Developmental Genetics of Loci at the Base of the *X* Chromosome of *Drosophila melanogaster*

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ABSTRACT

We have conducted a genetic and developmental analysis of the 26 contiguous genetic complementation groups within the 19D3-20F2 interval of the base of the *X* chromosome, a region of 34 polytene bands delimited by the *maroon-like* and *suppressor of forked* loci. Within this region there are four loci which cause visible phenotypes but which have little or no effect on zygotic viability (*maroon-like, little fly, small optic lobes* and *sluggish*). There are 22 loci which, when mutated, are zygotic lethals and three of these, *legless/runt, folded gastrulation* and *13E3*, have severe effects on embryonic development. In addition, three visible phenotypes have been defined only by overlapping deficiencies (*melanized-like, tumorous head*, and *varied outspread*). We have analyzed the lethal phases and maternal requirement of 58 mutations at 22 of the zygotic lethal loci by means of germline clone analysis using the dominant female sterile technique. Additionally, all lethal complementation groups, as well as a specific subset of deficiencies, have been studied histologically for defects in the development of the central and peripheral embryonic nervous systems.

DETAILED genetic analyses of specific segments of the *Drosophila melanogaster* genome provide critical information on the density of mutable loci and on the presence of clustered genes that may be related to specific developmental or structural functions. In some cases these analyses provide the only initial pragmatic avenue to our understanding of complex phenotypes. It is the characterization of a large number of mutations induced by different mutagens and isolated in different screens for visible mutants, steriles, lethals and behaviorals, for example, that allows an essentially complete description of the array of phenotypes that can be derived from a genetic unit. These genetic dissections are fundamental to understanding the roles that a specific gene may play during development and are also prerequisites for integrating the molecular biological data with the genetic and cell biological results. Such detailed studies on genomic segments have however only been attempted in very few small intervals of the genome. These include: subdivisions 2C to 3C (SHANNON *et al.* 1974; PERRIMON, ENGSTROM and MAHOWALD 1984b, 1985); division 10 (GEER, LISCHWE and MURPHY 1983); divisions 19 and 20 (SCHALET and LEFEVRE 1976); subdivision 21D to 22A (ROBERTS *et al.* 1985); division 35 (ASHBURNER, TSUBOTA and WOODRUFF 1982); division 36 (STEWARD and NUSSLEIN-VOLHARD 1986); division 37 (WRIGHT, BEWLEY and SHERALD 1976); subdivision 84B-C (LEWIS *et al.* 1980, CAVENER, OTTESON and KAUFMAN 1986); division 87 (GAUSZ *et al.* 1979); and division 93 (MOHLER and PARDUE 1984).

The proximal region of the *X* chromosome which occupies nearly one and a half polytene divisions spans the *maroon-like* to *suppressor of forked* interval from bands 19D3 through 20F2. It is recognized as a transition zone from conventional euchromatin in division 19 to β-heterochromatin in division 20 and this interval consists of approximately one and a half megabases of DNA (JOHN and MIKLOS 1988). It is particularly amenable to detailed genetic and developmental analyses because a large number of lethal and visible mutations as well as chromosomal rearrangements have been isolated and mapped to this area. Few of these loci, however, have been subjected to developmental, cell biological or molecular biological analyses. This 34 band interval holds one of the most complete arrays of mutagenized loci in the entire genome (LIFSCHYTZ and FALK 1968, 1969; LIFSCHYTZ and YAKOBOVITZ 1978; SCHALET and LEFEVRE 1973, 1976; LEFEVRE1981; KRAMERS *et al.* 1983; EEKEN *et al.* 1985; ZUSMAN, COULTER and GERGEN 1985; LEFEVRE and WATKINS 1986; SCHALET 1986; MIKLOS, KRAMERS and SCHALET 1986; GREEN, YAMAMOTO and MIKLOS 1987; MIKLOS *et al.* 1987, 1988). Furthermore the region is especially rich in mutations associated with putative neurological phenotypes such as *uncoordinated, uncoordinated-like, shaking, Passover, flightless, small optic lobes, sluggish, stoned* and *stress sensitive* and this has allowed us to examine the rela-

tionships between neurological mutants and essential genes. Finally, the 19D3 to 20F2 interval has been microdissected and microcloned and molecular entry points are thus available for most, if not all, of the genes between *maroon-like* and *suppressor of forked* (MIKLOS *et al.* 1988).

In this paper we have combined genetic complementation analysis, with lethal phase and germline clone analysis to study the genes within this region. Additionally, because many loci appear to be associated with neurological defects, we have also examined the effects of the available mutations, regardless of their lethal phases, on the organization of both the central and peripheral nervous system of the embryo.

MATERIALS AND METHODS

Strains: Table 1 lists the origins, characteristics and references to the rearrangements used in this study, and in Table 2 are listed the mutations we have analyzed. Most of the stocks used in this study were maintained using *FM6, FM7* or *Binsn* balancer chromosomes or kept in an *attached-X* (*C(1)DX, y f*) stock with appropriate duplication of the region usually involving the y^+Ymal^{106}, y^+Ymal^+ or B^sY chromosomes (SCHALET and LEFEVRE 1976). The *X*-linked dominant female sterile mutation *Fs(1)K1237* (or Ovo^{D1}, BUSSON *et al.* 1983; PERRIMON 1984) is maintained in an attached-*X* stock: *C(1)DX, y f/Y* females are crossed to *Fs(1)K1237* v^{24}/*Y* males. Descriptions of the stocks and balancer chromosomes, unless identified in the test, can be found in LINDSLEY and GRELL (1968) and in SCHALET and LEFEVRE (1976).

Germline clone analysis: Germline clones of zygotic lethals were generated using the dominant female sterile technique (PERRIMON and GANS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984a). Briefly, virgin females heterozygous for a zygotic lethal mutation, *FM6/lethal* or *FM7/lethal* were mated to *Fs(1)K1237/Y* males. At the end of the first larval instar the progeny of such a cross were irradiated with 1000 rads (Torrex 120D X-ray machine; 100 kV, 5 mA, 3-mm aluminum filter). Mitotic recombination in the germline of *lethal/Fs(1)K1237* females was detected by inspection for ovarian development in individual females. Those females which had germline clones were then isolated and crossed with two wild-type males (*OreR P2*). The eggs and progeny of these females were subsequently analyzed. The frequency of females carrying germline clones homozygous for a particular lethal varied from 5% to 8%. Since our analysis involved lethal mutations in the 19D-20F region located proximal to the dominant female sterile mutation (the ovo^D locus is located in 4E), mitotic recombination events occurring between ovo^D and the proximal-*X* region produce germ cells heterozygous for the lethal mutation. Such events were recovered at a frequency of about 10% of all mitotic recombination events and these were easily identified because male progeny were found from these females (PERRIMON, ENGSTROM and MAHOWALD 1984a). For each lethal tested in our study about 300 *lethal/Fs(1)K1237* females were examined. If no germline clones were detected, then a subset of these females were dissected in order to determine if there were defects in oogenesis (PERRIMON, ENGSTROM and MAHOWALD 1984a; see Table 2).

Analysis of embryonic phenotypes: Embryos were examined by four methods. (1) Embryonic cuticles were prepared according to the Hoyer's mount technique of VAN DER MEER (1977). Unhatched eggs were soaked in 50% Clorox bleach, heated at 65° in glycerol/acetic acid, and mounted in Hoyer's mountant. (2) Histological sections were prepared as described by MAHOWALD, CAULTON and GEHRING (1979). The vitelline membranes were removed using minor modifications of published procedures (MITCHISON and SEDAT 1983). Embryos were embedded in JB4 plastic (Polysciences) and serial 4-μm sections were cut using a Leitz 1516 microtome. These were stained with methylene blue with the slides being dried and mounted in Aquamount. (3) The patterns of the early nuclei divisions were examined using Hoechst DNA staining following removal of the vitelline membrane. (4) Immunochemical staining was performed as described by SMOUSE *et al.* (1988) with the localization of horseradish peroxidase being performed as published (GHYSEN *et al.* 1986). A polyclonal antisera against horseradish peroxidase (anti-HRP, JAN and JAN 1982) which labels all central nervous system (CNS) and peripheral nervous system (PNS) cell bodies and axons was used to look for global defects in the nervous system pattern. The *SOX2* monoclonal antibody (GOODMAN *et al.* 1984) which recognizes the cell bodies and axons of the entire PNS and a subset of CNS neurons, was also used to look for more subtle defects in neurogenesis.

The determination of the lethal phase of a particular genotype was performed as described by PERRIMON, ENGSTROM and MAHOWALD (1984a).

Genetic analyses: Single pair matings were performed on standard Drosophila medium at 22°–25°.

RESULTS

Genetic organization of the 19D3 to 20F2 region

Twenty-six complementation groups have so far been found within the 19D3-20F2 interval of the *X* chromosome (SCHALET and LEFEVRE 1976; LEFEVRE and WATKINS 1986; MIKLOS *et al.* 1987, 1988). Figures 1, 2 and 3 list these complementation groups with their approximate cytological locations as well as the positions of three loci identified by overlapping deficiencies (*melanized-like, varied outspread* and *tumorous head*). It is not known as yet whether these three phenotypes identify separate genes since no point mutations have yet been defined in them. The origins, and genetic limits, of the rearrangements used in this analysis are indicated in Table 1. The complementation map was established by an extensive crossing program involving over 10,000 crosses. The various unassigned alleles were not only crossed *intra* as well as *inter se*, but were tested against alleles at adjacent and nearby loci. The different alleles were also tested against a panel of over 100 published and unpublished chromosomal rearrangements such as deficiencies, duplications and translocations.

