Zygotic Lethals With Specific Maternal Effect Phenotypes in *Drosophila melanogaster*. I. Loci on the *X* Chromosome

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ABSTRACT

In order to identify all *X*-linked zygotic lethal loci that exhibit a specific maternal effect on embryonic development, germline clonal analyses of *X*-linked zygotic lethal mutations have been performed. Two strategies were employed. In *Screen A* germline clonal analysis of 441 mutations at 211 previously mapped *X*-linked loci within defined regions was performed. In *Screen B* germline clonal analysis of 581 larval and pupal mutations distributed throughout the entire length of the *X* chromosome was performed. These approaches provide an 86% level of saturation for *X*-linked late zygotic lethals (larval and pupal) with specific maternal effect embryonic lethal phenotypes. The maternal effect phenotypes of these mutations are described.

EMBRYONIC development in *Drosophila* requires maternal and zygotic gene products (see review by KONRAD *et al.* 1985; MAHOWALD and HARDY 1985; AKAM 1987; PERRIMON and MAHOWALD 1988). To identify genes that control specific embryonic developmental pathways, maternal genes have been detected via maternal effect lethal mutations isolated from screens for female sterility, while zygotic genes have been detected via screens for embryonic lethal mutations. These screens have relied on the assumption that the respective functions of such genes will be very tightly restricted to a developmental stage; *e.g.*, maternal genes will be expressed only during oogenesis and zygotic genes only during embryogenesis. Such screens have successfully identified genes that play central roles in embryonic patterning (NUSSLEIN-VOLHARD and WIESCHAUS 1980; NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; WIESCHAUS, NUSSLEIN-VOLHARD and JURGENS 1984; JURGENS *et al.* 1984; GANS, AUDIT and MASSON 1975; MOHLER 1977; PERRIMON *et al.* 1986; SCHUPBACH and WIESCHAUS 1986; NUSSLEIN-VOLHARD, FROHNHOFER and LEHMANN 1987). However, further genetic and molecular analysis of some of these genes has challenged the original assumption. Detailed genetic analyses have demonstrated that: (1) most female sterile mutations comprise hypomorphic alleles of zygotic lethals (PERRIMON *et al.* 1986); (2) a large number of genes that when mutated lead to embryonic lethality are maternally expressed (WIESCHAUS and NOELL 1986; PERRIMON and MAHOWALD 1988); and (3) when analyzed as germline clones most genes that when mutated lead to zygotic lethality are maternally expressed (GARCIA-BELLIDO and ROBBINS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984a; PERRIMON and MAHOWALD 1986a). Similarly, molecular analyses of some of these loci have supported the genetic results; *i.e.*, some maternal effect loci encode transcript(s) that are expressed during all developmental stages (*e.g.*, *fs(1)k10*: HAENLIN *et al.* 1985; *Toll*: GERTULLA, JIN and ANDERSON 1988).

To appreciate the complexity of the genetic control of development we require an accurate estimate of the number of genes involved in a developmental process. So far studies in *Drosophila* have characterized a small number of maternal and zygotic genes that perturb specific developmental processes; *e.g.*, 14 maternal and 7 zygotic genes are involved in the determination of the dorsoventral axis (reviewed by ANDERSON 1987); 18 maternal genes affect the anteroposterior axis of the embryo (reviewed by NUSSLEIN-VOLHARD, FROHNHOFER and LEHMANN 1987); 6 zygotic genes are implicated in the initial switch between either an epidermal or neural fate (LEHMANN *et al.* 1983); 20 zygotic genes are involved in embryonic segmentation (reviewed by AKAM 1987); and 7 zygotic genes are homeotic (reviewed by AKAM 1987). To date, most of the screens designed to detect genes involved in a specific developmental process have been restricted by the underlying assumption that genes playing a role maternally are only expressed during oogenesis and that genes playing a role during embryogenesis are only expressed zygotically. Indeed, by strictly holding to this assumption most genes acting in both maternal and zygotic developmental processes have not yet been identified. The use of germline clonal analysis should, in principle, allow us to analyze the maternal effect of all zygotic lethal mutations

(PERRIMON, ENGSTROM and MAHOWALD 1984a; PERRIMON and MAHOWALD 1986a). This approach allows us to identify two distinct classes of genes: (1) loci that express zygotic functions that are masked by the maternally-stored gene product and could not be identified in screens for embryonic lethal mutations and (2) zygotic lethal loci with specific maternal effects that could not be isolated in screens for female sterility since homozygosity for loss of function mutations in such loci would lead to preimaginal lethality. This paper describes a screen designed to identify most of these "cryptic" loci on the *X* chromosome.

MATERIALS AND METHODS

Strains: Duplications and deficiencies used in the mapping of the *X*-linked lethals mutations are listed in Table 1. The dominant female sterile mutation ovo^{D1} (or *Fs(1)K1237*) is maintained in an attached-*X* stock; *C(1)DX, y f/*$ovo^{D1}v^{24}$*/Y* (BUSSON *et al.* 1983; PERRIMON 1984).

The *FM3/w v* l^{44TS}*/*$B^{s}Y$ stock was used for mass production of virgins. The *Y* chromosome is labeled so that flies resulting from nondisjunction of the sex chromosome in the father or mother can be detected. l^{44TS} is a temperature-sensitive lethal mutation (KOMITOPOULOU *et al.* 1983).

Screens: Two strategies were used to analyze the maternal effects of *X*-linked zygotic lethal loci in germline clones. In *Screen A* collections of previously-mapped and available *X*-linked lethals were obtained from G. LEFEVRE and various other sources. The origin and references of these stocks is indicated in Table 2. The lethal phases and germline clonal phenotypes of these mutations were analyzed. These stocks were usually maintained as *FM7c*, *FM6* and *M5* balanced stocks (LINDSLEY and GRELL 1968) or in attached-*X* females with the appropriate duplication. In *Screen B* random *X*-linked lethals were generated following mutagenesis using ethyl methane sulfonate (EMS) or hybrid dysgenesis. The schemes in which these lethals were induced are shown in Figure 1 and the results from the various mutageneses are shown in Table 3. Germline clonal analysis of all late (larval and pupal) lethals was performed.

Mutagenesis: The two screens are described in Figure 1. For EMS screens (*A*), 2–3-day-old males of genotype *y w* sn^{3} f^{36a}*/Y* or *y f/Y* (symbolized as "*X*") and not starved before mutagenesis were fed EMS (according to the method of LEWIS and BACHER 1968). Batches of 15 mutagenized males were crossed to 20 *FM3/w v* I^{44TS} virgin females at 25°. Flies were transferred every day for a period of 4 to 5 days. When F_1 progeny began pupariation, the vials were shifted to 29° to ensure lethality of the *w v* I^{44TS} males. Because *FM3/Y* males die during embryonic stages only two classes of F_1 females were obtained. Single pair matings were established using one *FM3/X** virgin female with 2 *FM7c/Y* males. From among the F_2 progeny, vials with no *X*/Y* male progeny were kept and larval and pupal lethal mutations were selected by detecting dead larvae and pupae on the side of the vial. From among the F_3 progeny lethal lines were established using the same *FM7c* balancer by crossing *FM7c/X** virgin females to *FM7c/Y* males. Subsequently, the lethal phase of the mutation was rechecked.

For the hybrid dysgenesis screen (*B*), batches of 10 homozygous *y f* females from a strain of M cytotype were crossed to 5 males of P cytotype (*II2*). Crosses and progeny were maintained at 22° to reduce sterility in the F_1 females of genotype *y f/II2*. All subsequent crosses were done at 25°.

TABLE 1

Cytology and references of the deficiencies (*Df*) and duplications (*Dp*) used in the localization of the lethal mutations

Rearrangement	Cytology	Ref.[a]
Dp(1;Y)$y^{2}Y^{611}$	1A1 to 1B14; Y	1
Dp(1;Y)$y^{2}Y^{67g}$	1A1 to 2B17-18 and 20A3 to base; Y	1
Dp(1;3)w^{vco}	2B17-C1 to 3C4-5; 77D3-5; 81	1, 2
Dp(1;Y)w^{+303}	2D1-2 to 3D3-4; Y	1, 2
Dp(1;3)w^{+67k27}	3A5 to 3E8; 86E	1
Dp(1;2)^{51b}	3C2 to 3D5-6; 52E-F	1
T(1;2)w^{64b13}	3C2 to 5A1-2; 26D	1
T(1;2)rb^{+71g}	3F3 to 5E8; 23A15	1
Dp(1;3)sn^{13a1}	6C11 to 7C9; 79E	1
T(1;2)sn^{+72d}	7A8 to 8A5; 58E	1
Dp(1;Y)$v^{+}Yy^{+}$	1A1 to 1B2, 9F3 to 10C1-2, 20B to base; Y	1
Dp(1;Y)$v^{+}Yy^{+3}$	1A1 to 1B2, 9F3 to 10E3-4, 20B to base; Y	1
Dp(1;2)v^{75d}	9A2 to 10C2; 40-41	1
Dp(1;2)v^{+63i}	9E1 to 10A11; 56A	1, 3
T(1;3)v^{+74c}	9E3-4 to 11B12; 80-81	1
T(1;2)v^{65b}	10A1 to 11A7; 40-41	1
Dp(1;4)r^{+}	13F to 16A2; 102F2-3	1
T(1;2)r^{+75c}	14B13 to 15A9; 35D-E	1
T(1;3)f^{+71b}	15A4 to 16C2-3; 80-81	1
Dp(1;Y)$y^{+}Ymal^{106}$	1A1 to 1B2, 18F to base; Y	1
Df(1)A94	1E3 to 2B15	1
Df(1)S39	1E4 to 2B11-12	1
Df(1)Pdg-kz	2D3-4 to 2F5	2
Df(1)64C18	2E1 to 2; 3C2	2
Df(1)2F1-3A4	2F1 to 3A4	2
Df(1)X12	2F6-3A1 to 3B5	2
Df(1)HC194	3A1 to 3C4	4
Df(1)GAM201	3A4 to 3C2	4
Df(1)HA44	3C12 to 3E3	4
Df(1)N^{69h}	3C6 to 3D3-4	1
Df(1)ct^{J6}	6E1 to 7C1	1
Df(1)HA32	6E4-5 to 7A6	1
Df(1)ct^{J4}	7A2 to 7C1	1
Df(1)RF19	7A4-5 to 7B2-3	4
Df(1)GA34	7A5 to 7B3	4
Df(1)ct^{268-42}	7A5-6 to 7B8-C1	4
Df(1)ct^{4b1}	7B2-4 to 7C3-4	4
Df(1)RA2	7D10 to 8A4-5	1
Df(1)GE202	7D13 to 7E3	4
Df(1)HA11	7D14 to 7D22	4
Df(1)VE624	7E4 to 8B	4
Df(1)KA14	7F1-2 to 8C6	1
Df(1)v^{L15}	9B1 to 10A1	1
Df(1)v^{VAM19}	9E1 to 10A	4
Df(1)v^{JA22}	9F13 to 10A1	4
Df(1)KA7	10A9 to 10F10	1
Df(1)N71	10B5 to 10D4	1, 3
Df(1)DA622	10B8 to 10D2	3
Df(1)HA85	10B-C to 10F	1
Df(1)KA10	11A1 to 11A7	1
Df(1)HF396	18E1-2 to 20	1
Df(1)GA37	19E2 to 19F6	4
Df(1)N77	19E2 to 20F	4
Df(1)JA21	19E4 to 20F	4
Df(1)Q359	19E7 to 19F6	1
Df(1)VE696	19F1 to 20F	4
Df(1)C74	19F1 to 20A4	4
Df(1)DCB1-35b	19F1 to 20F	1

[a] References: (1) CRAYMER and ROY (1980); (2) PERRIMON, ENGSTROM and MAHOWALD (1984b); (3) PERRIMON and MAHOWALD (1986b); (4) G. LEFEVRE, personal communication.

