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l(1)hopscotch, a Larval–Pupal Zygotic Lethal with a Specific Maternal Effect on Segmentation in *Drosophila*

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The maternal and zygotic effect phenotypes of mutations at the *l(1)hopscotch (l(1)hop)* locus are described. *l(1)hop* is located in 10B6-8 on the salivary gland chromosome map and 17 alleles have been characterized. A complex complementation pattern is observed among the 17 alleles. The lethal phase of null alleles of *l(1)hop* occurs at the larval–pupal interface associated with a small disc phenotype. Embryos produced from homozygous *l(1)hop* germline clones show segment specific defects. The extent of these defects depends upon both the strength of the allele and the paternal contribution. In the most extreme case embryos exhibit defects associated with five segments T2, T3, A4, A5, and A8. In the less extreme phenotype defects are only associated with A5. Thus, activity of $l(1)hop^{+}$ is required both for the maintenance and continued cell division of diploid imaginal precursors and for the establishment of the full array of segments.

INTRODUCTION

Four hours after blastoderm formation, the *Drosophila* embryo is subdivided into 19 repeating units; 6 head, 3 thoracic, and 10 abdominal segments (Turner and Mahowald, 1977; Kaufman, 1983; Struhl, 1983). Each of these contain precursor cells for the larval and adult epidermis. While larval precursors differentiate into larval structures, adult precursors remain undifferentiated until pupariation after which they form the adult epidermis. The commitment of cells to their developmental fates occurs prior to segmentation. Nuclear transplantation (Zalokar, 1971; Illmensee, 1972) and gynandromorph analyses (Sturtevant, 1929; Garcia-Bellido and Merriam, 1969) indicate that cleavage nuclei, prior to the blastoderm, are totipotent. Both genetic (Wieschaus and Gehring, 1976b) and experimental (Simcox and Sang, 1983) analyses establish that commitment occurs during blastoderm formation. Furthermore, cell lineage studies have indicated that cell determination at the blastoderm stage is segment specific (Wieschaus and Gehring, 1976a).

Genetic analysis of segmentation of the *Drosophila* embryo has identified a set of genes which seem to be directly involved in the spatial organization of the metameric pattern. Based upon their developmental expressions and effects, these genes are classified into three broad groups: (1) maternal effect lethal (MEL) loci exhibit global effects on the axial coordinates of the embryo (Nüsslein-Volhard, 1979); (2) embryonic segmentation genes determine the number and polarity of segments (Nüsslein-Volhard and Wieschaus, 1980); and (3) homeotic genes specify segmental identity (Ouwenel, 1976; Garcia-Bellido, 1975). The embryonic phenotypes generated from these mutants support models which propose that gradients of morphogenetic molecules organized under maternal control serve as determinants to direct the expression of a set of zygotic genes that specify the determined state of segments and compartments (see review by Mahowald and Hardy, 1985).

We have recently provided evidence for the existence of a fourth group of genes which appear to play an important role in the control and embryonic development (Perrimon *et al.*, 1984; see Perrimon and Mahowald, 1986 for review). These genes are among the late zygotic lethal loci and exhibit specific maternal effect phenotypes on embryonic development when analyzed in germline clones. In this paper, we describe in detail the developmental genetics of one of these loci, named *l(1)hopscotch* because of its maternal effect on both thoracic and abdominal segments.

MATERIALS AND METHODS

Strains. The first two *l(1)hopscotch (l(1)hop)* alleles, $l(1)hop^{9P5}$ and $l(1)hop^{1PP7}$ were isolated during our analysis of the MEL phenotype of late lethals (Perrimon, Konrad, Engstrom, and Mahowald, unpublished). Subsequent mapping using deficiencies and duplications (Table 1) localized *l(1)hop* in the 10B area of the salivary gland chromosomes. *l(1)hop* was found to be allelic to *l(1)L4* (Lefevre, 1971). Ten additional alleles were obtained from Dr. G. Lefevre (Lefevre, 1971 and unpublished), four alleles from Dr. R. Voelker, and another allele, *msv1*, was obtained from Dr. B. Geer (Geer *et al.*,

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1983). Details on the origins of these mutations can be found in Table 2.

The dominant female sterile mutation, *Fs(1)K1237*, used to generate germline clones, is maintained in an attached-*X* stock: *C(1)DX,y f/Y* females crossed to *Fs(1)K1237* v^{24}*/Y* males. Descriptions of stocks and balancers, unless identified in the text, can be found in Lindsley and Grell (1968).

The unstable ring-*X* chromosome *R(1)2,In(1)*w^{vc}*/y w sp,In(1)dl-49/Y* was obtained from Dr. J. Hall. The frequency of loss of the ring-*X* chromosome is about 40%.

