DEVELOPMENTAL GENETICS OF THE 2C-D REGION OF THE DROSOPHILA *X* CHROMOSOME

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ABSTRACT

We have conducted a genetic and developmental analysis of genes within the 2C-D area of the *X* chromosome. Phenotypes of 33 mutations representing nine adjacent complementation groups including eight recessive lethals and one visible homeotic mutation (polyhomeotic) are described. Germline clonal analysis of the eight zygotic lethals has revealed three types of gene requirements: (1) normal activity at two pupal lethal loci (corkscrew and *C204*) and one larval lethal locus (ultraspiracle) is required for normal embryogenesis; (2) normal activity at three larval lethal loci (*DF967, VE651* and *Pgd*) is required for normal oogenesis; and (3) activity at only one locus (*EA82*), a larval lethal, appears to have no maternal requirement. Ambiguous results were obtained for the *GF316* lethal complementation group. Analysis of mitotic figures of the pupal lethals indicates that *C204* disrupts an essential mitotic function. This result correlates with the preblastoderm arrest observed among embryos derived from germline clones of *C204*. Embryos derived from germline clones of corkscrew (*csw*) exhibit a "twisted" phenotype. The recessive lethal ultraspiracle (*usp*) disrupts the organization of the posterior tip of the larva both zygotically and maternally: second instar *usp/Y* larvae derived from heterozygous *usp/+* mothers possess an extra set of spiracles, whereas *usp/Y* embryos derived from females possessing a germline clone (*usp/usp*) exhibit a localized ventral defect in the ninth or posterior eighth abdominal segment. Analysis of the phenotypes of deficiency-hemizygous embryos indicates the presence of an embryonic zygotic lethal locus, as yet unidentified, which produces central nervous system and ventral hypoderm degeneration. Additional information on the genetic organization of loci within the adjacent 2E area are also described. The implications of this analysis to our understanding of the maternal role of zygotic lethal loci in development are discussed.

ANALYSIS of genes whose mutations disrupt a specific developmental step may provide clues concerning the determinative events leading to embryonic pattern formation. In Drosophila, such genes are usually identified by mutations that result in abnormal embryonic phenotypes. It is proposed that a set of maternal genes called maternal effect lethal (MEL) mutations organize the overall embryonic pattern sequence, whereas zygotic genes (embryonic

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zygotic lethal mutations) interpret this maternal information (NUSSLEIN-VOLHARD 1979; NUSSLEIN-VOLHARD and WIESCHAUS 1980; GEHRING 1984).

Results from extensive mutagenesis experiments indicate that among all loci in Drosophila about 1% produce MEL phenotypes (MAHOWALD *et al.* 1984) and 2.5% produce embryonic lethal phenotypes (NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984) that dramatically disrupt embryonic patterning. Because the majority of loci (about 85%) in the Drosophila genome belong to the zygotic lethal class (SHANNON *et al.* 1972; HOCHMAN 1973), it is crucial for a full understanding of embryogenesis to determine their contributions. Evaluation of any maternal effects is one portion of such a study. By analyzing germline clones of zygotic lethals, we have recently provided evidence that genes with vital functions (*i.e.*, recessive lethals) also exhibit specific maternal effects similar to those exhibited by female sterile (MEL) and embryonic lethal mutations (PERRIMON, ENGSTROM and MAHOWALD 1984a,b). It is necessary to extend such analyses to a broad range of zygotic lethal loci to determine their maternal effects. One method of approach is to analyze in details specific areas of the genome. We have undertaken such an analysis on the *X* chromosome because of the availability of a dominant female sterile mutation, *Fs(1)K1237* (BUSSON *et al.* 1983; PERRIMON 1984). This mutation allows us to rapidly screen for developmental effects of a large set of zygotic lethal mutations when homozygous in the female germline. This approach combined with classical genetic analyses permits us to systematically analyze the contribution of sets of adjacent genes to oogenesis and embryonic development. We have previously described the developmental genetics of the 2E-F area (PERRIMON, ENGSTROM and MAHOWALD 1984b). In this paper the phenotypes of genes within 2C-D are described.

MATERIALS AND METHODS

Stocks: Except as mentioned in the legend of Table 3, all lethal mutations used in this analysis were obtained from G. LEFEVRE. Those designated by the letters E, V or D are ethylmethane sulfonate (EMS) induced. Others are X ray induced. All lethal mutations are kept either in *FM7* or *FM6* balanced stocks or in attached-*X* stocks *C(1)DX, y f/Y* carrying $Dp(1;Y)w^{+303}$ or $Dp(1;3)w^{vco}$. The dominant female sterile mutation *Fs(1)K1237* (or Ovo^{D1}) is maintained in males in an attached-*X* stock, *C(1)DX, y f/K1237* v^{24}/Y. Descriptions of balancers and mutations can be found in LINDSLEY and GRELL (1968). Flies were grown on standard Drosophila medium at 25 ± 0.5°. In this report all lethals and female steriles are abbreviated by omitting the prefix symbols *l(1)* and *fs(1)*, respectively.

Genetic analysis: Each recessive lethal mutation was tested for complementation with deficiencies, duplications and other lethal mutations as described by PERRIMON, ENGSTROM and MAHOWALD (1984b).

Methodology for evaluating the maternal effect of zygotic lethals: Lethal phases were determined by two methods: (1) the lethal phase of *1/Y* male offspring of heterozygous lethal-bearing mothers (*+/1*) and wild-type fathers (*+/Y*), and 2) lethal male (*1/Y*) offspring of attached-*X* mothers (*C(1)DX,y f /Y*) and lethal-duplication fathers ($l/Dp(1;Y)w^{+303}$). If *1/Y* progeny of cross 1 (derived from heterozygous mother) die at an earlier stage (or show a more extreme embryonic phenotype, in the case of embryonic zygotic lethals) than those obtained from cross 2, then it suggests a maternal requirement for the gene product involved (the maternal effect is detectable in mutant offspring). All lethal mutations were examined by germline clonal analyses as previously described (PERRIMON, ENGSTROM and MAHOWALD 1984a) utilizing the dominant female sterile technique

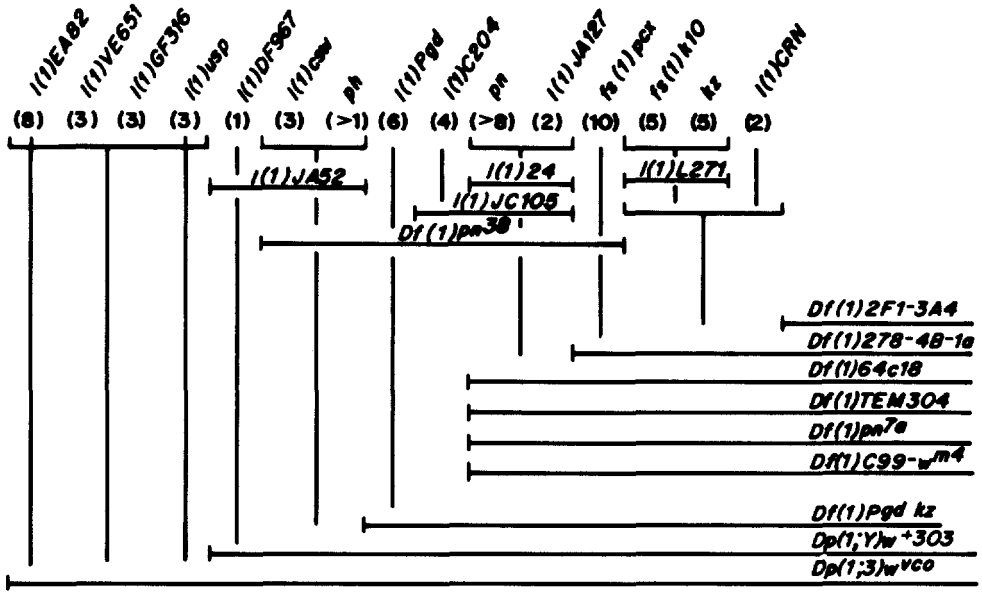

FIGURE 1.—Genetic map of the 2C-D area. Deficiencies and duplications are represented (see Table 1 for their breakpoints). Complementation groups are presented relative to their inclusion within these deficiencies and duplications. The relative order of genes grouped above open bars is unknown. The number of alleles at each complementation group is indicated in parentheses (see Table 3). *L271, l(1)24, JC105* and *JA52* are depicted as deficiencies based on their complementation patterns. Flies of genotype *JA52/Df(1)Pgd kz* are lethal, suggesting the presence of an additional, as yet unidentified, recessive lethal complementation group distal to *Pgd*. See text for the additional informations concerning the 2E area.

