizygous *crn* embryos illustrating the weakest (*a*) and strongest (*b*) expression. Embryos appear twisted along their longitudinal axis. The denticle pattern (not visible in these phase-contrast micrographs) is not affected by the mutation. ×150.

defects were also observed, probably due to the hypodosage of other genes inside this relatively large deficiency. These results indicate that there probably are no other embryonic lethal mutations disrupting the embryonic pattern in the interval 2E1 to 3C2-3. Therefore, the zygotic lethal phenotypes of hemizygous deficiencies support the order of complementation groups shown in Figure 2.

For convenience we have grouped the results of developmental analyses of the loci according to the effective lethal phase exhibited by hemizygous lethal individuals (embryonic, larval or pupal), their female sterility or their recessive visible phenotypes.

*Embryonic lethal loci*: Two loci within this genetic region show an embryonic lethal phase: "crooked neck" and "giant."

Two alleles of crooked neck (*crn*) have been characterized, *EA130* and *RC63*. All hemizygous embryos for either allele derived from heterozygous mothers exhibit the twisted phenotype shown in Figure 3. At least three other zygotic lethal loci in Drosophila have been shown to produce a similar, but stronger, phenotype; these include twist and snail on the second chromosome and twisted gastrulation on the *X* chromosome (C. NÜSSLEIN-VOLHARD and E. WIESCHAUS, personal communication; K. KONRAD, personal communication). The phenotype of female embryos heterozygous for *crn* alleles and *Df(1)64c18* exhibits a similar, but more extreme, phenotype, suggesting that these two alleles may not be amorphic (null), although hypodosage of other genes within *Df(1)64c18* may be responsible for the phenotypic differences. Germ line clonal analysis indicates that this locus is required for female germ line viability (Table 2).

We tested only one embryonic lethal allele of the giant (*gt*) locus: $gt^{X11}$. Hemizygous embryos derived from heterozygous mothers exhibit a gap in their segmentation. Abdominal segments 5 to 8 are frequently affected (Figure 4*c* and *d*), but occasionally reduced A5 or A8 denticle belts are found; A6 and

TABLE 2

*Germ line clone results*

| Mutation | DP | Lethal phase | *N* | *Ne* | *Nd* | *Nc* | Phenotype |
|---|---|---|---|---|---|---|---|
| *JA127* | + | L1-L2 | 216 | 0 | 216 | 1 | L |
| *JC105* | + | L1-L2 | 317 | 0 | 177 | 0 | L |
| *EC205* | − | L2 | 359 | 0 | 100 | 0 | L |
| *EA130* | + | E | 300 | 0 | 130 | 0 | L |
| *RC63* | + | E | 382 | 0 | 205 | 0 | L |
| *VA296* | + | L2 | 220 | 0 | 150 | 0 | L |
| *DF942* | + | L1-L2 | 293 | 0 | 270 | 0 | L |
| *DC776* | + | L1-L2 | 270 | 0 | 172 | 0 | L |
| *HA90* | + | L1-L2 | 286 | 0 | 106 | 0 | L |
| *L271** | + | L2-L3 | 310 | 0 | 100 | 0 | L |
| *VA172* | + | L3 | 450 | 24 | NT | NT | NME |
| *JC155* | + | L2-L3 | 502 | 0 | 191 | 0 | L |
| *DC798* | + | L2-L3 | 300 | 0 | 150 | 0 | L |
| *HF330* | + | L3 | 552 | 0 | 270 | 3 | L[a] |
| *HF311* | + | L2-L3 | 276 | 2 | 221 | 3 | L[a] |
| *EC226* | + | L2 | 324 | 0 | 126 | 0 | L |
| *EF462* | − | E | 266 | 0 | 194 | 0 | L |
| *D62** | NT | L2-L3 | 300 | 0 | 151 | 0 | L |
| *D72** | NT | L2-L3 | 310 | 0 | 161 | 0 | L |
| *EA75* | + | L3-P | 220 | 10 | NT | NT | MEL |
| $gt^{X11}$ | + | E | 151 | 11 | NT | NT | NME |
| *fs(1)pcx* | | E | 110 | 12 | NT | NT | GLD |
| *fs(1)k10* | | E | 200 | 18 | NT | NT | GLD |