All experiments were conducted at 25°C on standard *Drosophila* medium.

Analysis of germline clones. Germline clones were induced according to the dominant female sterile technique which utilizes the mutation *Fs(1)K1237* (or *Ovo*^{D1}) (Perrimon and Gans, 1983; Perrimon, 1984; Perrimon *et al.*, 1984). Flies were irradiated with a dose of 1000 rad (gamma-ray machine; Model GR-9 Co-60 irradiator) at the end of the first instar larval stage. Such conditions generate 4 to 7% mosaic females. In all experiments, flies carrying *l(1)hop* germline clones were analyzed individually to identify and eliminate distal mitotic recombination events (Perrimon and Gans, 1983) occurring between *l(1)hop* (located within chromosome bands 10B6-8) and *Fs(1)K1237* (located in 4E). About 10% of recombination events occurred distally.

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); (2) histological sections were prepared as described by Mahowald *et al.* (1979); (3) scanning electron micrographs were prepared as described by Turner and Mahowald (1976) after the vitelline membranes were removed according to the technique of Mitchison and Sedat (1983) as modified by Dequin *et al.* (1984); and (4) embryonic nervous systems were stained for acetylcholinesterase activity (Brown and Schubiger, 1981).

Analysis of zygotic phenotype. Lethal phase determination was performed as described by Perrimon *et al.* (1984) at 18, 25, and 29°C to check for temperature-sensitive mutations. Examination of diploid structures utilizing Hoechst DNA stain was performed as described in Perrimon *et al.* (1985a). Adult cuticular structures were incubated in 10% KOH at 65°C for at least 1 hr, dehydrated, and mounted in Canada Balsam medium.

To check for the presence of the abdominal 5 specific muscle the succino-dehydrogenase stain, as described by Lawrence and Johnston (1984), was utilized.

RESULTS

Genetic Mapping

Figure 1 shows the genetic map of the 10A11-10B10 area. The list of duplications and deficiencies used in this analysis is shown in Table 1. Six lethal complementation groups map between the proximal breakpoint of *Dp(1;2)*v^{+63i} (10A11) and the distal breakpoint of *Df(1)m-13* (10B6-8). The relative order of these six complementation groups has been established by meiotic recombination by Geer *et al.* (1983). *l(1)hop* is located between *l(1)dishevelled (l(1)dsh;* 34.50) and *l(1)disc-large (l(1)dlg;* 34.82) at meiotic position 34.61. The distal position of *l(1)hop* relative to *l(1)dlg* is further supported by the complementation pattern of *Df(1)DA622* with the zygotic lethal complementation groups of the 10B area (see Fig. 1). Cytologically *l(1)hop* is located distal to the *Df(1)DA622* distal breakpoint (10B8) and proximal to the 10B6-7 bands as shown by the rearranged allele of *l(1)VE874*, *l(1)VE874*^{GA118} which possesses a breakpoint in position 10B6-7 (Lefevre, personal communication; Perrimon, unpublished observations). Thus, cytologically *l(1)hop* maps to the 10B6-8 region of the salivary gland chromosomes. Results from the other stocks listed in Table 1 were consistent with this localization.

Lethal Phase and Zygotic Phenotype

We analyzed 17 alleles of the *l(1)hop* locus (Table 2). None appear cytologically rearranged (Lefevre, personal communication; Perrimon, unpublished observations).

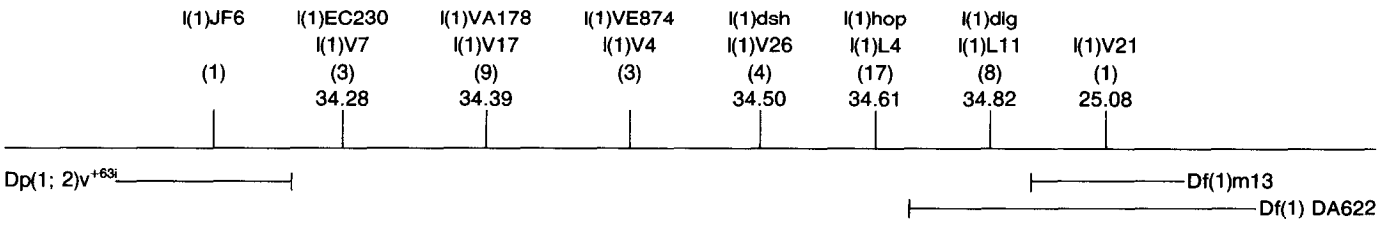

FIG. 1. Genetic map of the 10A11-B10 area. Lethal complementation groups are represented relative to their inclusion within the deficiencies (Df) and duplications (Dp). The number of alleles for each complementation group is shown in parenthesis. Two nomenclatures for each complementation group are shown: the upper names are used in this paper; the lower ones come from Geer *et al.* (1983). The meiotic position of the lethal complementation groups is from Geer *et al.* (1983). The identity of *l(1)disc large (l(1)dlg)* with *l(1) L11* and their location relative to deficiencies was determined by D. Sponaugle (personal communication).

