Genetic and Developmental Analysis of Polytene Section 17 of the *X* Chromosome of *Drosophila melanogaster*

Daniel F. Eberl,*[,1] Lizabeth A. Perkins,[†] Marcy Engelstein,[†] Arthur J. Hilliker* and Norbert Perrimon[†]

**Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada N1G 2W1, and [†]Department of Genetics and Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02115*

Manuscript received April 22, 1991
Accepted for publication November 22, 1991

ABSTRACT

Polytene section 17 of the *X* chromosome of *Drosophila melanogaster*, previously known to contain six putative lethal complementation groups important in oogenesis and embryogenesis, has here been further characterized genetically and developmentally. We constructed *fcl*$^{+}$*Y*, a duplication of this region, which allowed us to conduct mutagenesis screens specific for the region and to perform complementation analyses (previously not possible). We recovered 67 new lethal mutations which defined 15 complementation groups within *Df(1)N19* which deletes most of polytene section 17. The zygotic lethal phenotypes of these and preexisting mutations within polytene section 17 were examined, and their maternal requirements were analysed in homozygous germline clones using the dominant female sterile technique. We present evidence that an additional gene, which produces two developmentally regulated transcripts, is located in this region and is involved in embryogenesis, although no mutations in this gene were identified. In this interval of 37 to 43 polytene chromosome bands we have defined 17 genes, 12 (71%) of which are of significance to oogenesis or embryogenesis.

THE *X* chromosome is the most extensively characterized portion of the genome of the model higher eukaryote, *Drosophila melanogaster*. It comprises about 20% of the nuclear genome and deficiencies are available for about 77% (estimated from WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984) and duplications are available for approximately 68% (estimated from EBERL and HILLIKER 1988) of the euchromatic portion of the *X* chromosome. Euchromatin constitutes about 50% of the mitotic length of the *X* chromosome and contains 99% of the *X* chromosome gene loci (see HILLIKER, APPELS and SCHALET 1980). The availability of *X* chromosome duplications and deficiencies has been invaluable for conducting detailed genetic analyses of specific segments of the *X* chromosome. Such studies have allowed us to acquire a great deal of information on the nature of genetic organization in *D. melanogaster* (*e.g.*, JUDD, SHEN and KAUFMAN 1972), and have provided us with mutations that identify gene loci that are important in a variety of biological pathways or phenomena. Of particular interest to us are genes which are important in the regulation of oogenesis and embryogenesis.

Some regions of the *X* chromosome have not been extensively analyzed due to the lack of appropriate duplications. The chromosomal region defined by *Df(1)N19*, essentially polytene section 17, is one of these. Nevertheless, this region of the *X* chromosome contains six putative complementation groups important in the regulation of oogenesis or embryogenesis, namely, *unpaired* (*upd*), *porcupine* (*porc*), *foreclosed* (*fcl*), *fused* (*fu*), *phantom* (*phm*), and *exocephalon* (*exo*) (NÜSSLEIN-VOLHARD and WIESCHAUS 1980; WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984; EBERL and HILLIKER 1988; PERRIMON, ENGSTROM and MAHOWALD 1989), as well as one cloned DNA sequence containing a pair of alternative transcripts which show a highly specific developmentally regulated pattern of expression (NG *et al.* 1989). Complementation analysis was not possible, however, due to the lack of an appropriate duplication. Therefore, to further characterize the organization of this region we conducted a genetic and developmental analysis of polytene section 17 of the *X* chromosome.

MATERIALS AND METHODS

Genetic strains: Flies were raised on standard Drosophila media at 18°, 25° or 29°. Table 1 lists and briefly describes the preexisting *X* chromosome duplications and deficiencies employed in this study. It should be noted that we present revised cytological descriptions for 11 of these rearrangements. Preexisting lethal mutations isolated by other laboratories and falling within the region analyzed include *l(1)9130* and *porc*^{1517} (FERRUS *et al.* 1990) and *upd*^{YC43}, *upd*^{YM55} and *phm*^{XE07} (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984) and were obtained from the laboratories in which they were discovered. The *os*^{o}, *Bx*, *Bx*^{3} and *fu* mutations were obtained from the Bowling Green Drosophila Stock Center. The *FM3/w v l*^{us}*/B*^{S}*Yy*$^{+}$ strain is described by KOMITOPOULOU *et al.* (1983). The *X*-linked dominant female

[1] Current address: Department of Biochemical Pharmacology, State University of New York, Buffalo, New York 14260.

TABLE 1

X chromosome deficiencies and duplications employed in this study

Rearrangement	Cytology	References[a]	Source of stock
Df(1)os^{1A}	17A3; 17A6[b]	3	A. FERRUS
Df(1)os^{UE19}	17A5; 17A12	7	This work
Df(1)D2	17A7-9; 17B2	7	This work
Df(1)fu^{i3}	17A3-5; 18B4-C1[b]	1	B. LIMBOURG
Df(1)N19	17A3-6; 17F2-18A3[b]	2	Bowling Green
In(1)Cl^{L}y^{4R}	Deficient for 17A7-11; 18A3-4[b]	5	This work
Df(1)fu^{S4}	17A2-7; 18B7-11[b]	1	B. LIMBOURG
Df(1)fu^{E5}	17B3-C1; 17E7-F3[b]	1	B. LIMBOURG
Df(1)E160.2	17B3-C1; 18A4-7[b]	6	W. ENGELS
Df(1)E128	Deficient for 17B3-C2; 18AB[b]	6	W. ENGELS
Df(1)fu^{B10}	17C5-D1; 18A4-7[b,c]	1	B. LIMBOURG
Df(1)fu^{H4}	17C3; 17D2[b]	1	B. LIMBOURG
Dp(1;3)JC153	16E; 17A7-12; 99D3-8[b]	3,4	M. TANOUYE
Dp(1;3)fu^{+10}	17A; 17DE; 98B[b]	1	B. LIMBOURG
Dp(1;Y)W39 (=*fcl^{+}Y*)[d]	Duplicated for 16F; 18A5-7 and 19E5-7 to base	7	This work
T(1;Y)W32	16F1-4; Y^{S}	3,4	M. TANOUYE
T(1;Y)V7	16F1-4; Y^{S}	3,4	M. TANOUYE

[a] 1, BUSSON *et al.* 1988; 2, CRAYMER and ROY 1980; 3, FERRUS *et al.* 1990; 4, TANOUYE, FERRUS and FUJITA 1981; 5, STURTEVANT and BEADLE 1936; 6, ENGELS and PRESTON 1981; 7, this work.

[b] The cytology of these rearrangements has been reexamined (D. F. EBERL) and the revised breakpoints are reported here. For previous descriptions of the cytology, see the additional references given.

[c] There is also a tandem duplication, *Dp(1;1)17A; 19AB* superimposed on this deficiency, such that the deficiency is present in both copies.

[d] Cytologically, *Dp(1;f)W39* is identical to *fcl^{+}Y* in polytene chromosome squashes.

sterile *Fs(1)K1237* (or *ovo^{D1}*) (BUSSON *et al.* 1983; PERRIMON 1984) was utilized for germline clone analysis (see below). The *en-lacZ* strain was obtained from C. HAMA and T. KORNBERG. This strain carries a β-galactosidase (*lacZ*) gene under the control of the *engrailed* (*en*) regulatory regions on the second chromosome (HAMA, ALI and KORNBERG 1990). After the blastoderm stage, the *en-lacZ* strain expresses the β-galactosidase protein in patterns identical to the endogenous *en* protein (HAMA, ALI and KORNBERG 1990). Descriptions of other genetic strains and mutations are provided by LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1985, 1987, 1990).

Mutagenesis: To analyze the genetic organization of the region uncovered by *Df(1)N19*, we constructed a duplication, on the *Y* chromosome, of polytene section 17 of the *X* chromosome. The details of the construction of this duplication, *fcl^{+}Y* or *Dp(1;Y)W39*, are reported in the RESULTS section and are diagrammed in Figures 1 and 2.

A deletion encompassing a portion of section 17, *Df(1)os^{UE19}*, was constructed by irradiating females heterozygous for an *X* chromosome marked with *y cv v f car* and an Ore-R wild-type *X* chromosome. These females were crossed to *f os^{o} car/Y* males. Female progeny expressing the *os^{o}* recessive phenotype were further analyzed as putative deletions. Another mutation, *UE69*, recovered from this experiment, is an allele of *os-upd*.

Recessive lethal mutations falling within polytene section 17, as defined by *Df(1)N19*, were isolated in three different screens diagrammed in Figure 3. Screen A (Figure 3A) involved the selection of temperature sensitive lethals falling within the region defined by *Df(1)N19*. The lethal mutations were recessive lethal at 29° and hemizygous viable at 18°. Screens B and C (Figure 3, B and C) utilized *fcl^{+}Y* constructed in this study (see RESULTS for details of synthesis). This duplication allowed the recovery and complementation analysis of recessive lethals falling within polytene section 17. The mutagens employed were γ-radiation, ethyl methanesulfonate (EMS) and 1,2:3,4-diepoxybutane (DEB). A mutagenesis protocol based on that of LEWIS and BACHER (1968) was used for EMS (12 and 25 mM) and DEB (10 mM).