TABLE 2

***Screen A*: Germline clonal analyses of *X*-linked lethals**

Locus[a]	Location[b]	Lethal phase[c]	Germline clone analysis[d]					Origin[e]	Ref.[f]
			N	NGLC	ND	NGLC[d]	MEX		
EF463	1A4	P-A	250	10	NT	NT	NME	1	9
VE899	1A5	L3	300	0	100	0	L	1	9
EA106	1A6	L	200	10	NT	NT	NME	1	9
DA659	1A8	E	200	5	NT	NT	NME	1	9
VE859	1B5	P	200	6	NT	NT	NME	1	9
VA76	1B6	L3	200	7	NT	NT	VMEX	1	9
svr^{DC765}	1B7	L	100	4	NT	NT	NME	1	9
EF435	1B8	E	150	8	NT	NT	NME	1	9
VA208	1B10	E-L	190	10	NT	NT	NME	1	10
mul^{EF406}	1B13	Pol	300	0	100	0	L	1	9
VA23	1C3	Pol	280	15	NT	NT	NME	1	10
VE846	1D3	L	300	0	100	0	L	1	9
EA48	1E2	L3-P	300	0	100	0	L	1	9
VA209	1E2	L1-2	200	0	100	0	L	1	9
A102	1E3	L	180	8	NT	NT	NME	1	9
C24	1E4	E	200	8	NT	NT	NME	1	9
VE676	1E5	L	300	0	100	0	L	1	9
HC156	1F1	P	200	4	NT	NT	ME	1	9
DC793	1F2	L3	250	0	100	0	L	1	9
VA92	1F3	L-P	100	6	NT	NT	NME	1	9
VE804	1F4	L-P	100	3	NT	NT	MER	1	9
EA97	2A1	E	300	20	NT	NT	NME	1	9
A70	2A2	L3	300	0	100	0	L	1	9
A60	2A3	L1	100	0	130	0	L	1	9
br^{VE662}	2B6	L3	100	3	NT	NT	NME	1	9
npr^{1}	2B5	P	520	11	NT	NT	NME	2	10
npr^{2}	2B5	P	465	9	NT	NT	NME	2	10
dor^{VE915}	2B10	L3	300	10	NT	NT	AO	1	9
VE736	2A-B	E-L	220	9	NT	NT	VMEX	1	10
VA130	2B	L	360	0	150	0	L	1	10
VE810	2B	L2	300	0	100	0	L	1	9
VA177	2B	P	200	4	NT	NT	NME	1	9
VA335	2B	E	300	0	100	0	L	1	9
VE672	2B	L2	300	0	100	0	L	1	9
arm^{XK22}	2B17	E	300	0	100	5	AO	3	9, 11
EA82	2C3	L2-3	210	8	NT	NT	NME	1	12
VE651	2C4	L2-3	250	0	49	5	AO	1	12
GA55	2C8	L	190	10	NT	NT	NME	1	12
usp^{VE653}	2C9	L1-2	180	5	NT	NT	MER	1	12
DF967	2D1	L2-3	320	0	200	0	L	1	12
csw^{C114}	2D2	L3-P	190	11	NT	NT	ME	1	12
C204	2D3	L3-P	174	13	NT	NT	ME	1	12
Pgd^{VA55}	2D5	L2	350	0	100	5	AO	1	12
JC105	2E2	L1-2	317	0	177	0	L	1	13
kz^{VA296}	2E3	L2	220	0	150	0	L	1	13
crn^{EA130}	2F1	E	300	0	130	0	L	1	13
VA172	2F3	L3	450	24	NT	NT	NME	1	13
HF330	2F4	L3	552	0	270	3	L	1	13
EC226	2F5	L2	364	0	126	0	L	1	13
ph^{EA75}	2F6	L3-P	220	10	NT	NT	ME	1	14
gt^{X11}	3A1	E	151	11	NT	NT	NME	3	11, 13
tko^{VE691}	3A2	L3	300	0	100	0	L	1	9
zw1^{g31}	3A4	Pol	250	7	NT	NT	VMEX	4	9
zw8^{g10}	3A6	L	580	0	NT	NT	L	4	15
zw4^{DF944}	3A7	P	240	5	120	20	MER	1	9
zw10^{h10}	3A8	L	300	4	NT	NT	VMEX	4	9
zw2^{e21}	3A9	E-L	750	0	NT	NT	L	4	15
zw13^{13}	3A10	L	250	0	100	0	L	4	9, 15
zw3^{k22}	3B1	L	300	6	NT	NT	ME	4	1, 9, 15
zw6^{a25}	3B2-3	L1	790	10	NT	NT	NME	4	15
zw12^{h1}	3B3	L1	650	0	NT	NT	L	4	15

TABLE 2—Continued

Locus[a]	Location[b]	Lethal phase[c]	Germline clone analysis[d]					Origin[e]	Ref.[f]
			N	NGLC	ND	NGLC[d]	MEX		
zw7^{e3}	3B3-4	L1-2	700	0	NT	NT	L	4	9, 15
zw5^{s27}	3B3-4	L-P	500	0	NT	NT	L	4	15
zw11^{e5}	3B4	L2	300	0	NT	NT	L	4	9, 15
zw9^{k18}	3B4	L	520	0	NT	NT	L	4	9, 15
N^{VAN81}	3C7	E	250	10	NT	NT	MER	1	10
RA55	3D6	E-L	250	0	100	0	L	1	9
VE807	3E1	L1	300	0	150	0	L	1	9
VA148	3E2	E-L	600	0	100	5	AO	1	9
VE880	3E3	P	100	3	NT	NT	NME	1	9
ptg^{VAM201}	3E7	P-A	300	6	NT	NT	VMEX	1	9
l(1)ml	10*	L3	123	1	123	4	VMEX	5	10
hnt^{X001}	4D-E	E	200	5	NT	NT	NME	3	9, 11
svb^{YP17b}	4E	Pol	250	7	NT	NT	NME	3	9, 11
l(1)EN8	13*	L-P	166	9	166	15	VMEX	5	10
C214	6D1	L2-3	200	0	85	0	L	1	9
EA43	6D3	E-L	108	3	NT	NT	AO	1	9
VE921	6D4	L-P	175	6	NT	NT	NME	1	9
DF962	6D5	L-P	160	11	NT	NT	VMEX	1	9
VA179	6D6	P	215	18	NT	NT	VMEX	1	9
VA234	6D7	P	350	1	100	7	AO	5	10
DC737	6E2	E	280	6	NT	NT	NME	1	9
jnLX	6E-F	P	240	16	NT	NT	VMEX	6	9
ogre^{jnL3}	6E-F	L	250	0	150	0	L	6	9
jnL2	6E-F	E	150	15	NT	NT	NME	6	9
jnL1	6E-F	E	300	15	NT	NT	NME	6	9
EC251	6E4	E-L	300	22	NT	NT	NME	1	9
cm^{VEM119}	6E5	L	278	6	NT	NT	NME	1	9
JA9	7A3	L	220	7	NT	NT	NME	1	9
EF465	7A7	E-L	180	0	150	0	L	1	9
kf^{VE614}	7B1	E	100	0	90	0	L	1	9
EA68	7B4	L-P	270	18	NT	NT	NME	1	10
VA156	7B5	L-P	260	16	NT	NT	NME	1	10
VA175	7C1	L	158	7	154	8	VMEX	1	9
VA276	7C2	L3	600	0	100	0	L	1	10
VA293	7C	P	350	2	150	2	L	1	10
EA24	7C7	L	67	1	63	1	MER	1	9
GA41	7C8	E	176	0	100	0	L	1	9
DF948	7C9	L-P	165	10	144	12	VMEX	1	9
EF491	7D3	L	203	5	188	6	VMEX	1	9
VA333	7D4	L	223	3	200	9	VMEX	1	9
DC704	7D6	L	283	0	270	0	L	1	9
mys^{XB87}	7D1-5	E	300	7	NT	NT	MER	3	9, 11
fs(1)h^{k3}	7D1-6	L	400	0	200	0	L	1	10
VE607	7D8	L3	275	0	250	0	L	1	9
VA334	7D10	E-L	177	0	150	0	L	1	9
VA113	7D11	E-L	176	3	150	3	NME	1	9
EF421	7D12	E-L	233	3	150	4	NME	1	9
DA572	7D13	E	195	8	NT	NT	NME	1	9
VA86	7D16	E	90	0	80	0	L	1	9
VA40	7D18	L3	760	10	400	16	AO	1	10
sdt^{XN05}	7D22	E	230	8	NT	NT	NME	3	9, 11
EF520	7E4	E-L	188	1	176	1	L	1	9
VA293	7E5	L3-P	415	2	211	2	L	1	9, 10
DF980	7E7	L	71	0	66	1	L	1	9
VA142	7F1	E	310	15	NT	NT	NME	1	9
VE727	7F4	L-P	140	1	132	2	VMEX	1	9
VA195	7F8	L3-P	600	0	195	0	L	1	10
VE661	8A1	L2-3	300	5	300	16	AO	1	10
JA18	8A2	L	330	0	200	0	L	1	9
otd^{XC86}	8A3	E	180	7	NT	NT	NME	1	9, 11
d-deg 11	27.2*	L-P	250	0	100	0	L	7	9
d-deg-3	27.5*	P	250	0	100	0	L	7	9
d-deg 9	29.7*	L3	300	5	NT	NT	NME	7	9
ras^{DC819}	9E3	L	300	0	80	2	AO	1	9

TABLE 2—Continued

Locus[a]	Location[b]	Lethal phase[c]	Germline clone analysis[d]					Origin[e]	Ref.[f]
			N	NGLC	ND	NGLC[d]	MEX		
DC701	9E4	L1-2	300	0	90	3	AO	1	9
EA79	9F1	P-A	200	9	NT	NT	VMEX	1	9
EA61	9F7	P-A	100	3	NT	NT	NME	1	9
VE828	9F9	E	300	0	100	0	L	1	9
EA86	9F11	P	270	0	135	0	L	1	9
DF923	10A2	P	200	15	NT	NT	NME	1	9
VE772	10A4	L2	300	4	NT	NT	NME	1	9
DA514	10A5	Pol	111	1	111	1	L	1	10
DC812	10A6	L1-2	100	3	NT	NT	NME	1	9
VE774	10A7	E-L1	300	10	NT	NT	MER	1	9
EF444	10A8	L	200	5	NT	NT	VMEX	1	9
rtv^{VE745}	10A9	E	300	7	NT	NT	NME	1	9, 11
discless1	35*	L3	300	0	100	0	L	7	9
VE821	10A10	E	270	0	100	0	L	1	9
JF6	10A11	E	300	0	100	0	L	1	9
VE874	10B3	Pol	200	10	NT	NT	NME	1	9
EC230	10B4	L1-2	150	0	NT	NT	L	1	9
VA178	10B5	L3	200	8	NT	NT	AO	1	9
dsh^{v26}	10B6	L	200	5	NT	NT	MER	1	16
hop^{VA85}	10B7	L	200	9	NT	NT	MER	1	17
dlg^{111}	10B8	L-P	200	8	NT	NT	MER	1	18
DC705	10B13	L	200	7	NT	NT	AO	1	9
VA188	10B14	L-P	200	1	20	1	AO	1	9
L3	10B15	Pol	250	0	100	0	L	1	9
L9	10B16	L3-P	250	0	100	0	L	1	9
C248	10B17	E-L	200	5	NT	NT	VMEX	1	9
Rpll215	10C2-3	L	320	0	320	10	AO	1	19
DF912	10C5	E	300	0	100	0	L	1	9
HA10	10C6	L1-2	200	0	100	0	L	1	9
VE623	10C8	E-L	300	1	110	1	L	1	9
DC833	10C10	L2-3	220	6	220	10	VMEX	1	10
C154	10D1	L	300	0	100	0	L	1	9
VE742	10D7	L-P	100	6	NT	NT	ME	1	9
VE817	10E4	E	300	0	100	0	L	1	9
DF939	10E6	L1-2	300	0	100	0	L	1	9
DF978	10F1	L1-2	300	20	NT	NT	NME	1	9
VE603	10F2	L1-2	300	3	NT	NT	AO	1	9
EA17	10F4	P-A	200	8	NT	NT	NME	1	9
VE755	10F6	L3-P	300	10	NT	NT	MER	1	9
VA147	10F9	Pol	100	0	100	0	L	1	9
A29	10F10	Pol	300	0	100	0	L	1	9
L13	11A2-3	E	300	10	NT	NT	NME	1	20
tsg^{RF32}	11A2-3	E	200	7	NT	NT	NME	1	20
L2	11A6	L-P	200	8	NT	NT	VMEX	1	20
l(1)11Da	11D	L-P	300	0	NT	NT	L	7	1
l(1)11Db	11D	L-P	400	1	100	5	AO	7	1
l(1)11Dc	11D	P-A	300	0	NT	NT	L	7	1
l(1)11Eb^{7}	11E	L	300	0	NT	NT	L	7	1
l(1)11Ec^{1}	11E	P	200	5	NT	NT	NME	7	1
l(1)11Ed^{1}	11E	L-P	300	0	NT	NT	L	7	1
l(1)11Fa^{1}	11F	E-L	300	0	NT	NT	L	7	1
l(1)11Fb^{2}	11F	E-L	300	0	100	0	L	7	1
crt^{2}	11F	P-A	200	0	NT	NT	L	7	1
d-deg 10	46.7*	P	300	0	100	0	L	7	9
d-deg-4	52*	L-P	160	5	NT	NT	NME	7	9
d-het 2	57*	P	300	0	100	0	NME	7	9
d-small 9	57*	P	200	5	NT	NT	NME	7	9
upd^{YM55}	17A-B	E	180	9	NT	NT	NME	3	9, 11
VA197	18F5	L	200	0	100	0	L	1	21
VA132	19A3	P-A	250	5	NT	NT	VMEX	1	21
VA624	19C5	E-L1	190	5	NT	NT	NME	1	21
run^{HM449}	19E	E	250	17	NT	NT	NME	8	11, 21
Pas^{EC201}	19E3	L1-2	200	5	NT	NT	NME	1	21