TABLE 1
LIST OF DEFICIENCIES AND DUPLICATIONS USED[a]

Rearrangement	Cytology	References
Df(1)N71	Df(1)10B5; 10D4	Craymer and Roy (1980)
Df(1)DA622	Df(1)10B8; 10D2	G. Lefevre, pers. comm.
Df(1)m-13	Df(1)10B6-8; 11A	T. Goralski, pers. comm.
Dp(1;Y)v^+Yy^+	Dp(1;Y)9F3 to 10C1-2; 20B to base; 1A1 to 1B2;Y	Craymer and Roy (1980)
Dp(1;Y)v^+Yy^+3	Dp(1;Y)9F3 to 10E3-4; 20B to base; 1A1 to 1B2;Y	Craymer and Roy (1980)
Dp(1; 2)v^{+63i}	Dp(1; 2)9E1 to 10A11; 56A	Craymer and Roy (1980)
T(1; 2)v^{65b}	T(1; 2)10A1 to 11A7; 40-41	Craymer and Roy (1980)
T(1; 3)v^{74c}	T(1; 3)9E3-4 to 11B12; 80-81	Craymer and Roy (1980)

[a] For each rearrangement the cytology and references are indicated.

In addition, none appear to show thermosensitive or coldsensitive effects (results not shown). Determination of the lethal phases of hemizygous *l(1)hop/Y* and homozygous *l(1)hop/l(1)hop* progeny indicates that 16 of the 17 alleles (all except $l(1)hop^{msv1}$) have the same lethal phase (Tables 2 and 3). Of the progeny derived from heterozygous mothers, 20% die during larval stages (L2-3) while the remaining 5% die during early pupal stages. In Table 3, only results for one of the 16 larval-pupal lethals ($l(1)hop^{VA85}$) are shown. Dead larvae have a normal external cuticular pattern. All larval diploid structures, however, that were examined following staining with the Hoechst DNA stain (imaginal discs, ovaries and testes, hindgut and foregut imaginal ring, and proliferation centers of the brain) are reduced in size, similar to that observed for *l(1)pole hole* (Perrimon *et al.*, 1985a), (results not shown). When tested over deficiencies, these 16 *hopscotch* mutations behave as amorphs

TABLE 2
l(1)hopscotch MUTATIONS

Mutation	Mutagen	Lethal phase	NGLC	References[a]
9P5	EMS	L-P	28	1
1PP7	EMS	L-P	12	1
VA85	EMS	L-P	57	2
VA275	EMS	L-P	34	2
DC764	EMS	L-P	31	2
VA312	EMS	L-P	25	2
VA108	EMS	L-P	15	2
VE666	EMS	L-P	26	2
GA32	XR	L-P	22	2
HC257	XR	L-P	95	2
C111	XR	L-P	178	2
L4	EMS	L-P	15	2
msv1	EMS	L-P-A	175	3
M4	ENU	L-P	23	4
M13	ENU	L-P	41	4
M38	ENU	L-P	31	4
M75	ENU	L-P	16	4

Note. The origin of *l(1)hop* mutations is indicated. All *l(1)hop* hemizygous males are recovered over the duplication *Dp(1;Y)*v^+Yy^+. For each allele, the lethal phase (larval, L; Pupal, P) and number of females carrying a germline clone (NGLC) is indicated. An "A" in the lethal phase column indicates that adults were obtained. Originally, $l(1)hop^{DC764}$ and $l(1)hop^{VA312}$ exhibited earlier lethal phase attributable to second site mutations.

[a] Abbreviations used: ethylmethane sulfonate (EMS), *N*-ethyl-*N*-nitrosourea (ENU) or X rays (XR); (1) Perrimon, Konrad, Engstrom, and Mahowald, unpublished; (2) Lefevre (1971) and unpublished observations; (3) Geer *et al.* (1983); (4) Voelker, unpublished observations.