Four loci within this region were found to have visible phenotypes and to have little or no effect on zygotic viability. Furthermore, no lethal alleles were found at these loci; (*maroon-like, little fly, small optic lobes*, and *sluggish*). Twenty-two of the 26 loci are represented by zygotically lethal alleles and among these 22 loci, viable alleles have been found at 6 of

TABLE 1

Origin, cytology, genetic extent and references to the deficiencies (*Df*), duplications (*Dp*) and proximal aneuploid segregants from the T(X; Y) translocations used in this study

Rearrangement[a]	Origin[b]	Deficient or duplicated for the region	References[c]
Df(1) mal^{17}	X-ray	*mal–mell*	7, 16, 20, 21
Df(1) mal^{10}	X-ray	*mal–mell*	7, 8, 16, 20, 21
Df(1) 16-3-35	Neutron	*mal–shak-B*	7, 16, 17, 21, 25
Df(1) N77	X-ray	*mell–su(f)*	1
Df(1) GA37	X-ray	*leg–LB20*	1, 21
Df(1) GA40	X-ray	*leg–su(f)*	1, 21
Dp(1; Y) mal^{102}	X-ray	*leg–su(f)*	16, 27, 35
Df(1) B57	X-ray	*leg–unc*	7, 8, 16, 17
Df(1) LB6	mito-C	*shak-B–eo*	7, 8, 16, 20, 21
Dp(1; Y) mal^{171}	X-ray	*shak-B–su(f)*	16, 27, 36
Dp(1; f) mini-2	MR	*R-9-28–su(f)*	18
Dp(1; f) mini-ring	MR	*R-9-28–su(f)*	18
Df(1) LB7	mito-C	*R-9-28–su(f)*	4
Df(1) 26B	P-M	*R-9-28–eo*	19, 21
*Df(1) 17-351**	Neutron	*R-9-28–LB20*	16, 17, 21
Df(1) A118	X-ray	*R-9-28–vao*	7, 8, 16, 17
Df(1) HC279	X-ray	*R-9-28–vao*	1
Df(1) 17-489	Neutron	*EC235–su(f)*	16, 17, 21
*Df(1) A53**	X-ray	*EC235–eo*	16
Df(1) runt^{1112}	EMS	*mal–vao*	19, 21, 27
Df(1) T2-14A	^{3}HdC	*EC235–vao*	7, 8, 16, 17, 20, 21
*Df(1) Q539**	EMS	*vao–LB20*	7, 8, 16, 20
Df(1) LB23	EMS	*vao–su(f)*	7, 8, 16, 20, 21
Df(1) D43L1	^{137}Cs	*unc–su(f)*	7, 8, 16, 20, 21
Df(1) GA33	X-ray	*unc–uncl*	1, 21
Df(1) S54	X-ray	*unc–eo*	16, 17, 20, 21
Dp(1; Y) B101	X-ray	*lfl–su(f)*	21
Dp(1; f) mini-77	MR	*lfl–su(f)*	18
Df(1) DCB1-35b	^{3}HdC	*lfl–su(f)*	7, 8, 16, 20, 21
Df(1) C74	X-ray	*lfl–intro*	1
Df(1) 16-129	Neutron	*lfl–fli*	8, 16, 17, 21
Df(1) GA104	X-ray	*fli–LB20*	16, 17
Df(1) 2/19B	MR	*fli–LB20*	15
Df(1) 17-257	Neutron	*fli–eo*	8, 16, 17, 21
Df(1) GE263	X-ray	*fli–eo*	16, 17
Df(1) JA117	X-ray	*A112–LB20*	16, 17, 20, 21
Df(1) JC77	X-ray	*A112–eo*	16, 17, 20, 21
Df(1) HM44	HMS	*mal–A112*	16, 17
Df(1) 17-137	Neutron	*eo–su(f)*	8, 16, 21
Df(1) JC4	X-ray	*en–su(f)*	16, 17, 20, 21
Dp(1; Y) B154	X-ray	*eo–su(f)*	21
Df(1) JC12	X-ray	*eo–su(f)*	1, 21
Dp(1; Y) Y126	X-ray	*eo–su(f)*	8, 16
Df(1) GA22	X-ray	*wap–su(f)*	16, 17, 21
Dp(1; f) mini-6	MR	*wap–su(f)*	18
Dp(1; f) 3	X-ray	*wap–su(f)*	7, 16, 21
Dp(1; Y) B108	X-ray	*wap–su(f)*	21
Dp(1; Y) B109	X-ray	*wap–su(f)*	21
Df(1) 16-2-13	Neutron	*wap–uncl*	16, 17, 21
Df(1) DCB1-35c	^{3}HdC	*wap–intro*	8, 16, 17, 20, 21
Df(1) EA113	EMS	*uncl–su(f)*	16, 17
*Df(1) JA27**	X-ray	*fog–su(f)*	16, 17, 20, 21
Df(1) HM430	HMS	*fog–20*	5
Df(1) HF359	X-ray	*EA41–su(f)*	1, 17
Dp(1; Y) B^{S}Y	X-ray	*EA41–su(f)*	8, 16, 17
Df(1) 17-148	Neutron	*20–su(f)*	16, 21
Df(1) GA131	X-ray	*sph–su(f)*	16, 17

[a] Note: * denotes that a rearrangement is associated with a distal lethal(s) which is not covered by a *Ymal^{+}*-type chromosome.

[b] Nomenclature: P-M refers to hybrid dysgenesis and MR to Mutation-Recombination induced rearrangements; tritiated deoxycytidine (^{3}HdC), cesium-137 (^{137}Cs), mitomycin C (mito-C).

[c] References: 1, G. LEFEVRE (unpublished data); 2, G. MIKLOS (unpublished data); 3, D. KUHN (unpublished data); 4, A. SCHALET (unpublished data); 5, KRAMERS *et al.* (1983); 6. LIFSCHYTZ and FALK (1969); 7, SCHALET and LEFEVRE (1976); 8, SCHALET and LEFEVRE (1973); 9, LEFEVRE (1981); 10, LIFSCHYTZ and FALK (1968); 11, SCHALET (1986); 12, LINDSLEY and GRELL (1968); 13, KOANA and HOTTA (1978); 14, HOMYK and SHEPPARD (1977); 15, EEKEN *et al.* (1985); 16, LINDSLEY and ZIMM (1986); 17, MIKLOS *et al.* (1987); 18, GREEN, YAMAMOTO and MIKLOS (1987); 19, ZUSMAN, COULTER and GERGEN (1985); 20, LINDSLEY and ZIMM (1985); 21, LINDSLEY and ZIMM (1987); 22, MIKLOS *et al.* (1988); 23, MIKLOS *et al.* (1984); 24, FISCHBACH and HEISENBERG (1981); 25, YAMAMOTO and MIKLOS (1987); 26, YAMAMOTO *et al.* (1987); 27, GERGEN and WIESCHAUS (1986); 28, J. MERRIAM (personal communication); 29, MARKOW and MERRIAM (1977); 30, MIKLOS, KRAMERS and SCHALET (1986); 31, G. SCHLOSSER, U. BOSCHERT and K. F. FISCHBACH (unpublished data); 32, ZUSMAN and WIESCHAUS (1985); 33, THOMAS and WYMAN (1984); 34, L. E. KELLY (unpublished data); 35, SCHALET and FINNERTY (1968); 36, LIFSCHYTZ and YAKOBOVITZ (1978).

TABLE 2

Germline clone analysis of mutations within the 19D3-20F2 area[a]

Locus and mutation	Origin	Lethal phase[b]	Germline clone analysis[c]				Maternal expression	Ref.[d]
			N	NGLC	ND	NCL		
maroonlike								
mal	EMS	V	—	—	—	—	ME	7
legless/runt								
HM449	HMS	E	250	17	—	—	NME	5
VE751A	EMS	E	250	15	—	—	NME	1
shaking-B								
EC201	EMS	L1-2	200	5	—	—	NME	1
HM437	HMS	E-L	150	10	—	—	NME	5
E81	EMS	L1-2	190	15	—	—	NME	6, 7
R-9-28								
R9-28	EMS	L1-2	200	9	—	—	MELR	6, 7, 16
LB2	EMS	L	400	26	—	—	MELR	8
*EC242**	EMS	L1-2	300	0	200	0	L	1, 16
151	X-ray	L	200	0	NT	NT	L	7, 8, 9, 16
EC235								
EC235	EMS	L	300	0	250	0	L	1, 16
HM435	HMS	L1-2	180	0	150	0	L	5, 16
D83	MR	L	400	0	150	0	L	15
little fly								
lf	EMS	V	—	—	—	—	NME	7
uncoordinated								
DC803	EMS	P-A	150	7	—	—	NME	1
16-3-212	Neutron	P-A	130	6	—	—	NME	7
27E2	X-ray	P-A	170	9	—	—	NME	4
little flylike								
HM46	HMS	E-L1	300	0	120	0	L	5
B-56	X-ray	L	150	0	150	0	L	6, 7, 10
DF970	EMS	L1-2	200	0	100	0	L	1
B-214								
DA689	EMS	P	400	20	—	—	MEL	1, 16
flightless								
W-2	EMS	L2-3	250	11	—	—	MEL	6, 10
EN3	X-ray	L	140	12	—	—	MEL	12, 16
EF498	EMS	P	200	15	—	—	MEL	1
HC183	X-ray	P	200	12	—	—	MEL	1
DA534	EMS	P-A	200	8	—	—	MEL	1
l^3	EMS	V	—	—	—	—	NME	14
O^2	EMS	V	—	—	—	—	NME	13
small optic lobes								
KS58	EMS	V	—	—	—	—	NME	17, 24
sluggish								
EE85	EMS	V	—	—	—	—	NME	17
A112								
8-1	Spont.	L	150	0	120	0	L	11, 16
17-62	Neutron	E-L	300	0	100	0	L	1, 4
*GF314**	X-ray	E-L	200	0	150	1	L	1, 16
LB20								
LB20	EMS	L-P	150	0	150	1	L	8, 16
C27	X-ray	P	150	0	160	0	L	1, 16
*DA618**	EMS	L-P	250	0	100	1	L	1

[a] Nomenclature on the origin of the alleles: Ethyl methanesulfonate (EMS), hycanthone methanesulfonate (HMS), diethyl-sulfate (DES), spontaneous (Spont.), [^{3}H]thymidine (^{3}H-Thy), Mutation-Recombination (MR). An "*" indicates the presence of additional lethal mutation(s) on the lethal chromosome not covered by duplication(s) of the region such as the *Ymal*$^+$ type.

[b] Nomenclature on the lethal phase: The lethal phase (LP) 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; A: adults die shortly after emergence; V, viable.

[c] 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; P, perdurance; A "—" refers to not tested.

[d] References: See legend of Table 1.

TABLE 2

Germline clone analysis of mutations within the 19D3-20F2 area[a]

Locus and mutation	Origin	Lethal phase[b]	Germline clone analysis[c] N	NGLC	ND	NCL	Maternal expression	Ref.[d]
extra organs								
A7	^{3}H-Thy.	L2	150	10	—	—	MEL	8
DC726	EMS	E-L	250	6	—	—	MELR	1
S1	Spont.	P-A	300	12	—	—	MEL	3
EA13	EMS	L	250	11	—	—	MEL, AO	1
wings apart								
D48	MR	P-A	150	12	—	—	MEL	15
Q217	EMS	P-A	140	14	—	—	NME	6, 10
introvert								
Q56	EMS	P	100	7	—	—	NME, P	6, 10, 16
DC789	EMS	L1-2	200	0	80	1	P	1
19-98	Spont.	P	100	4	—	—	NME	11, 16
uncoordinated-like								
VA228	EMS	L1-2	200	12	—	—	MEL, AO	1
R-10-10	EMS	P-A	180	5	—	—	NME	6, 10
folded gastrulation								
114	X-ray	E	170	10	—	—	NME	8, 16
EA41								
*EA41**	EMS	L	200	0	150	0	L	1, 16
A72	X-ray	L	300	0	150	0	L	1, 16
stoned								
8P1	X-ray	V	110	8	—	—	NME	8, 16
VE720	EMS	L1-2	200	7	—	—	NME	8, 16
13E3								
13E3	X-ray	E	150	13	—	—	NME	8, 16
S60	X-ray	E-L1	200	4	—	—	MELR	1, 16
20								
20	X-ray	L	200	0	100	0	L	8, 16
VA97	EMS	L-P-A	200	4	—	—	NME	1, 16
sparse hairs								
VE829	EMS	L-P	400	0	150	0	L	1
S1	Spont.	L	300	0	150	0	L	4
suppressor of forked$^{pale\ bristles}$								
R-9-18	EMS	P	170	11	—	—	NME	6, 10
suppressor of forked								
S2	Spont.	L1-2	300	0	140	0	L	4
X2	X-ray	L-P	300	0	120	0	L	6, 10
3DES	DES	L-P	300	0	100	0	L	8

them (*shaking-B, flightless introvert, stoned, suppressor of forked$^{pale\ bristles}$* and *suppressor of forked*). Finally a set of deficiencies within the region has been identified that in *trans*-heterozygotes give rise to the three visible phenotypes *melanized-like, varied outspread* (SCHALET and LEFEVRE 1976), and *tumorous head* (D. KUHN and G. L. G. Miklos, unpublished data).