TABLE 2—Continued

Locus[a]	Location[b]	Lethal phase[c]	Germline clone analysis[d]					Origin[e]	Ref.[f]
			N	NGLC	ND	NGLC[d]	MEX		
R-9-28	19E4	L	200	0	100	0	MER	8	21
EC235	19E5	L	300	0	250	0	L	1	21
unc^{DC803}	19E8	P-A	150	7	NT	NT	NME	1	21
lfl^{DF970}	19F1	L1-2	200	0	100	0	L	1	21
DA689	19F2	P	400	20	NT	NT	AO	1	21
fli^{EF498}	19F3	P	200	15	NT	NT	ME	1	21
$A112^{8-1}$	19F4	L	150	0	120	0	L	8	21
LB20	19F5	L-P	150	0	150	1	L	8	21
eo^{A7}	20A2	L2	150	10	NT	NT	ME	8	21
wap^{Q217}	20A3	P-A	150	12	NT	NT	NME	8	21
$Q56^{19-98}$	20A4	P	100	4	NT	NT	NME	8	21
$uncl^{VA228}$	20A5	L1-2	200	12	NT	NT	VMEX	1	21
fog^{114}	20A3-B	E	170	10	NT	NT	NME	8	21
$EA41^{A72}$	20A-B	L	300	0	150	0	L	8	21
stn^{VE720}	20B3	L1-2	200	7	NT	NT	NME	1	21
13E3	20C1	E	150	13	NT	NT	NME	8	21
20^{VA97}	20C3	Pol	200	4	NT	NT	NME	1	21
sph^{VE829}	20E1	L-P	400	0	150	0	L	1	21
$su(f)^{pb}$	20E	P	170	11	NT	NT	NME	1	21
$su(f)^{S2}$	20F1	L	300	0	140	0	L	8	21
d-deg 12	65.8*	L3-P	200	4	100	0	L	7	9
mus105	NT	L-P	250	0	100	0	L	7	9
mus109	NT	L-P	200	3	NT	NT	VMEX	7	9

[a] Genetic nomenclature: *svr* (*silver*), *mul* (*multiple*), *br* (*broad*), *npr* (*non-pupariation*), *dor* (*deep-orange*), *arm* (*armadillo*), *usp* (*ultaspiracle*), *csw* (*corkscrew*), *Pgd* (*Phosphoglucodehydrogenase*), *kz* (*kurz*), *crn* (*crooked neck*), *ph* (*pole hole*), *gt* (*giant*), *tko* (*technical knockout*), *zw* (*zeste-white*), *N* (*Notch*), *ptg* (*pentagon*), *l(1)ml* (*l(1)melano-like*), *hnt* (*hindsight*), *svb* (*shaven baby*), *cm* (*carmine*), *sdt* (*stardust*), *kf* (*kinked femur*), *ct* (*cut*), *mys* (*l(1)myospheroid*), *fs(1)h* (*fs(1)homeotic*), *otd* (*orthodenticle*), *ras* (*raspberry*), *rtv* (*retroactive*), *dsh* (*dishevelled*), *hop* (*hopscotch*), *dlg* (*discs-large-1*), *tsg* (*twisted gastrulation*), *crt* (*crumpled tips*), *upd* (*unpaired*), *run* (*runt*), *Pas* (*Passover*), *unc* (*uncoordinated*), *lfl* (*little flylike*), *fli* (*fightless*), *eo* (*extra organs*), *wap* (*wings apart*), *uncl* (*uncoordinated like*), *fog* (*folded gastrulation*), *stn* (*stoned*), *sph* (*sparse hairs*), $su(f)^{pb}$ (*suppressor of forked*$^{pale\ bristle}$), *su(f)* (*suppressor of forked*).

[b] Location: The cytological location of each lethal complementation group is indicated. In some cases only the meiotic location is known (indicated by an *)) We have used GEORGE LEFEVRE's lethal designation, except where allelisms to named loci have been proven. The *l(1)* designation has, in most cases been omitted for brevity.

[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; A, adult (dies shortly after emergence); V, viable.

[d] Nomenclature on the germline clonal analysis: Maternal expression (MEX) was determined by germline clonal 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 is the number of females dissected; NGLC[d] is the number of females determined to have a germline clone by dissection. The maternal expression (MEX) has been subdivided according to the phenotype of the homozygous lethal germline clones: L, lethal; AO, abnormal oogenesis; ME, maternal effect lethal phenotype; MER, rescuable maternal effect lethal phenotype; VMEX, variable maternal expression; NME, no maternal effect; NT, not tested.

[e] Origin of the *X*-linked lethals: (1) G. Lefevre; lethals that begin with letters E, V or D are EMS induced; the others are X-ray induced. (2) I. KISS; (3) E. WIESCHAUS; (4) L. ROBBINS; (5) Bowling Green Stock Center; (6) T. CLINE; (7) B. BAKER; (8) G. MIKLOS.

[f] References: (9) This study; (10) PERRIMON, ENGSTROM and MAHOWALD (1984a); (11) WIESCHAUS and NOELL (1966); (12) PERRIMON, ENGSTROM and MAHOWALD (1985b); (13) PERRIMON, ENGSTROM and MAHOWALD (1984b); (14) Perrimon, Engstrom and MAHOWALD (1985a); (15) GARCIA-BELLIDO and ROBBINS (1983); (16) PERRIMON AND MAHOWALD (1987); (17) PERRIMON and MAHOWALD (1986b); (18) PERRIMON (1988); (19) MORTIN, PERRIMON and BONNER (1985); (20) K. KONRAD, personal communications; (21) PERRIMON, SMOUSE and MIKLOS (1989).

From among the F_1 progeny batches of 5 dysgenic F_1 *y f/ II2* virgin females were mated with *M5/Y* males. From among the F2 progeny single pair matings of *M5/X** virgin females with 2 *FM7c/Y* males (of M cytotype) were established and their progeny examined to detect *X*-linked lethal mutations. Subsequent selection and establishment of mutant stocks was similar to that of the EMS screen with the exception that most P-induced mutations were stabilized in a P cytotype.

Mapping the lethal mutations: Zygotic lethal mutations which yielded interesting phenotypes in germline clonal analysis were mapped using overlapping duplications and deficiencies that covered various regions of the *X* chromosome (Table 1). Lethal mutations which could not be covered by duplications were mapped by meiotic recombination using a marked *X* chromosome (*sc ec cv ct*^{6} *g*^{2} *f*). Descriptions of stocks and balancers not given in the text can be found in LINDSLEY and GRELL (1968).

Germline clonal analysis: Germline clones of zygotic lethal mutations were generated using the dominant female sterile technique (PERRIMON and GANS 1983; PERRIMON, ENGSTROM and MAHOWALD 1984a). This technique uses the dominant female sterile mutation ovo^{D1}. Females heterozygous for ovo^{D1} do not lay eggs. To induce homozygous germline clones for a zygotic lethal mutation, virgin females heterozygous for the lethal are mated with males carrying

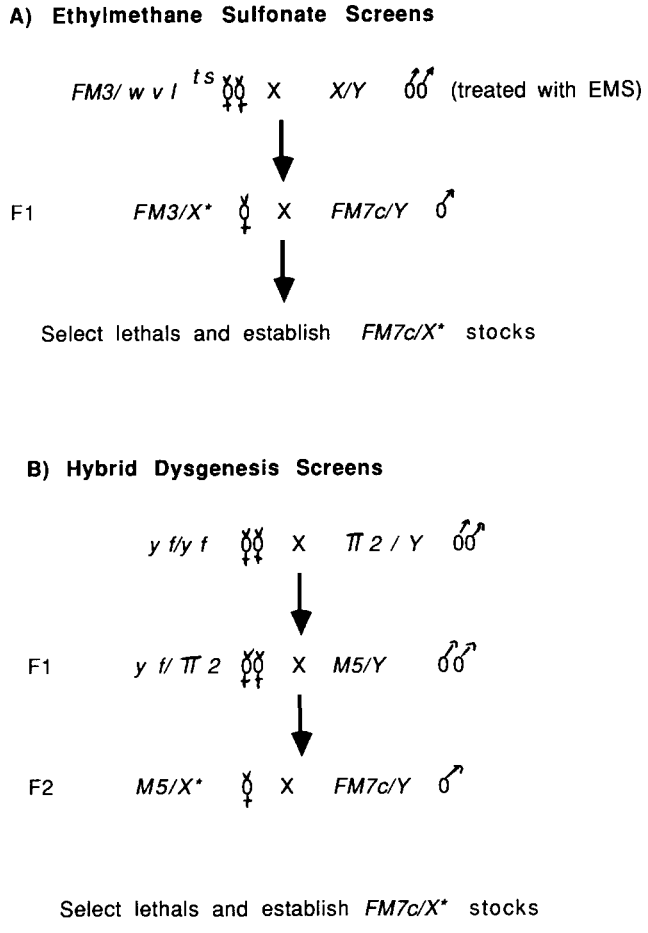

FIGURE 1.—Mating schemes to isolate X-linked lethal mutations.

the dominant female sterile mutation (*FM7/+ lethal* × *ovo*^{D1} *+/Y*). At the end of the first larval instar stage, progeny are irradiated with 1000 rads using a model GR-9 ^{60}Co irradiator or a Torrex 120D X-ray machine (100 kV, 5 mA, 3-mm aluminum filter) or a Clinac-18 X-ray machine. When *ovo*^{D1}/+ larvae are irradiated, X-ray-induced mitotic recombination events in the female germline generate germ cells of genotype +/+. The frequency of females carrying germline clones homozygous for the + chromosome varied from 5% to 10%. The loss of the *ovo*^{D1} mutation in these germ cells allows these cells to develop normally through oogenesis and produce eggs that develop into adults. Females of genotype + *lethal*/*ovo*^{D1} + that possess a germline clone were detected by the presence of a developed ovary which is easily scored by inspection of the abdomen. Each of these females were isolated and crossed with two wild-type males (*Oregon-R P2*). Their eggs and progeny were subsequently analyzed. For each lethal about 300 females of genotype + *lethal*/*ovo*^{D1} + were examined following irradiation. If no clones were detected, a subset of these were dissected in Ringer's solution in order to examine ovarian development. The morphology of the egg chambers from these developed ovaries was subsequently examined for potential defects in oogenesis.

Lethal mutations were located proximal to the dominant female sterile mutation may have mitotic recombination events occurring between the *ovo*^{D} locus (located in 4E) and the lethal resulting in germ cells heterozygous for the lethal mutation. These distal mitotic recombination events are identified by the emergence of adult males (PERRIMON, ENGSTROM and MAHOWALD 1984a) and were discarded and not included within the results.