(PERRIMON and GANS 1983; PERRIMON 1984). A maternal effect is indicated when *1/Y* progeny derived from germline clones die earlier than when derived from heterozygous mothers.

Embryonic phenotypes were examined using cuticular whole mounts (VAN DER MEER 1977).

DNA staining of ovaries and embryos: Ovaries were dissected in fresh Ringer's (EPHRUSSI and BEADLE 1936) solution and spread on slides coated with poly L-lysine (1 mg/ml) from Sigma Chemical Company. The Ringer's solution was removed and tissues were incubated for 5–10 min in a drop of Hoechst 33342 (1 μg/ml dissolved in Ringer's solution). The ovaries were washed in Ringer's, fixed in acetone at −20° for 5 min, washed again in Ringer's and mounted in 90% glycerol. Slides were inspected under epifluorescent illumination.

Patterns of nuclei in early embryos were analyzed by Hoechst-DNA staining, a technique modified from MITCHISON and SEDAT (1983) and described by DEQUIN, SAUMWEBER and SEDAT (1984).

Detection of mitotic figures: Whole brains were dissected from third instar larvae, treated 2 hr in colchicine and squashed individually on slides (GUEST and HSU 1973), and mitotic figures were examined and counted.

RESULTS

Genetic organization of 2C-D: We have analyzed 33 mutations representing nine complementation groups within the 2C-D region of the *X* chromosome. The complementation groups within 2C-D are delimited distally by the distal breakpoint of $Dp(1;3)w^{vco}$ and proximally by the distal breakpoints of *Df(1)64c18, Df(1)TEM304,* $Df(1)pn^{7a}$ and $Df(1)C99\text{-}w^{m4}$. Figure 1 shows a map

TABLE 1

List of rearrangements in the 2C-D area

Rearrangement	Cytology	References
Df(1)64c18	*Df(1)2E1-2;3C2*	CRAYMER and ROY (1980)
Df(1)TEM304	*Df(1)2E2-F1;3A4-6*	J. LIM (personal communication)
Df(1)pn^{7a}	*Df(1)2E1;3A4*	ILYINA *et al.* (1980)
Df(1)c99-w^{m4}	*Df(1)2E1-2;3C1-2*	G. LEFEVRE (personal communication)
Df(1)Pgd kz	*Df(1)2D3-4;2F5*	GERASIMOVA and ANANJEV (1972)
Df(1)pn^{38}	*Df(1)2D3-4;2E3*	SLOBODYANYUK and SEROV (1983)
Df(1)278.4B.1a	*Df(1)2E3-F3;3A5-B4*	T. WU (personal communication)
Df(1)2F1-3A4	*Df(1)2F1;3A4*	M. GREEN (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)

Cytotological breakpoints of duplications (Dp) and deficiencies (Df) used in mapping the loci in the region are given.

of the entire 2C-E region including a revision of the 2E region and Table 1 indicates the cytology of rearrangements used in this study.

Four distal complementation groups represented by 17 recessive lethal mutations are covered by *Dp(1;3)w*^{vco} but not by *Dp(1;Y)w*$^{+303}$. The *EA82* locus is represented by eight alleles, two of which (*EA82*^{HC207} and *EA82*^{C212}) are cytological rearrangements with breakpoints in salivary chromosome band 2C3 (G. LEFEVRE, personal communication); thus, this complementation group is at that position. Similarly, since one (*usp*^{KA21}) of the three alleles of the ultraspiracle (*usp*) locus is a rearrangement with a breakpoint in 2C9 (G. LEFEVRE, personal communication), this complementation group is located at that position. The two remaining complementation groups, *VE651* (three alleles) and *GF316* (three alleles) cannot be positioned as accurately but lie between the distal breakpoints of *Dp(1;3)w*^{vco} (2B17-C1) and *Dp(1;Y)w*$^{+303}$ (2D1-2).

Five complementation groups are located between the distal breakpoints of *Dp(1;Y)w*$^{+303}$ and *Df(1)64c18* (2E1-2). These groups are more accurately localized by the breakpoints of *Df(1)pn*^{38} (2D3-4;2E3) which does not uncover *DF967*, placing it in 2D1-2. The exact linear positions of corkscrew (*csw*) and polyhomeotic (*ph*) cannot be determined, but they lie between the distal breakpoints of *Df(1)pn*^{38} and *Df(1)Pgd kz* (2D3-4). This cytological location is supported by the fact that the *csw*^{KC16} allele of the *csw* locus is a rearrangement with a breakpoint in 2D3-5 (G. LEFEVRE, personal communication). The two more proximal loci, phosphoglucodehydrogenase (*Pgd*) and *C204*, are positioned in 2D4-6 with *C204* being more proximal because it is uncovered by *JC105* which also uncovers *pn* and *JA127* in 2E (PERRIMON, ENGSTROM and MAHOWALD 1984b).

In addition, we have characterized a mutation, *JA52*, which fails to complement three complementation groups in 2C-D (*DF967, csw* and *ph*). In our previous description of loci located proximal to *C204* (PERRIMON, ENGSTOM and MAHOWALD 1984b), we positioned prune (*pn*) distal to *JA127* based upon its inclusion in *Df(1)Pgd*^{35}. The present complementation analysis in 2C-D,

TABLE 2

Lethal phase comparison of Dp(1;Y)w^{+303}, Df(1)Pgd kz, JA52, Df(1)pn^{38} *and controls (yf)*

	N	*N* unh	%f unf	%f *y*	%f *y*$^+$
yf/yf × *yf/Y*	720	65	8.5	0.6	0
yf/yf × *yf/DpY*	220	48	2.3	20	0
XX/Y × *yf/Y*	123	42	33	1[a]	0
XX/Y × *yf/DpY*	397	228	47	14[a]	0
yf/Df(1)Pgd kz × *yf/Y*	420	156	9	4	27
XX/Y × *Df(1)Pgd kz/DpY*	121	68	27.2	10[a]	19[a]
yf/yf × *Df(1)Pgd kz/DpY*	184	82	26.6	22	2
yf/JA52 × *yf/Y*	148	43	4	2	24
XX/Y × *JA52/DpY*	194	142	55	11[a]	18
yf/yf × *JA52/DpY*	111	29	8	18	1[a]
yf/Df(1)pn^{38} × *yf/Y*	126	51	11	3	29
XX/Y × *RC63/DpY*	174	88	23	12[a]	14[a]

N, number of eggs examined; *N* unh, number of unhatched eggs; %f unf, percentage of embryos exhibiting no cuticle or unfertilized. The percentage of lethality of yellow and yellow$^+$ embryos is indicated as %f *y* and %f *y*$^+$, respectively, and was derived by *N y* (or *N y*$^+$)/*N* − *N* unf. Triplo-*X* individuals die at larval or pupal stages. *Df/Y* embryos were easily detected from *XX/DpY* dead embryos because the *XX* carry the embryonic marker yellow. *Dp(1;Y)w*$^{+303}$ (symbolized as *DpY*) has a partial diplo-lethal effect in male embryos and a partial triplo-lethal effect in female embryos (see Figure 2B).

[a] When crosses involved attached-*X* [*C(1)DX, y f/Y*] females (symbolized as *XX*), %f *y* and %f *y*$^+$ have been corrected by ¾ in order to take into account *Y/Y* embryos that were scored as unfertilized eggs.

however, reveals that our *Df(1)Pgd*^{35} stock is not a deficiency but is probably *l(1)35* (a recessive lethal allele of *Pgd*) which was induced on a prune-bearing chromosome (GVOZDEV *et al.* 1975). Therefore, the relative positions of *pn* and *JA127* are unknown. The present analysis allows us to position the female sterile locus *fs(1)*pecanex (*pcx*) distal to *fs(1)k10*, kurz (*kz*) and *l(1)* crooked neck because *pcx* is uncovered by *Df(1)pn*^{38} (2D3-4; 2E3). Because females of genotype *Df(1)pn*^{38}*/Df(1)278.4B.1a* are viable and female sterile (with the *pcx* phenotype), no other lethal complementation group exists between the two deficiency breakpoints.