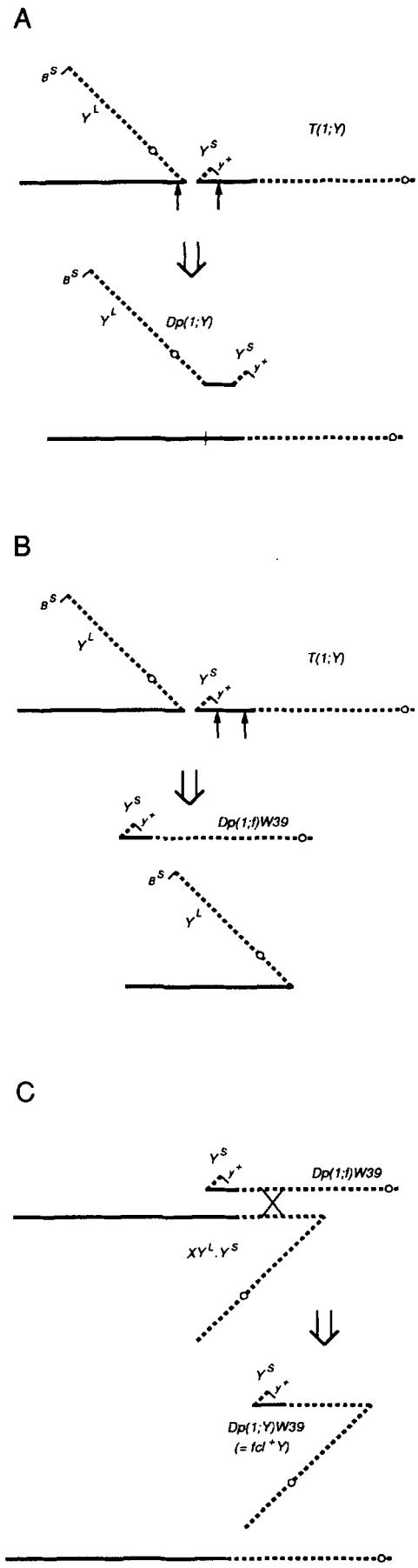
Complementation analysis: Complementation analyses were largely performed following the general procedure described by EBERL and HILLIKER (1988). Each of these schemes utilized one of *Dp(1;f)W39*, *Dp(1;Y)W39* (=*fcl^{+}Y*), *Dp(1;3)fu^{+10}* or *Dp(1;3)JC153* (see Table 1). Complementation analyses involving as one genetic variant a viable *Bx*, *os* or *fu* allele did not necessitate the use of an *X* duplication for complementation.

Cytology: Salivary gland preparations were made from larvae heterozygous for specific rearrangements and cytologically normal chromosomes. Salivary glands were dissected from third instar larvae in 45% acetic acid, transferred to a drop of 2% acetolacto-orcein on a siliconized slide, squashed immediately with a coverslip and examined using phase contrast optics. Breakpoints were determined using the polytene *X* chromosome map of BRIDGES (1938).

Germline clone analysis: Germline clones of zygotic lethal mutations were generated using the dominant female sterile technique (PERRIMON and GANS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984, 1989; PERRIMON *et al.* 1986).

Analysis of embryonic phenotypes: Embryonic cuticles were prepared according to the HOYER's mount technique of VAN DER MEER (1977). The determination of the lethal phase of a particular genotype was performed as described by PERRIMON, ENGSTROM and MAHOWALD (1984). Embryos were prepared for immunohistochemistry as previously described (SMOUSE and PERRIMON 1990).

***In situ* hybridization to polytene chromosomes and embryos:** Salivary gland polytene squashes (GALL and PARDUE 1971) were hybridized with random primed probes (FEIN-



BERG and VOGELSTEIN 1983) labeled with bio-16dUTP (ENZO Biochem). Signal detection was achieved with streptavidin-conjugated horseradish peroxidase followed by histochemical detection with aminoethylcarbazole. These reagents were purchased as a kit, DETEK 1-HRP, from ENZO Biochem. Wild-type Oregon R embryos were collected at 25° and aged until the desired developmental stages. *In situ* hybridization to whole mount embryos using digoxigenin-labelled probes was as described by TAUTZ and PFEIFLE (1989). Embryos were dehydrated through an ethanol series followed by acetone and mounted in Euparal.

Molecular biology: Molecular biology methods including DNA purification, DNA cloning, Southern analysis, DNA sequencing, and plaque hybridization were performed as described in SAMBROOK, FRITSCH and MANIATIS (1989) and AUSUBEL *et al.* (1990).

***P* element transformation:** Vectors used for *P* element transformations, pCaSpeR-2 and pCaSpeR-hs, carry the *white*$^+$ gene which allows the detection of transformants (THUMMEL, BOULET and LIPSHITZ 1988; C. THUMMEL, personal communication) and were kindly provided by C. THUMMEL. Injections were done into *y w/y w*; *Δ2-3 Sb/In(3)TM6* (ROBERTSON *et al.* 1988) precellular embryos. This strain constitutively synthesizes an endogenous transposase which is present in the ooplasm. Transformants were identified by rescue of their white eye color to near wildtype. Transformed lines were maintained with *In(1)FM7c*, *In(2LR)CyO*, or *In(3LR)TM3, Sb* balancer chromosomes.

RESULTS

Construction of a duplication for polytene chromosome section 17: Since polytene section 17 is on the *X* chromosome, a duplication for this region was required in order to screen for and to perform complementation tests among recessive lethals in the region. *Y*-linked *X* chromosome duplications are most useful because they segregate from the *X* chromosome in the male, facilitating analysis of *X* chromosome mutations.

To construct a *Y*-linked duplication for polytene section 17 we attempted to revert *X*;*Y* translocations with breakpoints in the *Shaker* (*Sh*) locus at 16F1-4 (TANOUYE, FERRUS and FUJITA 1981), using the

FIGURE 1.—Construction of a duplication for polytene chromosome section 17. The horizontal lines represent *X* chromosome material, while the diagonal lines represent B^SYy^+-derived chromosome material. Euchromatic regions are shown by solid lines, heterochromatic regions by dashed lines. (A) *Y*-linked duplications of the polytene section 17 region of the *X* chromosome were hoped to be recovered as imprecise reversions of the *T(1;Y)*s by a new translocation event with breakpoints at the positions indicated by arrows. The *T(1;Y)*s were irradiated in females because the two chromosomal elements produced in a rearrangement segregate from one another during female meiosis (see also Figure 2 and text). (B) A diagram of the deletion event, indicated by arrows, which occurred in the *X*-proximal element of *T(1;Y)W32* during the same experiment as in A, to produce the free duplication, *Dp(1;f)W39*. (C) A diagram showing the attachment of the free duplication, *Dp(1;f)W39*, to a *Y* chromosome by a mitotic crossover in male larvae, producing *Dp(1;Y)W39*, which is also called *fcl*$^+$*Y*. In each case (A to C) there are two chromosomal elements produced, which segregate at meiosis, so that only the upper product shown is recovered in any given offspring.

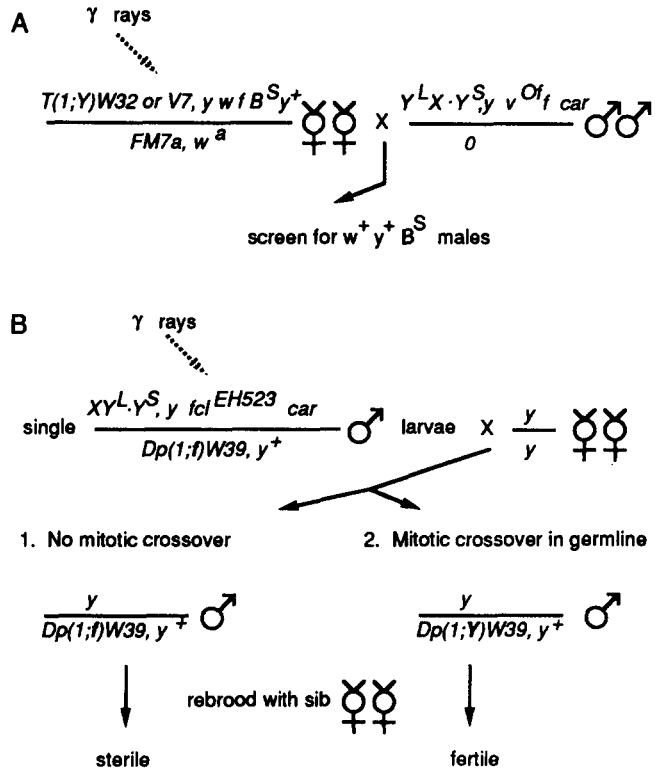


FIGURE 2.—Crosses used in the construction of *Y*-linked duplications. (A) These crosses were used to screen for reconstituted *Y* chromosomes that carry segments of the *X* chromosome by imprecise reversion of the translocations *T(1;Y)W32* and *T(1;Y)V7* (see also Figure 1, A and B). The $Y^L X \cdot Y^S$ chromosome carries inversions *In(1)EN* and *In(1)dl-49* (not shown in the figure) and can therefore serve as a balancer for rearrangements recovered in female offspring. (B) Mitotic recombination was induced in male larvae to attach the free duplication to the *Y* chromosome that is derived from the $XY^L \cdot Y^S$ chromosome (see Figure 1C). This attachment, resulting in the formation of *Dp(1;Y)W39*, also called *fcl*$^+$*Y*, was recognized by the production of an F_2 progeny, indicating fertile F_1 males.

scheme depicted in Figures 1 and 2. *T(1;Y)W32* and *T(1;Y)V7* were chosen because their *Y* chromosome breakpoints in the short arm do not disrupt any of the fertility factors. The translocations were irradiated in females because of the tendency to recover "half-translocations" from irradiated oocytes (ABRAHAMSON, HERSKOWITZ and MULLER 1954; reviewed by ROBERTS 1976). Thus, if the two translocation element dyads interchange, they will segregate from one another. One possible class of interchange would have as one product a *Y* chromosome duplication of polytene section 17 (Figure 1A). This interchange class would be found among *Y* chromosome reversions. Thus the reversion event allows the cosegregation of the *Y* chromosome moieties from the *X* chromosome moieties in the oocytes and is therefore identifiable in the F_1, allowing an efficient screen. The irradiated females were crossed to $Y^S X \cdot Y^L$ males (Figure 2A) so that infertile or partial *Y* chromosomes could still be recovered. Table 2 shows the results of this screen.