TABLE 3

***Screen B:* Germline clonal analysis of late zygotic lethals**

Dose EMS	*N chromosomes*	*N* lethals (%)	*N* tested in GLC
Screen L3-pupal lethals (P)			
0.005M	1364	130 (9.5)	35
0.025M	1221	342(28)	68
Screen L2-pupal lethals (PP)			
0.025M (1, 2, 3)	441	124 (28)	47
0.012M (4, 5, 6)	871	200 (23)	87
0.006M (7, 8, 9)	746	104 (14)	49
Screen larval-pupal lethals (PB)			
0.025M	1123	348 (31)	123
0.012M	1232	271 (22)	102
Screen hybrid dysgenesis for larval-pupal lethals (H)			
—	1366	97 (7.1)	70

Larval and pupal lethal mutations were induced using EMS or by P-M hybrid dysgenesis (see details in Figure 1). Different screens were performed using differing concentrations of EMS. In order to avoid the induction of multiple mutations rather low concentrations of EMS were used. The number of chromosomes analyzed and the frequency of induced X-linked lethal mutations is indicated. Finally, the number of lethals selected for germline clonal analysis is indicated. Each screen is designated by different letters (*i.e.*, PO, PP, PB or H) and the numbering system for each lethal line allowed the detection of identical mutations derived from premeiotic clusters.

Analysis of zygotic phenotypes: To determine the lethal phase of a zygotic lethal, the lethal mutation was first outcrossed with a wild-type strain (*Oregon-R P2*, referred to as +) to remove the balancer chromosome. Virgin heterozygous +/*lethal* progeny (from the cross *Balancer*/*lethal* × +/*Y*) were collected and crossed to wild-type +/*Y* males. Between 200 and 300 eggs (N) are subsequently collected from this cross over a period of 6 hours and lined up on a petri plate containing an agar-molasses medium. These eggs were incubated at 25° for a period of 24 hr, when the hatching rate was determined. All unhatched eggs (N^{unh}) were collected and dechorionated in 50% Clorox bleach after which the number of unfertilized (N^{unf}) and fertilized eggs were scored. Subsequently, cuticle preparations of the unhatched embryos were performed: unhatched eggs were subsequently washed in water, heated at 65° in glycerol/acetic acid and mounted in Hoyers media (VAN DER MEER 1977). If the number $N^{unh} - N^{unf}/N - N^{unf}$ was approximately 25%, the lethal mutation was classified as an embryonic lethal.

Classification of the lethal phase phenotypes: The hatched larvae from these plates were fed live yeast to ensure further development and examined every 2 days. During this time the number of dead larvae was scored as well as the number of pupae that formed. After 10 days of development, emerging adults were counted and the number of dead pupae scored. The zygotic lethal mutations have been subdivided into 7 classes according to the specific stage of lethality of the hemizygous progeny derived from heterozygous mothers: *class 1* lethals die during embryonic stages; *class 2* lethals die at the embryonic-larval transition; *class 3* lethals die as larvae (L1, L2 or L3); *class 4* lethals die at the larval-pupal transition; *class 5* lethals die as pupae; *class 6* lethals die at the pupal-adult transition (a fraction of the hemizygous progeny emerge and die shortly thereafter); and *class 7* lethals are polyphasic (no clear lethal phase can

be assigned). Examination for cuticular defects was performed for class 1 and class 2 zygotic lethal mutations.

RESULTS

Classification of the germline clone phenotypes: The phenotypes observed from homozygous germline clones of zygotic lethals have been classified into six groups. *Group 1: germline cell lethality.* This group combines two phenotypic classes. In the first case, no eggs were laid by any of the females thought to possess clones. Following dissection of these females no ovaries with vitellogenic oocytes were found. In the second case, very few eggs were laid by females carrying clones. Dissection revealed that very few clones were present in these females and that only in a small subset the ovarioles were fully developed. The frequency of these clones was very low (about 1% of + *lethal/ovo*^{D1} + females had a clone). This latter pseudo-lethal case was grouped with the germline cell lethals in order to simplify the classification. *Group 2: abnormal oogenesis.* This group includes females that possess a germline clone with specific defects during oogenesis. The phenotypes usually included collapsed eggs, small eggs, or eggs with defective appendages (*i.e.*, lack or fusion filaments). Also included are zygotic lethal mutations that produce very specific oogenic defects, *e.g.*, nurse cell defects or blocked vitellogenesis. *Group 3: strict maternal effect lethality.* In this group all the progeny derived from homozygous germline clones died during embryogenesis and exhibited a same terminal structural defect. The maternal effect did not appear to be influenced by the introduction of the wild-type copy of the gene from the father. *Group 4: rescuable maternal effect lethal phenotype.* In this group hemizygous progeny derived from homozygous germline clones died at a lethal phase earlier (usually as embryos) than the hemizygous progeny derived from heterozygous females. Introduction of the wild-type copy of the gene from the father influenced the development of the zygote to some extent. *Group 5: variable maternal expression.* In this group the gene that mutate to lethality was clearly maternally expressed. No specific or consistent phenotype was observed. In most cases both unhatched and/or defective eggs and larvae were obtained from homozygous germline clones. Unhatched embryos exhibited variable phenotypes. Included in this group are lethal mutations which exhibit the perdurance phenomenon (see PERRIMON, ENGSTROM and MAHOWALD 1984a). *Group 6: no maternal effect.* In this group hemizygous male progeny (*lethal/Y*) derived from homozygous (*lethal/lethal*) germline clones exhibited the same lethal phase as progeny derived from heterozygous (*lethal/+*) females. In addition, the number, morphology and fertility of female progeny derived from homozygous germline clones was normal.

TABLE 4

Summary of both *Screens A* and *B*

LP	L	AO	ME	MER	VMEX	NME	Total (%)
Screen A:							
E	10	1	0	2	0	18	31 (15)
E-L	8	2	0	1	2	5	18 (8)
L	43	11	2	5	10	16	87 (41)
L-P	12	2	4	3	5	5	31 (15)
P	4	2	2	1	2	11	22 (10)
P-A	2	0	0	0	3	5	10 (5)
Pol	5	0	0	0	1	6	12 (6)
Total	84	18	8	12	23	66	211
Screen B:							
L-P	158	34	67	91	95	136	581

The lethal phase has been subdivided into seven groups (see RESULTS): 1, embryonic lethals (E); 2, lethals that die at the embryonic-larval transition (E-L); 3, larval lethals (L); 4, lethals that die at the larval-pupal transition (L-P); 5 pupal lethals (P); 6, lethals that die at the pupal-adult transition (P-A); and 7, polyphasic lethals (Pol). The percentage of loci within each category is indicated. Nomenclature: see legend of Table 2.

Screen A: Germline clonal analysis of mapped *X*-linked loci: Germline clonal analyses of 441 mutations at 211 different loci were performed (see Table 2 for results and Table 4 for summary). These loci are predominantly located within the polytene cytogenetic intervals 1A4-3E8 (70 loci), 6D1-8A3 (46 loci), 9E3-11F (54 loci), and 18F5-20F1 (25 loci). These regions, covering approximately 35% of the *X* chromosome, are believed to be close to saturation for vital loci (LEFEVRE 1981; LEFEVRE and WATKINS 1986; our unpublished results).

One of the concerns of any saturation mutagenesis is the possibility that some of the mutations isolated may not be complete loss of function alleles (null or amorphic, MULLER 1932) but rather are hypomorphic mutations that lead to reduced gene activity. It is important to realize that germline clones may produce different phenotypes depending upon the severity of the particular allele. For example, an amorphic allele for a given locus may correlate with germ cell lethality, whereas a hypomorphic allele of the same locus may show a specific maternal effect lethal (MEL) phenotype. Alternatively, the amorph may show a MEL phenotype but the hypomorph will show no maternal effect. Previously, germline clonal analyses of a large number of mutations in specific regions of the *X* chromosome were performed in order to address this issue specifically (PERRIMON, ENGSTROM and MAHOWALD 1984b, 1985b; PERRIMON, SMOUSE and MIKLOS 1989). These studies have estimated that about 15% of the mutations at a specific locus do not correspond to an amorphic phenotype, thus demonstrating the importance of examining multiple alleles at a zygotic lethal locus before defining the germline clonal phenotype as amorphic. In *Screen A*, 441 mutations at

211 loci were examined and the number of alleles analyzed at specific loci varied between one and eight. At least two alleles were analyzed for 70% of the 211 loci examined. In Table 2 the allele that has been selected for representing the germline clone phenotype of a locus is with the strongest phenotype, which we assume is amorphic.

The collection of X-linked lethals analyzed (Tables 2 and 4) represents all lethal phases, with 15% dying as embryonic lethals (31 loci), 8% dying at the embryonic/larval interface (18 loci), 41% dying as larvae (87 loci), 15% dying at the larval/pupal interface (31 loci), 10% as pupae (22 loci). 5% dying at the pupal adult transition (10 loci) and 6% dying polyphasically (12 loci). The polyphasic alleles may represent hypomorphic alleles at loci for which a null might exhibit an earlier lethal phase. Table 4 summarizes the results of this screen according to the stage of lethality. Some interesting features emerge from this analysis. First, the number of loci that are not maternally required is high during both embryonic and pupal stages and drops significantly during larval stages (Figure 2). This reflects the large number of genes that encode embryonic-specific functions (*e.g.*, gastrulation genes: *twisted gastrulation* and *folded gastrulation*; segmentation genes: *Giant, orthodenticle, unpaired, runt*). Likewise, during pupal stages it reflects the large number of genes that encode pupariation-specific functions (*e.g.*, non-pupariation genes involved in ecdysone response: *npr 1* and *npr 2* loci; KISS, SZABAD and MAJER 1978). Second, the number of loci that are cell lethal in germline clones increases during larval stages. These loci most probably represent housekeeping functions, with lethality of hemizygous progeny derived from heterozygous females occurring during larval stages because there is sufficient maternal product stored in the egg to complete embryonic but not larval development. Third, the number of cell lethal loci during embryonic stages is low. This reflects the enrichment during embryonic stages of embryonic-specific functions. Additionally, the number of cell lethal loci is low at later stages (late larval and pupal). This is probably because hemizygous progeny derived from females heterozygous for a lesion that affects a housekeeping function will not possess enough maternal product to reach these late developmental stages.

Screen B: Germline clonal analysis of random X-linked lethal mutations: Since the production of germline clones of a large number of mutations is quite tedious, results from *Screen A* were used to choose the developmental stage with the highest frequency of mutations with maternal effects. Thus, examination of mutations which are lethal at this developmental stage should yield a number that exhibit specific maternal effect lethal (MEL) phenotypes. Results from screen A indicate that larval and pupal stages where NMEX-NL/total = 0.52 (calculated from Table 4) would likely yield the greatest enrichment for MEL mutations. Therefore, a second approach undertaken involved the isolation of a random set of 581 X-linked larval-pupal lethal mutations from a set of mutagenesis experiments and their subsequent analysis by the generation of germline clones (Figure 1, Table 3).

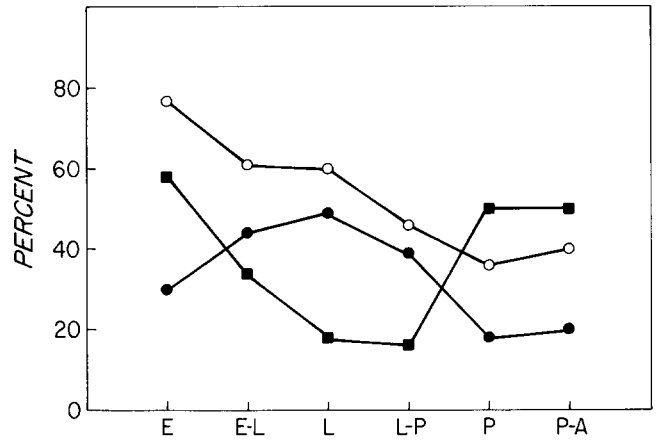

FIGURE 2.—Germline expression according to the stage of lethality. Three different graphs have been plotted according to the developmental stage of lethality of the mutations (see Table 4 for numbers): (black squares) 100 × (number of mutations which are not maternally required/number of total mutations examined); (black circles) 100 × (number of mutations that are germline cell lethal/number of total mutations examined); and (empty circles) 100× (number of mutations that are germline cell lethal/number of mutations that are maternally required).