Phenogenetic behavior of Dp(1;Y)w^{+303} (DpY): Because we used *Dp(1;Y)w*$^{+303}$ (referred to as *DpY*) in lethal phase determinations, it was necessary to evaluate the viability of flies carrying this duplication (Table 2). When *y f/y f* females are mated to *y f/DpY* males, *y f/DpY* sons have reduced viability. Only 61% of them are recovered (compared to the viability of their *y f/y f* sibling sisters). Because male and female progeny from the cross *y f/y f* × *y f/Y* show the same viability, *DpY* must be responsible for this reduced viability. To assess whether this *DpY* lethality was embryonic or not, hatchability frequencies were determined (Table 2). In the control no lethality is observed among progeny of *y f* mothers and *y f* fathers, but about 20% embryonic lethality is observed when

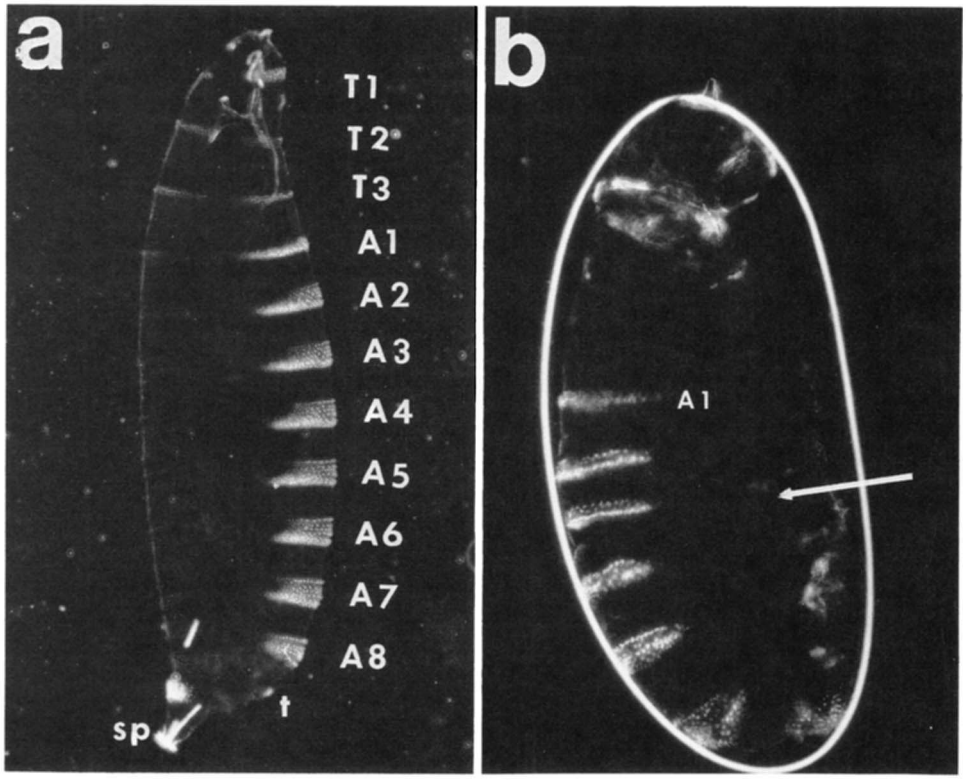

FIGURE 2.—The lethal phenotype of $Dp(1;Y)w^{+303}$. Dark-field photomicrograph of the lateral aspect of a wild-type larva is shown in (a). The thoracic (T1, T2, T3) and abdominal (A1 through A8) denticle belts are indicated, as are the posterior spiracles (sp) and anal tuft (t). Dead embryos of genotype $+/Dp(1,Y)w^{+303}$ exhibit a U-shaped phenotype (b) with abnormal head morphology and aminoserosal defects (arrow).

y f mothers are mated to *y f/DpY* fathers. This number correlates with the 61% recovery of adult males (compared to the sibling females). The phenotype exhibited by these unhatched embryos is consistent (Figure 2b). Defects seem to be associated with germ band shortening, with such embryos showing abnormal head involution and defects in the amnioserosa. To determine whether this lethality is specific to males or not, crosses involving attached-*X* mothers (*C(1)DX, y f/Y*) were performed. Again, a fraction of embryos (Table 2) do not hatch and exhibit the U-shaped phenotype shown in Figure 2b. In such crosses *Y/DpY* embryos are also produced; however, they do not reach cuticular formation stages and are scored as unfertilized. To take into account such embryos the frequency of embryonic lethality is corrected (% frequency × ¾) in Table 2. This corrected frequency is lower than that among *y f/DpY* males, indicating that females carrying an extra dose of *DpY* may be less sensitive to lethality than their sibling brothers. These results indicate the presence of a gene within $Dp(1;Y)w^{+303}$ which, when present in excess dosage, causes a partial dominant lethality both in males and in females. Furthermore, we found this dominant embryonic lethality to be temperature sensitive, *i.e.*, a higher fre-

quency of unhatched embryos are observed as the temperature is increased (results not shown).

A second characteristic of *Dp(1;Y)w*$^{+303}$ concerns the viability of mutant/*DpY* sons for the most distal locus (*DF967*) covered by this duplication. In the cross *y f/DF967* × *y f/DpY* the recovery of *DF967/DpY* males is only 34% (compared to the viability of their *y f/DpY* brothers). Similarly, *JA52/DpY* males are recovered at 26%. These results can be explained by position effect on the *DF967* gene (the first locus covered by *DpY*) since such poor recovery of mutant/*DpY* males is not observed at other lethal loci within 2C-D (results not shown).

Phenotype of embryos hemizygous for the deficiencies: We will only describe the embryonic lethal phenotypes of *JA52*, *Df(1)pn*^{38} and *Df(1)Pgd kz* since the phenotypes of the other deficiencies shown in Figure 1 can be found in PERRIMON, ENGSTOM and MAHOWALD (1984b).

To evaluate the influence of maternal effects of genes within the deficiencies, lethal phases were determined when the deficiency was maternally (*i.e.*, +/*Df* × +/*Y*) or paternally inherited (*i.e.*, *C(1)DX/Y* × *Df/DpY*). If the *Df/Y* embryonic phenotypes obtained from these two sets of crosses are compared, it can be determined whether the *Df* uncovers genes exhibiting maternal effects (*i.e.*, heterozygosity of at least one gene within the *Df* is responsible for differences observed in the resulting *Df/Y* progeny). It should also be noted that, if lethality among progeny derived from cross 1 is significantly higher than 25%, then heterozygosity of at least one gene within the *Df* is imposing a partially dominant lethality on other classes of progeny (*i.e.*, of genotype +/*Y*, *Df*/+ or +/+). Because *DpY* is used in the second set of crosses, it was expected that a class of dead embryos of genotype *C(1)DX/DpY* would be mixed with the *Df/Y* embryos. *C(1)DX/DpY* embryos were easily identified by utilizing the embryonic marker yellow (*y*) which is carried by the *C(1)DX* compound chromosome. *Df(1)pn*^{38} was analyzed using only cross 1 since we were unable to recover *Df(1)pn*^{38}*/DpY* males. This was probably due to the presence of another *X*-linked lethal on the *Df(1)pn*^{38} chromosome. In the case of *JA52* similar results are observed in both sets of crosses. Embryos hemizygous for *JA52* or *Df(1)pn*^{38} exhibit similar embryonic phenotypes (Figure 3); they possess no ventral cuticular structures. We do not presently have an embryonic lethal complementation group within *JA52* or *Df(1)pn*^{38} that exhibits this phenotype; however, the phenotype observed among the deficiency-bearing embryos suggests that an unidentified embryonic zygotic lethal locus does exist.