TABLE 2

Results of the screens to isolate duplications

	T(1;Y)W32 (*N* = 392)[a]		*T(1;Y)V7* (*N* = 184)		Total (*N* = 576)	
Exceptional class	Recovered	Fertile	Recovered	Fertile	Recovered	Fertile
B^S	102	NT[b]	21	18	123	NT
y^+	7	6	9	9	16	15
y^+ B^S	6	5	4	2	10	7
y^+ B^S Bx	1	0			1	0
y^+ B^S f^+	1	0			1	0
y^+ B^S f^+ Bx car^+			1	0	1	0
y^+ Bx	1	1			1	1
B^S f^+	2	2	1	0	3	2
B^S f^+ Bx car^+			1	0	1	0
B^S Bx car^+			1	0	1	0
Bx car^+	2	0	1	0	3	0

[a] *N* = number of bottles screened. There were about 70 male offspring and 100 female offspring per bottle. Therefore about 40,000 male offspring were screened.
[b] NT = not tested.

Among the exceptional male F_1 progeny were 10 which carried both the y^+ and B^S markers indicative of reversion. Of these, seven were fertile and transmitted a restored *Y* chromosome. Unfortunately, none of them rescued the *os* mutation, and therefore none of them carried a duplication that extended as far as the *os* locus. The y^+ exceptionals represented potential free *X* duplications. Only one of the 15 y^+ exceptionals, *Dp(1;f)W29*, rescued *os* but did not rescue any of the more proximal mutations (see Figure 5) and so had limited utility in this analysis.

Unexpectedly, the exceptional progeny that proved to be most useful was a y^+ Bx male (Table 2). The dominant Bx phenotype suggested that it carried a duplication that extended to at least the *Bx* locus. Further analysis demonstrated that it behaved genetically as a *Dp(1;f)* which did not possess the *Y* chromosome fertility factors, consistent with the absence of the B^S marker. This rearrangement, *Dp(1;f)W39*, was maintained in a *Dp(1;f)W39*, $y^+/Y^S X \cdot Y^L$, In(*1*)*EN In(1)dl-49*, *y* v^{Of} *f car* and *C(1)DX*, *y f*/ *Dp(1;f)W39*, y^+ strain. Complementation analysis demonstrated that *Dp(1;f)W39* rescued *os*, *fcl*, *fu* and *phm* mutations, as well as *Df(1)N19*, and cytological analysis revealed a deletion of 18A5–7 to 19E5–7 in the *X*-proximal element of *T(1;Y)W32* (see Figures 1B and 4A).

To facilitate complementation analysis, this free duplication, *Dp(1;f)W39*, was then modified by attaching it to a *Y* chromosome through γ ray induced mitotic recombination with an $XY^L \cdot Y^S$ chromosome (Figure 1C) using the scheme shown in Figure 2B. Male larvae of the constitution $XY^L \cdot Y^S$, *y* fcl^{EH523} *car*/ *Dp(1;f)W39* were irradiated at the first instar (24–48 hr after oviposition) with 1500 rad of γ-rays. At this stage the testes contain only spermatogonia (BODENSTEIN 1950). This allows some cell division to occur

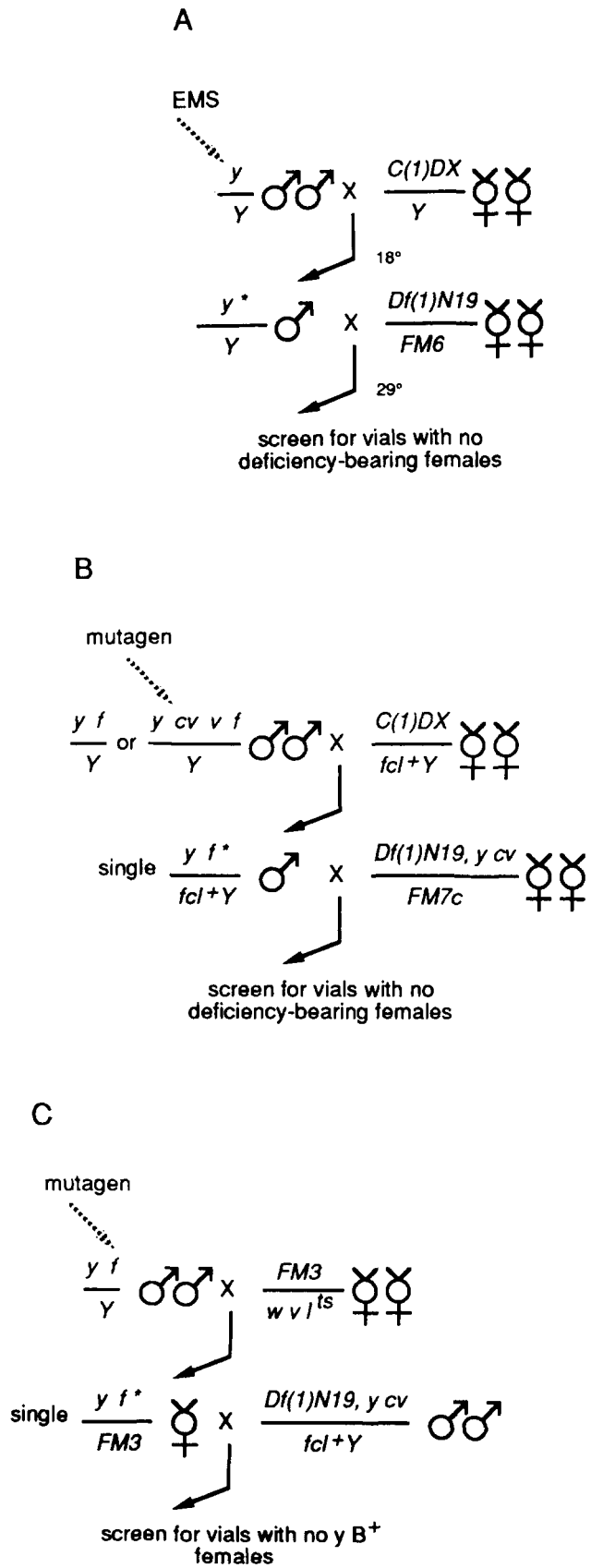


FIGURE 3.—Screens for lethal mutations in the polytene section 17 region. Shown are the screens performed for temperature sensitive lethals and mosaically recovered lethals (screen A) without the use of a duplication, and attached-*X* (screen B) and *FM3* (screen C) schemes performed for lethals or deficiencies using the *fcl*$^+$*Y* duplication that was constructed in this study.

after the crossover event before primary spermatocytes differentiate, thus increasing the number of sperm that can carry a particular recombinant chromosome. The resulting adult males were each crossed to two *y* virgin females. Parents were discarded after 7 days and the offspring were rebrooded into fresh vials. Production of an F_2 indicates a putative recombination event which attaches the *Y* chromosome moiety of $XY^L \cdot Y^S$ onto the duplication, thus making the duplication-bearing F_1 males fertile. Of the 360 irradiated larvae tested, 75 produced F_2 progeny. Only 23 of these were further examined and 4 were found to be *Y*-linked duplications which cover *fcl*. One of these was examined cytologically, was found to be similar to *Dp(1;f)W39* (see Figure 4A), and was named *Dp(1;Y)W39* and given the synonym *fcl*$^+$*Y* following the conventions established by BROSSEAU *et al.* (1961).

Two other exceptional males proved to carry duplications of the *forked* (*f*) locus. One, *Dp(1;f)W325*, is probably a deletion of the majority of the *FM7a* balancer chromosome since it is phenotypically y^{31d}, f^+ and *B* (not B^S), as *B* is derived from *FM7a*. Cytologically, very few bands are visible. The second, *Dp(1;Y)W73*, carries y^{31d}, f^+ and B^S, and cytologically carries 16A to 16F, as well as several bands which must be derived from the tip of the *FM7a* chromosome. This duplication is fertile in males without an additional *Y* chromosome, indicating that the *Y* chromosome breakpoint in the original *T(1;Y)W32* is beyond the most distal fertility factor on Y^S. The two duplications, *Dp(1;Y)W73* and *fcl*$^+$*Y*, therefore together cover all of polytene sections 16 and 17, and represent an increase from about 68% to 77% of the *X* chromosome euchromatin that is now covered with duplications.

Mutagenesis screens: Prior to construction of the duplication a screen was performed for recessive lethal mutations recovered as mosaics or temperature-sensitive (ts) lethals over *Df(1)N19* following the scheme shown in Figure 3A. This screen (screen A) is efficient first in that it eliminates in the first generation unconditional lethals, most of which fall outside the deficiency, and second that cultures which contain a lethal that is mosaic in the F_1, or a ts lethal, are automatically balanced. In this screen 67 mutations were recovered among 7478 fertile single pair matings; 19 were rescued by *fcl*$^+$*Y*, of which 9 proved to fall within section 17, though only 7 were within *Df(1)N19*. Of these seven mutations, three were ts, while the remaining four were complete lethals, suggesting that they were recovered as mosaics. Mosaics are known to be recovered at an appreciable frequency when using

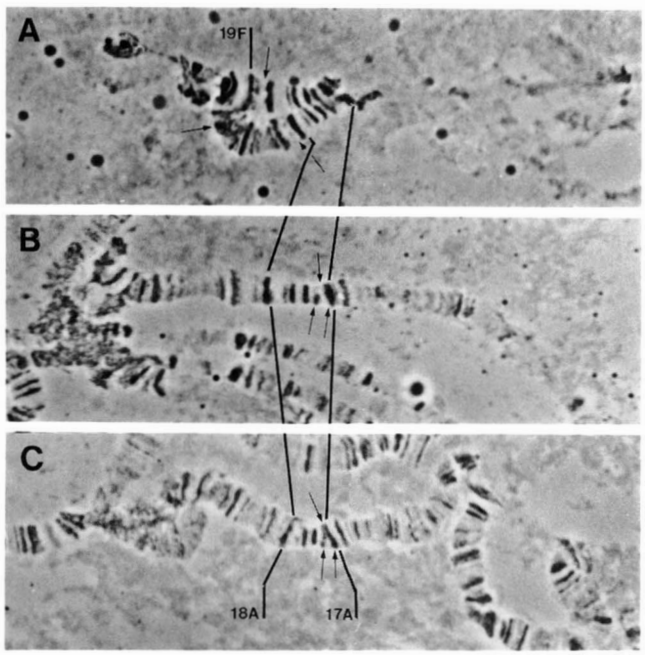


FIGURE 4.—Cytology of new rearrangements recovered in this study. In each case the normal *X* chromosome is the lower homolog, with the deleted segment delimited by arrows, and the upper homologue is the deleted element, with the new junction formed by the deletion event indicated by an arrow. (A) The *fcl*$^{+}$*Y* duplication, which has the same appearance as *Dp(1;f)W39*, is formed by deleting part of the *X*-proximal element of *T(1;Y)W32* (see Figures 1B, 2A and text). The normal *X* chromosome distal to 17A is stretched out to the right. (B) A *Df(1)D2* heterozygote. (C) A *Df(1)os*^{UE19} heterozygote.