All mutations (lethal and female sterile) were analyzed in germ line clones. Lethal alleles are grouped by complementation groups; those beginning with the letters *E*, *V* and *D* are EMS induced; the remaining are X-ray induced. Hybrid dysgenesis-induced lethals are indicated by an asterisk (*). The effective lethal phase(s) of recessive lethal mutations is indicated. If lethal males are recovered over $Dp(1;Y)w^{+303}$, + is indicated in the DP (duplication) column. The number of irradiated females (*N*), the number of females producing eggs (*Ne*) and the number of dissected females (*Nd*) containing clones (*Nc*) are shown. When *Nd* is less than *N*, *Ne* is included in *Nc*. Finally, the phenotype of germ line clones is indicated: no maternal effect (NME), maternal effect lethal (MEL), lethal in germ line clone (L). For the two female sterile mutations *fs(1)pcx* and *fs(1)k10*, the results indicate that both mutations disrupt a germ line function (GLD). Nomenclature: Embryonic (E), larval instars (L1, L2, L3) and pupal (P) lethal phase. NT, Not tested.

[a] See text.

A7 are always missing (see also HONISH and CAMPOS-ORTEGA 1982). Germ line clone results clearly indicate that *gt* has no maternal effect; *i.e.*, the phenotype of embryos derived from females possessing a homozygous germ line clone is similar to the hemizygous embryos derived from heterozygous moth-

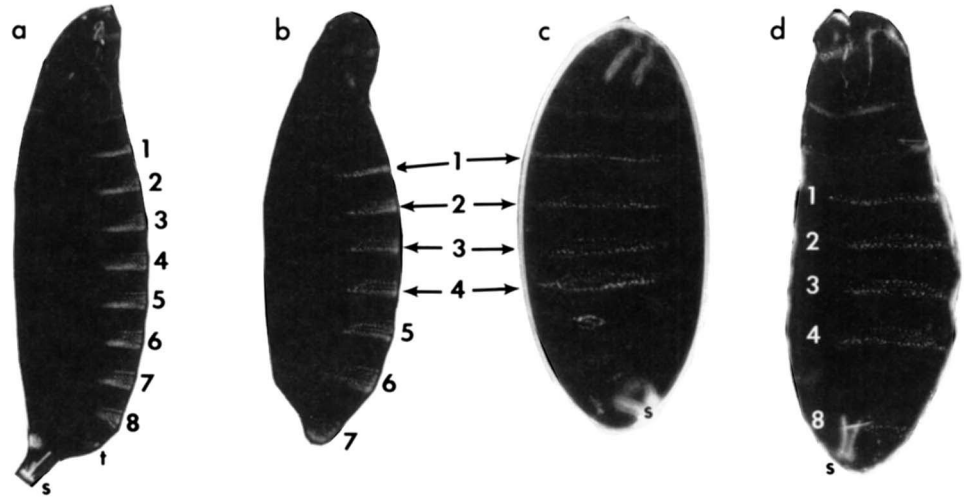


FIGURE 4.—Embryonic phenotype of giant (*gt*) and *l(1)*pole hole (*l(1)ph*). Dark-field images of 24-hr wild type (*a*, lateral aspect), *l(1)ph* (*b*, lateral aspect) and *l(1)gt*$^{X11}$ (*c* and *d*, ventral aspects). Pole hole embryos are derived from germ line clones. The pole hole embryo possesses normal segmentation from anterior (top) through the seventh abdominal segment (7), but most of the more posterior structures including the spiracles and anal tuft (see s and t, respectively, in a are missing. The giant$^{X11}$ embryos possess normal segmentation from anterior (top) through the fourth abdominal segment (4, in *c* and *d*) and normal posterior structures, but 5 to 8 are missing. Occasionally, partial fifth and eighth abdominal belts are present. s = spiracles, t = anal tuft, 1 through 8 = abdominal denticle belts. ×150. The same embryonic phenotype of *gt*$^{X11}$ embryos derived from germ line clones or heterozygous mothers indicates that *gt*$^{X11}$ has no maternal effect.