TABLE 3
ZYGOTIC LETHAL PHASE OF *l(1)hopscotch*

Cross	*N*	Stage of lethality (%) E	L	P	f_A (%)
VA85/+ × +/Y	200	2	20	7	0
VA85/+ × VA85/DpY	210	1	24	3	0
VA85/+ × Df/DpY	194	3	26	4	0
Df/+ × VA85/DpY	168	2	28	1	0
msv1/+ × +/Y	180	3	6	10	15
msv1/+ × msv1/DpY	190	4	7	12	9
msv1/+ × Df/DpY	192	2	9	21	0
Df/+ × msv1/DpY	185	5	12	19	0
msv1/+ × C111/DpY	410	1	5	21	0
msv1/+ × M13/DpY	215	2	6	16	3
msv1/+ × GA32/DpY	210	2	6	16	4
msv1/+ × VA275/DpY	206	1	4	15	10
msv1/+ × VA85/DpY	398	3	2	8	17
msv1/+ × M4/DpY	250	1	2	3	23
msv1/+ × M38/DpY	200	1	3	1	25
msv1/+ × M75/DpY	310	0	2	2	24

Note. To characterize the various *l(1)hop* alleles three sets of crosses were performed. Heterozygous females for a *hopscotch* allele were crossed to: (1) wild-type males (+/Y); (2) males bearing the same *l(1)hop* allele (*l(1)hop/Dp(1;Y)*v^+Yy^+ symbolized as *l(1)hop/DpY*); (3) males bearing a deficiency for the hopscotch locus *Df(1)N71/Dp(1;Y)*v^+Yy^+3 (symbolized as *Df/DpY*). Results are only shown for two *hopscotch* alleles $l(1)hop^{VA85}$ and $l(1)hop^{msv1}$. All other alleles gave results similar to $l(1)hop^{VA85}$. Finally, transallelic complementation were performed. Results are shown for four mutations, all other alleles gave results similar to $l(1)hop^{C111}$. N is the number of eggs analyzed (no more than 5% of unfertilized eggs were observed in each crosses). The stage of mortality (E, embryonic; L, larval; and P, pupal) is indicated. Frequencies are calculated as the number of dead progeny/the number of fertilized eggs ×100. The frequency of emerging mutant progeny (f_A) is indicated. All the emerged flies were female sterile (see text). f_A represent the number of emerged mutant/the number of fertilized eggs ×100 A "+" chromosome in females is an *FM7* chromosome. The +/Y males are wild-type *Ore R P2*. These chromosomes allow us to detect the hemizygous mutant or transheterozygous progeny without ambiguity.

(null; Muller, 1932) inasmuch as there is no change in the zygotic lethal phase between hemizygous (*l(1)hop/Y* and *l(1)hop/Df*) or homozygous *hopscotch*. Examples of such lethal phase are shown in Table 3 for one allele $l(1)hop^{VA85}$.

A series of crosses, utilizing deficiencies and duplications of the locus, clearly indicates that $l(1)hop^{msv1}$ is a hypomorphic mutation (Table 3). Both $l(1)hop^{msv1}/Y$ and $l(1)hop^{msv1}/l(1)hop^{msv1}$ have a later lethal phase than the other alleles, with 15% male and 9% female flies emerging. The female homozygotes show poorer viability with only one third living more than 2 days. The hypomorphic nature of this allele is confirmed by the result that $l(1)hop^{msv1}/Df$ females (Table 3, crosses 7 and 8) do not emerge. Furthermore, whereas emerged $l(1)hop^{msv1}/Y$ males are morphologically normal, 40% of $l(1)hop^{msv1}/l(1)hop^{msv1}$ and 85% of $l(1)hop^{msv1}/Df$ (removed from the pupal cases) flies have major defects, e.g., one leg or wing missing, or small eyes, etc. These results clearly indicate that the $l(1)hop^{msv1}$ is hypomorphic. Analysis of the paternal rescue of the maternal effect indicates that *l(1)hop* is dosage compensated (see below). If the gene is dosage compensated then both $l(1)hop^{msv1}$ hemizygous males and homozygous females should have the same viability. However, our results indicate that $l(1)hop^{msv1}$ homozygous females are less viable than $l(1)hop^{msv1}$ hemizygous males. The observed reduction in female viability may reflect the inability of the mutant gene activity to keep pace with the general rate of female development which is typically more rapid than that of males.

To determine whether the maternal dosage of $l(1)hop^{+}$ influenced the zygotic lethal phase of *hopscotch* progeny (Perrimon *et al.*, 1985a), lethal phases were determined under two conditions: (1) $+/l(1)hop$ crossed to $+/Y$ males; and (2) $C(1)DX, yf/Y$ crossed with $l(1)hop/DpY$ males. If mutant progeny derived from cross 1 exhibit an earlier lethal phase than progeny derived from cross 2, then a maternal effect can be attributed to the heterozygous females. No significant differences were observed when all *hopscotch* alleles were tested by these crosses (results not shown).