The germline clonal analysis of these loci (Table 4) correlates well with the results from screen A (Figure 2). Among these 581 mutations, the frequency of mutations that are germline cell lethals is 27% (compared to 25% in *Screen A*) and 23% of the loci are not maternally expressed (compared to 30% in *Screen A*). Mutations that produce interesting germline clone phenotypes were mapped (a phenotype was judged interesting if the oogenic or embryonic phenotype observed was reproducible and specific). For mapping, each mutation was first crossed to a set of duplications that collectively covered about 45% of the X chromosome (see Table 1 for stocks). Once a lethal mutation was determined to be covered by a large duplication, more precise mapping was pursued by complementation tests with smaller deficiencies and duplications and/or mutations that yield similar phenotypes. Finally, complementation tests with mutations obtained from *Screen A* were performed to determine whether the newly induced mutations identified additional complementation groups. Lethal mutations that could not be mapped using duplications were mapped meiotically and their cytological location deduced from both their meiotic position and their complementation pattern with duplications (see MATERIALS AND METHODS).

Germline clone phenotypes of larval and pupal zygotic lethal mutations: The different phenotypes recovered during germline clonal analysis of zygotic lethal loci have been subdivided into various categories which are described below. Table 5 is a list of all *X*-linked loci with specific phenotypes. In Figures 3 and 4 the phenotypes of mutations that produce abnormal embryos are shown. Appropriate references for each locus are listed in Table 2.

Mutations that affect oogenesis: Phenotypes involving alterations in oogenesis observed in germline clones have been classified into five groups. (1) Mutations that affect nurse cell development: homozygous germline clones of *Phosphoglucodehydrogenase* (*Pgd*) produce less than 15 nurse cells. In clones of *VE651* the nurse cells degenerate at an early stage. (2) Mutations that block oogenesis: no eggs are laid and abnormal postvitellogenic oocytes have been observed in clones of *RpII215*, *VA148* and *l(1)11Db*. (3) Mutations that alter egg shape: mutations at *armadillo* (*arm*), *raspberry* (*ras*) and *VE603* produce very small eggs in homozygous germline clones. Germline clones of *VA178* produce small eggs which have fused filaments. Two mutations, *DC701* and *VA188*, produce the "*dumpless*" phenotype in homozygous germline clones; the developmental program of nurse cells in these mutants seems to be delayed compared to that of follicular cells. The eggshell is secreted around the oocyte when nurse cells have not yet "dumped" their cytoplasm into the oocyte. Approximately, 80% of the eggs derived from homozygous germline clones of *DA689* have either no chorionic filaments or filaments which are fused dorsally and displaced posteriorly. There is no effect on egg size and about 4% of the embryos develop but are U-shaped (due to the apparent failure of germband retraction) with extreme head defects. (4) Mutations that produce collapsed eggs and fused filaments (*PB7u*, *14P11*, *10P4*, *6PP7*, *12P3*, *5P4*) or collapsed eggs with an overall wild-type eggshell pattern (*deep-orange*, *VA234*, *EA42*, *VA40*, *VE661*, *6PP19*, *DC705*, *4P1*, *PB2v*): some of these mutations probably perturb some step(s) of vitellogenesis or choriogenesis since no evident oogenesis defects are observed. The eggs derived from homozygous germline clones are flaccid when laid or collapse after egg laying. (5) Mutations which produce eggs that appear unfertilized: germline clones of two mutations (*11P11* and *VE742*) produce normal eggs that do not show signs of embryonic development.

Mutations that affect embryonic development: These have been classified into 6 groups according to the cuticular phenotype of the mutant embryos derived from homozygous germline clones. These maternal effect phenotypes are associated with: (1) poor cuticular development, (2) the twisted phenotype, (3) the neurogenic phenotype, (4) the dorsal open phenotype, (5) segmentation defects and (6) subtle cuticular defects.

Maternal effect associated with poor cuticular development (*C204*, *l(1)discs-large-1*, *flightless*): *l(1)C204* (*C204*, 2D3, 5 alleles). *C204/Y* progeny derived from heterozygous mothers exhibit a small disk phenotype and examination of neuroblast mitotic figures indicates that *C204* disrupts an essential mitotic function. Defects associated with the maternal effect of *C204* occur prior to blastoderm formation and lead to poorly developed cuticle, indicating that the wild-type gene product is stored maternally and required during the early, rapid cleavage divisions.

l(1)discs-large-1 (*dlg-1*, *10B8*, *9 alleles*)). Larvae that lack zygotic $dlg\text{-}1^{+}$ gene activity die at the larval-pupal transition and have an aberrant growth of imaginal cells. Embryos lacking both maternal and zygotic activity of $dlg\text{-}1^{+}$ show defects in neurogenesis and morphogenesis that result in very abnormal embryos with very poor cuticle differentiation. Although differentiated, most tissues are misshapen. This maternal effect is rescuable to some extent. Interestingly, one allele, $dlg\text{-}1^{HF321}$, is a temperature-sensitive hypomorphic mutation. Embryos derived from homozygous $dlg\text{-}1^{HF321}$ females exhibit defects associated with dorsal closure and head involution that resemble the "dorsal open" phenotype described below.

l(1)flightless (*fli*, *19F3*, *9 alleles*). *fli/Y* hemizygous progeny derived from heterozygous mothers die during larval and pupal stages. Embryos derived from *fli* homozygous germline clones are very poorly differentiated. Defects are detectable soon after blastoderm formation when abnormal folds are apparent and mesoderm invagination is clearly abnormal. At later stages only patches of epidermis are present. This maternal effect does not appear to be influenced by the introduction of a paternal wild-type copy of the gene.

Maternal effect associated with a twisted phenotype (*corkscrew*, *9PP12*, *8PP9*, *PB8P*, *R-9-28*, *extra organ*): *l(1)corkscrew* (*csw*, *2D2*, *4 alleles*). *csw/Y* hemizygous progeny derived from heterozygous mothers die as pupae and exhibit a small disk phenotype. All embryos derived from *csw/csw* germline clones exhibit a twisted phenotype. This phenotype is fully penetrant and not influenced by the introduction of wild-type sperm.

l(1)R9-28 (*R9-28*, *19E4*, *5 alleles*). Mutants at this locus are larval lethals. They yield a rescuable maternal effect lethal phenotype in homozygous germline clones. The embryonic phenotypes from germline clones include variable head defects and dorsal closure defects. A fraction of the embryos are normal.

l(1)extra organs (*eo*, *20A2*, *5 alleles*). Mutants at this locus are larval lethals. In germline clones they yield a fully penetrant maternal effect lethal phenotype.

TABLE 5

Summary of X-linked zygotic lethals which show specific phenotypes in germline clones

Locus	Location	*Screens*		Lethal phase	Germline clone phenotype
		A	*B*		
Abnormal oogenesis					
deep-orange	2B10	2	1	L3	Collapsed eggs
armadillo	2B15-17	2	1	E	Small eggs
VE651	2C4	3	1	L2-L3	Nurse cell degeneration
Pgd	2D5	6	+	L2	Abnormal number of nurse cell nuclei
VA148	3E2	1	+	E-L	Abnormal migration of follicle cells
*PB7u**	3E8-6C11 (4.5)	−	1	L-P	Collapsed eggs, fused filaments
14P11	6C11-7A8	1	+	L-P	Collapsed eggs, fused filaments
EA43	6D3	1	+	E-L	Collapsed eggs
VA234	6D7	1	+	P	Collapsed eggs
VA40	7D18	1	1	L3	Collapsed eggs
VE661	8A1	1	+	L2-3	Collapsed eggs
6PP19	9A2-E1	1	+	L-P	Collapsed eggs
10P4	9E3-10C2	1	+	L-P	Collapsed eggs, fused filaments
11P11	9A2-E3	−	2	L-P	Few eggs are laid, no development
raspberry	9E3	2	1	L	Small eggs
DC701	9E4	1	+	L1-2	Dumpless phenotype
VA178	10B5	1	+	L3	Small eggs, fused filaments
DC705	10B13	1	+	L	Collapsed eggs
VA188	10B14	1	+	L-P	Dumpless phenotype
Rp11215	10C2-3	5	+	L	Abnormal postvitellogenic oocytes
VE742	10D7	2	+	L-P	No development
VE603	10F2	2	1	L1-2	Small eggs
*l(1)*11Db	11D	1	+	L-P	Blocked oogenesis
*6PP7**	11B12-13F (51)	1	1	L-P	Collapsed eggs, fused filaments
12P3	13F-16A2	1	+	L-P	Collapsed eggs, fused filaments
5P4	15E3-16C2	1	+	L-P	Collapsed eggs, fused filaments
*4PI**	16A2-18F (57)	−	1	L-P	Collapsed eggs, some look normal
*PB2v**	16A2-18F (57)	−	1	L-P	Collapsed eggs
DA689	19F2	1	+	P	Egg filaments abnormally located
Strict maternal effect lethal phenotype (no hatching)					
HC156	1F1	2	+	P	Segmentation defects with holes
corkscrew	2D2	3	1	L3-P	Twisted embryos
C204	2D3	4	1	L3-P	Preblastoderm defect
pole-hole	2F6	6	3	L3-P	Acron and telson missing
zeste-white-4	3A7	4	1	P	Neurogenic
zeste-white-3	3B1	2	1	L	Naked embryos
*9PP1**	3E8-6C11 (11)	−	1	L-P	Fusion of some segments
*9PP12**	3E8-6C11 (14)	−	1	L-P	Twisted embryos with head defects
*1PP22**	3E8-6C11 (8)	−	1	L-P	Ventral hypoderm missing
6P6	4B	−	1	L-P	Neurogenic
10P11	7D-F	+	1	L-P	Few eggs developed, segment fusion
8PP9	7A8-7C9	+	1	L-P	U-shaped embryos with holes
*1P3**	11B12-13F	−	1	L-P	Neurogenic
*5PP1**	11B12-13F (50)	−	1	L-P	Ventral holes in the cuticle
5PP10	13F-16A2	−	1	L-P	Segmentation defects with holes
flightless	19F3	7	2	P	Gastrulation defects
extra-organ	20A2	4	1	L2	Twisted embryos with abnormal cuticle
Maternal effect rescuable (hatching lead to females)					
VE804	1F4	1	+	L-P	Overall pattern is normal
ultraspiracle	2C9	3	+	L1-2	Defects in most posterior segment
Notch	3C7	5	2	E	Neurogenic
3P5	3A5-E8	+	1	L-P	Dorsal open
1PP18	7C9-8A5	1	2	L-P	Dorsal open
EA24	7C7	1	+	L	Neurogenic
myospheroid	7D1-5	2	1	E	Dorsal open
*IP2**	8A5-9A2 (25)	−	1	L-P	Defective number of segments
*PB18w**	8A5-9A2 (28)	−	1	L-P	Dorsal open
3PP4	9A2-E3	−	1	L-P	Ventral holes
VE774	10A7	3	1	E-L1	Poor cuticle development, head missing
dishevelled	10B6	4	1	L	Segment polarity

TABLE 5—Continued

Locus	Location	*Screens*		Lethal phase	Germline clone phenotype
		A	*B*		
hopscotch	10B7	19	2	L	Defective number of segments
discs-large-1	10B8	7	2	L-P	Poorly differentiated embryos
VE755	10F6	4	1	L3-P	Neurogenic
*12P2**	11B12-13F (39)	–	1	L-P	Poor cuticle differentiation
*7P1**	11B12-13F (40)	–	2	L-P	Dorsal open
*porcupine**	16E-17B (59)	–	1	L	Segment polarity
*PB8P**	16A2-18F (60)	–	1	L-P	U shaped embryos with head defect
fused	17C-E	–	2	P	Segment polarity
R9-28	19E4	4	1	L	Twisted embryos with holes in the cuticle

The name of each locus, its location, lethal phase and germline clone phenotype is indicated. A "*" indicates that the cytological location of the mutation has been deduced from the meiotic position (in parenthesis) and the complementation pattern with duplications. The number of alleles obtained from both *Screens A* and *B* is indicated. A "+" indicates that loci obtained from one screen should have been identified by the other screen. A "−" indicates that the locus defined from screen B is outside the region analyzed from *Screen A*. A mutation has a strict maternal effect lethal phenotype if no progeny hatch, likewise, a mutation has a rescuable maternal effect if female adult progeny are obtained from homozygous germline clones.