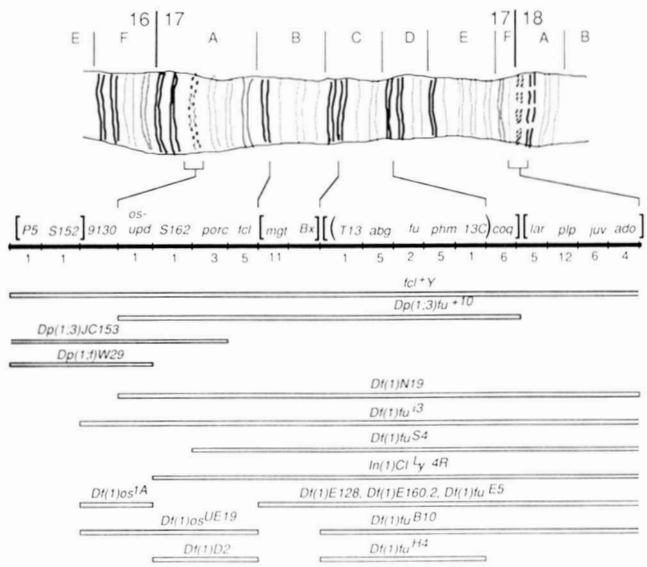


FIGURE 5.—Cytogenetic map of polytene section 17. The complementation groups we have identified are shown along the thick horizontal line below the polytene chromosome map. Parentheses and square brackets indicate complementation groups whose left-right order is unknown. Below each complementation group is presented the number of new alleles recovered in the present study. The extents of duplications (shaded rectangles) and deficiencies (open rectangles) as determined by complementation are indicated in the lower part of the figure. Those rearrangements which may extend beyond the region shown are indicated with open ends. See Table 1 for a list of the cytological breakpoints of these rearrangements.

chemical mutagens (see review by LEE 1976).

From the mutagenesis screens involving the use of the *fcl*$^{+}$*Y* duplication (screens B and C) the recovery of lethal mutations falling within *Df(1)N19* are as follows: 51 lethals from 16,250 fertile matings for EMS, 4 lethals from 3387 fertile matings for γ-radiation and 5 lethals from 3393 fertile matings for DEB.

Figure 5 presents a summary of our cytogenetic analysis of the region corresponding to polytene section 17. All together, 67 new mutations were recovered from these various screens, and together with the preexisting lethals (Table 3) a total of 84 mutations within *Df(1)N19* were characterized genetically and developmentally. The specific region of intense analysis is defined by *Df(1)N19* and extends from 17A2–6 to 17F2–18A3. We have been able to identify 15 lethal complementation groups in this region (the screens were not designed to recover male or female sterile mutations). Thus, with the *Bx* locus, there are 16 gene loci defined by mutations in this interval of 37–43 polytene chromosome bands (BRIDGES 1938).

Genetic and developmental characteristics of individual complementation groups within *Df(1)N19*: Let us now consider each complementation group beginning with the most distally located. Details of the origin, embryonic phenotype and germline clone phenotype of each allele are presented in Table 3 (see also Figure 6).

outstretched small eye-unpaired (os-upd): WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984), in their search for embryonic lethal loci in the *X* chromosome, identified two mutations, *upd*^{YC43} and *upd*^{YM55}, that meiotically map to positions 58.7 and 59.3, respectively. Because these mutations had similar mutant embryonic phenotypes they were assigned to the same putative complementation group, *unpaired* (*upd*). Using *fcl*$^{+}$*Y* we were able to demonstrate allelism between these two mutations. We have isolated one additional allele, *upd*^{UE69}. Further, we found that *upd* mutations are allelic to the three visible *outstretched small eye* (*os*) mutations (see LINDSLEY and GRELL 1968). The detailed genetic analysis of this complementation group will be reported elsewhere (R. BINARI, K. MCCALL and N. PERRIMON, manuscript in preparation). Cytologically, the *os-upd* locus is in 17A5–8, inclusive, based on its inclusion within *Df(1)os*^{UE19} but not *Df(1)D2* (Table 1; Figures 4 and 5). Phenotypic analysis of *os-upd* mutant embryos show that the most consistent defect observed is a deletion of the fifth abdominal denticle belt and a disruption of the posterior mid-ventral region of the fourth abdominal denticle belt (Figure 6B). This defect alone does not produce embryonic lethality as mutant larvae, pupae and sometimes adults are able to develop

lacking this abdominal region (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984). In addition, other segments such as the second and third thoracic segments and the eighth abdominal segment are sometimes deleted. The head is usually involuted and most head structures are present (Figure 6B). The upd phenotype is similar to the maternal effect phenotype of the larval-pupal zygotic lethal *hopscotch* (PERRIMON and MAHOWALD 1986). Germline clone analysis of *os-upd* alleles indicate that *os-upd*$^+$ function is not required for germline viability and that *os-upd* does not have a maternal effect (Table 3).

S162: We isolated only one allele in this complementation group and found that this late larval lethal allele does not have a maternal effect in homozygous germline clones (Table 3). For this and other complementation groups represented by only a single lethal mutation we have not assigned a descriptive name. A single allele may have a phenotype not representative of most other possible alleles.

porcupine (porc): The first *porc* allele, *porc*^{PB16}, was identified in a screen to analyze the maternal effect of *X*-linked zygotic lethal mutations (PERRIMON, ENGSTROM and MAHOWALD 1989). A subsequent allele, *porc*^{15175}, was isolated by FERRUS *et al.* (1990) in their genetic analysis of the *Shaker* (*Sh*) region and we isolated three additional alleles in our screens (Table 3). Hemizygous *porc/Y* progeny derived from heterozygous females mostly die during pupariation. However, hemizygous *porc/Y* embryos derived from homozygous germline clones exhibit a rescuable maternal effect lethal phenotype similar to the embryonic lethal phenotype of the *wingless* segment polarity gene (Figure 6C; PERRIMON, ENGSTROM and MAHOWALD 1989). This maternal effect is fully paternally rescuable since *porc*/+ germline clone-derived progeny are perfectly normal and lead to normal and fertile adult females.

foreclosed (fcl): Two mutations identified by EBERL and HILLIKER (1988), *fcl*^{EH244b} and *fcl*^{EH523}, were placed into a putative complementation group based on similar map position and the same phenotype. This phenotype is characterized by the lack of internal organogenesis evidenced by a large dorsal yolk plug, and the naked and poorly differentiated cuticle (Figure 6D), though mouthparts and posterior spiracles are usually well developed. We have confirmed allelism between these mutations and identified five new *fcl* alleles in this study. With the exception of two of the new mutations, *fcl*^{2C} and *fcl*^{G26}, which are lethal at the larval stage, all new alleles show the same embryonic lethal phenotype (Table 3). To examine whether thoracic and abdominal segmentation is perturbed in these embryos lacking discernible cuticle markers, we examined the expression pattern of the segmentation gene *engrailed* (*en*) using the *en-lacZ* gene described in the MATERIALS AND METHODS. No defects were detectable in the striped pattern of β-galactosidase expression (data not shown) indicating that *fcl* affects a late embryonic process. The *fcl* gene is required for germline development. All alleles are lethal in germline clones, except *fcl*^{1C} and *fcl*^{G26} which perturb late oogenesis since the few eggs that are laid have in most cases fused chorionic appendages (Table 3).

maggot (mgt): We have isolated 11 alleles in this complementation group. All are clearly larval lethal with the exception of *17C* which is a polyphasic lethal (Table 3). The wild-type function encoded by this complementation group is required for germline development since homozygosity in the female germline for the alleles tested either produce germline lethality or perturb oogenesis (Table 3). In the case of two alleles, *3X* and *8C*, the few eggs laid have, in most cases, fused chorionic filaments.

Beadex (Bx): We obtained no alleles of *Bx*, which is defined by dominant alleles that are homozygous viable (see LINDSLEY and GRELL 1968), and recessive alleles that give a heldup wings phenotype (see discussion of *heldup-a* by LINDSLEY and ZIMM 1985).