Genetic and developmental characteristics of individual loci

The *maroon-like* locus (*mal*): Mutations at this locus are fully viable and both hemi- and homozygous *mal* flies exhibit a brownish eye color. Additionally, the larval malpighian tubes can be short, bloated and irregularly formed (SCHALET and LEFEVRE 1976; LINDSLEY and GRELL 1968). Flies genetically hemi- or homozygous for *mal* have brownish eyes if they are derived from *mal/mal* mothers but the eyes are wild type if they are derived from *mal/+* mothers. Therefore mutations at the *mal* locus have a maternal effect phenotype (Table 2). Furthermore, *mal* mutations affect the activity of a large number of dehydrogenases and oxidases (see review by O'BRIEN and MACINTYRE 1978).

The *melanized-like* region (*mell*): Females transheterozygous for the two overlapping deficiencies (*Df(1)mal^{10}* and *Df(1)N77*, have a body color which is darker than normal, and which is especially noticeable in the thorax. Additionally, the abdominal tergites have slightly transverse wrinkles (SCHALET and LEFEVRE 1976). The *melanized-like* phenotype is not as extreme in *Df(1)mal^{17}/Df(1)N77* individuals.

The *legless* locus (*leg*): The alleles tested for complementation at this locus include: *AA33, YE96, HM449, B2/13.1, HA14, VE726, DC839, LB5*, and *VE751A* (G. LEFEVRE, unpublished data; A. Schalet,

mal	*MAROONLIKE*	19D
mell	*MELANIZEDLIKE*	
leg/run	*LEGLESS/RUNT*	
shak-B	*SHAKING-B*	
R-9-28		19E
EC 235		
lf	*LITTLE FLY*	
vao	*VARIED OUTSPREAD*	
unc	*UNCOORDINATED*	
lfl	*LITTLE FLYLIKE*	
B-214		
fli	*FLIGHTLESS*	
sol	*SMALL OPTIC LOBES*	19F
slg	*SLUGGISH*	
A112		
LB20		
tuh	*TUMOROUS HEAD*	
eo	*EXTRA ORGANS*	
wap	*WINGS APART*	
intro	*INTROVERT*	
uncl	*UNCOORDINATEDLIKE*	
fog	*FOLDED GASTRULATION*	
EA41		20
stn	*STONED*	
13E3		
20		
sph	*SPARSE HAIRS*	
su(f)^{pb}	*SUPPRESSOR OF FORKED pale bristles*	
su(f)	*SUPPRESSOR OF FORKED*	

FIGURE 1.—List of the loci in the 19D3-20F2 region of the *X* chromosome. The designation(s) of each complementation group(s) (middle column), their symbols (left column) and their approximate cytological locations (right most column) is indicated. In some cases the proximal-distal orientation of complementation groups has yet to be resolved (*lfl–B214*; *sol–slg*; *tuh–eo*; *EA41–stn*, *su(f)^{pb}–su(f)*).

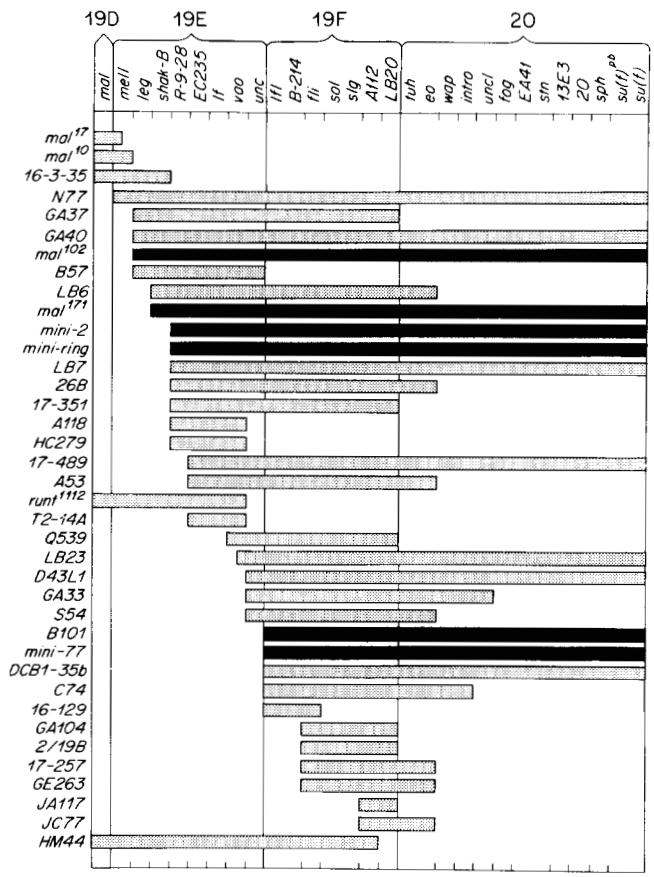

FIGURE 2.—Genetic map of the 19D3-20F2 region of the *X* chromosome. The extents of the deficiencies are schematized by boxes. Duplications as well as the proximal parts of *T(X;Y)* translocations are shown as filled boxes. For the breakpoints of these rearrangements see Table 1. Complementation groups are presented relative to their inclusion within these deficiencies and duplications. *Note*: The extent of *mal^{17}* has been drawn midway into the *mell* locus since the phenotype of *mal^{17}/N77* heterozygotes is not as severe as that of *mal^{10}/N77* heterozygotes. Similarly, deficiency *LB23* has been drawn half way into the *vao* locus. The proximal-distal orientation of the *tuh-1* region relative to the *eo* locus has also not yet been determined (D. T. KUHN and G. L. G. MIKLOS unpublished data).

unpublished data; KRAMERS *et al.* 1983; SCHALET and LEFEVRE 1976; GERGEN and WIESCHAUS 1985, 1986; this work). Most alleles at this locus are embryonic lethals and exhibit a "pair rule" phenotype (NUSSLEIN-VOLHARD and WIESCHAUS 1980). It transpires that *runt* and *legless* are alleles at the same locus and we have retained the original nomenclature for the locus. Some mutations at this locus give rise to adult mutant flies which can have one or both metathoracic legs absent (the *legless* phenotype). Embryos mutant for lethal alleles of *leg/run* have deletions of the odd numbered denticle bands and partial mirror image duplications of the remaining structures. More detailed analysis of *leg/run* embryos indicates that the structures which have been deleted arise from the A (anterior) and P (posterior) compartments of the even numbered abdominal segments and the S (separation) compartment of the odd numbered abdominal segments (the evidence for at least three compartments per segment was postulated by MEINHARDT 1982, 1984). Anti-horseradish peroxidase staining shows that the loss of *leg/run* function also results in deletion of at least half the normal segmental ganglia or neuromeres in the CNS (Figure 4, A and D). This is no doubt due to the fact that *leg/run* is expressed and required at the cellular blastoderm stage in cells which will serve as precursors to both epidermis and CNS. The surviving CNS tissue sometimes fuses to form a continuous nerve cord, but it is always poorly organized and contains no commissural fascicles and very few longitudinal fascicles. The amount of CNS tissue which survives clearly represents less than half the normal number of neuromeres. This is presumably because the boundaries of cells deleted by *leg/run* do not correspond to segmental boundaries, but rather span both segmental and parasegmental boundaries (GERGEN and WIESCHAUS 1985, 1986) and thus delete CNS cells from all segments. Many of the pair rule genes are expressed later in development in segmentally repeated patterns in the CNS, and are required

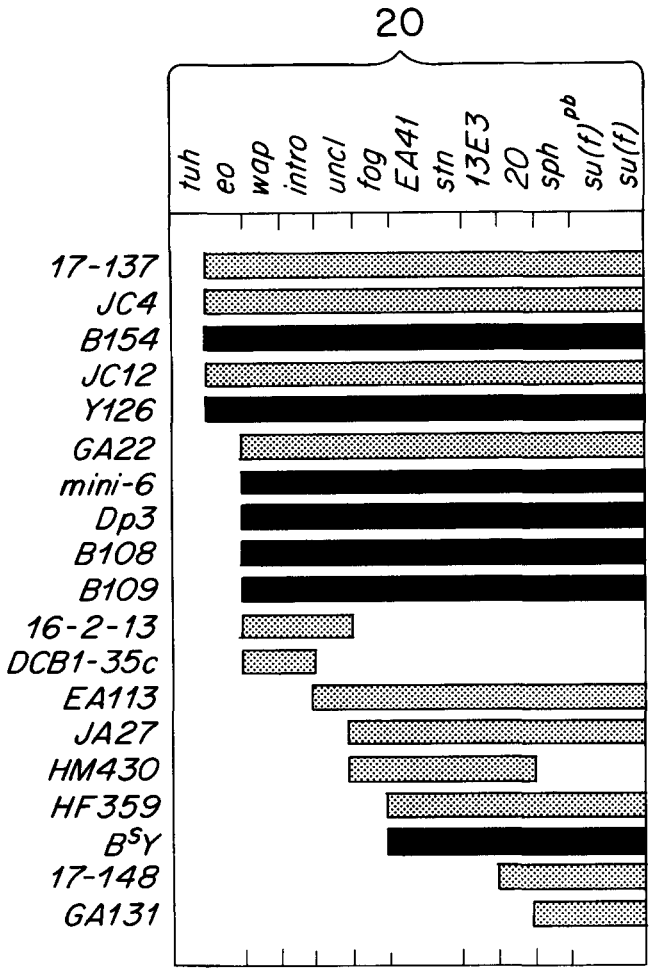

FIGURE 3.—Genetic map of the 20 region of the *X* chromosome. See legend of Figure 2 for the nomenclature.

for normal development of the neurons expressing them (DOE *et al.* 1988; DOE, SMOUSE and GOODMAN 1988). This suggests that an alternative explanation for the CNS phenotype of *leg/run* embryos is that the mutant CNS pattern is in fact the sum of at least two effects, including the early pattern deletions in alternating segment-wide units, and later in development, CNS-specific pattern defects in every segment.