Transallelic Complementation

Complementation analysis between *hopscotch* alleles indicates that all the 16 amorphic alleles fail to complement each other. All heterozygous combinations of amorphic alleles produce the same zygotic lethal phase as seen in homozygous *l(1)hop* flies. However, some complementation is observed between the hypomorphic allele, $l(1)hop^{msv1}$ and some of the amorphs. The nine amorphic alleles (referred to as Class A amorphs), $l(1)hop^{9P5}$, $l(1)hop^{DC764}$, $l(1)hop^{VA312}$, $l(1)hop^{VA108}$, $l(1)hop^{VE666}$, $l(1)hop^{HC257}$, $l(1)hop^{C111}$, $l(1)hop^{1PP7}$, and $l(1)hop^{L4}$, produce the same phenotype over a deficiency and in trans with $l(1)hop^{msv1}$. The seven remaining alleles (referred to as Class B amorphs) complement, to some extent $l(1)hop^{msv1}$. Four of them; $l(1)hop^{M13}$; $l(1)hop^{GA32}$, $l(1)hop^{VA275}$, and $l(1)hop^{VA85}$ complement respectively to 12, 16, 40, and 68% (Table 3). The last three alleles $l(1)hop^{M4}$; $l(1)hop^{M38}$, $l(1)hop^{M75}$ fully complement $l(1)hop^{msv1}$. All the emerged flies are morphologically normal although female sterile. The pattern of $hopscotch^{+}$ activity obtained from the transallelic complementation analysis has been summarized in Fig. 2.

Sterility of $l(1)hop^{msv1}$ is Somatic in Origin

The phenotype of adult $l(1)hop^{msv1}$ hemizygous flies has been described by Dybas *et al.* (1983). Hemizygous $l(1)hop^{msv1}/Y$ males have rudimentary testes usually devoid of gametes. However, even when gametes are present, they do not mature properly. Females hemizygous or homozygous for $l(1)hop^{msv1}$ have atrophic gonads and no egg chambers are detectable. Interestingly, the class B amorphic alleles show considerable complementation with $l(1)hop^{msv1}$. Flies heterozygous with any of these alleles and $l(1)hop^{msv1}$, sometimes lay eggs which, however, never develop. The eggs appear unfertilized and possess an abnormal shape, i.e., small, with a clear chorion and chorionic filaments either partially fused or missing. $l(1)hop^{msv1}/l(1)hop^{GA32}$, $l(1)hop^{msv1}/l(1)hop^{M13}$, and $l(1)hop^{msv1}/l(1)hop^{M38}$ females exhibit an extreme ovarian phenotype and almost never lay eggs; $l(1)hop^{msv1}/l(1)hop^{VA275}$ and $l(1)hop^{msv1}/l(1)hop^{M75}$ lay few eggs (respectively, 10 and 17% compared to wild-type egg production); and, $l(1)hop^{msv1}/l(1)hop^{VA85}$ and $l(1)hop^{msv1}/l(1)hop^{M4}$ lay a lot of eggs (respectively, 55 and 65% compared to wild type). Since eggs of normal shape are obtained when all the *hopscotch* alleles are analyzed in germline clones (see below), the gonadal defects, at least in females, are not germline dependent. These observations, in combination with the defects in imaginal precursors described earlier, suggest that the ovarian abnormalities are associated with effects of the mutation on the follicle cells.

Gynandromorph Analysis

The production of gynandromorphs is useful in determining whether embryonic or larval lethality is as-

$$\frac{\text{Class A or B}}{\text{Class A or B}} = \frac{\text{Class A or B}}{\text{Df}} < \frac{\text{msv1}}{\text{Class A}} = \frac{\text{msv1}}{\text{Df}} < \frac{\text{msv1}}{\text{msv1}} < \frac{\text{msv1}}{\text{Class B}} < \frac{\text{msv1}}{+} = \frac{+}{+}$$

FIG. 2. Complementation pattern and gene product activity at the *hopscotch* locus. The measure of *hopscotch* gene activity is schematized according to the lethal phase, viability and fertility phenotype of transallelic combinations of the *l(1)hop* alleles. For details on the heterogeneity observed among class B amorphs see Table 3 and text.

sociated with any particular region of the organism (Hotta and Benzer, 1973; Zusman and Wieschaus, 1985). Two alleles of *l(1)hop* were tested, *l(1)hop*^{9P5} and *l(1)hop*^{VE666}. In the first experiment (*FM7/y w sn l(1)hop*^{9P5}*f* crossed with Ring-X males) 52 *FM7/Ring X* gynandromorphs were obtained; and, in the second (*FM7/y l(1)hop*^{VE666}*f* crossed with Ring-X) 64 *FM7/Ring X* gynandromorphs were found. In both cases no *l(1)hop/Ring X* gynandromorphs were obtained. These results indicate that we cannot attribute a lethal focus to lack of *hopscotch* gene activity and that gynanders suffer severe developmental defects that lead to inviability.