Differences in phenotypes are observed among embryos produced by cross 1 *vs.* cross 2 when *Df(1)Pgd kz* is analyzed. When *Df(1)Pgd kz* is maternally inherited, the phenotype of unhatched embryos is quite variable. These embryos possess poorly differentiated cuticles with ventral cuticular holes, and, in addition, they have a twisted phenotype which can be attributed to the crooked neck (*crn*) locus (PERRIMON, ENGSTROM and MAHOWALD 1984b). However, when unhatched embryos derived from cross 2 are examined, they do not exhibit any major pattern defects. Since *crn* is located within *Df(1)Pgd kz*, this last result suggests that *crn* embryos derived from the cross *C(1)DX/Y* × *crn/*

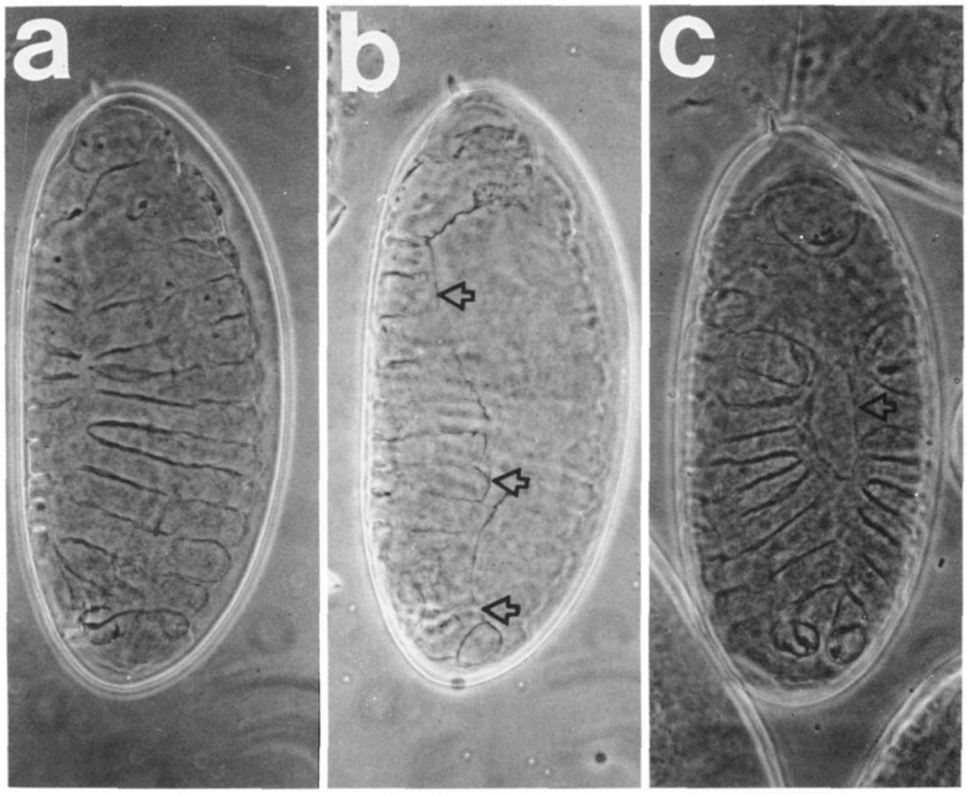

FIGURE 3.—Embryonic phenotype of *JA52* and *Df(1)pn*^{38} hemizygous embryos. Note the poor cuticle differentiation and lack of all ventral structures (right side of arrows in b). (a) and (b) are two views of the same embryo showing lacks of all ventral structures. Dorsal defects in the amniosera are also found occasionally in such embryos (arrow in c).

DpY should not exhibit the twisted phenotype. Since this is indeed the case for *crn*^{RC63} (Table 2), maternal heterozygosity of *crn* must be responsible for the twisted phenotype. This is also supported by our previous work showing that *crn* is required for germline viability (PERRIMON, ENGSTROM and MAHOWALD 1984b). Because *Df(1)Pgd kz* embryos derived from cross 1 exhibit other defects, there must be another, as yet unidentified, gene(s) inside the deficiency that exhibits a maternal effect detectable in mutant offspring. Alternatively, additivity of maternal effects of genes within the deficiency may be responsible for such phenotypes.

No significant dominant lethality due to the maternal haplo-insufficiency of genes within *Df(1)Pgd kz, 1(1)JA52* or *Df(1)pn*^{38} is observed since the expected fraction of unhatched embryos is obtained from females heterozygous for those deficiencies (Table 2).

Larval lethal loci: We identified five larval lethal loci within the 2C-D area. These are *EA82, VE651*, ultraspiracle (*usp*), *DF967* and mutations at the Phosphoglucodehydrogenase (*Pgd*) locus (Table 3).

Eight alleles of the *EA82* complementation group were characterized. Two

TABLE 3

Germline clone analysis of mutations within 2C-D area

Mutation	Dp	Lethal phase	N	Ne	Nd	Nc	Phenotype
C204	+	L3-EP	174	13	NT	NT	MEL
HC262	+	L3-EP	213	9	NT	NT	MEL
11P3	+	L3-EP	50	4	NT	NT	MEL
A17	+	EP	210	7	NT	NT	NME
Pgd^{n1}	+	L2	350	0	100	5	AO
Pgd^{n2}	+	L2	300	0	140	3	AO
VE618	−	L2-L3	310	0	100	6	AO
135	+	L2-L3	250	0	120	2	AO
VA55	+	L1-L2	350	0	120	3	AO
DF958	+	L2-L3	763	0	763	30	AO
[a]*ph*^{k}	+	L2-L3-P	160	5	NT	NT	NME
JA52	+	E	275	0	150	0	L
C114	+	L3-EP	190	11	NT	NT	MEL
VA199	+	L3-EP	200	10	NT	NT	MEL
DF967	+	L2-L3	320	0	200	0	L
VE653	+	L1-L2	180	5	NT	NT	MEL
VE849	+	E-L1-L2	220	6	NT	NT	MEL
GF316	+	E	270	8	NT	NT	MEL
GA55	+	L2-L3	190	10	NT	NT	NME
DF943	+	L-EP-A	170	7	NT	NT	NEM
VE651	+	L2-L3	250	0	49	5	AO
VA56	−	L1-L2	350	0	248	2	AO
VE782	+	L2-L3-P-A	150	0	85	3	AO
EA82	+	L2-L3	210	8	NT	NT	NME
EA43	+	L2-L3	210	10	NT	NT	NME
GA17	−	E	300	11	NT	NT	NME
A115	+	L1-L2-L3	NT	NT	NT	NT	NT
VE692	−	L3	160	7	NT	NT	NME
HC288	+	L2-L3-EP	250	9	NT	NT	NME
HC207	+	E-L1-L2-L3-EP	185	0	130	8	AO

Lethal alleles are presented by complementation groups. When lethal males are recovered over *Dp(1;Y)w*$^{+303}$ (loci proximal to *usp*) or over *Dp(1;3)w*^{vco} (loci distal to *DF967*) a "+" is indicated in the duplication (Dp) column. The lethal phase(s) of each recessive lethal mutation is indicated as follows: E, embryonic; L1, L2, L3, larval instars; P, pupal; A, adult. *N*, number of irradiated females; *Ne*, number of females producing eggs; *Nd*, number of females dissected; *Nc*, number of females containing clones. The phenotypes of the homozygous lethal germline clones are: L, lethal in germline clones; AO, abnormal oogenesis; MEL, maternal effect lethal; NME, no maternal effect. NT, not tested.

We previously analyzed four mutations (*Pgd*^{DF958}, *usp*^{VE653}, *DF967* and *EA82*^{VE692}) and obtained similar results (PERRIMON, ENGSTROM and MAHOWALD 1984a). One slight difference was observed. In the earlier study, mutant *usp*^{VE653} also produced abnormal eggs. In this study, the stocks we used were "cleaned" and no such eggs were found.

Pgd^{n1} and *Pgd*^{n2} were obtained from A. C. CHRISTENSEN, *ph*^{k} from K. KONRAD and *C204*^{11P3} was EMS induced by us.