The PNS of embryos is composed of a number of discrete and highly organized neuronal cell clusters which are found in segment specific patterns (Figure 5A; GHYSEN *et al.* 1986; CAMPOS-ORTEGA and HARTENSTEIN, 1985). The PNS defects which are associated with two pair rule mutations have been examined (DOE and GOODMAN 1985; HARTENSTEIN 1987). In *fushi tarazu* (*ftz*) mutant embryos it has been reported that the normal number and pattern of PNS neurons were formed in the surviving segments, and that these clusters of sensory neurons simply stretched the distance between them in response to the nearly doubled size of the segment. It was concluded in the second report, however, that the mutations *ftz* and *paired* give rise to deletions of PNS pattern elements corresponding to the deleted regions of cuticle, with a fusion of the surviving PNS pattern elements to form composite segments. From the latter analysis it was possible to conclude compartmental origins of the PNS pattern elements; in particular, the most distinct cell type, the lateral chordotonal cells of the abdominal segments, apparently derive from the posterior compartments. We have found, however, that the PNS in *leg/run* embryos departs dramatically from this simple pattern of deletion and fusion. As shown in Figure 5B, there are four to five composite segments with PNS cells per *leg/run* embryo, and there are twice the normal number of chordotonal cells per composite segment. In many cases (*e.g.*, Figure 5, E, F and G) the clusters of chordotonals appear to be mirror image duplications. Even in these cases, the axons of all chordotonals of a given composite segment appear to fasciculate together and project to the CNS via normal dorsoventral projections.

There are several possible explanations for the differences between *leg/run* and the other pair rule genes examined. It is possible for example that the boundaries of the pattern elements deleted by *leg/run* have been incorrectly interpreted, or that the compartmental origin of the chordotonals is more complex than suggested, and that the double clusters represent fusions of chordotonals from adjacent clusters. It is also possible that the deletion of pattern elements in *leg/run* juxtaposes compartment boundaries in such a way that mirror image duplications of the chordotonal precursors are produced.

Germline clone analysis of two amorphic *leg/run* alleles (*HM449* and *VE751A*) was performed (Table 2). It is found that *run/Y* hemizygous progeny are similar in phenotype to those derived from heterozygous *run/+* females and no effect on the *run/+* progeny is observed indicating that *legless/runt* has no maternal effect. WIESCHAUS and NOELL (1986) using combinations of amorphic and hypomorphic temperature sensitive *run* alleles obtained evidence that *runt* has a very slight maternal effect phenotype.

The *shaking-B* locus (*shak-B*): Both viable and lethal alleles have been found at this complex locus (MIKLOS *et al.* 1987). The *Passover* and *shaking-B* alleles are the best characterized of these in an electrophysiological sense and both alleles have been found to disrupt the connectivity of the giant fiber circuit (THOMAS and WYMAN 1984; D. BAIRD, R. J. WYMAN, J. DAVIES and G. L. G. MIKLOS, unpublished data). Adult flies mutant for *shaking-B* or *Passover* are hyperactive and have difficulty flying, they also exhibit uncoordinated leg movements under ether anaesthesia (D. BAIRD, R. J. WYMAN, J. DAVIES and G. L. G. MIKLOS, unpublished data). The null alleles are larval lethals but some viable alleles exist. The alleles tested for complementation include: *R-9-29*, *E81*,

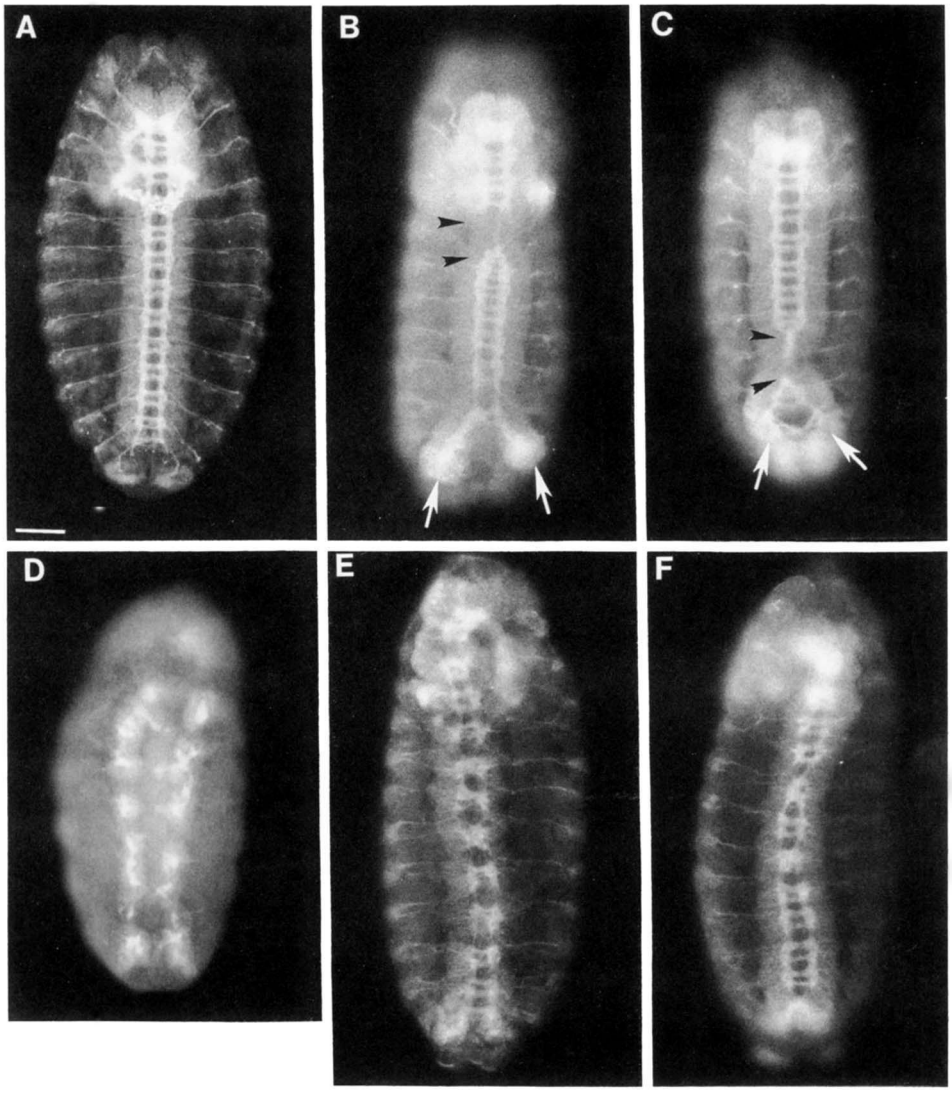

FIGURE 4.—The CNS phenotypes of *folded gastrulation*, *legless/runt* and *Df(1)HF359* embryos. All embryos are stained with fluorescein conjugated anti-HRP; ventral views are shown and anterior is to the top. Scale bar = 10 μm. (A) A wild-type embryo at approximately 13–14 hr; every segment but the last (A9) contains two horizontal commissures; A9 has only one commissure. (B) and (C) *fog* mutant embryos. The white arrows indicate the split in the ventral nerve cord, and the black arrowheads indicate gaps in the cord. Both embryos have protruding brain lobes as well (not visible in this focal plane). (D) *leg/run* mutant embryo. Note the small size of the embryo and the greatly reduced and disorganized CNS. (E) and (F) *Df(1)HF359* mutant embryos. In both examples the CNS of multiple segments is disorganized and the brain lobes are protruding. In each embryo there are some neuromeres with nearly normal morphology. The slight twist around the longitudinal axis is an artefact of mounting.

EC201, *L41*, *shak-B*^{2}, *Pas*, *TH73*, *EF535*, *17-360* and *HM437* (G. LEFEVRE, unpublished data; A. SCHALET, unpublished data; KRAMERS *et al.* 1983, SCHALET and LEFEVRE 1976; HOMYK and SHEPPARD 1977; THOMAS and WYMAN 1984; T. HOMYK, unpublished data; this work). We have examined three zygotic lethal alleles at this locus, namely *EC201, HM437* and *E81* (Table 2). Both *EC201* and *E81* are clearly larval lethal and mutant embryos derived from heterozygous females do not show CNS or PNS defects. About a fourth of the mutant progeny derived from heterozygous *HM437/+* females die during embryonic stages. Mutant embryos for the three alleles do not exhibit any cuticle, central and peripheral nervous system defects and germline clone analysis of these alleles indicates that mutations at this locus do not have a maternal effect.

The *R-9-28* locus: To date, only lethal alleles have been found at this locus. The alleles tested for complementation include: *R-9-28*, *GA71*, *151*, *DA507*, *EC242*, *AF2/19*, and *LB2* (G. LEFEVRE, unpublished data; A. SCHALET, unpublished data; D. G. N. KRAMERS, unpublished data; SCHALET and LEFEVRE 1976; this work). In this study we have analyzed four larval lethal alleles in detail and none of the mutant embryos derived from heterozygous females exhibit defects in the CNS or PNS. Ambiguous results however are obtained from the germline clone analysis (Table 2): two mutations *EC242* and *151* are cell lethals in homozygous germline clones, whereas *R-9-28* and *LB2* exhibit a rescuable maternal effect lethal phenotype. The embryonic phenotypes include both head and dorsal closure defects. Additionally, while most of the embryos are usually twisted, a small fraction are also phenotypically normal.