Hopscotch Shows a Unique Maternal Effect

The role of *hopscotch* in oogenesis can be readily determined by examining the development of clones of homozygous *hop* oocytes. Embryos derived from homozygous *hop* germline clones display a range of phenotypes that depend upon the allele examined and the paternal contribution. The less extreme phenotype of *hopscotch* embryos consist of a single defect in the abdominal segmentation. These embryos lack the denticle belt corresponding to abdominal segment five. The missing denticle belts corresponds to abdominal segment 5 (A5) based on examination of the interdenticle phenotype (Fig. 3E). *Hopscotch* larvae that display this phenotype (Fig. 3F) usually hatch and lead to viable adults (see below). Some of these *hopscotch* larvae also present minor defects in the thoracic region. In Fig. 4A is shown an *hopscotch* embryo in which there is a fusion of dorsal metathoracic hairs with what appear to be the dorsal abdominal 1 hairs.

In the more extreme *hopscotch* embryos three regions are affected; (1) A5 is missing but this defect can extend to A4. The most frequent defects associated with A4 is the deletion of the posterior most region of the denticle (Figs. 3B, D). Dorsally in the area corresponding to posterior A4, embryos present a very irregular pattern of hairs (arrow in Fig. 3C). (2) The denticle belt of T2 is deleted and these defects can extend to T3 (Fig. 4B). (3) A8 is reduced in size (Fig. 3D) and sometimes deleted; in the most extreme cases the posterior spiracles are also defective. In rare embryos partial fusion of A6 and A7 is observed.

The extent of these defects varies depending upon the allele examined and the paternal contribution. The extreme phenotype is observed from germline clones of class A and B alleles that have not received the paternal wild-type copy of the gene. Embryos that have received the wild-type copy exhibit a range of defects from the more to the less extreme. Only a very small fraction (see below) of the rescued progeny are able to hatch and lead to adults. Germline clones of the hypomorphic allele *l(1)hop*^{msv1} lead to the less extreme phenotype and a large fraction of the embryos hatch (see below).

Examination of hatching larvae derived from a female carrying a *hopscotch* germline clone crossed to a wild-type male indicates that only larvae with normal thoracic cuticle (Fig. 3F) develop to pupal stages and occasionally eclose (see next section for rescuability dosage).

Zygotic Rescue and Dosage Compensation

To analyze further the effect of the paternal rescue on the *hopscotch* maternal effect, four sets of crosses were performed for all *hopscotch* alleles. The results for only three alleles are presented in Table 4 (results for the remaining alleles are similar to those of *l(1)hop*^{C111}). Females carrying *l(1)hop* germline clones were crossed to four genetically different males: (1) *+/Y*, wild-type males; (2) *+/DpY*, males carrying the wild-type allele of *l(1)hop*$^{+}$ on both the *X* and *Y* chromosomes; (3) *Df/DpY*, males carrying a deficiency of *hopscotch* on the *X* and a duplication on the *Y* chromosomes; and (4) *+/Y; +/DpA*, males carrying a duplication of *l(1)hop* on an autosome. The results of Cross 1 indicate that the *l(1)hop* MEL phenotype is partially rescuable; i.e., introduction of a wild-type copy of *l(1)hop*$^{+}$ via the sperm is able to partially compensate for the lack of maternal *l(1)hop*$^{+}$ gene activity. However, even these rescued progeny exhibit defects in both their larval and adult abdominal segments. The paternal rescue in Cross 1 leads to only 1% of the female progeny reaching adulthood. However, 14% of the male progeny rescued with *l(1)hop*$^{+}$ gene activity (Cross 2) become adults. These sex-specific differences in rescue suggest that *hopscotch* is dosage compensated. The third set of crosses were performed to analyze the phenotypes of *l(1)hop/Df* female progeny derived from germline clones. These exhibit the typical *hopscotch* phenotype, except that the cuticle shows less differentiation than usual (results not shown): These cuticle defects are probably due to haplo-insufficiency of other genes within the deficiency. Finally, the fourth set of crosses analyzes the rescue when two wild-type copies are introduced via the sperm. The rescue of *l(1)hop/+; +/DpA* females is greater than that observed for *l(1)hop/+* females, but, surprisingly, *l(1)hop/Y; +/DpA* males are not recovered. Similar results were obtained when two duplications (*Dp(1;2)v*^{65b} and *Dp(1;3)v*^{74c}) of the 10B area were tested (Table 4).