ers. Many other recessive embryonic lethal alleles of *gt* are known. The viable allele of *gt* (LINDSLEY and GRELL 1968) was tested over *gt*$^{X11}$ and *Df(1)64c18*, and in both cases similar effects on viability, developmental delay and adult size were observed, indicating that *gt*$^{X11}$ behaves as a null mutation and the viable *gt* allele behaves as a hypomorphic (reduced wild-type activity) mutation (results not shown).

*Larval lethal loci*: Multiple alleles of five loci exhibited larval lethal phases (*JA127*, lethals of the *kz* locus, *VA172*, *HF330* and *EC226*). Four of these proved to be germ line lethal by clonal analysis (Table 2), and we obtained ambiguous results for the other (*VA172*).

Three alleles of the *JA127* lethal complementation group (*JA127*, *EC205*, *JC105*), which were all first to second instar lethals, were tested. In each instance no germ line clones (*i.e.*, no vitellogenic egg chambers) were found, although more than 100 irradiated females heterozygous for each allele were dissected and examined.

All of the alleles of the kurz lethal complementation group (*VA296*, *DF942*, *DC776*, *HA90*, *L271*) of this locus, with the exception of the recessive visible marker kurz (*kz*), are first to second instar larval lethal. Surprisingly, many first instar larvae are found alive after as long as 1 wk but do not molt. The *kz* allele is homozygous and hemizygous viable and exhibits a recessive Minute-like bristle phenotype (LINDSLEY and GRELL 1968). The original stock of *kz*

possessed a nearly wild-type phenotype, but outcrosses produced both an easily classifiable bristle phenotype and delayed development, indicating that *kz* expression is sensitive to genetic background. Females, heterozygous for *kz* and a deficiency (*Df(1)64c18*) or any lethal allele, occasionally emerged but only after a delay of several days. All lethal alleles of this locus were cell lethal in germ line clones. These results, as well as the phenotype of *kz*, suggest that *kz* is an hypomorphic mutation. A common phenotype of all recessive lethal alleles is slow larval development, which correlates with the slow growth of individuals hemi- or homozygous for the *kz* allele.

All three alleles of the *VA172* lethal complementation group were lethal at the second to third larval instar stages. Germ line clone analysis of *VA172* did not show any maternal effect, whereas *JC155* as well as *DC798* were found to be germ line lethal. The fact that *VA172* does not exhibit a maternal effect suggests that both the *JC155* and *DC798* chromosomes carry another lethal mutation. Since *JC155* and *DC798* males were recovered when they contained the duplications $Dp(1;Y)w^{+303}$ and $Dp(1;3)w^{vco}$, respectively (see Table 2), the second lethal would have to be covered by these duplications. Another possibility is that *VA172* represents a hypomorphic allele of the gene, whereas *JC155* and *DC798* behave as amorphic mutations. Since all three alleles exhibit the same zygotic lethal phase (Table 2), we cannot as yet distinguish between these two hypotheses.

The two alleles of the *HF330* lethal complementation group (*HF330* and *HF311*) tested were similar both for their lethal phase and for the terminal phenotype in their germ line clones. The few clones (two) of *HF311* which produced a small number of eggs may reflect phenomena that we previously observed and discussed as a progressive phenotypic degeneration (PERRIMON, ENGSTROM and MAHOWALD 1984 and see DISCUSSION). The terminal phenotype found upon dissection of these females was arrested during early oogenesis.