T13: The single allele at the *T13* locus produces lethality soon after eclosion. Adults have an outstretched, canopy wing phenotype. The *X* chromosome carrying *T13* shows substantial non-disjunction from the balancer in *T13/FM6* females but disjoins regularly from *fcl*$^+$*Y* in males. The polytene *X* chromosome of *T13* appears normal, but the basis of the elevated frequency has not been further examined. Germline mosaics of this allele indicate that *T13* is not required for germline viability and does not have a maternal effect (Table 3). Though not tested, *T13* may be allelic to certain *heldup* (*hdp*) mutations recovered by ENGELS and PRESTON (1981) which MATTOX and DAVIDSON (1984) have classified as *hdp-b* alleles, all of which are rearrangements with one break in 17C2–3 induced by mobilization of a *P* element in that location.

ambiguous (abg): We have identified five mutations in this complementation group. They produce lethality at either embryonic, larval or pupal stages. Ambiguous results are however obtained in germline clone analysis (Table 3). Three alleles, *D13, S12* and *T232*, do not exhibit a maternal effect, whereas the *13D* allele shows a rescuable maternal effect and *7C* perturbs an essential step of oogenesis. Two classes of embryos are obtained from *13D* homozygous germline clones, half show very poor cuticle development while the other half have a normal cuticle pattern.

fused (fu): Many mutations of *fu* are known (LINDSLEY and GRELL 1968; KING 1970; WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984; BUSSON *et al.* 1988; PERRIMON, ENGSTROM and MAHOWALD 1989). We have recovered two new alleles in this study. Null

TABLE 3

Germline clone analysis of mutations within polytene section 17

Locus and mutation[a]	Screen[b]	Mutagen	Lethal phase[c]	Germline clone analysis[d]				
				N	NGLC	ND	NCL	Conclusion
outstretched small eye-unpaired (os-upd)								
YC43	6	EMS	E	200	7	—	—	NME
YM55	6	EMS	E-L-P	200	8	—	—	NME
UE69	7	γ-ray	E-L-P	200	6	—	—	NME
S162								
S162	1	EMS	L3	200	9	—	—	NME
porcupine (porc)								
PB16	3	EMS	P	240	11	—	—	MELR
i8	2	EMS	P	240	11	—	—	MELR
15175	5	X-ray	P	250	31	—	—	MELR
G18	2	γ-ray	P	190	9	—	—	MELR
2E	2	EMS	P	200	10	—	—	MELR
foreclosed (fcl)								
D18	2	DEB	E	150	0	80	0	L
3D	2	EMS	E	225	0	100	0	L
2C	2	EMS	L	170	0	100	0	L
G26	2	γ-ray	L	150	10	—	—	AO
1C	2	EMS	E	210	5	—	—	AO
EH244b	4	EMS	E	519	0	166	0	L
EH523	4	EMS	E	283	0	—	—	L
maggot (mgt)								
3C	2	EMS	L1	240	0	100	0	L
12C	2	EMS	L	150	0	100	0	L
D14	2	DEB	L2	150	0	100	0	L
G28	2	γ-ray	L	250	0	100	0	L
3E	2	EMS	L1	200	0	100	0	L
16N	2	EMS	L1	200	0	100	0	L
3X	2	EMS	L	225	0	100	5	AO
8C	2	EMS	L1-2	250	9	—	—	AO
21D	2	EMS	L1-2	250	0	100	0	L
17C	2	EMS	E-L-P	—	—	—	—	—
1E	2	EMS	L1-2	—	—	—	—	—
Beadex (Bx)	No lethal alleles							
T13								
T13	1	EMS	A	200	12	—	—	NME
ambiguous (abg)								
D13	2	DEB	EP	200	5	—	—	NME
S12	1	EMS	EP	200	12	—	—	NME
T232	1	EMS	L	200	10	—	—	NME
13D	2	EMS	L-P	200	12	—	—	MELR
7C	2	EMS	E-L-P	220	12	—	—	AO
fused (fu)								
1PP7	3	EMS	P	200	5	—	—	MELR, AO
9P2.3	3	EMS	P	180	6	—	—	MELR, AO
D12	2	DEB	P	100	8	—	—	MELR, AO
5N	2	EMS	P	200	5	—	—	MELR, AO
phantom (phm)								
EH162b	4	EMS	E	300	0	100	0	L
16C	2	EMS	E	200	6	—	—	AO
XE07	6	EMS	E	300	0	100	6	AO
EH603b	4	EMS	E	300	0	100	5	AO
EH740b	4	EMS	E	400	0	80	5	AO
EH320	4	EMS	E	144	11	—	—	NME
EH354	4	EMS	E	272	9	—	—	NME
15D	2	EMS	E	200	6	—	—	NME
R32	1	EMS	E	200	8	—	—	NME
29N	2	EMS	E	250	8	—	—	NME
T112[a]	1	EMS	L-P	150	8	—	—	NME
13C								
13C	2	EMS	L2	200	7	—	—	NME

Locus and mutation[a]	Screen[b]	Mutagen	Lethal phase[c]	Germline clone analysis[d]				
				N	NGLC	ND	NCL	Conclusion
coquilled (coq)								
7E	2	EMS	A	200	8	—	—	MEL
31D	2	EMS	A	225	8	—	—	MEL
12B	2	EMS	P-A	150	8	—	—	MEL
10C	2	EMS	A	150	2	100	0	MEL
19D	2	EMS	A	200	10	—	—	MEL
2D	2	EMS	A	225	3	50	0	AO
larvaphile (lar)								
5C	2	EMS	L	250	0	100	0	L
6B	2	EMS	L2	225	0	100	0	L
6	2	EMS	L2	240	0	100	0	L
17D	2	EMS	L2	200	0	—	—	L
P131[ts]	1	EMS	L3	200	0	100	0	L
polyphase (plp)								
16D	2	EMS	E	200	9	—	—	NME
23D	2	EMS	E	225	9	—	—	NME
6E	2	EMS	E	225	6	—	—	NME
1B	2	EMS	E	200	15	—	—	NME
37D	2	EMS	E	225	12	—	—	NME
18N	2	EMS	E	250	4	—	—	NME
4E	2	EMS	L	225	5	—	—	NME
9C	2	EMS	E-L-P	165	11	—	—	NME
7B	2	EMS	LP	225	14	—	—	NME
2X	2	EMS	E-L	225	8	—	—	MEL
15C	2	EMS	P	250	12	—	—	MELR
27N	2	EMS	P	200	7	—	—	MELR
juvenile (juv)								
5E	2	EMS	L3	200	5	—	—	AO
G17	2	γ-ray	L3	200	7	—	—	AO
14C	2	EMS	L1-2	225	8	—	—	AO
10B	2	EMS	L	195	9	—	—	AO
2B	2	EMS	E	180	10	—	—	AO
26N	2	EMS	L-P	250	3	100	0	AO
adolescent (ado)								
4P1	3	EMS	L-P	200	10	—	—	AO
3B	2	EMS	P	150	10	—	—	AO
25D	2	EMS	L-P	225	5	—	—	AO
9B	2	EMS	L	270	21	—	—	AO
Other mutations analyzed in this study								
Df(1)os^{UE19}	7	γ-ray	E	250	0	100	0	L
Df(1)D2	2	DEB	E	200	0	100	0	L
Df(1)N19		X-ray	E	250	0	100	0	L
91930	5	X-ray	P	150	25	—	—	NME

[a] A "*ts*" indicates that the mutation is temperature sensitive. All lethal phases were determined at 25°.

[b] References: 1, this work, screen A; 2, this work, screens B or C; 3, PERRIMON, ENGSTROM and MAHOWALD (1989); 4, EBERL and HILLIKER (1988); 5, FERRUS *et al.* (1990); 6, WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984); 7, this work, induced by γ-rays in females (see text).

[c] Nomenclature on the lethal phase: The lethal phase of each mutation is indicated: E, embryonic; L, larval stages (L1 refers to the first instar larval stage, L2 to the second and L3 to the third); P, pupal stage; LP, late pupal; EP, early pupal; A, adults die shortly after eclosion.

[d] Nomenclature on the germline clone analysis. Maternal expression was determined by germline clone analysis. *N* is the number of females of genotype *Fs(1)K1237 v*^{24}*/lethal* analyzed for the presence of germline clones. NGLC corresponds to the number of females possessing a germline clone. ND, number of females dissected; NCL, number of females found to possess a clone after dissection. The maternal expression has been subdivided according to the phenotype of the homozygous lethal germline clones. These categories are: L, lethal; AO, abnormal oogenesis; NME, no maternal effect; MEL, maternal effect lethal phenotype; MELR refers to a paternally rescuable maternal effect lethal phenotype; a "—" refers to not tested. All experiments were done at 25°.

fu mutations produce lethality during the pupal stage (BUSSON *et al.* 1988) and weaker mutations are adult viable, exhibiting a range of defects including fusion of veins, outspread wings and female sterility (LINDSLEY and GRELL 1968). Eggs derived from females homozygous for a viable *fu* allele or from homozygous germline clones of a *fu* lethal allele have a partially rescuable maternal effect (COUNCE 1956;

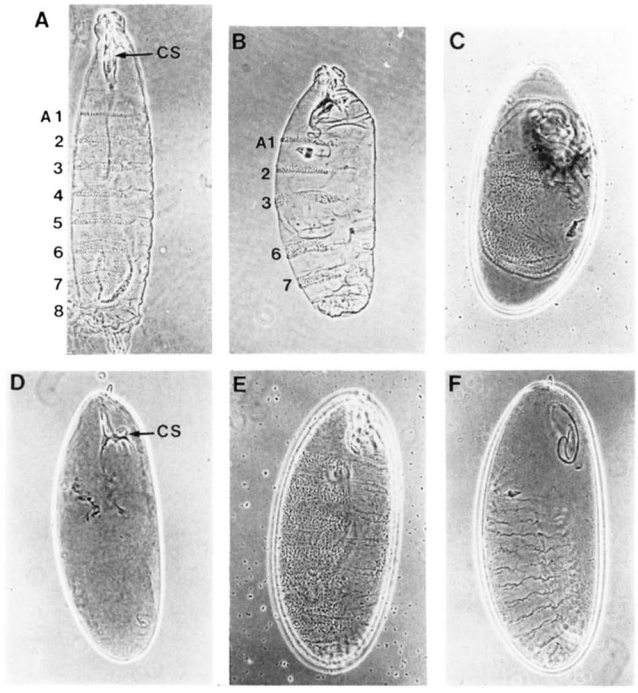


FIGURE 6.—Cuticle phenotypes. (A) A phase contrast micrograph of a ventral view of a wild-type embryo. The cephalopharyngeal skeleton (CS) and eight abdominal segments A1–8 are indicated. (B) A *Df(1)os*^{1A} embryo showing the unpaired phenotype. (C) A *porcupine* embryo derived from germline clones homozygous for *porc*^{G18}. (D) A *foreclosed* embryo (*fcl*^{1C}). (E) A *fused* embryo derived from germline clones homozygous for *fu*^{D12}. (F) A *phantom* embryo (*phm*^{16C}). Anterior is up in all figures.