Two mutations (*7P1* and *2PP1*) have been assigned to the *7P1* complementation group since they had similar lethal phases, germline clone phenotypes and meiotic positions (40 and 42, respectively).

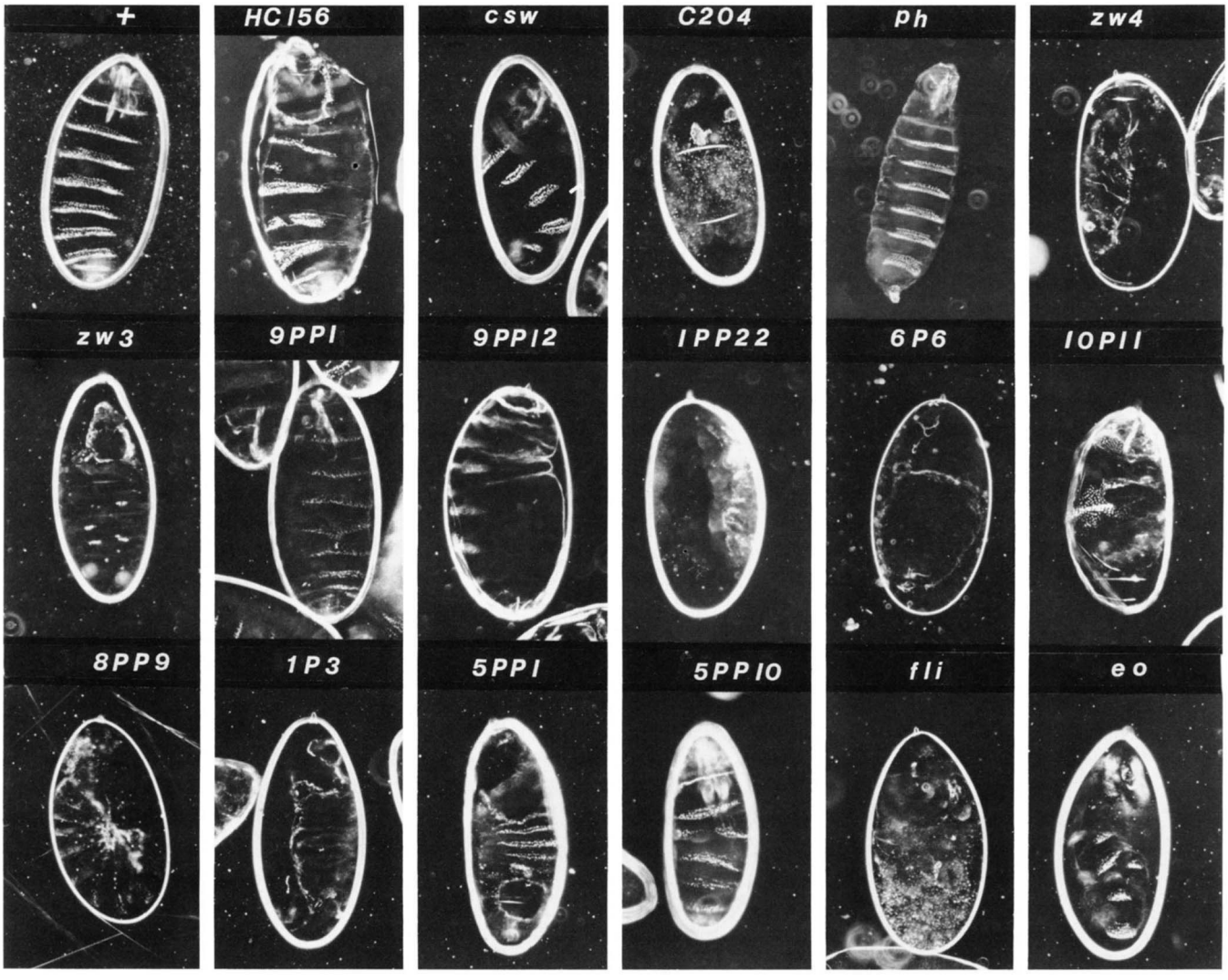

FIGURE 3.—Zygotic lethals with a strict maternal effect lethal phenotype. Dark field photographs of cuticle preparations of wild-type and mutant embryos are shown. The identity of each mutant is indicated by the allele designation in the upper part of each photograph. Nomenclature: *wild type* (+), *corkscrew* (*csw*), *pole hole* (*ph*), *zeste-white 4* (*zw4*), *zeste-white 3* (*zw3*), *flightless* (*fli*), *extra-organs* (*eo*).

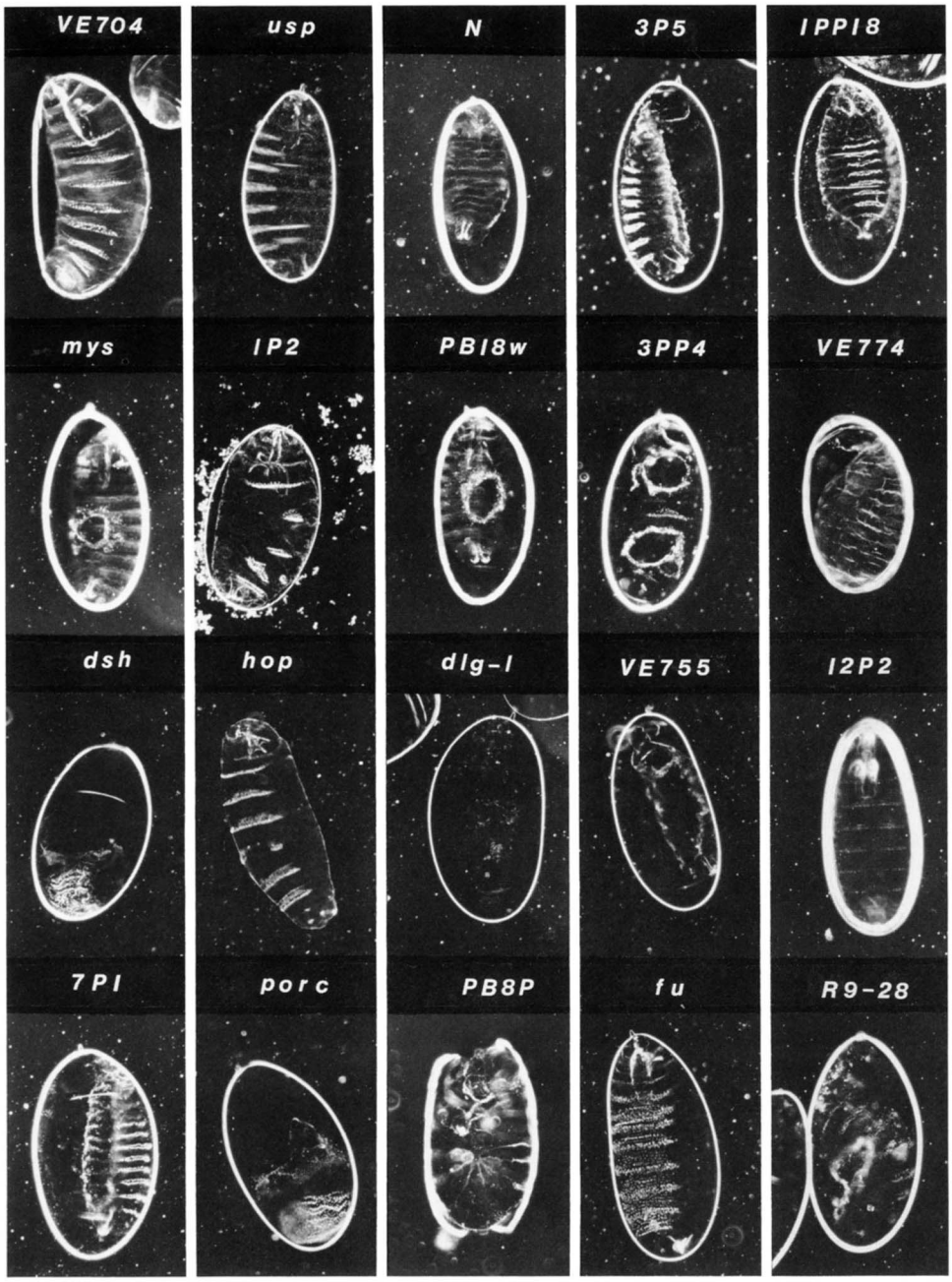

FIGURE 4.—Zygotic lethals with a rescuable maternal effect lethal phenotype. Nomenclature: *ultraspiracle* (*usp*), *Notch* (*N*), *myospheroid* (*mys*), *dishevelled* (*dsh*), *hopscotch* (*hop*), *discs-large-1* (*dlg-1*), *porcupine* (*porc*), *fused* (*fu*).

Most embryos are U-shaped as a result of incomplete germ-band retraction, and show poor cuticular differentiation and variable holes in the ventral epidermis.

Three loci—*l(1)9PP12* (3E8-6C11), *l(1)8PP9* (7A8-C9), and *l(1)PB8P* (16A2-18F)—are each represented by only one allele. These alleles are larval-pupal lethal mutations and yield similar maternal effect lethal phenotypes that lead to twisted embryos. In most cases these embryos also have head defects as well as holes in their epidermis. In the case of *l(1)PB8P* introduction of the paternal wild-type copy of the gene rescues the maternal effect.

Maternal effect associated with a neurogenic phenotype (*zeste-white-4*, *Notch*, *6P6*, *VE755*, *1P3*): Five loci which yield a neurogenic phenotype in germline clones have been characterized. Their neurogenic phenotype, which consists of hypertrophy of the nervous system at the expense of ventral, lateral and anterior ectodermal precursors (WRIGHT 1970), was examined in embryonic sections and by antibody staining against horseradish peroxidase (HRP) in whole mounts (results not shown). These embryos contain only the most dorsal and posterior cuticle (Figures 3 and 4).

l(1)zeste-white-4 (*zw4*, *3A7*, *5 alleles*). Hemizygous progeny derived from heterozygous females die during larval stages. Embryos derived from homozygous germline clones have a neurogenic phenotype that for

some alleles does not appear to be influenced by the introduction of the wild-type gene from the father. The fecundity of the females that possess *zw4* homozygous germline clones decline with time that is correlated with oogenic defects. For example, five females bearing germline clones of $zw4^{DF944}$ produced 15 eggs between days 2–3 postemergence, 8 eggs from days 3 to 4, 6 eggs from days 5 to 6 and 0 eggs from days 5 to 10. Although the number of eggs produced was rather small, each embryo exhibited the neurogenic phenotype. Similar results are obtained with three other *zw4* alleles. Subsequent analysis of the ovaries of these flies revealed disorganized egg chambers and tumorous-like overgrowth of nurse cells.

l(1)Notch (*N, 3C7, 7 alleles*). Null mutations at the *N* locus are embryonic lethal and germline clone analysis of *N* has indicated that the gene is maternally expressed (JIMENEZ and CAMPOS-ORTEGA 1982; PERRIMON, ENGSTROM and MAHOWALD 1984a; WIESCHAUS and NOELL 1986). Two late zygotic lethal mutations were recovered from *Screen B* that represent hypomorphic *N* alleles. These two new alleles of *N* are larval-pupal lethal mutations and exhibit a rescuable neurogenic phenotype in germline clones. Resulting embryonic phenotypes ranged from very severe to weakly neurogenic phenotypes in which embryos have a portion of the ventral cuticle.

l(1)6P6 (*6P6, 4B, 2 alleles*). Hemizygous progeny derived from heterozygous females die at the larval-pupal interface. All embryos derived from homozygous germline clones have a neurogenic phenotype. However, as in germline clones of *zw4*, there is a decline in fecundity over time that is correlated with oogenic defects accompanying the neurogenic phenotype in germline clones. Interestingly, the hypomorphic female sterile mutation *fs(1)107* (PERRIMON *et al.* 1986) has been shown to be allelic to *6P6*. *fs(1)107* is a rescuable maternal effect lethal mutation that exhibits a neurogenic phenotype. Interestingly, unlike *6P6*, the decline in fecundity is not observed in females homozygous for *fs(1)107*.

l(1)VE755 (*VE755, 10F6, 5 alleles*). Hemizygous progeny derived from heterozygous females die at the larval-pupal interface. Hemizygous embryos derived from homozygous germline clones have a strong neurogenic phenotype which is paternally rescuable.

l(1)1P3 (*1P3, 11B12-13F, 1 allele*). Hemizygous progeny derived from heterozygous females die at the larval-pupal interface. All embryos derived from homozygous germline clones have neurogenic phenotypes which range from the very severe to the weak neurogenic phenotype, in which embryos have a partial ventral cuticle. Embryos exhibiting the weak neurogenic phenotype may correspond to those that have received a wild-type copy of the locus paternally.