All four alleles of the *EC226* lethal complementation group (*EC226*, *EF462*, *D62*, *D72*) were cell lethal in germ line clones. With the exception of *EF462*, all of the lethals exhibited a second to third instar lethality. *EF462* was found to be an embryonic lethal in which embryos had a normal cuticular morphology. Since we have not been able to recover *EF462* males over $Dp(1;Y)w^{+303}$, the *EF462* chromosome probably contains a second lethal mutation located outside $Dp(1;Y)w^{+303}$ that is responsible for the embryonic lethality.

*Pupal lethal locus*: One complementation group within this region showed pupal lethality: *l(1)*pole hole (*EA75*).

One allele of this locus (*EA75*) has already been described in a previous paper (PERRIMON, ENGSTROM and MAHOWALD 1984). This locus contains five known alleles (N. PERRIMON, L. ENGSTROM and A. P. MAHOWALD, unpublished data). All alleles exhibit late larval to early adult lethality, and the amorphic alleles have a maternal effect. Some of the embryos derived from germ line clones show the pole hole or torso phenotype (Figure 4*b*). The genetic and developmental analysis of this locus will be described elsewhere (N. PERRIMON, L. ENGSTROM and A. P. MAHOWALD, unpublished data).

*Female sterile loci*: Two female sterile loci are found in this chromosomal region: *fs(1)*pecanex and *fs(1)k10*.

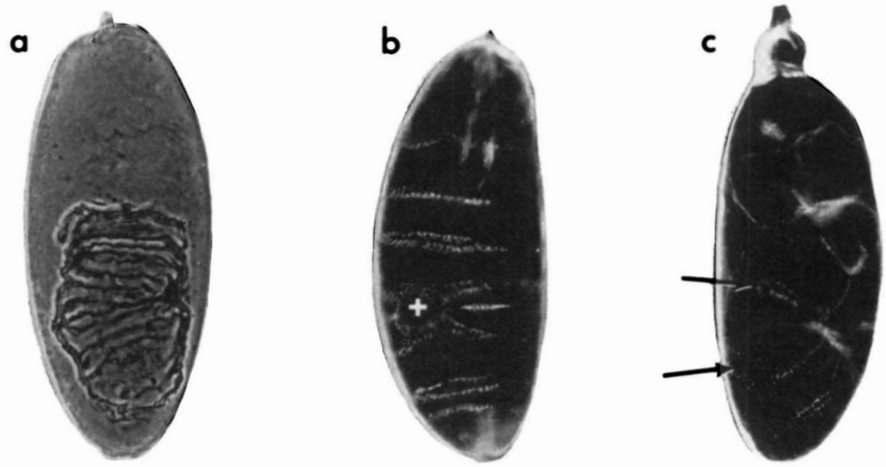


FIGURE 5.—Embryonic phenotype of pecanex (*pcx*) and *k10* embryos. Photomicrographs of 24-hr *pcx* (*a*, phase contrast), a partially rescued *pcx* (*b*, dark field) and *k10* (*c*, dark field) embryos. The *pcx* embryo possesses only wrinkled, dorsal cuticle due to nervous tissue hypertrophy. The partially rescued *pcx* embryo (about 20% of the embryos derived from homozygous *pcx* mother crossed by wild-type males) appears normal except for ventral holes (+). The *k10* embryo (*c*, dark field) consists of a long twisted tube containing dorsal and variable amount of ventral (denticle belts at arrows). Note that the anterior "bottle neck" containing the micropyle (P) resembles the shape seen in the forming egg chamber (see WIESCHAUS, MARSH and GEHRING 1978). Similar phenotypes were obtained when *pcx* and *k10* embryos were derived from homozygous germ line clones within a wild-type soma, establishng and confirming the germ line dependence of these female sterile mutations. ×150.