When the progeny of females carrying homozygous germline clones for the hypermorphic allele *l(1)hop*^{msv1} are analyzed, the results are similar to those obtained for *l(1)hop*^{C111}. All of the results are consistent with the hypomorphic effect of *l(1)hop*^{msv1}; i.e., more rescue is usually observed in all crosses (Table 4). Like, *l(1)hop*^{C111}, the fourth cross exhibits a poor rescue of *l(1)hop*^{msv1}*/Y;*

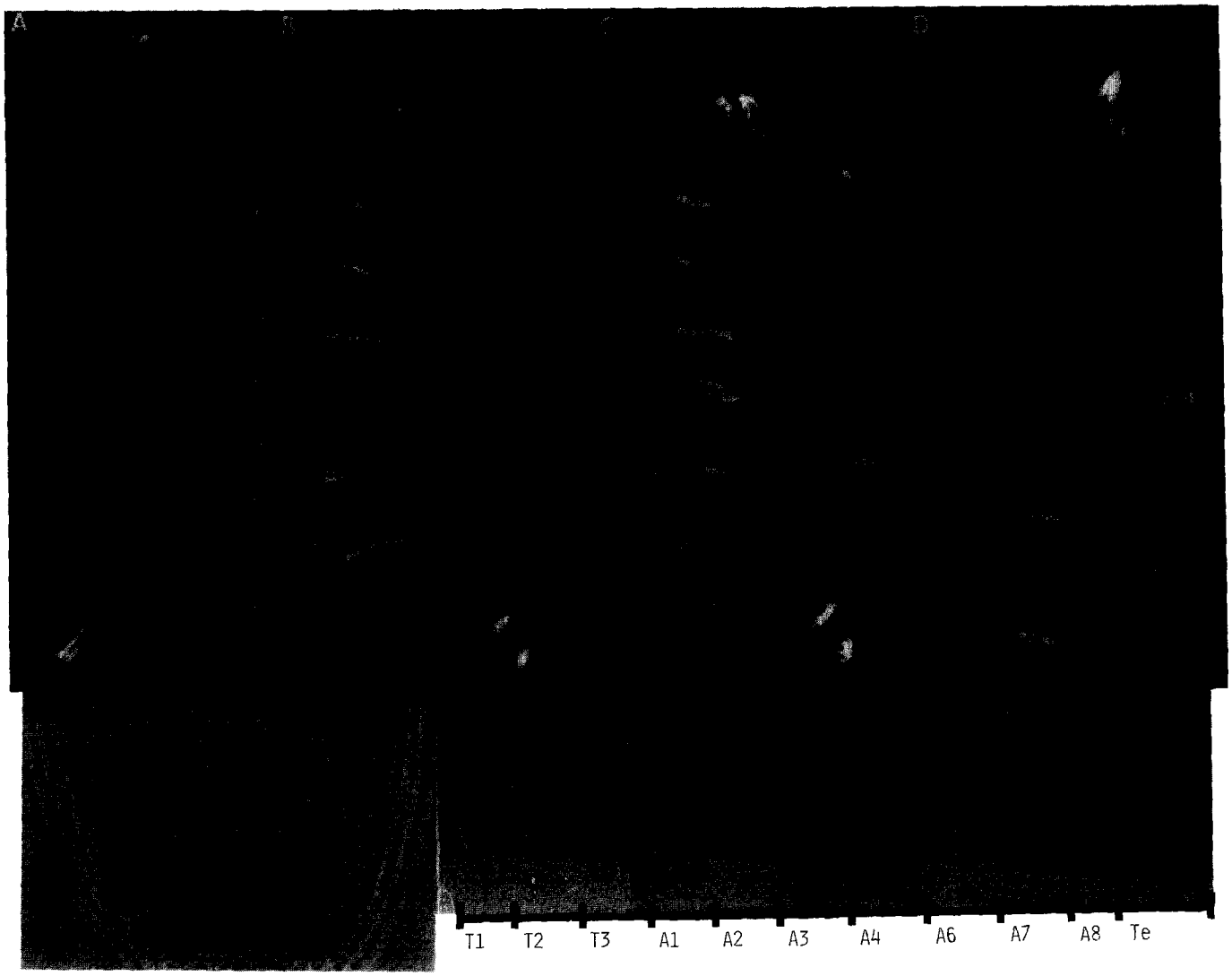

FIG. 3. Abdominal defects of germline clone derived *hopscotch* embryos. (A) is a dark-field micrograph of a wild-type embryo showing eight abdominal dentical belts. *Hopscotch* embryos (B) are missing abdominal segment 5. Dorsal defects in the same area are also visible (C, arrow). The embryo in (D) exhibits defects in the abdominal segments 4 and 8 (D). (E) is a phase-contrast micrograph of an *hopscotch* embryo showing the abdominal region spanning segments 4 to 8. Note the irregular pattern of naked cuticle between A4 and A6 (arrows). In (F) a third instar *l(1)hop/+* larvae derived from a germline clone is shown. Except for the absence of A5, the pattern is normal. Note in this larvae the presence of the anterior spiracles. Abbreviations: Thoracic (T1 to T3) and abdominal (A1 to A8) segments.