[a] The polyhomeotic allele we tested (*ph*^{k}) is subvital; larval and pupal lethality is observed.

of them, $EA82^{C212}$ and $EA82^{HC207}$, are the cytological rearrangements mentioned earlier. Five alleles (*EA82*, $EA82^{EA43}$, $EA82^{A115}$, $EA82^{VE692}$ and $EA82^{HC288}$) have a larval lethal phase and exhibit no maternal effect in germline clonal analysis. Two mutations, $EA82^{VE692}$ and $EA82^{GA17}$ are apparently associated with additional X-linked lethal(s) since no viable offspring are recovered when they are covered by a duplication (Table 3). $EA82^{GA17}$ has an embryonic lethal phase (no embryonic pattern abnormalities were detected in such embryos) and $EA82^{VE692}$ has a larval lethal phase. Neither exhibit a maternal effect in homozygous germline clones.

An interesting feature is observed in the case of the $EA82^{HC207}$ rearrangement. $EA82^{HC207}$ complements all zygotic lethals in the 2C area with the exception of mutations at the *EA82* locus. Moreover, $EA82^{HC207}/Y;Dp(1;3)w^{vco}$ males are viable, indicating that $EA82^{HC207}$ is probably mutant only at the *EA82* locus. Determination of its lethal phase indicates lethality at various stages (Table 3). About 5% of embryos fail to hatch and show a very abnormal embryonic pattern similar to that exhibited by the reverse polarity loci (NUSSLEIN-VOLHARD and WIESCHAUS 1980). Each segment appears to be duplicated in a mirror-image pattern (result not shown). $EA82^{HC207}$ involves a chromosomal rearrangement in which a segment of the *X* chromosome is inverted with a breakpoint at 2C3 (*In(1)2C3-7B1*; G. LEFEVRE, personal communication). Because of the proximity of the reverse polarity embryonic lethal loci armadillo (*arm*, located within $Dp(1;Y)y^2y^{67g}$ at 2B15-17; WIESCHAUS, NUSSLEIN-VOLHARD and JURGENS 1984), we suggest that the abnormal $EA82^{HC207}/Y$ embryos found are the result of a position effect of the inverted piece of chromosome at position 2C3 on the nearby *arm* gene. Germline clonal analysis of $EA82^{HC207}$ indicates that germ cells homozygous for $EA82^{HC207}$ cannot produce viable eggs. Oogenesis is blocked at a late stage. Similarly, E. WIESCHAUS (personal communication) has observed that normal function of arm^+ is required for correct oogenesis. This observation supports the idea that the phenotype of $EA82^{HC207}$ is due to a position effect on the *arm* gene.

We examined three alleles at the *VE651* complementation group. Two of them are recovered over $Dp(1;3)w^{vco}$ ($VE651^{VE782}$ and *VE651*), *VE651* shows a larval lethal phase and $VE651^{VE782}$ appears to be a polyphasic lethal. The mutation $VE651^{VA56}$ exhibits a larval lethal phase but, since $VE651^{VA56}$ males carrying $Dp(1;3)w^{vco}$ are lethal, another lethal is probably present on the $VE651^{VA56}$ chromosome; therefore, we cannot determine the effective lethal phase of $VE651^{VA56}$. Nurse cell degeneration is observed (results not shown) when these three alleles are analyzed in germline clones, suggesting that normal function at this locus is required for oogenesis.

The ultraspiracle locus is representated by three alleles; one (usp^{KA21}) is a rearrangement and has been mentioned earlier. Two alleles (usp^{VE653} and usp^{VE849}) exhibit larval lethal phases. *usp/Y* first instar larvae derived from *usp/+* females exhibit no morphological abnormalities, whereas second instar larvae possess two sets of posterior spiracles. It is known that during larval development a new set of spiracles with distinct morphology is formed at each molt (RUHLE 1932; BODENSTEIN 1950). First instar spiracles have two openings,

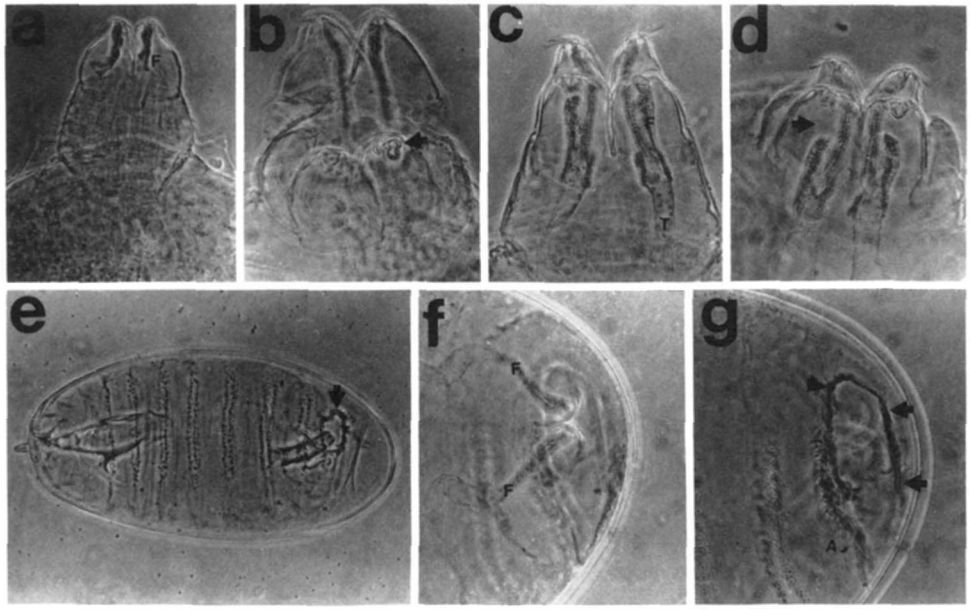

FIGURE 4.—Zygotic and germline clone phenotype of ultraspiracle (*usp*). Wild-type larvae have two posterior spiracles (a), each possessing internal filzkorper (f). In second instar larvae hemizygous for *usp* derived from *usp*/+ mothers an extra set of spiracles is observed (b) exhibiting three apertures (arrow). The filzkorper corresponding to the extra spiracles may be connected (c) or not connected (d) to the apertures. T indicates the trachea with which filzkorper (F) are continuous. Half the embryos derived from the homozygous *usp* germline clones exhibit a localized cuticular defect posterior to the eighth abdominal denticle belt (e). This defect is ventrally localized (g). (f) and (g) are two focal planes of the same embryo.

whereas those of second instar possess three. *usp*/*Y* second instar larvae possess a set of second instar spiracles with the typical three apertures. In addition, the first instar set of spiracles are present more posteriorly (Figure 4b–d). The two sets of spiracles are connected with filzkorper, which in turn are connected with internal trachea (Figure 4c). Occasionally, incomplete filzkorper are observed (Figure 4d). When *usp* alleles usp^{VE653} and usp^{VE849} are analyzed in germline clones, half of the progeny (Table 4, Figure 4e–g) show a localized ventral defect posterior to the eighth abdominal (A8) denticle belt (Figure 4e–g). We do not know at the present time whether these defects occur in posterior A8 or in the A9 segment. The other half of germline clonally derived embryos hatch. Although a large fraction of these larvae die at various stages (Table 4), without exhibiting the *usp* phenotype, some develop into adult females with normal viability and fertility.

We have only one allele at the *DF967* complementation group. Lethal phase determination indicates that *DF967* is a larval lethal. No clones among irradiated + *K1237*/*DF967* + females were found (200 were dissected) which indicates that lack of $DF967^{+}$ activity is lethal to the germline either during larval development or during early oogenesis in adults. Similarly, no viable germline clones are obtained when the mutation *JA52*, which behaves as a three-gene

TABLE 4

Comparison of lethal phase of usp^{VE653} *zygotes produced by heterozygous mother with those produced by homozygous germline clone mothers*

Cross	*N*	*N* unh	*N* unf	%E	*N*L	*N*P	%L
yf/VE653 × *yf/Y*	378	12	4	2.14	72	58	19.4
VE653/VE653 × *yf/Y*	218	141	40	56.7	71	4	94

Almost no embryonic lethality is detected when *usp/Y* individuals are derived from heterozygous mothers. When present, most lethality occurs during larval stages. When derived from germline clones homozygous for *usp*, half the embryos fail to hatch. Those that do not hatch exhibit the embryonic phenotype described in Figure 4e–g. The half that hatch die at later larval stages and may even produce viable female progeny, indicating that the maternal effect is rescuable. *N*, number of eggs examined; *N* unh, number of unhatched eggs; *N* unf, number of unfertilized eggs; *N*L, number of larvae examined; *N*P, number of pupae formed from the number of larvae (*N*L) transferred; %E and %L, respectively, the frequency of embryonic and larval lethality observed.

deficiency, is analyzed in germline clones. Because *DF967* is one of these three complementation groups, it is possible that the absence of fertile *JA52/JA52* germline clones is due to the lack of $DF967^{+}$ activity. Germline clones of *csw* and *ph* also uncovered by *JA52* produce eggs as described below.