The *fs(1)*pecanex (*pcx*) mutation was discovered in our laboratory (L. ENGSTROM, F. R. TURNER, J. CAULTON, C. KERN and A. P. MAHOWALD, unpublished data) during an EMS screen for female steriles. Subsequently, nine additional alleles were identified among *fs* mutations from MOHLER's (1977) collection. D. MOHLER (personal communication) also found that *pcx* is allelic to *mel(1)R* described by ROMANS, HODGETTS and NASH (1976). Based on unstained whole mounts the phenotype of *pcx* embryos, as described by ROMANS, HODGETTS and NASH (1976), included normal early development but subsequent disruption of most morphological systems. Subsequently, the phenotype has been shown to exhibit a hypertrophy of the nervous system (MAHOWALD 1983, Figure 5*a* and *b*) similar to the rescuable female sterile mutation, almondex (SHANNON 1972), and reminiscent of zygotically lethal neuralizing mutations (JIMENEZ and CAMPOS-ORTEGA 1982; LEHMANN *et al.* 1983). The *pcx* alleles are genetically rescuable, *i.e.*, *pcx/pcx* females crossed by wild-type males produce adult female offspring (however, this rescue is not fully penetrant; see Figure 5*b*), whereas *pcx/pcx* females crossed by *pcx* males produce only embryos with the characteristic *pcx* phenotype. However, ROMANS, HODGETTS and NASH (1976) described one allele (*mel(1)R°*) that was not genetically rescuable. Germ line clonal analysis establishes that the *pcx* phenotype is germ line dependent. Because of its phenotypic similarities to almondex, including a possible background-sensitive eye phenotype, the locus has been named pecanex. Since at least ten alleles exist and since females heterozygous for *pcx*

and all of the lethal mutations in the 2E2-F3 area are fertile, we conclude that the *pcx* locus probably cannot be mutated to a lethal state.

Five alleles of the *fs(1)k10* (*k10*) female sterile mutation exist. The first one, *k10*, was discovered by WIESCHAUS, MARSH and GEHRING (1978), and, subsequently, three alleles were found by D. MOHLER and one by L. ENGSTROM. All alleles are similar in phenotype and show the same phenotype over *Df(1)64c18*. Furthermore, *k10* complements all lethals of the 2E-F area with the exception of *L271*, which has been discussed earlier. These results suggest that like *pcx*, *k10* is probably a gene that cannot be mutated to a lethal state. We confirmed the result of WIESCHAUS, MARSH and GEHRING (1978), using the dominant female sterile technique, that *k10* is germ line dependent (Table 2). This mutation causes a dorsalization of egg chambers during oogenesis and, subsequently, of the chorion (WIESCHAUS, MARSH and GEHRING 1978). In addition the few embryos that develop from such eggs (about 1%) exhibit a partial dorsalization (WIESCHAUS 1980) with more severe effects on the anterior than on the posterior pattern (Figure 5*c*).

*Recessive-visible loci*: Two loci in this chromosomal region contain recessive-visible alleles.

No lethal alleles of the prune (*pn*) locus have been recovered. Since at least 12 alleles of *pn* exist (LINDSLEY and GRELL 1968), *pn* is probably not mutable to a lethal state. It should be noted that both *JC105* and *l(1)24* which uncover the lethal complementation group *JA127* also uncover the *pn* locus. *pn* appears to be a pure recessive visible mutation with no detectable maternal effect.

The kurz (*kz*) visible mutation has been shown by the preceding data to be an allele of a recessive lethal complementation group.

## DISCUSSION

The results of this study indicate that the ten bands within the region of the *X* chromosome between 2E1 and 3A1 contain at least 11 complementation groups. Eight of the 11 groups (73%) are mutable to lethal states, two to female sterility and one to a recessive visible (eye color) state only. We identified hybrid dysgenesis-induced lethal alleles at four loci. The average of 4.7 alleles for each lethal complementation group in the 2E-F region suggests that most of the complementation groups have been identified. However, it must be pointed out that in the extensive studies of the 3A1-3C1 interval 41 lethal mutations defined ten complementation groups (JUDD, SHEN and KAUFMAN 1972), but later 344 mutations defined 15 lethal complementation groups (cited in LEFEVRE 1981).