Table 3). Among the eggs that develop past the early cleavage stages, those which have not received a *fu*$^{+}$ gene from their father exhibit a segment polarity phenotype (Figure 6E; NÜSSLEIN-VOLHARD and WIESCHAUS 1980), others exhibit either a weak segment polarity phenotype or are normal and lead to normal and fertile females. Additionally, females with homozygous germline clones often develop ovarian tumors, a phenotype previously observed from females homozygous for the viable *fu* alleles (KING 1970). PREAT *et al.* (1990) have shown by molecular characterization that the *fu*$^{+}$ gene product is a serine/threonine protein kinase.

phantom (phm): WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984) assigned five putative alleles to this complementation group on the basis of cuticle phenotype. EBERL and HILLIKER (1988) assigned three additional alleles to this group, and two mutations to the putative complementation group *exocephalon* (*exo*) on the basis of whole mount phenotype. However, allelism could not be tested due to the lack of an appropriate duplication. Using *fcl*$^{+}$*Y* to test allelism among these mutations we have found that all alleles tested (all of our mutations and the representative allele, *phm*^{XE07}, provided by E. WIESCHAUS) belong to the same complementation group, which will henceforth be called *phm*. Five new alleles were recovered in the present study. All alleles with the exception of the temperature sensitive allele *phm*^{T112} are embryonic lethals (Table 3). The phm phenotype consists of poorly differentiated cuticle lacking denticle bands (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984; Figure 6F). Additionally, the embryo is contracted posteriorly with a large opening in the head region. The embryonic defects associated with *phm* embryos occur late during embryonic development and we did not detect any defect in the expression pattern of *en* (data not shown) using the *en-lacZ* fusion gene (see MATERIALS AND METHODS).

Germline clone analysis of *phm* alleles indicates that maternal expression is dependent upon the allele analyzed (Table 3). One mutation, *phm*^{EH162b}, produces germline lethality, four alleles fail to produce normal eggs, and six others have no maternal effect. These results indicate that the *phm* locus encodes diverse functions, some of which are required for oogenesis and others during embryogenesis. Moreover, the maternal *vs.* the zygotic requirements for this gene can be dissected by the various mutations. Nevertheless, *inter se* crosses performed between all alleles at three different temperatures (18°, 25° and 29°) failed to reveal any interallelic complementation (P. REINAGEL and N. PERRIMON, unpublished).

13C: The only mutation recovered in this complementation group is a second instar larval lethal allele that does not have a maternal effect in homozygous germline clones (Table 3).

The above five complementation groups all fall within the interval 17C3 to 17D2, inclusive, as defined by *Df(1)fu*^{H4} (Figure 5). No other rearrangement breakpoints are available within this interval and therefore the left-right order of these groups is unknown.

coquilled (coq): This complementation group falls within *Df(1)fu*^{B10} and *Dp(1;3)fu*$^{+10}$ but not within *Df(1)fu*^{H4}. No rearrangement breakpoints serve to determine the left-right of this group with *Df(1)fu*^{H4}. Hemizygous individuals carrying any of the six alleles die as late pupae or early adults. The adults show a "scalloped-like" phenotype in the wings, hence the name *coquilled*. All alleles tested in germline clone analysis have a fully penetrant maternal effect lethal phenotype. Many eggs appear unfertilized and some are defective; embryos are arrested at various developmental stages (Table 3).

Mutations falling within *Df(1)N19* but not rescued by *Dp(1;3)fu*$^{+10}$ fall into four complementation groups (Figure 5) and are described below.

larvaphile (lar): All five alleles in this complementation group are larval lethals and produce germline lethality in germline clones (Table 3).

polyphase (plp): We have isolated 12 alleles which can produce lethality at either embryonic, larval or

pupal stages (Table 3). Embryos carrying the embryonic lethal alleles have a normal cuticular morphology and none of these alleles exhibit a maternal effect. Other alleles cause lethality at later stages, and two of these show a rescuable maternal effect. Dead embryos derived from homozygous germline clones of these two alleles have a normal morphology. A maternal effect lethal dorsalizing phenotype seen in germline clones of the *2X* allele was found to be attributable to a second site mutation in *fs(1)gastrulation defective.*

juvenile (juv): Hemizygous progeny derived from mothers heterozygous for any of the six alleles recovered in this complementation group die before pupariation at the end of the third instar stage. All the alleles tested in germline clones perturb oogenesis (Table 3). Among the eggs that are laid some have fused filaments, others are smaller or collapsed.

adolescent (ado): Three new mutations were recovered here which are alleles of the *4P1* mutation described by PERRIMON, ENGSTROM and MAHOWALD (1989). These mutations are larval-pupal lethals. Mutant pupae are often smaller than wildtype. Normal activity of this gene is required for oogenesis since all mutations tested by germline clone mosaics produce defective eggs (Table 3). Among the eggs derived from females with germline clones a range of phenotypes including fused chorionic filaments, small size or collapsed.

Embryonic phenotypes of deficiencies in polytene section 17: Analysis of the phenotypes of deficiencies within a genetically saturated region can indicate if mutations in some loci have been missed by the genetic screens. If genetic saturation of a region is obtained then the zygotic lethal phenotype of a specific deficiency should resemble the phenotype of the lethal loci contained within it (see for example WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984) although this may be confounded by genetic interactions. For example, such analyses are of particular importance since they may reveal the presence of genetically complex situations in which multiple transcription units have to be deleted to detect an embryonic phenotype (see for example DURA *et al.* 1987).

To determine whether the phenotype of the deficiencies correspond to the zygotic lethals of the region, we analyzed the stage of lethality and phenotypes of most of the deficiencies in polytene section 17. As listed in Table 4 the phenotypes of embryos carrying *Df(1)os^{1A}*, *Df(1)os^{UE19}*, *Df(1)D2*, *Df(1)E128*, *Df(1)fu^{E5}*, or *Df(1)fu^{H4}* resemble the phenotypes of those embryonic lethal loci *upd*, *fcl* or *phm* that they encompass. This indicates that no other early acting genes within these deficiencies are likely to have been missed in the mutagenesis screens.

The embryonic phenotypes of the two deficiencies *Df(1)N19* and *Df(1)fu^{S4}* is less straightforward since

TABLE 4

Embryonic phenotype of the deficiencies

Deficiency	Expected phenotype	Observed phenotype
Df(1)N19	upd, fcl, phm	No cuticle
Df(1)os^{1A}	upd	upd
Df(1)os^{UE19}	fcl, upd	fcl
Df(1)D2	fcl	fcl
Df(1)fu^{S4}	fcl, phm	No cuticle
Df(1)E128[a]	phm	phm
Df(1)fu^{E5}	phm	phm
Df(1)fu^{H4}	phm	phm

[a] The phenotype of *Df(1)E128/Y* embryos is more extreme than *phm*. This is most likely due to the presence of additional lethals located proximally which are not included in *Df(1)N19*.

hemizygous embryos for either of these deficiencies show no cuticle differentiation (Table 4). These deficiencies uncover both *fcl* and *phm*, and it is most likely that deleting these two genes is responsible for the "no cuticle" phenotype. Nevertheless we can not rule out the possibility that there is an additional gene, falling between *fcl* and the *3C* complementation group so that it is not deleted by the smaller deletions, that also affects cuticle differentiation and that we have failed to mutate. Since the lack of cuticle in *Df(1)N19* embryos makes it difficult to assess the possibility that it deletes an additional gene that affects segmentation, we examined the pattern of expression of *en*$^+$ (using the *en-lacZ* strain), as a segmentation marker, in *Df(1)N19* embryos, and found that it is similar to *upd* embryos (see Figure 8C). Therefore, it is unlikely that there are any other segmentation genes in this region.

The *bangles* and *beads* (*bnb*) gene: Previously, a cDNA (and thus a putative gene) which showed a segmental expression pattern in both the epidermis and nervous system was isolated and mapped by *in situ* localization to region 17E on the *X* chromosome (NG *et al.* 1989). An *in situ* hybridization to *Df(1)N19/+* polytene chromosomes showed that this DNA is located within the region defined by the deficiency since an hybridization signal was detected on only one homolog, within the deficiency loop (data not shown). We herein name this gene *bangles and beads* (*bnb*) due to both its banded epidermal and punctate neural expression patterns (NG *et al.* 1989). We have further localized *bnb*$^+$ within chromosomal bands 17E3–9 by *in situ* hybridization.

The organization of the *bnb* gene is shown in Figure 7. Two developmentally regulated transcripts are encoded from the 17 kb of genomic DNA shown. Sequence analysis of the longest cDNA, *KZ30*, reveals two polyadenylation signals which can account for the two transcripts revealed by Northern analysis (NG *et al.* 1989). *KZ30* is encoded by two exons with a 1.6-kb intron being positioned immediately 5′ to the start of translation.

To examine whether our screen identified muta-

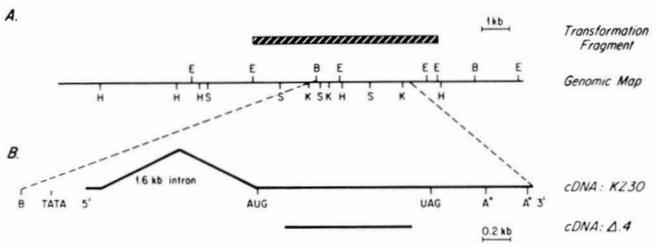


FIGURE 7.—Organization of the *bnb* gene. The extent of the DNA fragment used for P-element transformation and two *bnb* cDNAs, KZ30 (NG *et al.* 1989) and Δ.4 (KNUST *et al.* 1987), is indicated. The enzymes used to establish the restriction map are: *Hin*dIII (H), *Eco*RI (E), *Sal*I (S), *Kpn*I (K). Further sequencing of the *bnb* genomic region and the cDNAs revealed some discrepancies with the original sequence (NG *et al.* 1989). The amino acids at position 112 through 153 and 168 through 178 should be:

```
112 AIPEKKTLPEEAKPAQENAPVEAE
    KKQEKTARTEAEPTVEAQP 153
168 ANAEVQKQVVD      178
```

These changes do not affect the region of homology with the mammalian protein gap-43 (NG *et al.* 1989). Searches with the modified sequence to updated databases fail to identify any significant homology (outside the "Gap-box," NG *et al.* 1989) between *bnb* and other protein or nucleotide sequences contained in these databases.