The *EC235* locus: So far, only lethal alleles have been found at this locus. The alleles tested for complementation include: *EC235*, *48-1*, *5-7*, *11/27*, *D76*,

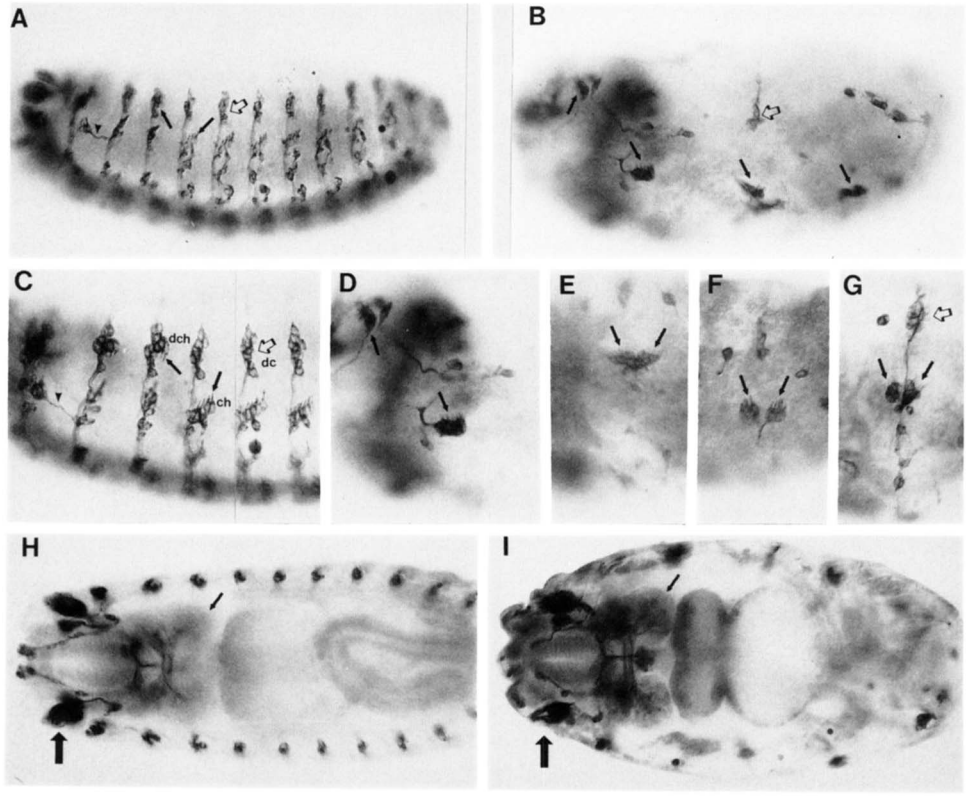

FIGURE 5.—PNS phenotypes of *legless/runt* embryos. All embryos are stained with the *SOX2* monoclonal antibody (GOODMAN *et al.* 1984). (A)-(G) are lateral views; anterior to the left and dorsal to the top; (H) and (I) are dorsal views with anterior to the left. (A) Wild-type embryo; the arrowhead points to the crossover axons of the lateral chordotonal organs in T1; the upward arrow points to the dorsal chordotonal organs of T3; the downward arrow points to the lateral chordotonal organs of A1; and the white arrow points to the dorsal cluster of A2. (B) *leg/run* mutant embryo; the orientation and coded arrows are essentailly the same as in wild type. There are four recognizable clusters of chordotonals, including a large dorsal cluster of cells with ventrally pointing dendrites (upward black arrow) and three lateral clusters of cells with dorsally pointing dendrites (downward pointing arrows). There is also a single dorsal cluster typical of abdominal segments (white arrow) and a fifth cluster of unidentified cells in the terminal region (unmarked). (C) Higher magnification of a wild type embryo similar to the one in (A). The same code for the arrows applies; dch = dorsal chordotonal organs; ch = lateral chordotonal organs; dc = dorsal cluster. (D)–(G) *leg/run* embryos. (D) is a higher magnification of the embryo in (B) to show more clearly the enlarged dorsal cluster of ventrally pointing chordotonals presumably derived from T1/T2 (upward arrow); the downward arrow points to an enlarged cluster of lateral chordotonals apparently derived from T3/A1. (E)–(G) *leg/run* mutant embryos showing apparent mirror-image duplications or lateral fusions of clusters of lateral chordotonals. In each case there are approximately twice the normal number of cells (5), with the dendrites of half the cells pointing in either direction (black arrows). In (G) there is a dorsal cluster (white arrow) which sends axons ventrally to fasciulate with the axons of the lateral cluster. (H) Dorsal view of a wild type embryo showing the sensory neurons of the antennal-maxillary complex (large arrow) and one of the brain lobes (small arrow). (I) Dorsal view of a *leg/run* embryo indicating that the head sensory structures (large arrow) and the brain lobes (small arrow) develop normally.

D83, *VE909*, and *HM435* (G. LEFEVRE, unpublished data; A. SCHALET, unpublished data; EEKEN *et al.* 1985; ZUSMAN, COULTER and GERGEN 1985; this work). The three alleles which we have examined (*EC235*, *HM435* and *D83*) are early larval lethals and no defects could be detected in either the CNS or PNS in hemizygous mutant embryos derived from heterozygous females. All three mutations produce lethality in homozygous germline clones (Table 2).

The *little fly* locus (*lf*): This phenotype is observed in only one mutation. Females that are homozygous for the *little fly* mutation are smaller than normal and their eclosion is generally delayed (A. SCHALET, unpublished data; SCHALET and LEFEVRE 1976). Homozygous *little fly* flies are fully viable and fertile indicating that the gene has no significant maternal effect (Table 2). The little fly phenotype is also uncovered in some females *trans*-heterozygous for two overlapping deficiencies: *Df(1)T2-14A* and *Df(1)Q539* (Figures 2 and 3). Since only a proportion of these *trans*-heterozygous exhibit this phenotype it may well be that the distal *Df(1)Q539* breakpoint either encoaches somewhat on the *little fly* locus, or is involved in a position effect phenonomenon. For the present therefore we have drawn the genetic breakpoints of *DF(1)Q539* and those of *Df(1)A118, Df(1)HC279, Df(1)runt*^{1112} and *Df(1)T2-14A* as in Figures 2 and 3. We believe it more prudent to leave the resolution of the various breakpoints to molecular analyses utilizing the microclones that are available in this area (MIKLOS *et al.* 1988).

The *varied outspread* region (*vao*): Females *trans*-

heterozygous for the two overlapping deficiencies *Df(1)Q539* and *Df(1)A118* not only have poor viability but the survivors have the eye pigment unevenly distributed. Furthermore the wings can be wrinkled and some "material" often extrudes from the vagina (SCHALET and LEFEVRE 1973, 1976). As with the case of the *melanized-like* locus, it is not possible to further evaluate the status of this region since no mutations are available within it.

The *uncoordinated* (*unc*) locus: Only semilethal alleles have been isolated at this locus and surviving flies are unable to walk because of a lack of coordination in their leg movements. The wings of survivors may be held up and frequently curled at the tips. Death usually occurs shortly after eclosion as the flies become mired in the medium. The alleles which have been tested for complementation include: *16-3-212*, *27E2*, *S86*, *S95*, *W-5*, *DC803*, *GE230*, and *JC8* (G. LEFEVRE, unpublished data; A. SCHALET, unpublished data; SCHALET and LEFEVRE 1976; LINDSLEY and GRELL 1968; this work). We have analyzed three *unc* alleles (*DC803*, *16-3-212* and *27E2*) in detail. The lethal phase of all three occurs during pupariation or shortly after emergence, and no defects were detected in either the CNS or PNS of the mutant embryos derived from heterozygous females. Germline clone analysis of all three alleles indicates that the *unc* gene is not maternally required (Table 2).

The *little flylike* locus (*lfl*): Both semilethal and lethal alleles exist at this locus. Surviving flies hemi- and homozygous for some alleles are smaller than normal and their eclosion is generally delayed. The allelic combinations between some *little flylike* mutations give rise to surviving individuals who can have rough eyes, absent bristles, sparse thoracic hairs and abnormal wing phenotypes. The alleles which have been tested for complementation include: *B-96*, *B-56*, *EF446*, *DF970*, and *HM46* (G. LEFEVRE, unpublished data; KRAMERS *et al.* 1983; SCHALET and LEFEVRE 1976; this work). We have examined three lethal alleles, two of which *B-56* and *DF970*, are clearly larval lethals. No defects could be detected in either the CNS or PNS of hemizygous progeny derived from heterozygous females. The remaining allele we have tested, *HM46* has an earlier lethal phase in which dead embryos are found but they do not exhibit any cuticle, CNS or PNS defects. All three *little flylike* lethals are cell lethal in germline clones (Table 2).

The *B-214* locus: To date, only lethal alleles have been found at this locus. We have examined the only available allele *DA689* and found it to be a pupal lethal mutation with no defects in either the CNS or PNS of mutant *DA689/Y* embryos. Germline clone analysis of *DA689*, however revealed a fully penetrant, maternal effect lethal phenotype (Table 2) in which about 80% of the eggs derived from homozygous germline

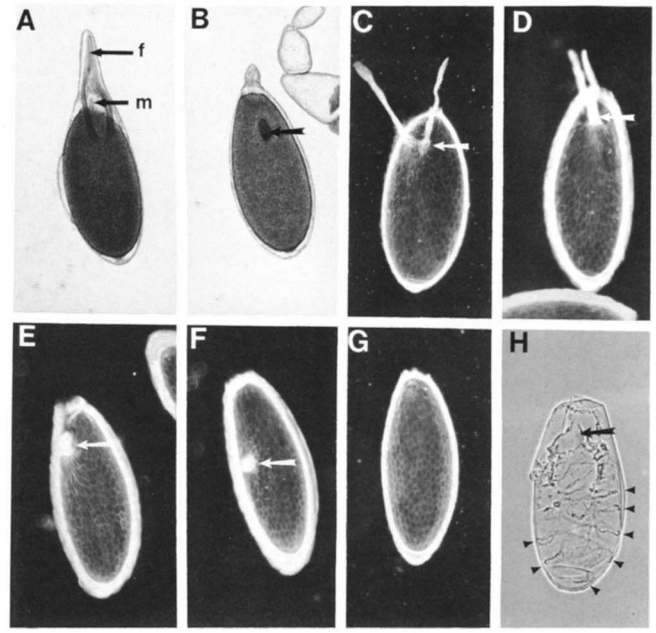

FIGURE 6.—Maternal effect phenotype of the *DA689* allele. A is a dorsal view of a wild type stage 14 egg chamber (KING 1970). Note the well differentiated chorionic filaments (f) and the micropile (m). The egg chamber is still surrounded by the follicular cell epithelium. B is a dorsal view of a homozygous stage 14 *DA689* oocyte. Note the position of the chorionic filaments on the dorsal side of the egg (arrow). Some of the eggs deposited from females possessing a germline clone homozygous for *DA689* have an almost wild type phenotype where the only detectable defects are at the base of the chorionic filaments (arrow in C), others have partially fused filaments (arrow in D). A complete fusion of the filaments is shown in E and F (indicated by arrows). As the phenotype becomes more extreme the position of these chorionic filaments becomes more posteriorly located on the dorsal side of the egg (compare E and F). Finally, a fraction of the eggs have no filaments (G). A small fraction of the eggs develop and have a very abnormal and variable phenotype. An example of such an embryo is shown in H. The embryo is U-shaped as indicated by the position of the segments (indicated by arrow heads) and very severe head defects are present (arrow).

clones had either no chorionic filaments or filaments which were fused dorsally and displaced posteriorly. We examined 138 eggs derived from germline clones homozygous for *DA689* (Figure 6). Twenty-eight (20%) had 2 chorionic filaments with slight defects at their base (Figure 6C), 30 (22%) had fused filaments (Figure 6D); 52 (38%) had the chorionic appendages located in the middle of the egg shell on the dorsal side (Figure 6, E and F), and 28 (20%) had no chorionic filaments whatsoever (Figure 6G). About 4% of the embryos develop to a stage where they produce cuticle; these embryos are U-shaped as a result of defective germ band retraction and have extreme head defects (Figure 6H).