Based on our analysis of the loci within this relatively small chromosomal region, four classes of genes with significant effects on early development were identified: (1) strict maternal effect lethal, (2) maternal effect rescuable, (3) recessive embryonic lethals with specific effects on the developmental pattern and (4) recessive lethals with maternal effects on development (characterized by germ line clone analysis). The loci with significant influences on embryonic development occur in two clusters, one at 2E3 and another at 2F6-3A1. During complementation analysis no interactions among genes within these regions

were found. Further genetic and molecular analyses may reveal whether the proximities of these developmentally influential genes have any significance or not.

*Class 1*: We characterized one mutation *fs(1)k10* with a strict maternal effect phenotype. The *fs(1)k10* alleles result in a "dorsal-like" phenotype as described by WIESCHAUS, MARSH and GEHRING (1978) and WIESCHAUS (1980). At least ten female sterile loci in Drosophila exhibit a similar phenotype (ANDERSON and NÜSSLEIN-VOLHARD 1983). Two such loci have been extensively studied: dorsal, on the second chromosome (NÜSSLEIN-VOLHARD 1979), and gastrulation defective, on the *X* chromosome (KONRAD and MAHOWALD 1984). This dorsalized phenotype has been interpreted as either a defect in positional information in the egg (NÜSSLEIN-VOLHARD 1979) or a failure to form ventral invaginations (KONRAD and MAHOWALD 1984). The dorsalization of *k10* embryos appears to be a function of the anterior-posterior axis as has been noted in gastrulation-defective embryos (K. KONRAD, personal communication) but not in dorsal embryos (ANDERSON and NÜSSLEIN-VOLHARD 1983). Because *k10* is unique among the female sterile mutations producing dorsalized embryos in that its effects are visible during oogenesis, it may be one of the earliest acting genes influencing dorsal-ventral embryonic pattern determination in Drosophila.

*Class 2*: We characterized the *pcx* locus that belongs to the class of neurogenic loci as a rescuable maternal effect mutation. Among such loci two rescuable female steriles, almondex (*amx*) (SHANNON *et al.* 1972) and pecanex (*pcx*), and six embryonic lethals (LEHMANN *et al.* 1983) have now been identified. Three of these neuralizing genes are expressed maternally and zygotically as shown by germ line clone analysis of Notch mutations (JIMENEZ and CAMPOS-ORTEGA 1982; PERRIMON, ENGSTROM and MAHOWALD 1984) and by the zygotic rescue of *amx* and *pcx*.

*Class 3*: Two embryonic recessive lethal loci were characterized. Germ line clone analysis revealed that one of them, *crn*, is required for germ line viability. This is quite surprising since most embryonic recessive lethals with effects on embryonic patterning do not exhibit maternal effects (LAWRENCE, JOHNSTON and STRUHL 1983; E. WIESCHAUS, personal communication; N. PERRIMON, unpublished observations) as illustrated here for the *gt* locus.

*Class 4*: We characterized one recessive lethal, with a pupal lethal phase, that exhibits maternal effects on embryonic development. *l(1)*pole hole is functionally required both maternally and zygotically and will be described elsewhere (N. PERRIMON, L. ENGSTROM and A. P. MAHOWALD, unpublished results).