We have examined six alleles at the Phosphoglucodehydrogenase (*Pgd*) locus, all of which exhibit a very similar phenotype. They have a larval lethal phase and disrupt germline development when homozygous in germline clones. Germline abnormalities analyzed by Hoechst staining (Figure 5) indicate very abnormal numbers and patterns of nurse cell nuclei.

Pupal lethal loci: corkscrew (csw) *and* C204: The two nonrearranged alleles of *csw* that we analyzed (csw^{C114} and csw^{VA199}) exhibit lethal phases at the larval-pupal transition (Tables 3). Dissection of third instar larvae hemizygous for either of these two alleles reveals a small disk phenotype. Examination of neuroblast squashes reveals dramatic reductions in the numbers of mitotic figures, but we cannot detect defects in chromosome morphology. When these two alleles are analyzed in homozygous germline clones, both exhibit a maternal effect lethal phenotype. All clonally derived embryos exhibit either a U-shaped or twisted phenotype (Figure 6). These phenotypes are not influenced either by the paternal genotype or by shifting homozygous germline clone bearing females to 29°C.

The second pupal lethal locus is represented by four alleles. Three (*C204*, $C204^{HC262}$ and $C204^{11P3}$) show a lethal phase at the larval-pupal boundary and exhibit a small disk phenotype. The fourth allele, $C204^{A17}$, has an early pupal lethal phase and has imaginal disks that appear normal. Analysis of mitotic figures of the mutant *C204* reveals a drastic effect on both the number of mitotic figures and the morphology of the metaphase chromosomes, including abnormal condensation of the chromosomes (Figure 7). When analyzed in germline clones, the three small-disk alleles exhibit a similar maternal effect lethal phenotype which is not observed in the case of $C204^{A17}$. Embryos appear to arrest prior to the blastoderm stage as demonstrated by Hoechst-DNA staining (Figure 7f). When such embryos are allowed to develop further, patches of cuticle are observed (Figure 7d). The three alleles producing this phenotype

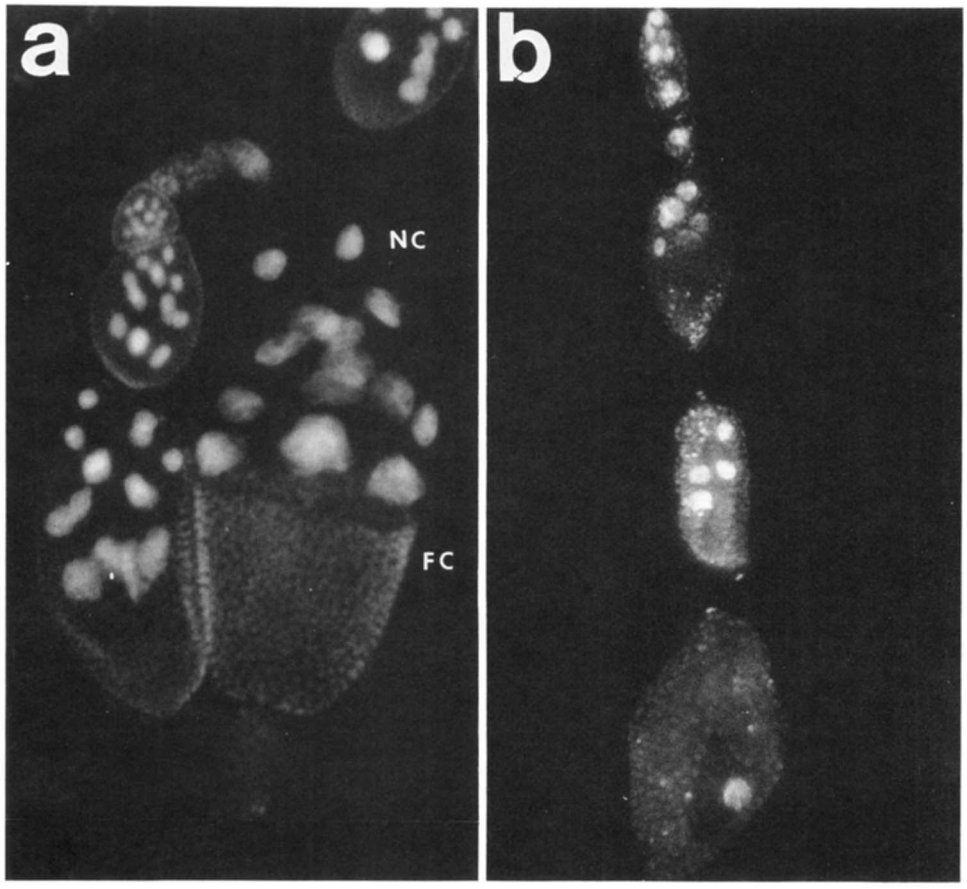

FIGURE 5.—Ovarioles of *Pgd* germline clones. Hoechst staining of a wild-type ovariole (a) with each egg chamber possessing 15 nurse cells (NC) and one oocyte derived from the germline and somatically derived follicle cells (FC). Egg chambers produced by germlines homozygous for *Pgd* mutations (b) possess abnormal numbers of nurse cells due either to abnormal germline cell divisions or to nurse cell degeneration.

exhibit no temperature effects and no paternal rescue. The findings that the mutation $C204^{A17}$ exhibits no maternal effect and possesses a later zygotic lethal phase suggest that $C204^{A17}$ is a hypomorphic allele of this locus.

Visible locus: polyhomeotic (ph): Within the 2C-D area only one complementation group, polyhomeotic (*ph*) exhibits a visible phenotype (DURA, BROCK and SANTAMARIA 1985). The mutation we characterized (ph^k) is subvital. Lethal phase determinations show that some hemizygous ph^k/Y males derived from $+/ph^k$ mothers die during larval and pupal stages. No obvious homeotic transformations are detectable among the dead larvae. Males that do survive exhibit a variable extra-sex comb phenotype in which meso- and metathoracic legs carry sex combs characteristic of prothoracic legs. In addition, the Miscadestral pigmentation (*MCP*) phenotype (LEWIS 1978), a partial transformation of A4 toward A5 is observed. Females heterozygous for ph^k and either

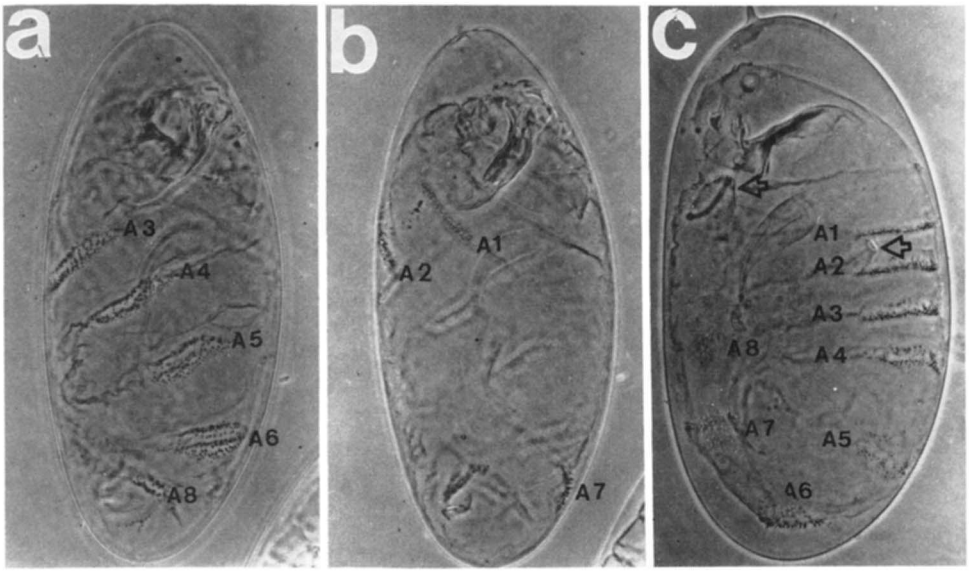

FIGURE 6.—Phenotype of embryos derived from germline clones of *l(1)csw*. Two phenotypes are observed: twisted (a and b) or U-shaped (c). (a) and (b) are two focal planes of the same embryo. Note the abnormal lateral separation of the posterior spiracles (c, arrows) in this embryo. Embryos derived from germline clones homozygous for *GF316* also exhibited a twisted and U-shaped phenotype. The *GF316* twisted phenotype was less pronounced than that in case of *csw* (a and b), but the U-shaped phenotype was identical (c).