Potential neurogenic mutations (*1PP22, EA24, 3PP4, 5PP1*): Mutations at four loci—*l(1)1PP22* (3E8-6C11), *l(1)EA24* (7C7), *l(1)3PP4* (9A2-E3), and *l(1)5PP1* (11B12-13F)—all of which are late zygotic lethals, lead to embryos that are missing some ventral epidermal structures in germline clones. These defects may correspond to the neurogenic phenotype, but none were checked for neural hypertrophy (either by sectioning or staining with antibody against HRP) since only one allele at each locus was available and few eggs were obtained from homozygous germline clones.

Dorsal open phenotype (*3P5, 1PP18, myospheroid, PB18w, 7P1*): Five loci were isolated which in germline clones lead to embryos which do not complete dorsal closure and result in a "dorsal open" or "*topless*" (PERRIMON and MAHOWALD 1986a) phenotype. Mutations at three loci—*l(1)3P5* (3A5-E8, 1 allele), *l(1)1PP18* (7C9-8A5, 3 alleles) and *l(1)7P1* (11B12-13F, 2 alleles)—have a similar lethal phase (during larval and pupal stages) and exhibit a similar fully rescuable maternal effect lethal phenotype. The dorsal open phenotype is very extreme with all the dorsal structures of the embryo missing. A temperature-sensitive hypomorphic allele of *l(1)discs-large-1* has a similar maternal effect lethal phenotype (PERRIMON 1988).

Mutations at two loci—*l(1)myospheroid* (*mys*, 7D1-5, 3 alleles) and *PB18w* (8A5,9A2, 1 allele)—yield a similar maternal effect lethal phenotype. *mys* is an embryonic lethal and *l(1)PB18w* is a larval and pupal lethal. Hemizygous embryos derived from germline clones have a large hole on their dorsal side. However, the phenotype is not as extreme as that of the previously described three loci. In both cases the maternal effect is fully rescuable by the introduction of a wild-type copy of the locus paternally.

Loci that affect segmentation pattern (*HC156, ultraspiracle, pole-hole, zeste-white 3, 10P11, 1P2, dishevelled, hopscotch, 5PP10, porcupine, fused*): *l(1)ultraspiracle* (*usp, 2C9, 3 alleles*). *usp/Y* hemizygous second instar larvae derived from heterozygous females exhibit two sets of posterior spiracles, apparently due to the retention of first instar cuticle around the second instar spiracles. When *usp* alleles are analyzed in germline clones, hemizygous progeny show a localized defect posterior to the eighth abdominal denticle belt. The remaining germline clone-derived embryos hatch and develop into adult females with normal viability and fertility.

l(1)pole-hole (*ph, 2F6, 9 alleles*). Mutations at the *ph* locus exhibit a larval-pupal lethal phase attributable to extremely small imaginal disks. Germline clone analysis reveals two embryonic phenotypes. First, *ph/Y* embryos derived from homozygous *ph/ph* germline clones exhibit a poorly defined cuticle. However, a second phenotype is observed in paternally rescued

ph/+ embryos in which all structures posterior to abdominal 7 (the telson) and some head structures (the acron) are missing. The phenotype of these latter embryos is indistinguishable from that seen in embryos displaying the *torso* phenotype (NUSSLEIN-VOLHARD, WIESCHAUS and JURGENS 1982; DEGELMANN *et al.* 1986).

l(1)zeste-white-3 (*zw3, 3B1, 3 alleles*). Hemizygous progeny derived from heterozygous females die during early larval stages. Embryos derived from homozygous germline clones are missing most of their denticle belts, and most head structures are defective. The phenotype resembles that of *naked* embryos (JURGENS *et al.* 1984). This maternal effect is fully penetrant and does not appear to be influenced by the introduction of the wild-type allele from the father.

l(1)10P11 (*10P11, 7C9-8A5, 1 allele*). Hemizygous progeny derived from heterozygous females die during larval and pupal stages. The segment defect resulting from germline clones of *10P11* appears to correspond to the fusion of A2 through A7. Only a few eggs developed from these clones, but all displayed a large ventral segment. No paternal rescue was observed, indicating that this phenotype was the result of a maternal deficiency of the *10P11* gene product. This embryonic phenotype is quite similar to that resulting from the maternal effect *knirps-like* genes (NUSSLEIN-VOLHARD, FROHNHOFER and LEHMANN 1987).

l(1)1P2 (*1P2, 8A5-9E2, 1 allele*). Hemizygous progeny derived from heterozygous females die during larval and pupal stages. Embryos derived from germline clones exhibit segmentation pair-rule segmentation defects (NUSSLEIN-VOLHARD and WIESCHAUS 1980). First instar larvae are missing the entirely or portions of T3, A2, A4 and A6. Although sibling embryos consistently display defects in this set of segments, there is slight variability in the number of defective segments in each embryo, as well as in the severity of the defect within these segments. The deletion pattern phenotype of *1P2* embryos is quite similar to that of *even-skipped*, a pair-rule embryonic lethal mutation which deletes odd-numbered thoracic segments (T1, T3) and even-numbered abdominal segments (A2, A4, A6, A8). However, T1 and T2 are not affected by *1P2*. This MEL phenotype showed partial paternal rescue. The wild-type allele, introduced via sperm, resulted in hatching of half the embryos with a few emerging as adult females. Variable segment defects were observed in these rescued offspring (*e.g.*, lack of halteres and metathoracic legs).

l(1)hopscotch (*hop, 10B7, 21 alleles*). *hop/Y* offspring from heterozygous mothers die as larvae. *hop/Y* embryos derived from homozygous *hop* germline clones exhibit segmentation defects. Mainly T2, T3 and A5 segments are missing. The introduction of a wild-type copy of *hop* by the sperm rescues the thoracic defects, but not the abdominal defects. Such *hop*/+ embryos occasionally lead to viable and fertile adults that are missing one abdominal segment.

Mutations at four loci on the *X* chromosome yield the segment polarity phenotype in which the embryonic cuticle shows continuous fields of sparse denticles, sometimes of reverse polarity, occupying the more anterior section of the normally naked region (NUSSLEIN-VOLHARD and WIESCHAUS 1980). One of them is an embryonic lethal (*armadillo*) and the others are late zygotic lethals (*dishevelled, porcupine* and *fused*).

l(1)armadillo (*arm, 2B15-17, 3 alleles*). Previously isolated amorphic *arm* alleles produce abnormal eggs in germline clones which fail to develop, revealing an absolute requirement for *arm* gene product during oogenesis (WIESCHAUS and NOELL 1986; PERRIMON and MAHOWALD 1987). In *Screen B* a new allele of *arm* ($arm^{H8.6}$) was isolated which shows a conditional embryonic segment polarity phenotype. At 18°, males hemizygous for this allele survive to late larval stages and possess wild-type cuticle. Because *Screen B* was performed for late lethal mutations, hypomorphic alleles of embryonic lethals have been recovered. At 25° and 29°, the cuticle of hemizygous embryos possesses a continuous field of sparse denticles, sometimes of reverse polarity, occupying the more anterior section of the normally naked intrasegmental region (a weak segment polarity phenotype). Germline clones of the temperature-sensitive $arm^{H8.6}$ allele produce eggs which undergo embryonic development. This maternal effect is fully rescuable paternally at all temperatures. The cuticles of hemizygous males derived from mothers that possess such germline clones exhibit a strong segment polarity phenotype at all temperatures which is identical to that of *dishevelled* embryos (KLINGENSMITH, NOLL and PERRIMON 1989).

l(1)dishevelled (*dsh, 10B6, 5 alleles*). *dsh/Y* progeny derived from heterozygous mothers die during larval stages. When progeny derived from homozygous *dsh* germline clones are analyzed, *dsh/Y* embryos exhibit a segment polarity phenotype and fail to form any segmental or parasegmental boundaries. *dsh*/+ germline clone-derived progeny are perfectly normal and lead to normal adult females.

l(1)porcupine (*porc, 16E-17B, 1 allele*). *porc/Y* hemizygous progeny derived from heterozygous females die during larval stages. When analyzed in germline clones *porc/Y* embryos exhibit a segment polarity phenotype identical to that of germline clone-derived *dsh* embryos. *porc*/+ germline clone-derived progeny are perfectly normal and lead to normal adult females.

l(1)fused (*fu, 17C-E, 2 alleles*). Two late pupal lethal alleles of *fused* were recovered. A similar rescuable maternal effect phenotype was observed in both cases.

A fraction of the eggs arrest at early cleavage stages. Among the remaining eggs the maternal effect is paternally rescuable although this rescue is not fully penetrant. Embryos that do not hatch exhibit a segment polarity phenotype.

Mutations at three loci—*l(1)HC156* (1F1, 2 alleles), *l(1)9PP1* (3E8-6C11, 1 allele), and *l(1)5PP10* (13F-16A2, 1 allele)—exhibit a larval and/or pupal lethal phase. When examined in homozygous germline clones, all three exhibit maternal effects and lead to embryos with various segmentation defects. These include missing segments [*l(1)HC156* and *l(1)5PP10*)] and fusion of segments (*l(1)HC156*). These phenotypes are usually associated with death of some epidermal derivatives, suggesting that the segment fusions might be the result of localized cell death. The maternal effect of these three loci does not appear to be influenced by the introduction of the wild-type copy of the gene paternally.

Loci that affect late cuticle differentiation (*VE804, VE774, 12P2*): Mutations at three different loci—*l(1)VE804* (1F4, 1 allele), *l(1)VE774* (10A7, 4 alleles) and *l(1)12P2* (11B12-13F, 1 allele)—exhibit rescuable maternal effects that become apparent late during embryogenesis. Hemizygous progeny derived from germline clones of *l(1)VE804* die during late embryogenesis with no apparent defects. They die during larval and pupal stages if derived from heterozygous mothers. The maternal effect associated with mutations at the *l(1)VE774* locus leads to embryos that differentiate poor cuticle structure and which are missing some head structures. Hemizygous progeny derived from heterozygous mothers usually die during early larval stages. Finally, embryos derived from germline clones of *l(1)12P2* differentiate few aberrant denticles similar to those of *shaven-baby* mutant embryos (WIESCHAUS, NUSSLEIN-VOLHARD and KLUDING 1984).

DISCUSSION

Level of saturation: The goal of these experiments was to saturate the *X* chromosome for late zygotic lethal mutations that exhibit very specific maternal effect lethal phenotypes in germline clones. The level of saturation for such analyses is always difficult to estimate because they rely on the assumption that all loci are equally mutable and that all mutations lead to the same phenotype (for various calculations see: GANS, AUDIT and MASSON 1975; MOHLER 1977; NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; WIESCHAUS, NUSSLEIN-VOLHARD and JURGENS 1984; JURGENS *et al.* 1984; PERRIMON *et al.* 1986; LEFEVRE and WATKINS 1986). A prerequisite for such a calculation is to know the size of the target; *i.e.*, the number of vital loci potentially identifiable by mutations on the *X* chromosome.