In a previous germ line clonal analysis of recessive lethal mutations at 48 loci on the *X*-chromosome, 73% of these loci exhibited effects on germ line function (PERRIMON, ENGSTROM and MAHOWALD 1984). We have two concerns when we use the germ line clone technique to demonstrate maternal effects: (1) that an individual mutation may not represent the null state of the gene and (2) that a second lethal mutation may be present on the same chromosome as the lethal being examined. In this study we have performed germ line clonal analyses on multiple alleles at each locus and have found that these concerns

must indeed be considered. For example, the *VA172* mutation may represent an hypomorphic lethal allele, and the embryonic lethality of *EF462* compared with other alleles must be due to a second mutation located outside of $Dp(1;Y)w^{+303}$. These two exceptional alleles represent 9% of the 23 lethal alleles tested. Because of this low frequency, our (PERRIMON, ENGSTROM and MAHOWALD 1984) estimate of frequencies of lethals with either no maternal effect or maternal effect or germ line lethality was probably not significantly influenced by the fact that we tested only one allele per locus.

In our previous study we (PERRIMON, ENGSTROM and MAHOWALD 1984) identified several mutations that exhibited a progressive degeneration of germ line function in clones that we interpreted as being due either to a perdurance of wild-type product in germ line clones, to residual (hypomorphic) activity of the lethal mutation or both. In this study we found an example in which a few clones of one allele (*HF311*) produced some eggs at first, but then regressed to previtellogenic arrest (germ line lethality), whereas no clones of another allele (*HF330*) produced eggs. The different behavior of these alleles in germ line clones must reflect differences in functional levels.

The combined techniques of careful traditional genetics, morphological examination and germ line clone analysis employed here should prove useful in the study of other areas of the genome of Drosophila. Indeed, such combined studies may be necessary if we are to understand the genetic contributions to development of zygotic lethal genes.

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## LITERATURE CITED

ANDERSON, K. V. and C. NÜSSLEIN-VOLHARD, 1983 Genetic analysis of dorsal-ventral embryonic pattern in *Drosophila*. In: *Primers in Developmental Biology*, Edited by G. MALACINSKI and S. BRYANT. Macmillan Press, New York. In Press.

BRIDGES, C. B., 1938 A revised map of the salivary gland X-chromosome of *Drosophila melanogaster*. J. Hered. **29:** 11–13.

CRAYMER, L. and E. ROY, 1980 New mutants. Drosophila Inform. Serv. **55:** 200–204.

GERASIMOVA, T. I. and E. V. ANANJEV, 1972 Cytogenetical localization of structural gene *Pgd* for 6-phosphogluconate dehydrogenase in *Drosophila melanogaster*. Drosophila Inform. Serv. **48:** 93.

HONISH, S. and J. A. CAMPOS-ORTEGA, 1982 *Giant* a segment specific defect during embryonic development. Drosophila Inform. Serv. **58:** 76–77.

JIMENEZ, F. and J. A. CAMPOS-ORTEGA, 1982 Maternal effects of zygotic mutants affecting early neurogenesis in *Drosophila*. Wilhelm Roux' Arch. **191:** 191–201.

JUDD, B. H., M. W. SHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the *X* chromosome of *Drosophila melanogaster*. Genetics **71:** 139–156.

KAUFMAN, T. C., M. P. SHANNON, M. W. SHEN and B. H. JUDD, 1975 A revision of the cytology

and ontogeny of several deficiencies in the 3A1-3C6 region of the *X* chromosome of *Drosophila melanogaster*. Genetics **79:** 265–282.

KONRAD, K. D. and A. P. MAHOWALD, 1984 Genetic and developmental approaches to understanding determination in early development. pp. 167–188. In: *Molecular Aspects of Early Development*, Edited by G. MALACINSKI and W. KLEIN. Plenum Press, New York.

LAWRENCE, P. A., P. JOHNSTON and G. STRUHL, 1983 Different requirements for homeotic genes in the soma and germ line of *Drosophila*. Cell **35:** 27–34.

LEFEVRE, G., 1981 The distribution of randomly recovered x-ray induced sex-linked genetic effects in *Drosophila melanogaster*. Genetics **99:** 461–480.