Df(1)pn^{38} or *JA52* are lethal, suggesting that *ph*^{k} is a hypomorphic allele. The lethal phase of *ph*^{k}/*Y* males derived from germline clones homozygous for *ph*^{k} was the same as those derived from heterozygous mothers, indicating that *ph*^{k} has no maternal effect. However, since *ph*^{k} may be a hypomorphic mutation, a stronger *ph* allele might exhibit a maternal effect.

Ambiguous phenotypes at the GF316 *complementation group:* There are three alleles in this group, each of which exhibits a different lethal phase. *GF316* has an embryonic lethal phase. Hemizygous embryos derived from heterozygous mothers do not exhibit any obvious pattern abnormality. *GF316*^{GA55} is a larval lethal and *GF316*^{DF943} exhibits a polyphasic lethal phase, with occasional emerging males having no obvious abnormality. Half of the progeny derived from homozygous *GF316* germline clones have a twisted or U-shaped phenotype (Figure 6C), whereas the other half hatch and give rise to viable and fertile progeny. This indicates that this MEL phenotype can be paternally influenced. In contrast *GF316*^{GA55} and *GF316*^{DF943} do not exhibit any MEL phenotype.

DISCUSSION

The developmental genetics of the 2C3-D6 segment of the *X* chromosome represented by 14 bands on the salivary gland chromosome (BRIDGES 1938) is reported. We analyzed the contribution of nine loci to oogenesis, embryogenesis and adult development. Eight of the nine complementation groups are

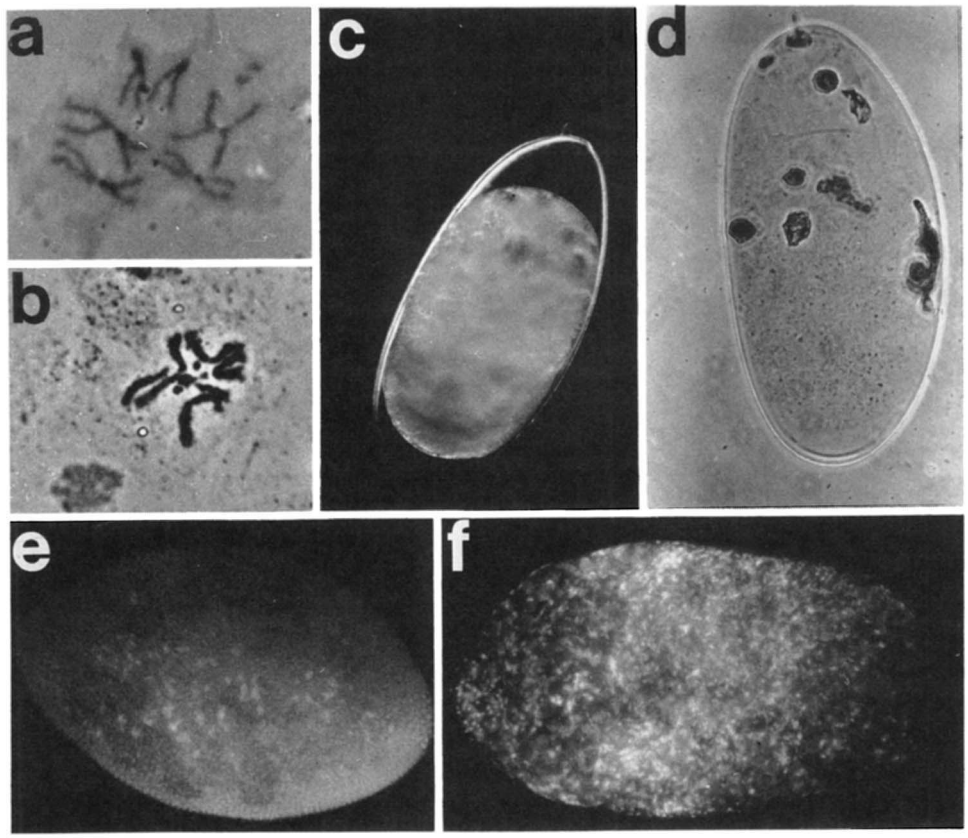

FIGURE 7.—Phenotypes of the *l(1)C204* locus. Mitotic figures as found in brain squashes of wild-type (a) and *C204* (b) larvae. Note that separation of homologs does not occur in the mutant (b). Mature (24 hr) homozygous *C204* germline clone-derived embryos are shown in dark-field photomicrograph of an unfixed dechorionated embryo (c) and in a phase-contrast photomicrograph of a cuticular (Hoyer's) preparation (d). Note the shortened embryo in (c) and the small patches of cuticle in (d). Hoechst-stained blastoderm stage wild-type (e) and homozygous *C204* germline clonally derived (f) embryos indicates the preblastoderm defect.

mutable to lethal states, and we found an average of 3.6 alleles in each lethal complementation group. This average suggests that most lethal complementation groups have been identified in this area (however, see discussion by LEFEVRE 1981 and discussion about *JA52* below).

Three loci disrupt the embryonic pattern when analyzed in germline clones. Mutations in the *C204* gene disrupt a function essential for cell division. Analysis of mitotic figures indicates that the *C204* gene product is required for normal chromosome physiology. The organization of preblastoderm nuclear divisions is greatly disrupted in embryos derived from germline clones, probably due to the lack of maternally derived gene product essential for nuclei division or for maintenance of chromosome integrity. This preblastoderm arrest is comparable to the phenotype exhibited by a set of female sterile mutations (ZALOKAR, AUDIT and ERK 1975). It was previously observed that loci

essential for normal mitotic functions exhibit maternal effects (BAKER, SMITH and GATTI 1972). The second locus, corkscrew, confers a specific, fully penetrant MEL phenotype on embryonic development. Embryos derived from such germline clones exhibit a twisted phenotype. Activity at the corkscrew locus is necessary for normal division of diploid cells in larvae. This phenotype is quite similar to that observed among several different embryonic zygotic lethal loci, such as twist, snail and twisted gastrulation (NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; K. KONRAD, personal communication). Because twist and snail disrupt ventral furrow formation (SIMPSON 1984), we examined gastrulation in germline clone-derived *csw* embryos. Ventral furrows and internal musculature are found in corkscrew embryos, suggesting that the terminal twisted phenotype must be related to some other defect. This in turn suggests that the twisted phenotype can be produced via more than one morphogenetic disruption. Finally, we characterized ultraspiracle which confers a specific rescuable MEL phenotype on the posterior tip of the embryo. Zygotically, *usp* appears to be required for normal morphogenesis of the posterior spiracles. The relationship between the maternal effect and the zygotic phenotypes is not clear because the location of the structures affected zygotically *vs.* maternally differ. The posterior spiracles originate from the dorsal side of the eighth abdominal segment (TURNER and MAHOWALD 1976), whereas the maternal effect lethal defect appears to be localized in the posterior ventral eighth or ninth segment. Moreover, it is difficult to understand why germline clonally derived larvae, which have received a wild-type dose of *usp*, die at various stages; it is possible that internal structures are also affected.