Two maps of the *X* chromosome polytene bands have been established. The first one (BRIDGES 1935) described 725 bands, but was later reexamined (BRIDGES, 1938) and expanded to 1012 bands. Some regions of the *X* chromosome have been extensively characterized and are believed to be close to saturation for vital loci. Thus, using the distribution of vital loci over these large regions it is possible to calculate the number of *X*-linked vital loci identifiable by mutations and compare this number to the number of polytene bands [N = (number of vital loci/number of polytene bands) × 100]. The best studied regions on the *X* chromosome are intervals 1A1 to 2B17 (35 vital loci and 61 bands; $N = 57\%$; LEFEVRE 1981; LEFEVRE and WATKINS 1986; G. LEFEVRE, personal communication); 2C3 to 3A1 (15 vital loci and 25 bands; $N = 60\%$; LEFEVRE 1981; PERRIMON, ENGSTROM and MAHOWALD 1984b, 1985b; LEFEVRE and WATKINS 1986); 3A2 to 3E7 (20 vital loci and 38 bands; $N = 52\%$; SHANNON *et al.* 1972; LEFEVRE 1981; LEFEVRE and WATKINS 1986; G. LEFEVRE, personal communication); 9E3 to 11A6 (46 vital loci and 90 bands; $N = 51\%$; LEFEVRE 1981; LEFEVRE and WATKINS 1986; G. LEFEVRE, personal communication); and the proximal *X* chromosome from 18F5 to 20F1 (25 vital loci and 49 bands; $N = 51\%$; LEFEVRE 1981; LEFEVRE and WATKINS 1986; PERRIMON, SMOUSE and MIKLOS 1988). These five regions cover 26% of the *X* chromosome and the average number of vital loci per number of bands is $N = 53.6\%$ (141 vital loci for 263 bands). Although it is clear that the distribution of vital loci along the polytene *X* chromosome is variable when small regions are analyzed (LEFEVRE and WATKINS 1986), it is interesting that over these relatively larger intervals N is quite constant. Thus, by extrapolation there are approximately 540 vital loci among the 1012 bands on the *X* chromosome. By using numbers obtained from Tables 2 and 4 it is possible to estimate that among loci that mutate to zygotic lethality on the *X* chromosome there are 15% of embryonic lethals (81 loci), 8% are dying at the embryonic/larval interface (43 loci), 41% are larval lethals (221 loci), 15% are dying at the larval-pupal interface (81 loci), 10% as pupae (54 loci), 5% are dying at the pupal-adult transition or shortly after emergence (27 loci), and 6% are polyphasic (32 loci).

The best criterion for saturation of a particular region is that each locus within the region is identified by a large number of alleles. These data are available from Table 5. It should be noted that loci that yield defects in oogenesis have not been examined in as much detail as those that lead to the production of defective eggs; also, the level of saturation has only been determined for loci with maternal effect phenotypes. We find that 20 loci were identified from *Screen A* and 34 from *Screen B*, for a total of 38

independent loci. Within the defined regions of the *X* chromosome analyzed (Table 2), only three loci have been obtained from *Screen B* which should have been identified in *Screen A*. Similarly, four loci identified from *Screen A* should have been obtained from *Screen B*. These results suggest that the present collection of such mutations is nearly complete. To calculate the level of saturation, we have assumed that the regions of the *X* chromosome analyzed in *Screen A* are now fully saturated. These regions encompass 360 bands or 35% of the *X* chromosome. Other *X*-linked regions have been examined only in *Screen B*. In this screen early lethals (embryonic and embryonic-larval) as well as polyphasic lethals have been eliminated. Thus, these screens target 72% of the vital loci, and the total number of *X*-linked larval-pupal loci required for saturation is estimated to be 390. A total of 581 larval/pupal lethal mutations were analyzed, indicating that the saturation level achieved with *Screen B* is about 78% ($m = 581/390 = 1.5$ and $P(0) = m^0e^{-m} = e^{-1.5} = 0.22$). When *Screens A* and *B* are considered together the overall level of saturation is 86% [$(0.35 \times 1 + 0.65 \times 0.78) \times 100 = 86\%$]. In conclusion, we estimate that *Screens A* and *B* have reached an 86% level of saturation for *X*-linked zygotic lethals (larval and pupal) with specific maternal effect lethal phenotypes.

The embryonic phenotypes: The focus of this study is the analysis of loci that exhibit a maternal effect lethal phenotype resulting in abnormal embryogenesis. The array of embryonic phenotypes obtained resemble those described from screens for embryonic lethal and female sterile mutations (see review by KONRAD *et al.* 1985; MAHOWALD and HARDY 1985; PERRIMON and MAHOWALD 1987; AKAM 1987). Although most phenotypes are unique to single loci (*e.g.*, *l(1)pole hole*, *l(1)zeste-white-3*, *l(1)hopscotch*), four phenotypes have been frequently obtained: twisted, neurogenic, dorsal open and segment polarity phenotypes.

Mutations at six loci (*corkscrew*, *9PP12*, *8PP9*, *PB8P*, *R-9-28*, *extra organ*) lead to twisted embryos when examined in homozygous germline clones. Similarly, twisted embryonic phenotypes have been described for mutations that affect the determination of the dorsoventral axis (*e.g.*, *dorsal* dominant phenotype, NUSSLEIN-VOLHARD 1979; *twist* and *snail*, SIMPSON 1983). Although the mutant embryos described here have not yet been examined for a defective ventral furrow, it is possible that these mutations affect mesoderm invagination. Interestingly, all but one (*R-9-28*) of the maternal effects associated with these zygotic lethal loci are not paternally rescuable, indicating that the function of these genes is required early in embryonic development (as the dorsalizing loci). KONRAD, GORALSKI and MAHOWALD (1988) proposed that the dorsalized phenotype arises progressively during development as a result of abnormal morphogenesis rather than being specified at blastoderm formation. Therefore, two different developmental mechanisms may lead to a dorsalized or twisted embryonic phenotype: mutations in genes that affect establishment of the dorsoventral axis and mutations in genes involved in mesoderm invagination. If this phenotype can be obtained in numerous ways, it may explain why so many loci can generate these phenotypes (there are at least 14 maternal and 7 zygotic genes reviewed by ANDERSON 1987). By extrapolation 30 (if we assume that the *X* chromosome represents one fifth of the fly genome) late zygotic lethal loci in the *Drosophila* genome will yield the twisted phenotype.

Mutations at five loci have been isolated that, as germline clones, lead to an hypertrophy of the central nervous system at the expense of the ventral, lateral and anterior ectodermal precursors: the "neurogenic" phenotype. Only one of these, *Notch*, has been previously described (WRIGHT 1970; LEHMANN *et al.* 1983). To date only eight neurogenic loci, all zygotically expressed, have been described in Drosophila. Two of them are *X*-linked loci, *almondex* (*amx*, LEHMANN *et al.* 1983) and *pecanex* (*pcx*, PERRIMON, ENGSTROM and MAHOWALD 1984b, LABONNE and MAHOWALD 1985), which are rescuable maternal effect mutations isolated from female sterile screens. Additionally, LEHMANN *et al.* (1983) describes six embryonic lethal loci which when mutated lead to the neurogenic phenotype. Five of these have a maternal effect and are also expressed in imaginal disks (DIETRICH and CAMPOS-ORTEGA 1985). The present analysis indicates that on the *X* chromosome there is at least seven loci that lead to the neurogenic phenotype (*pcx*, *zw4*, *N*, *6P6*, *amx*, *VE755*, *1P3*), suggesting by extrapolation that in the Drosophila genome there are probably as many as 35 loci that can be altered so as to generate this phenotype. Interestingly, all of the previously identified loci (except possibly *big-brain*, DIETRICH and CAMPOS-ORTEGA 1985) are pleiotropic and are both maternally and zygotically expressed. No loci have yet been found to be uniquely expressed at the time of the switch between the epidermal and neural fates. The pleiotropy of these loci may reflect their role in multiple developmental pathways, only one of which is associated with the developmental decision to become an epidermoblast.

Five loci have been identified that lead to embryos with a dorsal open or "*topless*" (PERRIMON and MAHOWALD 1986a) phenotype. This phenotype is characterized by defective dorsal closure and head involution, collapse of the somatic musculature and usually an oversized central nervous system. This phenotype is also associated with mutations in at least seven embryonic lethal loci (WIESCHAUS, NUSSLEIN-VOL-

HARD and JURGENS 1984; NUSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; JURGENS *et al.* 1984). This phenotype is also observed as the maternal effect of a temperature-sensitive allele at the *l(1)discs-large-1* locus (PERRIMON 1988). The defects associated with *l(1)dlg*, together with the fact that one of these loci, *l(1)myospheroid*, encodes the vertebrate integrin β-subunit protein (MACKRELL *et al.* 1988) suggest that mutations at these loci may perturb cell-cell adhesion.

Mutations at four *X*-linked loci are associated with a segment polarity phenotype (*arm, dsh, porc, fu*) in which some pattern elements within each segmental unit are deleted. Additionally, four autosomal embryonic lethal loci with a segment polarity phenotype have been described (NUSSLEIN-VOLHARD and WIESCHAUS 1980). Extrapolation from the present analysis suggests that there may be as many as 20 genes in the Drosophila genome that can be altered so as to produce this phenotype. All four *X*-linked loci are maternally expressed even though their maternal expression is irrelevant since they all are rescuable by introduction of the paternal wild-type allele.

The best understood segment polarity gene is *wingless* (BAKER 1987, 1988) the product of which is presumably secreted since it is homologous to the *int-1* proto-oncogene (RIJSEWIJK *et al.* 1987; CABRERA *et al.* 1987; PAPKOFF, BROWN and VARMUS 1987). It is likely that at least some other members of the segment polarity class are involved in processing, receiving or transducing the *wg* signal. The large number of loci that exhibit a similar phenotype may reflect the number of components involved in this process.

General considerations and implications for the analysis of Drosophila embryonic development: As described in the introduction, the isolation of genes that control embryonic developmental decisions has previously been restricted to those genes that are nominally specific to either oogenesis or embryogenesis. The formal validity of these "focused" screens has certainly been challenged by further genetic and molecular analysis of some of these genes. Developmental pleiotropism is a common feature of Drosophila genes. For example, some mutations that are intimately involved in establishing the anteroposterior and dorsoventral axes are expressed at various stages of zygotic development (*e.g., Toll*, GERTULLA, JIN and ANDERSON 1988), while segmentation genes are not only expressed during segmentation of the germ band but also at later stages in the central nervous system (see review by DOE and SCOTT 1988). The present analysis demonstrates that most genes are pleiotropic. If we assume that pleiotropy is a general developmental feature then two distinct classes of genes emerge from analysis of the maternal effect of zygotic lethals. In class I are embryonic functions that can be provided embryonically by the maternally stored gene product and which cannot be isolated from screens for embryonic lethal mutations. The irrelevance of their maternal expression to embryonic development is exemplified by the paternal rescue of the maternal effect phenotype (*e.g.*, genes such as *dishevelled*; PERRIMON and MAHOWALD 1987). In class II are maternal effect functions which cannot be isolated from screens for female sterility because the gene is also required during zygotic development (*e.g.*, genes such as *l(1)C204* and *l(1)corkscrew*; PERRIMON, ENGSTROM and MAHOWALD 1985b). Interestingly, not all mutations fall within these two groups. There are cases in which only some aspects of the maternal effect are rescuable by the introduction of the wild-type allele of the gene. For example, for *l(1)pole hole* only the poorly differentiated phenotype can be rescued by the paternal introduction of the wild-type gene (PERRIMON, ENGSTROM and MAHOWALD 1985a). Similarly the defect in segment A5 cannot be paternally rescued for *l(1)hopscotch* (PERRIMON and MAHOWALD 1986b). It is possible that such differences reflect the combination of multiple maternal effects.

The present analysis indicates that by using germline clonal analysis it is possible to identify additional genes that affect embryonic development than would be recognized in standard screens for embryonic lethal and female sterile mutations. However, this analysis is not yet exhaustive. It is possible that among the zygotic lethal mutations that are also germline cell lethals, there exists a set of genes that exhibit very specific maternal effects on embryonic development. We do not have access to these genes because homozygosity in germline cells during larval or pupal development is not compatible with cell viability. One way to overcome the problem of cell viability during larval and pupal development might be to generate germline clones of these mutations at a later stage (*e.g.*, in the adult female after germ cells have completed their divisions; see PERRIMON 1984).

This paper demonstrates that screens to detect all zygotic lethal mutations with specific maternal effect lethal phenotypes, although tedious, are formally possible. Obviously it will be important to conduct similar experiments on the autosomes to obtain a complete description of the loci that affect embryonic patterning in *Drosophila*. The present analysis increases the number of loci specifically implicated in gastrulation, neurogenesis and segmentation processes. To understand the control of these developmental events we will need to comprehend the function of these genes together with all other previously characterized loci (listed in AKAM 1987). It is only the detailed analysis of these loci at both the genetic and molecular level that will clarify the functions they provide during embryonic development.

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