LEHMANN, R., F. JIMENEZ, U. DIETRICH and J. A. CAMPOS-ORTEGA, 1983 On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. Wilhelm Roux' Arch. **192:** 62–74.

LEWIS, E. G., 1978 A gene complex controlling segmentation in Drosophila. Nature **276:** 565–570.

LEWIS, R. A., T. C. KAUFMAN, R. E. DENELL and P. TALLERICO, 1980a Genetic analysis of the Antennapedia Gene Complex (ANT-C) and adjacent chromosomal regions of *Drosophila melanogaster*. I. Polytene chromosome segments 84B-D. Genetics **95:** 367–381.

LEWIS, R. A., B. T. WAKIMOTO, R. E. DENELL, and T. C. KAUFMAN, 1980b Genetic analysis of the Antennapedia Gene Complex (ANT-C) and adjacent chromosomal regions of *Drosophila melanogaster*. II. Polytene chromosome segments 84A-84B1,2. Genetics **95:** 383–397.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

MAHOWALD, A. P., 1983 Genetic analysis of oogenesis and determination. pp. 349–363. In: *Time, Space and Pattern in Embryonic Development*, Edited by JEFFERY, W. RAND and R. A. RAFF. Alan R. Liss, Inc., New York.

MOHLER, J. D., 1977 Developmental genetics of the *Drosophila* egg. I. Identification of 50 sex-linked cistrons with maternal effects on embryonic development. Genetics **85:** 259–272.

NÜSSLEIN-VOLHARD, C., 1979 Maternal effect mutations that alter the spatial coordinates of the embryos of *Drosophila melanogaster*. pp. 185–211. In: *Determinants of Spatial Organization*, Edited by S. SUBTELNY and I. R. KONIGSBERG. Academic Press, New York.

PERRIMON, N., L. ENGSTROM and A. P. MAHOWALD, 1984 Analysis of the effects of zygotic lethal mutations on the germ line functions in *Drosophila*. Dev. Biol. In Press.

PERRIMON, N. and M. GANS, 1983 Clonal analysis of the tissue specificity of recessive female sterile mutations of *Drosophila melanogaster* using a dominant female sterile mutation, *Fs(1)K1237*. Dev. Biol. **100:** 365–373.

ROMANS, P., R. B. HODGETTS and D. NASH, 1976 Maternally influenced embryonic lethality: allele specific genetic rescue at a female fertility locus in *Drosophila melanogaster*. Can. J. Genet. Cytol. **18:** 773–781.

SHANNON, M. P., 1972 Characterization of the female-sterile mutant *almondex* of *Drosophila melanogaster*. Genetica **43:** 244–256.

SHANNON, M. P., T. C. KAUFMAN, W. M. SHEN and B. H. JUDD, 1972 Lethality patterns and morphology of selected lethal and semi-lethal mutations in the zeste-white region of *Drosophila melanogaster*. Genetics **72:** 615–638.

SORSA, V. and A. O. SAURA, 1980 Electron microscopic analysis of the banding pattern in the salivary gland chromosomes of *Drosophila melanogaster*: division 1 and 2 of X. Hereditas **92:** 73–83.

VAN DER MEER, J., 1977 Optical clean and permanent whole mount preparations for phase contrast microscopy of cuticular structures of insect larvae. Drosophila Inform. Serv. **52:** 160.

WIESCHAUS, E., 1980 A combined genetic and mosaic approach to the study of oogenesis in *Drosophila*. pp. 85–94. In: *Development and Neurobiology of Drosophila*, Edited by O. SIDDIQI, P. BABU, L. M. HALL and J. C. HALL. Plenum Press, New York.

WIESCHAUS, E., J. L. MARSH and W. J. GEHRING, 1978 *fs(1)K10*, a germ-line dependent female sterile mutation causing abnormal chorion morphology in *Drosophila melanogaster*. Wilhelm Roux' Arch. **184:** 75–82.

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