The present analysis provides evidence for the presence of an embryonic zygotic lethal locus in 2D2-3 whose phenotype appears to be degeneration of all structures ventral to the tracheal pits. Sections of embryos at 12 hr of age reveal that extensive cell death occurs in the central nervous system as well as in the ventral ectoderm (N. PERRIMON, unpublished results). The phenotype observed in Hoyer's mounts is very similar to that of the second chromosome-linked embryonic zygotic lethal "fizzy" described by NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984). It is interesting that no locus producing this phenotype has been identified in attempts to saturate the *X* chromosome for embryonic lethal loci (WIESCHAUS, NUSSLEIN-VOLHARD and JURGENS 1984) or in extensive screens for zygotic lethals in the 2C-D area (LEFEVRE 1981; G. LEFEVRE, personal communication). This probably indicates that this gene has a low mutability rate. Another possibility is that the embryonic phenotype exhibited by *JA52* and $Df(1)pn^{38}$ is synthetic in nature and is caused by the simultaneous removal of two or more genes located between the proximal breakpoint of *JA52* and the distal breakpoint of $Df(1)pn^{38}$. Because of the cytology of $Df(1)pn^{38}$ and *Df(1)Pgd kz*, this locus must be located within the 2D2-4 area.

There are many advantages to analyzing the phenotype of zygotic lethals systematically. Analysis of many alleles at one locus allows us to determine whether the phenotypes we observe are due to a hypomorphic (reduced activ-

ity) or amorphic (no activity) genic effect. The systematic characterization of all mutations available permits one to analyze all possible phenotypes involved in any developmental step that involves embryonic, larval and adult patterning. Such an approach also allows one to determine whether a set of adjacent loci share common mutant phenotypes or not. There is no apparent global relationship between all of the loci analyzed so far in the 2C-F area. However, some small clusters exist. For example, two homeotic like loci (*usp* and *ph*) are in close proximity to each other, and the two female sterile loci [*fs(1)pcx* and *fs(1)k10*] and two loci affecting embryonic segmentation [*l(1)ph* and giant] are similarly close, indicating that some regional and functional relationship may exist.

Another advantage of this analysis is the potential to identify interacting genes. However, no transinteraction between adjacent loci in the 2C-F area was found. The failure to identify clusters of loci producing similar or related phenotypes or of *trans*-interacting loci may not be too surprising since very few clusters of functionally related genes have been identified in Drosophila. It is possible that most developmental genes are in fact autonomously regulated.

The present analysis confirms earlier indications (GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984a,b) that many lethal genes have germline functions and that some impose specific MEL phenotypes on embryonic development. It is interesting that among the four loci found in the 2C-F area that exhibit MEL phenotypes [*i.e.*, ultraspiracle (*usp*), corkscrew(*csw*), *C204* and *l*(1)pole hole (*l(1)ph*)], three are pupal lethal loci [*csw*, *C204* and *l(1)ph*] and are associated with small-disk phenotypes. Further studies will be necessary to determine the significance of this observation.

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LITERATURE CITED

BAKER, B. S., D. A. SMITH and M. GATTI, 1982 Region-specific effects on chromosome integrity of mutations at essential loci in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **79:** 1205–1209.

BODENSTEIN, D., 1950 The postembryonic development of *Drosophila*. pp. 275–364. In: *Biology of Drosophila*, Edited by M. DEMEREC. Hafner Publishing Company, New York.

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

BUSSON, B., M. GANS, K. KOMITOPOULOU and M. MASSON, 1983 Genetic analysis of three dominant female sterile mutations located on the *X* chromosome of *Drosophila melanogaster*. Genetics **105:** 309–325.

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

DURA, J-M., H. W. BROCK and P. SANTAMARIA, 1985 *Polyhomeotic*: a gene of *Drosophila melanogaster* required for correct expression of segmental identity. Mol. Gen. Genet. **198:** 213–220.

DEQUIN, R., H. SAUMWEBER and J. W. SEDAT, 1984 Proteins shifting from the cytoplasm into the nuclei during early embryogenesis of *Drosophila melanogaster*. Dev. Biol. **104:** 37–48.

EPHRUSSI, B. and G. W. BEADLE, 1936 A technique of transplantation in *Drosophila.* Am. Nat. **70:** 218–225.

GARCIA-BELLIDO, A. and L. G. ROBBINS, 1983 Viability of female germ-line cells homozygous for zygotic lethals in *Drosophila melanogaster.* Genetics **103:** 235–247

GEHRING, W. J., 1984 Homeotic genes and the control of cell determination. pp. 3–32. In: *Molecular Biology of Devevelopment,* Proceedings of the ICN-UCLA Conference, Steamboat Springs, 1984, Edited by R. FIRTEL and E. DAVIDSON. Alan R. Liss, New York.

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

GUEST, W. C. and T. C. HSU, 1973 A new technique for preparing Drosophila neuroblast chromosomes. Drosophila Inform. Serv. **50:** 193.

GVOZDEV, V. A., S. A. GOSTIMSKY, T. I. GERASIMOVA, E. S. DUBROVSKAYA and O. YU. BRASLAVSKAYA, 1975 Fine structure of the 2D3-2F5 region of the *X*-chromosome of *Drosophila melanogaster.* Mol. Gen. Genet. **141:** 269–275.

HOCHMAN, B., 1973 Analysis of a whole chromosome in *Drosophila.* Cold Spring Harbor Symp. Quant. Biol. **38:** 581–589

ILYINA, O. V., A. V. SOROKIN, E. S. BELYAEVA and I. F. ZHIMULEV, 1980 Drosophila Inform. Serv. **55:** 205.

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

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

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

MAHOWALD, A. P., K. KONRAD, L. ENGSTROM and N. PERRIMON, 1984 Genetic approaches to early development. pp. 185–197. *Molecular Biology of Development,* Proceedings of the ICN-UCLA Conference, Steamboat Springs, 1984, Edited by R. FIRTEL and E. DAVIDSON. Alan R. Liss, New York.

MITCHISON, T. J. and J. W. SEDAT, 1983 Localization of antigenic determinants in whole *Drosophila* embryos. Dev. Biol. **99:** 261–264.

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

NUSSLEIN-VOLHARD, C. and E. WIESCHAUS, 1980 Mutations affecting segment number and polarity in *Drosophila.* Nature (Lond.) **287:** 795–801.

NUSSLEIN-VOLHARD, C., E. WIESCHAUS and H. KLUDING, 1984 Mutation affecting the pattern of the larval cuticle in *Drosophila melanogaster.* I. Zygotic loci on the second chromosome. Wilhelm Roux Arch. **192:** 267–282.

PERRIMON, N., 1984 Clonal analysis of dominant female sterile, germline-dependent mutations in *Drosophila melanogaster.* Genetics **108:** 927–939.

PERRIMON, N., L. ENGSTROM and A. P. MAHOWALD, 1984a Analysis of the effects of zygotic lethal mutations on the germ line functions in *Drosophila.* Dev. Biol. **105:** 404–414.

PERRIMON, N., L. ENGSTROM and A. P. MAHOWALD, 1984b Developmental genetics of the 2E.F. region of the Drosophila *X* chromosome: a region rich in "developmentally important" genes. Genetics **108:** 559–572.

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.

RUHLE, H., 1932 Das larvale Tracheensystem von *Drosophila melanogaster*: Meigen und seine Variabilitat. Z. Wiss. Zool. **141:** 159–245.

SHANNON, M. P., T. C. KAUFMAN, W. M. SHEN and B. H. JUDD, 1974 Lethality patterns and morphology of selected lethal and semi-lethal mutations in the zeste-white region of *Drosophila melanogaster*. Genetics **72:** 615–638.

SIMPSON, P., 1984 Maternal and zygotic gene interactions during formation of the dorsoventral pattern in Drosophila embryos. Genetics **105:** 615–632.

SLOBODYANYUK, S. and O. SEROV, 1983 Variations in the expression of the gene *Pgd* due to the effect of chromosomal rearrangements in *Drosophila melanogaster*. Mol. Gen. Genet. **191:** 372–377.

TURNER, R. F. and A. P. MAHOWALD, 1976 Scanning electron microscopy of *Drosophila melanogaster* embryogenesis. III. Formation of the head and caudal segments. Dev. Biol. **68:** 96–106.

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., C. NUSSLEIN-VOLHARD and G. JURGENS, 1984 Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. III. Zygotic loci on the *X*-chromosome and fourth chromosome. Wilhelm Roux Arch. **193:** 296–307.

ZALOKAR, M., C. AUDIT and I. ERK, 1975 Developmental defects of female sterile mutants of *Drosophila melanogaster*. Dev. Biol. **47:** 419–432.

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