The *flightless* locus (*fli*): Both viable and lethal alleles exist at this locus. Females homozygous for the viable alleles (*fli* I^1, I^2, I^3 and *fli* O^2) are fertile but these flies are unable to fly (the flightless phenotype). Alleles tested for complementation include: *W-2*,

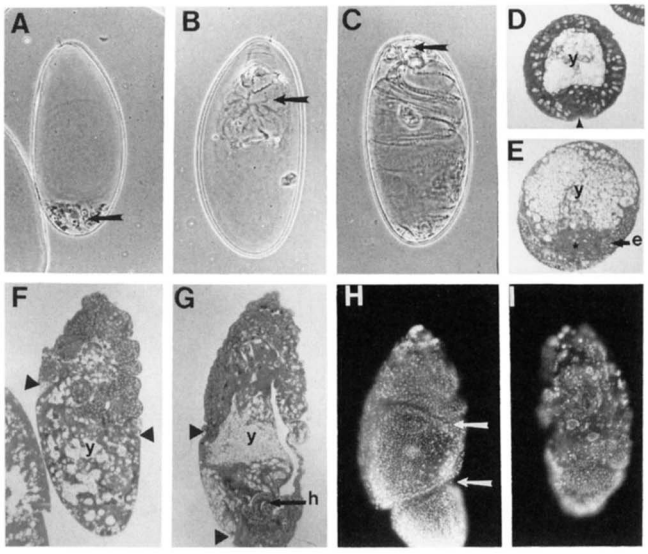

FIGURE 7.—Maternal effect lethal phenotype of alleles at the *flightless* locus. A and B are embryos derived from homozygous germline clones of *W-2*. Note the poor cuticle development (indicated by arrows). Embryos derived from homozygous germline clones of *DA534* develop more structures than observed for *W-2* (C). These embryos are usually twisted with various cuticle defects (segment fusion and holes in the cuticle). Note that there are head defects in this embryo (arrow). D and E are cross sections of embryos derived from germline clones of *W-2*. At 3.5 hours of development, abnormal gastrulation occurs (note the strange invagination of the mesoderm at the site of the arrowhead). At about 10 hr of embryonic development only patches of epidermis are differentiated surrounding part of the yolk. Internally, there are masses of disorganized cells (indicated by asterisks). F and G show longitudinal sections of *W-2* (F) and *DA534* (G) embryos derived from germline clones. In F, cellularization only occurred in the most anterior domain; note the large region of yolk (posterior to the arrowheads). In G, which is an 11 hour old embryo, only anterior and posterior regions have differentiated. No differentaition is detectable in the region delimited by the arrow heads but structures such as the hindgut have differentiated (ventral is to the left and anterior is up). H and I illustrate embryos derived from *W-2* homozygous germline clones stained with Hoechst DNA stain. The gastrulation defects are evident in H, an embryo at 4 hours of development, as indicated by the abnormal furrows (pointed by arrows). I is an older embryo, and only the most posterior region of the embryo appears to be correctly cellularized. Nomenclature: epidermis (e); hindgut; yolk (y).

GA105, *EN3*, *DA534*, *D44*, *18-80*, *fli O²*, *fli I³*, *EF498*, and *HC183* (G. LEFEVRE, unpublished data; H. G. DE COUET and G. L. G. MIKLOS. unpublished data; SCHALET and LEFEVRE 1976; KOANA and HOTTA 1978; HOMYK and SHEPPARD 1977; EEKEN *et al.* 1985; this work). Zygotic lethal alleles at this locus die during larval or pupal stages and have a fully penetrant maternal effect lethal phenotype (Table 2). None of these mutant embryos derived from heterozygous females has CNS or PNS defects.

The severity of the maternal effect lethal phenotype varies with the *flightless* allele analyzed. For example, embryos derived from *W-2* homozygous germline clones are very poorly differentiated (Figure 7, A and B). In contrast, embryos derived from females carrying germline clones of *DA534* develop further (Figure 7C). In *W-2* embryos, defects are detectable soon after blastoderm formation when abnormal folds become apparent (Figure 7H). Mesoderm invagination is clearly abnormal (Figure 7D) and at later stages only patches of epidermis are present (Figure 7, E and I). Embryos derived from *DA534* germline clones lack most of the epidermis from the abdominal segments (Figure 7G). Embryos derived from females carrying germline clones of the *EF498* allele on the other hand exhibit a range of phenotypes from extreme (such as *DA534*) to almost wild type. This maternal effect does not appear to be influenced by the introduction of wild type copy of the gene from the father.

The *small optic lobes* locus (*sol*): This is a structural brain mutant in which the cell number in the medulla cortex is reduced by about half, and in which the volume of the medulla and lobula complex neuropils is also reduced to about half. Additionally, some aspects of visual orientation behavior are defective (FISCHBACH and HEISENBERG 1981). No lethal alleles are known at this locus even though a large number of viable alleles have been identified (MIKLOS *et al.* 1987). Females homozygous for *sol* are fertile indicating that the gene has no significant maternal effect (Table 2).

The *sluggish-A* locus (*slg*): Mutant flies at this locus are defective in their movements toward a light source. The optomotor response is weak or nonexistent and there can be severe motor debilitation and uncoordinated behavior in combinations with certain deficiencies (MARKOW and MERRIAM 1977). Only one allele is known at this locus *EE85*, and it was induced by ethyl methanesulfonate (A. GHYSEN and S. BENZER, unpublished data; MIKLOS *et al.* 1987, Table 2). Females homozygous for *slg* are homozygous fertile indicating that the gene has no significant maternal effect.

The *A112* locus: So far only lethal alleles have been uncovered at this locus and alleles which have been tested for complementation include: *A112*, *11P1*, *17-62*, *GF314*, and *8-1* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976; SCHALET and FINNERTY 1968; MIKLOS, KRAMERS and SCHALET 1986; this work). We have analyzed in some detail three alleles at this locus all of which are lethal at the embryonic-larval interface. Dead embryos derived from heterozygous females have no obvious cuticle, CNS or PNS defects. It is only in some embryos mutant for *GF314* that some variable head defects are found and these are probably due to a second site lethal which is carried by *GF314* distally on the *X* chromosome. No homozygous germline clones are obtained from any of the three alleles tested indicating that mutations at this locus are germ cell lethal (Table 2).

The *LB20* locus: At this locus only lethal alleles

have been found. Alleles tested for complementation include: *LB20*, *DA618* and *C27*. (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976: MIKLOS, KRAMERS and SCHALET 1986; this work). All three alleles which have been analyzed exhibit a lethal phase during the larval-pupal stages. None of these mutant embryos derived from heterozygous females have any CNS or PNS defects. Germline clone analysis reveals that these mutations cause germ cell lethality (Table 2).

The *tumorous head-1* region (*tuh-1*): The distalmost mutant site in the Bithorax-Complex on chromosome three is *tumorous-head-3* (*tuh-3*). *Tuh-3* gives rise to head defects only in the presence of the recessive maternal effect allele *tuh-1*^{h} which is located at the base of the *X* chromosome (KUHN and PACKERT 1988; LINDSLEY and GRELL 1968). Thus *tuh-3; tuh-1*^{h} individuals have asymmetrical growths of various sizes on the head in which eye-antennal structures are replaced by posterior abdominal tergites and genital structures. In flies of the appropriate autosomal genotype we have found that the *tuh-1* region is uncovered by the two deficiencies *Df(1)GA37* and *Df(1)JC4* (D. T. KUHN and G. L. G. MIKLOS, unpublished data). Females trans-heterozygous for these deficiencies are viable and fertile.

The *extra organs* (*eo*) locus: Only lethal alleles have been found at this locus. When some of these are made heterozygous with the duplication bearing *Y* chromosome (*y*$^{+}$*Ymal*^{126}) flies are found in which the legs may be branched or completely duplicated, in which the antennae or aristae can be duplicated or triplicated, and in which the eyes and wings may be malformed. It is this combination of duplicated characteristics which gives the locus its name. The alleles tested for complementation include: *A7*, *DC801*, *17-260*, *17-36*, *47-2*, *EA13*, *DC726*, and *S1* (G. LEFEVRE, unpublished data; A. SCHALET, unpublished data; SCHALET and LEFEVRE 1976; SCHALET 1986; ZUSMAN, COULTER and GERGEN 1985; this work). We examined four alleles of *extra-organs* for this study. The lethality of hemizygous progeny from heterozygous mothers ranges from embryonic to late pupal stages according to the allele examined: *A7* and *EA13* are larval lethals; *DC726* is an embryonic to larval lethal, and *S1* is a pupal lethal (with some escapers). None of these mutant embryos derived from heterozygous mothers exhibit any CNS or PNS defects. Germline clone analysis (Table 2) however indicates that the gene is maternally required. The *DC726* allele, for example, has a rescuable maternal effect lethal phenotype, and produces embryos with variable phenotypes such as head defects and ventral holes. Both the *S1* and *A7* alleles have fully penetrant maternal effect lethal phenotypes. In particular, most embryos are U-shaped as a result of incomplete germ-band retraction, and have poor cuticle differentiation and ventral holes. The *EA13* allele has a phenotype similar to *S1* but in addition females usually develop ovarian tumors after approximately 6 days.

The *wings apart* locus (*wap*): Only lethal alleles are known at this locus. We have analyzed two alleles, *Q217* and *D48* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976; EEKEN *et al.* 1985; SCHALET 1986; this work). Mutant embryos derived from heterozygous mothers exhibit no CNS or PNS defects. Hemizygous *wap/Y* males and *wap/deficiency* females derived from heterozygous females usually die during late pupal stages. However, some progeny emerge from the pupal case and die shortly after eclosion. Their thorax is darker than normal and they usually have their wings set slightly apart and it is this phenotype for which the locus is named. Ambiguous results are obtained from the germline clone analysis (Table 2). On the one hand, germline clones homozygous for the MR-induced allele (*D48*) produce eggs that never develop, while on the other hand the EMS induced allele (*Q217*) does not have a maternal effect.

The *introvert* (*intro*) locus: Only lethal alleles exist at this locus and the alleles tested for complementation include *Q56*, *VA42*, *DC789*, and *19-98* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976; SCHALET 1986; G. SCHLOSSER, G. BOSCHERT and K. F. FISCHBACH, unpublished data; this work). Two of the mutations at this locus, *Q56* and *19-98* behave as pupal lethals while one *DC789* gives rise to larval lethality. None of the mutant embryos derived from heterozygous females has any defects in the CNS or PNS. Furthermore, pupae hemizygous for either the *Q56* or the *19-98* alleles are very abnormal with the head remaining inverted inside the thorax of the pupa. It is for this characteristic that G. SCHLOSSER, G. BOSCHERT and K. F. FISCHBACH (unpublished data) have assigned the locus designation. In addition individuals fail to contract at the beginning of pupation and remain slender like third instar larvae. While most thoracic structures do develop, eye development is very rudimentary. Germline clone analysis (Table 2) indicates that very few small clones are recovered for the *DC789* allele. In the case of the two pupal lethal alleles, *Q56* and *19-98*, clones are recovered at a normal frequency. For the first few days no maternal effect is observed, however, after about 4 days brown eggs are found with variable embryonic defects. Finally collapsed eggs and lethality of the homozygous germline clones is observed. We have previously described such variable phenotypes as the result of a perdurance effect (PERRIMON, ENGSTROM and MAHOWALD 1984a).