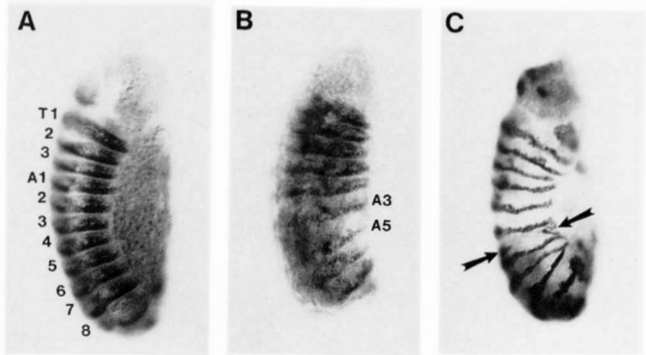


FIGURE 8.—Expression pattern of *bnb*. (A) The RNA expression pattern of *bnb* in a wildtype embryo. (B) The RNA expression pattern of *bnb* in a *Df(1)N19/Y* embryo that carries the autosomal transformant *23F9RIP*. The A4 stripe of *bnb* expression is deleted due to the absence of *unpaired*$^+$ (see C and text). *In situ* hybridizations to whole mount embryos in A and B are with digoxygenin labeled probes. (C) A *Df(1)N19/Y* embryo carrying the second chromosomal insertion *en-lacZ*. The stripe of *engrailed* expression corresponding to A4 is partially deleted (indicated by arrows). This defect is due to the absence of *unpaired*$^+$ function uncovered by the deficiency.

tions in the *bnb* gene we built several transformant lines that carry the DNA region containing the *bnb* gene. If the transformants can rescue the mutant phenotype(s) associated with mutations within any one of the complementation groups, then this locus identifies the *bnb* gene. Following partial *Eco*RI digestion of a λ EMBL3 clone (Figure 7) containing approximately 17 kb of genomic DNA, a 6.7-kb fragment was isolated and subcloned into a pCaSpeR-2 transformation vector (THUMMEL, BOULET and LIPSHITZ 1988). The transformant fragment contained 6.7 kb of genomic DNA from the *bnb* region with 2.6 kb upstream of the TATA box and 1.0 kb downstream of the second polyadenylation site (Figure 7).

To determine whether the transformants (symbolized as $P[bnb^+]$) contained sufficient regulatory elements to rescue a mutant phenotype, whole mount *in situ* hybridization to *Df(1)N19* embryos that carry $P[bnb^+]$ on the second chromosome were performed (Figure 8). Flies from two separate transformant lines were tested and in both cases the epidermal expression of the *bnb* gene (the "bangles" phenotype) was observed in *Df(1)N19/Y*; $P[bnb^+]$/+ male embryos indicating that sufficient regulatory region was present in the transformant to direct *bnb* epidermal expression. The neural expression of the *bnb* gene (the "beads" phenotype) is also detected but it is more difficult to assess whether the pattern of expression resembles wild type since *Df(1)N19* embryos deteriorate considerably during late embryogenesis. These whole mount *in situ* experiments indicate that the *bnb* gene on the *P* element transposon most likely is expressed in the correct expression pattern and should at the very least rescue a mutation within the *bnb* gene.

We tested whether the lethality associated with mutations within *Df(1)N19* could be rescued by $P[bnb^+]$. A minimum of two alleles from each complementation group, when available, were tested for rescue. No evidence was found for rescue of the lethality of any mutation tested indicating either that bnb^+ does not encode a vital function or that we have not recovered a mutation in this gene.

Although we have been unable to identify any mutations in the *bnb* gene, we still wanted to gain some insight into its possible function. Therefore, the phenotypes of embryos in which *bnb* is overexpressed or ubiquitously expressed were examined. The cDNA *KZ30* was inserted into the vector pCaSpeR-hs, a *P* element vector with *a white*$^+$ selectable marker used for expressing open reading frames under heat shock control. It contains both hsp70 promoter and 3′ regions flanking the inserted cDNA (C. THUMMEL, personal communication). When 0–6-hr embryos were heat shocked 21.4% of the embryos died; when 6–12-hr embryos were heat shocked there was 14.9% lethality which is significantly higher than heat shocked control embryos (Table 5). When the cuticles and central nervous systems of the heat shocked embryos carrying this construct were examined defects ranging from mild to severe were observed (Figure 9). Mild cuticle defects included disarranged or missing denticle bands whereas severe cuticle defects were observed in severely twisted embryos entirely lacking ventral denticles. Mild CNS defects included reduced or missing commissural tracts along with widened or thicker longitudinal tracts. Severe CNS defects were observed where commissures were almost entirely missing and

TABLE 5

Heat shock lethality of *hs-KZ30* flies

	Non-heat shocked 0–6-hr embryos		Heat shocked 0–6-hr embryos		Heat shocked 6–12-hr embryos	
	No. eggs	Percent lethal	No. eggs	Percent lethal	No. eggs	Percent lethal
hs-KZ30	615	4.5	1331	21.4	1239	14.9
Control	219	4.1	253	4.0	172	0.005

Appropriately aged embryos, either 0–6-hr or 6–12-hr embryos were heat shocked at 37° for 1 hr. Embryos 0–6 hr old apparently are more sensitive to heat shock than older, 6–12-hr embryos, likely indicating a developmental process during this time that is very sensitive to overexpression of *bnb* gene product. Five independent homozygous *hs-KZ30* insertion lines were analyzed for these experiments. Since the results were similar in each experiment, the results were pooled.

longitudinal tracts were grossly thicker. Since the phenotypes observed in these experiments appear to be related to the bnb^+ expression patterns, it is likely that bnb^+ plays an important role during development.

DISCUSSION

We have constructed a duplication, fcl^+Y, which allowed a genetic characterization of polytene section 17. This duplication enabled us to test by complementation the allelism of known mutations in this region. The *phm* and *exo* mutations were found to define a single complementation group, although the zygotic and germline phenotypes of the various mutations demonstrate that this may be a more complex locus. Allelism in all the other previously postulated complementation groups has been confirmed. Furthermore, in a series of new mutagenesis screens we have found new alleles of each of the putative lethal complementation groups that were previously known to reside in this interval. We have also identified ten new gene loci, six of which were found to be important in oogenesis or embryogenesis, and two others of which each had some alleles that affected these processes. Within the region there is an additional genetic element, bnb^+, which functions during embryogenesis. This single copy putative gene is defined by a cloned DNA sequence which contains two alternative transcripts (NG *et al.* 1989). None of the mutations recovered in our study appears to correspond to this gene, suggesting either that the mutagenesis has failed to saturate the region or that this gene is not essential for viability. Alternatively, failure to identify mutations in *bnb* may be due to redundancy of functional pathways as has been proposed, for example, in the genetic interactions between *abl* and *fas I* (ELKINS *et al.* 1990). Finally, no new alleles of *Bx* were recovered.

In summary, a total of 17 genes have been located within the region defined by *Df(1)N19* which is comprised of 37–43 polytene chromosome bands. Of these 17 genes, 12 (71%) influence or are expressed during oogenesis or embryogenesis. Therefore this may be a region that is enriched in genes of developmental significance regarding oogenesis and embryogenesis. We do not claim to have saturated this interval, although we obtained multiple new alleles for 11 of the 15 lethal loci. Thus this study has provided a comprehensive cytogenetic map and developmental characterization of polytene section 17. Most of this region has been cloned (MATTOX and DAVIDSON 1984; MARIOL, PREAT and LIMBOURG-BOUCHON 1987; NG *et al.* 1989; FERRUS *et al.* 1990) which, in conjunction with material generated in this study, will

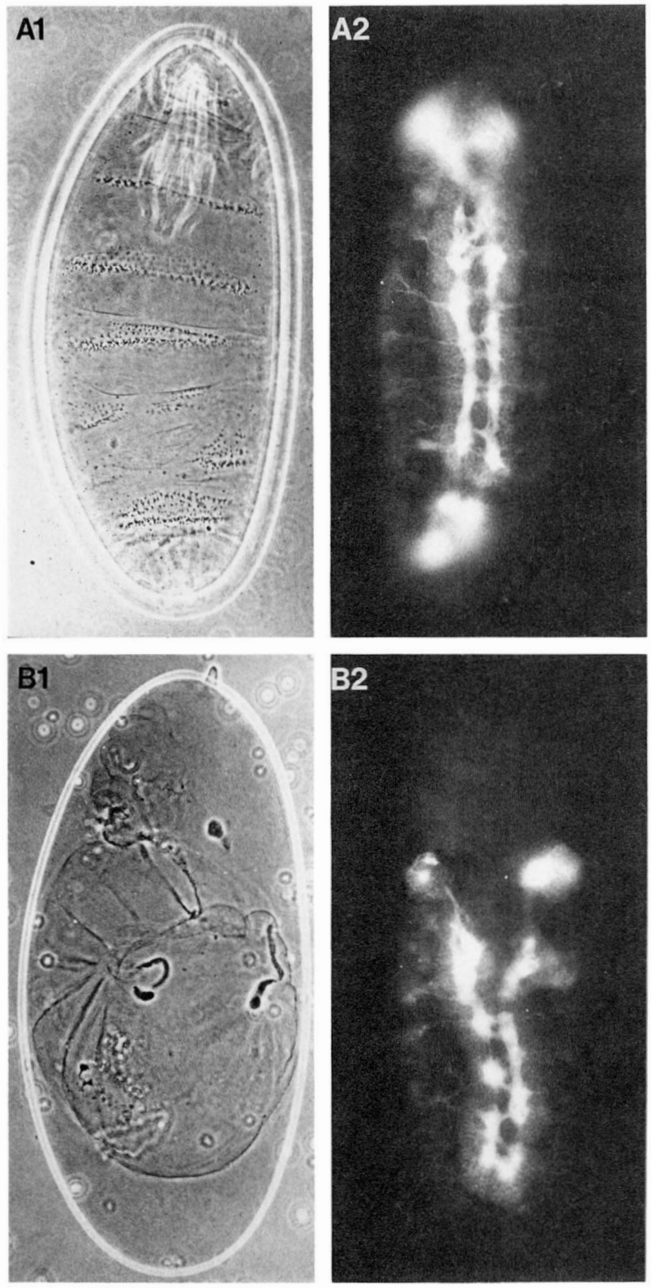


FIGURE 9.—Heat shock phenotype of *bnb*. Mild (A1) and severe (B1) cuticle defects. Mild (A2) and severe (B2) CNS defects, as revealed with anti-HRP.

allow a detailed molecular genetic analysis of the polytene section 17 region.