The *uncoordinated-like* locus (*uncl*): Semilethal and lethal alleles exist at this locus and we have analyzed two of them; *R-10-10*, and *VA228* (G. LE-

FEVRE, unpublished data; SCHALET and LEFEVRE 1976; this work). Neither of these mutations have zygotic effects on the CNS or PNS and both exhibit a different lethal phase as well as leading to different results in homozygous germline clones. The *VA228* mutation is an early larval lethal and germline clones of this allele at first produce a few dead embryos of apparently normal morphology. In addition, some very abnormal eggs are produced that do not develop. Dissection of the ovaries that possess the homozygous germline clones reveals the presence of abnormal egg chambers. The other *unc* allele, *R-10-10*, is a pupal lethal. Occasionally, flies emerge that are unable to walk because of lack of coordination in their leg movements and death usually occurs shortly after eclosion. Germline clone analysis of the *R-10-10* allele indicates that this mutation does not exhibit a maternal effect. It is also likely that the difference observed in germline clones between these two alleles is related to the strength of the allele. An allele such as *VA228* which is probably a strong amorph exhibits a maternal effect while a weak hypomorphic allele such as *R-10-10* does not.

The *folded gastrulation* locus (*fog*): We analyzed the *114* allele at the *fog* locus in detail (SCHALET and LEFEVRE 1976; ZUSMAN and WIESCHAUS 1985; this work). Embryos mutant for *fog* show variable phenotypes which fall into several overlapping classes. The earliest detectable defect is the appearance of supernumerary folds at gastrulation (ZUSMAN and WIESCHAUS 1985). Older embryos often twist around the longitudinal axis for one complete turn. Embryos at 11–14 hr fall into two classes: those with very poor differentiation of all internal tissues and those with a range of defects, the two most consistent of which are a dorsal, anterior hole through which the brain lobes protrude and a split in the posterior-most segments of the CNS (Figure 4, B and C). The split in the ventral nerve cord is accompanied by the protrusion of the midgut through the developing neuromeres, the surviving CNS cells in this region continue to develop and extend axons which are confined to the split hemiganglion. A gap often occurs in the ventral nerve cord, but its size and position are variable. When present, the gap is often followed posteriorly by a few segments of nearly normal CNS. In addition, older embryos sometimes have a slight twist around the longitudinal axis and deep folds in the ventral epidermis. The PNS and the position of identified CNS neurons appear normal in the least affected segments. It is not clear if these different classes of embryos represent different stages in the development of embryos exhibiting a single early defect, or if they represent variability in the severity of the phenotype due to differing levels of the *fog* gene product. Thus, the gastrulation defect may represent nearly complete loss of the *fog* product early in embryogenesis, while the less severely affected embryos may complete gastrulation relatively normally, but then develop specific defects as the requirement for the *fog* gene product is not met in later development. This would indicate that *fog* is required for normal gastrulation and, perhaps independently, for normal development of the head and tail regions. We have confirmed using germline clone analysis that the *fog* locus does not have a maternal effect (Table 2) as already pointed out by ZUSMAN and WIESCHAUS (1985).

The *EA41* locus: Only lethal alleles exist at this locus. We have analyzed two alleles; *A72*, and *EA41* (G. LEFEVRE, unpublished data; L. E. Kelly, unpublished; this work). Both are larval lethals and produce lethality in homozygous germline clones (Table 2). Mutant embryos derived from heterozygous mothers exhibit no CNS or PNS defects. One of the alleles, *EA41*, is associated with a second site lethal since *EA41* males are not recovered over a duplication of the region.

The *stoned* locus (*stn*): Both viable and lethal alleles exist at this locus and those tested for complementation include: *8P1*, *VE814*, stn^{ts1}, stn^{C}, *PH1*, *30A2*; and *VE720* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976; MIKLOS *et al.* 1987; ZUSMAN, COULTER and GERGEN 1985; KELLY 1983; this work). Flies mutant for a viable allele (*e.g.*, stn^{ts1}) have uncoordinated wing and leg movements at the restrictive temperature of 29°. These flies have an unusual jump response to a light-off stimulus. We have analyzed three lethal alleles: *PH1* is an embryonic lethal allele, *VE720* leads to early larval lethality, and *8P1* is viable. None of these three mutations have zygotic defects in the CNS or PNS. Germline clone analysis of *VE720* and *8P1* indicate that neither alleles are maternally required (Table 2).

The *13E3* locus: Two alleles at this locus have been examined; *13E3* and *S60* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976; this work) and both are embryonic-larval lethals. Dead mutant embryos do not show any obvious defects either in the cuticle or in their central and peripheral nervous systems. Ambiguous results are however obtained in germline clone analysis (Table 2). The *13E3* allele does not exhibit a maternal effect, whereas the *S60* allele has a poorly rescuable maternal effect lethal phenotype. Dead embryos derived from *S60* homozygous germline clones have variable defects in which the head does not involute and a range of segmentation defects are present.

The *20* locus: Only lethal alleles exist at this locus. We have examined two such alleles; *20* and *VA97* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976; this work). The *20* allele is a larval lethal while *VA97* is a polyphasic lethal. Mutant embryos derived

from heterozygous mothers exhibit neither CNS nor PNS defects. Ambiguous results are obtained from germline clone analysis (Table 2): while *20* is lethal in homozygous germline clones, *VA97* does not exhibit a maternal effect. It is likely that this difference between alleles is again related to the strength of the allele, as described for the *uncl* locus. A strong amorphic allele such as *20* exhibits a maternal effect while a probable weak hypomorphic allele such as *VA97* does not.

The *sparse hairs* locus (*sph*): Both semilethal and lethal alleles exist at this locus. Survivors may have sparse thoracic hairs and rough eyes. Additionally, the wings can be extended and their margins are incised. The alleles tested for complementation include: *4P1*, *VE829*, and *S1* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1973, 1976; this work). We have examined two alleles at this locus, *VE829*, a larval-pupal lethal, and *S1*, a larval lethal (Table 2). Neither of these mutations affect the CNS or PNS zygotically and both alleles are cell lethal when examined in homozygous germline clones.

The *suppressor of forked* complex: Both viable and lethal alleles exist at the *suppressor of forked*$^{pale\ bristles}$ (*su(f)*^{pb}) locus. Flies which are hemi- or homozygous for *f su(f)*^{pb} have nearly wild-type bristles, but their bristles and hairs are pale yellow and thread-like. Females of the genotype *su(f)*^{pb}/deficiency usually die just prior to eclosion (SCHALET and LEFEVRE 1976), indicating that the viable *su(f)*^{pb} alleles are hypomorphs of a zygotic lethal. We analyzed one pupal lethal allele at this locus: *R-9-18* (SCHALET and LEFEVRE 1976; this work) and have found that it does not have a maternal effect in homozygous germline clones (Table 2) nor does it have any zygotic effects on the development of the CNS and the PNS.

Both viable and lethal alleles also exist at the *suppressor of forked* (*su(f)*) locus. Viable alleles are characterized by their suppression of the *forked* (*f*) phenotype such that flies hemi- or homozygous for *f su(f)* have nearly wild-type bristles. Interestingly, females which are *su(f)/deficiency* have a *Minute-like* phenotype at 24–25° but are lethal at 29–30° (SCHALET and LEFEVRE 1976). We have analyzed three lethal alleles: *3DES*, *S2* and *X2* (G. LEFEVRE, unpublished data; SCHALET and LEFEVRE 1976; this work). All are larval lethals as well as being lethal in germline clones (Table 2). None of these mutations have zygotic effects on the CNS or PNS.

The effect of *Df(1)HF359* on the development of the embryonic nervous system

A series of deficiencies of the *maroon-like* to *suppressor of forked* region were also examined using anti-HRP and *SOX2* antibodies to determine if there were additional genes required for CNS or PNS development that may have been missed by looking at the various point mutations. We deliberately selected deficiencies which did not uncover either the *legless* or *fog* genes: These deficiencies were *A118*, *T2-14A*, *Q539*, *16-129*, *DCB1-35c*, *HF359* and *GA131*. All deficiencies (see Table 1 and Figures 2 and 3 for breakpoints) that we examined were phenotypically normal except for *Df(1)HF359* which removes seven lethal complementation groups: *EA41*, *stn*, *13E3*, *20*, *sph*, *su(f)*^{pb}, and *su(f)*. This deficiency does not remove *fog* or *legless/runt*, yet does produce a striking disorganization in the embryonic CNS (Figure 4, E and F), a result also found by J. B. THOMAS and C. S. GOODMAN (unpublished data). This phenotype includes misplaced cell bodies and grossly misrouted axons. The normal pattern of horizontal commissures is particularly sensitive to perturbation and is usually replaced by fused or unconnected bundles of axons. The intersegmental and segmental nerves develop normally, as do some of the fascicles composing the longitudinal connectives. Sensory neurons in the head and in the thoracic and abdominal segments are misplaced or missing. As in *fog* embryos, the brain lobes often protrude through a dorsal hole in the epidermis and a small fraction of embryos (approximately 5%) either differentiate very poorly, or do not complete germ band retraction. However, unlike *fog*, the majority of embryos hemizygous for the deficiency gastrulate normally, and twisted embryos are never seen. The CNS defects can be present in virtually every segment, although there are usually some normal neuromeres in most mutant embryos.

None of the lethal complementation groups within *Df(1)HF359* produce by themselves a phenotype resembling that of the deficiency. Additionally, *Df(1)GA131* which removes the three most proximal complementation groups *sph*, *su(f)*^{pb} and *su(f)* does not show any defects in the CNS and PNS. There are at least three possible explanations. (1) The phenotype is due to a single locus and none of the point mutations that we have examined are null for that locus; (2) it is conceivable that the phenotype is the result of a variable position-effect of heterochromatin on the *fog* locus; or (3) the loss of 2 or more lethal complementation groups uncovered by the deficiency is required to produce the phenotype.

DISCUSSION

The 19D3 to 20F2 region of the *X* chromosome contains 34 polytene chromosome bands (BRIDGES 1938) and a minimum of 25 genetic complementation groups. It must be borne in mind however, that the poorly banded nature of division 20, which is β-heterochromatic, is particularly refractory to cytological analysis (SCHALET and LEFEVRE 1973, 1976) and neither we nor others (LEFEVRE 1981) have been able to achieve the band counts obtained by BRIDGES. The

proximal part of subdivision 19F is also difficult to analyze microscopically, and it is largely for these reasons that the boundaries of the duplications and deficiencies used in our analyses have been given in genetic terms (Table 1). Despite these reservations, the base of the *X* represents one of the most extensively studied and systematically saturated regions of the Drosophila genome.

The detailed genetic analyses that we and others have carried out on this region have revealed a number of complexities. It is still difficult, for example, to exactly define precisely the number of genetic complementation groups with which we are dealing (LIFSCHYTZ and YAKOBOVITZ 1978).

Viable loci: So far there are four loci for which lethal alleles have yet to be found. These are *maroon-like, little fly, small optic lobes*, and *sluggish*. Two of these loci, namely *little fly* and *sluggish*, have only been defined on the basis of one allele, whereas multiple alleles occur at the other two loci.

Lethal loci: Nine loci have been found in which all the mutant alleles cause total lethality of the organism. These are *R-9-28, EC235, B-214, A112, LB20, folded gastrulation, EA41, 13E3* and *20*. Another six loci are represented by both lethal and semilethal alleles; in these cases it is the phenotypes of the resulting escapers that have been used to designate the loci. These are *legless, uncoordinated, little flylike, wings apart, uncoordinated-like* and *sparse hairs*.

Loci with lethal and viable alleles: Four loci have both lethal alleles as well as ones which give rise to visible phenotypes. They are *shaking-B, flightless, introvert* and *stoned*.

Loci of a more complex nature: Two regions at the base of the *X*, namely *suppressor of forked* and *extra organs*, are more difficult to classify. The *suppressor of forked*$^{pale\ bristles}$ and *suppressor of forked* complementation groups both have visible as well as lethal alleles, but the complex complementation results found by SCHALET and LEFEVRE (1976) led these authors to conclude that they were dealing with interallelic complementation. Thus, the *su(f)* complex may represent one or two loci. The *extra organs* locus was originally designated as such because some of the lethal alleles when combined with $y^{+}Ymal^{126}$ chromosome gave rise to duplicated structures such as legs and antennae. The $y^{+}Ymal^{126}$ chromosome thus rescues the lethality but gives rise to a mutant phenotype of varying expressivity.

Other subregions at the base of the X chromosome: Three regions have been defined on the basis of overlapping deficiency analysis and it is not yet possible to tell whether they represent separate loci, or whether the resultant phenotypes are caused by chromosomal breakpoints encroaching upon existing gene landscapes. These regions harbor *melanized-like, varied outspread*, and *tumorous head*. The *tumorous-head-1* region is actually found in natural populations in distinct common forms, $tuh\text{-}1^{g}$ and $tuh\text{-}1^{h}$. Neither of these are mutant chromosomes *per se*, but cause head and genital abnormalities when combined with *tuh-3*, a mutation which is located in the abdominal part of the *Bithorax Complex*. There is also a mutant, *short egg* (WIESCHAUS, AUDIT and MASSON 1981) in which the shape of the egg is less elongated and more rounded. The mutation giving rise to this phenotype has been mapped within *Df(1)JA117* (E. WIESCHAUS and G. L. G. MIKLOS, unpublished data). It is not yet known, however, whether this is a visible allele of an existing lethal complementation group or whether it will define a new locus.

If we treat the *suppressor of forked* complex as a single complementation group, then the base of the *X* contains a minimum of 25 such units. If the *melanized-like, varied outspread*, and *tumorous head* regions all ultimately reach the status of *bona fide* separate loci, then this minimum figure rises to 28. It should be noted however that whilst the base appears saturated for lethal loci it is unlikely to be saturated for all loci which have behavioral phenotypes or for loci which are involved in male sterility (LIFSCHYTZ and YAKOBOVITZ 1978). For instance, electrophysiological analyses of flies mutant for the *shaking-B* region in band 19E3 have revealed that deletions of the adjacent but not contiguous 19E5-6 area, have an effect on the output of the giant fiber circuit (D. BAIRD, R. J. WYMAN, J. DAVIES and G. L. G. MIKLOS, unpublished data). Thus there may well be another nonlethal neurological locus in 19E5-6.

It should also be noted that the nonlethal *small optic lobes* locus was discovered in a sophisticated neuroanatomical screen (FISCHBACH and HEISENBERG 1981) and there may well be more such loci for which the appropriate screens have yet to be done. Thus the 19D3-20F2 region probably contains a minimum of 25 loci in a maximum of 34 bands. This density of loci is similar to most, but not all, other regions of the *Drosophila* genome (SHANNON *et al.* 1974; LEFEVRE and WATKINS 1986).

It is known from previous data, drawn from elsewhere in the *D. melanogaster* genome, that at least 85% of all loci, when mutated, lead to lethality of the zygote (PERRIMON and MAHOWALD 1986). These zygotic lethal loci are classified according to when the mutation causes the death of hemi- and homozygous progeny derived from heterozygous mothers. Thus approximately 15% of all loci are viables. In this study there are 26 loci which have been characterized using point mutations (taking the *su(f)* complex as two genes). Among these loci, four (or 18%) are viables. These are *maroon-like, little fly, small optic lobes*, and

sluggish; and this figure is thus in good accord with previous estimates.

When the remaining 22 lethal loci are treated as a group it is found that 3 of these 22 (or 14%) cause death in the embryonic stages. These loci are *legless/runt, fog* and *13E3*. Mutations in 13 of the loci (or 59%) cause death in the larval stages. These are *shaking-B, R-9-28, EC235, little flylike, flightless, A112, extra-organs, uncoordinated-like, EA41, stoned, 20, sparse hairs* and *suppressor of forked*. Mutations in six of the loci (27%) cause death in the pupal stages. These are *uncoordinated, B-214, LB20, wings apart, introvert* and *suppressor of forked*$^{pale\ bristles}$. These numbers from loci at the base of the *X* chromosome of 14, 59 and 27% of embryonic-larval-pupal percentages are in good agreement with those of 20, 54 and 26% determined by PERRIMON and MAHOWALD (1986) on random sampling of loci from the entire chromosome. Furthermore, division 20, which is entirely β-heterochromatic, has an embryonic-larval-pupal lethal distribution of 17, 58 and 25% which is again in agreement with the percentages from the remainder of the *X*. Thus, although division 20 has an unusual molecular and cytological signature (MIKLOS *et al.* 1988), it nevertheless contains an array of loci, which in terms of their lethal mutational spectrum is unremarkable.

Germline clone analysis of the 22 loci which can mutate to lethality, reveals that seven have no maternal effects of note. These are *legless, shaking-B, uncoordinated, introvert, folded gastrulation, stoned* and *suppressor of forked*$^{pale\ bristles}$. Four loci are ambiguous in this regard, *wings apart, uncoordinated-like, 13E3* and *20*. The remaining 11 loci are maternally required and among these, at least seven are cell lethal in homozygous germline clones. These are *EC235, little flylike, A112, LB20, EA41, sparse hairs* and *suppressor of forked*. In the remaining four cases where ambiguous results are obtained from the germline clones, it is probable that the source of the ambiguity reflects differences in the strength of the alleles used, or the fact that some lethals at the base of the *X* also carry a second site mutation not covered by the *Ymal*$^{+}$ duplication chromosomes used in our analyses.

In the case of the *uncoordinated-like* and *20* loci, for example, the differences are probably related to the relative strengths of the alleles used. Thus, *unc*^{VA228} which causes death in the early larval instars as well as causing abnormal oogenesis, is probably closer to being an amorphic allele than *unc*$^{R\text{-}10\text{-}10}$ which causes lethality near the pupal-adult interface. Similarly, at locus *20*, the X-ray-induced allele *20* causes larval death and germline clone lethality, whereas the EMS-induced allele *VA97* is a polyphasic lethal; and does not give rise to a maternal effect.

Thus is it likely that the strong alleles at a particular locus (amorphs) produce maternally expressed effects, while weaker alleles (hypomorphs) are much milder in their actions. These results are again in excellent agreement with previous studies (PERRIMON, ENGSTROM and MAHOWALD 1984a, b, 1985; PERRIMON and MAHOWALD 1986).

Our analysis has also identified two new loci which are late zygotic lethals that exhibit specific maternal effect lethal phenotypes when examined in germline clone analysis. Interestingly, these two complementation groups *B-214* and *flightless* are close together on the chromosome. First, the maternal effect phenotype of the *DA689* allele of *B-214* primarily affects the correct formation of the egg. The chorionic filaments observed in such mutant eggs are quite similar to those produced by mutations in the two female sterile mutations *gurken* and *torpedo* (SCHUPBACH 1987). However, in eggs derived from germline clones of *DA689*, the aeropyles are not affected. In the second complementation group, namely *flightless*, the maternal effect of the lethal alleles appears to perturb gastrulation processes.

All 22 lethal complementation groups at the base of the *X* have been analyzed for defects in the development of the central and peripheral nervous systems. Except for *folded gastrulation* and *legless/runt*, which are described above, none of the lethal complementation groups produced specific and reproducible defects in the embryonic nervous system. Initially, this is somewhat surprising since there are several loci in this region which are associated with neurological disorders. The *shaking-B* and *Passover* alleles at the *shaking B* locus disturb the connectivity of the giant fiber circuit (THOMAS and WYMAN 1984); the *stoned* locus has mutations which cause abnormal jump responses to "light-off" stimuli (KELLY 1983); the *small optic lobes* locus (FISCHBACH and HEISENBERG 1981) has mutations that cause specific degeneration of neurons in the medulla and lobula complexes; the *sluggish* locus gives rise to abnormal phototactic behavior (MARKOV and MERRIAM 1977) and the *uncoordinated, uncoordinated-like* and *flightless* loci exhibit phenotypes which might be expected to have a significant neuromuscular component.

We examined lethal alleles of all the loci except *small optic lobes* and *sluggish* and no overt, nor indeed specific defects could be found in the embryonic nervous system using the probes described. It is probable therefore that the behavioral defects described in the adult flies are either not related to cell defects in the embryonic nervous system, or that such cell defects are not detectable using the probes and methodology employed here.

There is a precedent for behavioral mutations in *D. melanogaster* being directly (*Shaker*: PAPAZIAN *et al.* 1987; KAMB, IVERSON and TANOUYE 1987) or indirectly (*tko*: ROYDEN, PIRROTTA and JAN 1987) associ-

ated with some aspect of neuronal cell physiology, and not with a structural defect in the organization or wiring of the nervous system. It is also interesting in this respect, that of the 7 loci that could have potential for neurological studies, all but two have both lethal and visible alleles. Thus, most of the viable alleles which give rise to interesting neurological phenotypes belong to loci which can mutate to lethality. Indeed the viable alleles that do occur amongst the 22 zygotic lethal loci probably represent hypomorphic alleles of genes required for essential functions. An alternative possibility is that some of these genes encode multiple functions some of which are required only in the adult. Mutations in different functions of the same gene would thus result in different phenotypes from the same genetic unit.

Finally, the biochemical characterization of the genes affecting both behavior and development at the base of the *X* will further elucidate which aspects of function are affected by them. Since the base of the *X* chromosome has been microdissected and microcloned (MIKLOS *et al.* 1988) and since chromosomal walks have been initiated in a number of subregions (MIKLOS *et al.* 1984; DAVIES, PIRROTTA and MIKLOS 1987; YAMAMOTO *et al.* 1987; DE COUET *et al.* 1987) the molecular analyses of the genes causing interesting neurological disorders as well as those involved in different significant developmental processes now seem within reach.

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