We are grateful to A. FERRUS, O. PONGS, J. MERRIAM, D. BUSSON, B. LIMBOURG-BOUCHON, E. WIESCHAUS, W. ENGELS and M. TANOUYE and the Bowling Green Stock Center for sending us stocks, and to ELIZABETH KNUST for providing the *bnb* cDNA, *Δ.4*. We thank PAMELA REINAGEL and RICH BINARI for communicating unpublished results and THERESA CAPACI and ANTHONY AVRUCH for help with the DNA sequencing. This work was supported by the Howard Hughes Medical Institute and National Institutes of Health grant to N.P., and a Natural Sciences and Engineering Research Council of Canada grant to A.J.H.

Note added in proof: The nucleotide sequence of the *Drosophila melanogaster bangles and beads (bnb)* gene reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence databases under the accession number X63828.

LITERATURE CITED

ABRAHAMSON, S., I. H. HERSKOWITZ and H. J. MULLER, 1954 Genetic proof for half-translocations derived from irradiated oocytes of *Drosophila melanogaster*. Genetics **39:** 955.

AUSUBEL, F. M., R. BRENT, R. E. KINGSTON, D. D. MOORE, J. A. SMITH, J. G. SEIDMAN and K. STRUHL, 1990 *Current Protocols in Molecular Biology*. Wiley Interscience, New York.

BODENSTEIN, D., 1950 The postembryonic development of Drosophila, pp. 275–367 in *Biology of Drosophila*, edited by M. DEMEREC. John Wiley & Sons, New York.

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

BROSSEAU, G. E., B. NICOLLETI, E. H. GRELL and D. L. LINDSLEY, 1961 Production of altered Y chromosomes bearing specific sections of the X chromosome in Drosophila. Genetics **46:** 339–346.

BUSSON, D., 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.

BUSSON, D., B. LIMBOURG-BOUCHON, M.-C. MARIOL, T. PREAT and C. LAMOUR-ISNARD, 1988 Genetic analysis of viable and lethal *fused* mutants of *Drosophila melanogaster*. Roux's Arch. Dev. Biol. **197:** 221–230.

COUNCE, S. J., 1956 Studies on female-sterility genes in *Drosophila melanogaster*. II. The effects of the gene *fused* on embryonic development. Z. Indukt. Abstammungs-Vererbungsl. **87:** 462–481.

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

DURA, J.-M., N. B. RANDSHOLT, J. DEATRICK, I. ERK, P. SANTAMARIA, J. D. FREEMAN, S. J. FREEMAN, D. WEDDELL and H. W. BROCK, 1987 A complex genetic locus, *polyhomeotic*, is required for segmental specification and epidermal development in D. melanogaster. Cell **51:** 829–839.

EBERL, D. F., and A. J. HILLIKER, 1988 Characterization of *X*-linked recessive lethal mutations affecting embryonic morphogenesis in *Drosophila melanogaster*. Genetics **118:** 109–120.

ELKINS, T., K. ZINN, L. MCALLISTER, F. M. HOFFMANN and C. S. GOODMAN, 1990 Genetic analysis of a Drosophila neural cell adhesion molecule: interaction of fasciclin I and Abelson tyrosine kinase mutations. Cell **60:** 565–575.

ENGELS, W. R., and C. R. PRESTON, 1981 Identifying P factors in Drosophila by means of chromosome breakage hotspots. Cell **26:** 421–428.

FEINBERG, A. P., and B. VOGELSTEIN, 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:** 6–13.

FERRUS, A., S. LLAMAZARES, J. L. DE LA POMPA, M. A. TANOUYE and O. PONGS, 1990 Genetic analysis of the *Shaker* gene complex of *Drosophila melanogaster*. Genetics **125:** 383–398.

GALL, J. G., and M. L. PARDUE, 1971 Nucleic acid hybridization in cytological preparations. Methods Enzymol. **21:** 470–480.

HAMA, C., Z. ALI and T. B. KORNBERG, 1990 Region-specific recombination and expression are directed by portions of the *Drosophila engrailed* promoter. Genes Dev. **4:** 1079–1093.

HILLIKER, A. J., R. APPELS and A. SCHALET, 1980 The genetic analysis of D. melanogaster heterochromatin. Cell **21:** 607–619.

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.

KING, R. C., 1970 *Ovarian Development in Drosophila melanogaster*. Academic Press, New York.

KNUST, E., U. DIETRICH, U. TEPASS, K. A. BREMER, D. WEIGEL, H. VÄSSIN and J. A. CAMPOS-ORTEGA, 1987 EGF homologous sequences encoded in the genome of *Drosophila melanogaster*, and their relation to neurogenic genes. EMBO J. **6:** 761–766.

KOMITOPOULOU, K., M. GANS, L. H. MARGARITIS, F. C. KAFATOS and M. MASSON, 1983 Isolation and characterization of sex-linked female sterile mutants in *Drosophila melanogaster* with special interest to eggshell mutants. Genetics **105:** 897–920.

LEE, W. R., 1976 Chemical mutagenesis, pp. 1299–1341 in *The Genetics and Biology of Drosophila*, Vol 1c, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

LEWIS, E. B., and F. BACHER, 1968 A method of feeding ethyl methane sulfonate (EMS) to Drosophila males. Drosophila Inform. Serv. **43:** 193.

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

LINDSLEY, D., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*. Part 1: Genes A–K. Drosophila Inform. Serv. **62.**

LINDSLEY, D., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*. Part 3: Rearrangements. Drosophila Inform. Serv. **65.**

LINDSLEY, D., and G. ZIMM, 1990 The genome of *Drosophila melanogaster*. Part 4: Genes L–Z, balancers, transposable elements. Drosophila Inform. Serv. **68.**

MARIOL, M.-C., T. PREAT and B. LIMBOURG-BOUCHON, 1987 Molecular cloning of fused, a gene required for normal segmentation in the *Drosophila melanogaster* embryo. Mol. Cell. Biol. **7:** 3244–3251.

MATTOX, W. W., and N. DAVIDSON, 1984 Isolation and characterization of the *Beadex* locus of *Drosophila melanogaster*: a putative cis-acting negative regulatory element for the *heldup-a* gene. Mol. Cell. Biol. **4:** 1343–1353.

NG, S.-C., L. A. PERKINS, G. CONBOY, N. PERRIMON and M. C. FISHMAN, 1989 A *Drosophila* gene expressed in the embryonic CNS shares one conserved domain with the mammalian GAP-43. Development **105:** 629–638.

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

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, 1984 The effects of zygotic lethal mutations on female germ-line functions in *Drosophila*. Dev. Biol. **105:** 404–414.

PERRIMON, N., L. ENGSTROM and A. P. MAHOWALD, 1989 Zygotic lethals with specific maternal effect phenotypes in *Drosophila melanogaster*. I. Loci on the *X* chromosome. Genetics **121:** 333–352.

PERRIMON, N., and M. GANS, 1983 Clonal anslysis 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.

PERRIMON, N., and A. P. MAHOWALD, 1986 *l(1)hopscotch*, a larval-pupal lethal with a specific maternal effect on segmentation in *Drosophila*. Dev. Biol. **118:** 28–40.

PERRIMON, N., D. MOHLER, L. ENGSTROM and A. P. MAHOWALD, 1986 *X*-linked female sterile loci in *Drosophila melanogaster*. Genetics **113:** 695–712.

PREAT, T., P. THEROND, C. LAMOUR-ISNARD, B. LIMBOURG-BOUCHON, H. TRICOIRE, I. ERK, M.-C. MARIOL and D. BUSSON, 1990 A putative serine/threonine protein kinase encoded by the segment-polarity *fused* gene of *Drosophila*. Nature **347:** 87–89.

ROBERTS, P. A., 1976 The genetics of chromosome aberration, pp. 67–184 in *The Genetics and Biology of Drosophila*, Vol. 1a, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. JOHNSON-SCHLITZ, W. K. BENZ and W. R. ENGELS, 1988 A stable source of *P* element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SMOUSE, D., and N. PERRIMON, 1990 Genetic dissection of a complex neurological mutant, *polyhomeotic*, in *Drosophila*. Dev. Biol. **139:** 169–185.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

TANOUYE, M. A., A. FERRUS and S. C. FUJITA, 1981 Abnormal action potentials associated with the *Shaker* locus of *Drosophila*. Proc. Natl. Acad. Sci. USA **78:** 6548–6552.

TAUTZ, D., and C. PFEIFLE, 1989 A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. Chromosoma **98:** 81–85.

THUMMEL, C. S., A. M. BOULET and H. D. LIPSHITZ, 1988 Vectors for *Drosophila* P-element-mediated transformation and tissue culture transfection. Gene **74:** 445–456.

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

WIESCHAUS, E., C. NÜSSLEIN-VOLHARD and G. JÜRGENS, 1984 Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. III. Zygotic loci on the X-chromosome and fourth chromosome. Roux's Arch. Dev. Biol. **193:** 296–307.

Communicating editor: T. SCHÜPBACH