DpA/+ males. We obtained similar results when two duplications (*Dp(1;2)v*^{65b} and *Dp(1;3)v*^{74c}) were used. Therefore, it seems possible that dosage compensation of the genes within the duplications is affected. This may be due to a position effect variegation phenomena associated with the duplication.

Among the 16 larval-pupal alleles all, except *l(1)hop*^{VA85}, exhibit the same paternal rescue of the maternal effect as *l(1)hop*^{C111}. Although, no differences in the embryonic pattern of *hopscotch* embryos derived from homozygous *l(1)hop*^{VA85} germline clones were detectable, almost no adults were obtained (Table 4). The complementation pattern of *l(1)hop*^{VA85} with the *l(1)hop*^{msv1} allele indicates that a gene product is probably produced by both mutant genes. It is possible, therefore, that the poor rescue observed reflects an antimorphic effect of the maternally inherited *l(1)hop*^{VA85} gene product and the zygotically introduced *l(1)hop*$^{+}$ gene product. Such antimorphic effects exhibited by recessive mutations have also been observed for mutations in the RNA polymerase II locus (Mortin *et al.*, 1985).

Segmental Abnormalities Are Seen at Segmentation

In wild-type embryos gastrulation starts after the cellular blastoderm stage (6000 nuclei) by formation of

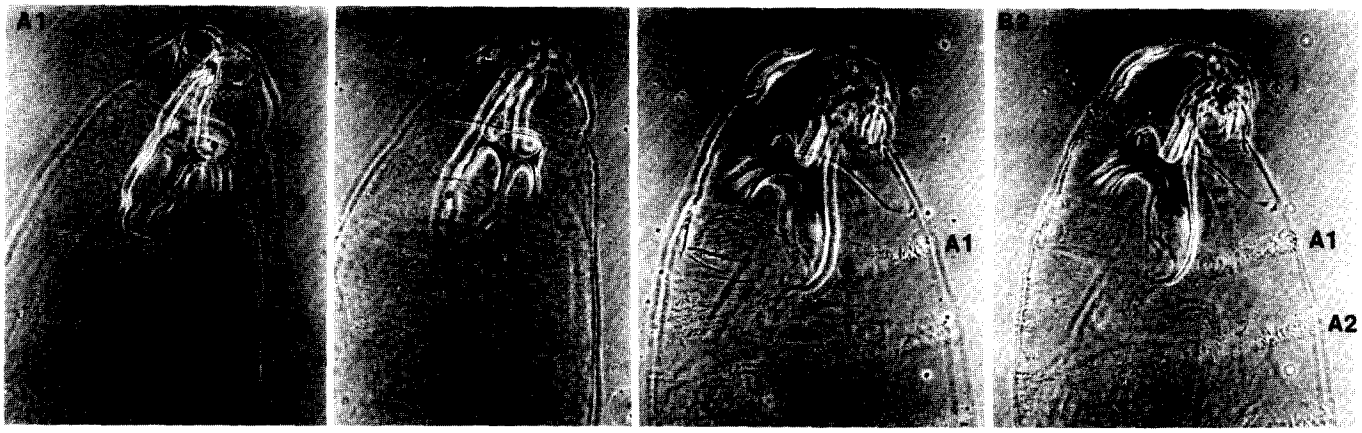

FIG. 4. Thoracic defects of germline clone derived *hopscotch* embryos. The larvae shown in (A) at two focal planes exhibit a subtle defect (arrow) between the dorsal metathoracic and A1 hairs. In the two focal planes of (B) the larvae is missing T2 and T3 thoracic denticle belts. The arrow in B1 indicates the lateral fusion between the ventral A1 denticle belt and (probably) the dorsal metathoracic hairs.

the ventral and cephalic furrows. Endoderm is internalized anteriorly (anterior midgut and stomodeal invagination) and posteriorly (posterior midgut and proctodeal invaginations). Pole cells are carried internally by the posterior midgut invagination. The amnioserosa forms during these morphogenetic movements. At 7 hr the extended germ band has completed segmentation and by 9 hr the germ band has shortened. Along with germ band shortening the nervous system, already organized into ganglia, starts to condense and will continue to shorten until 13 hr of development is reached. A8, 9, and 10, become rearranged to form the terminal abdominal segments. The gnathocephalic appendages and clypeolabrum involute resulting in the characteristic cryptocephalic dipteran larva (Turner and Mahowald, 1977, 1979).

Scanning electron micrographs of *hopscotch* embryos derived from germline clones of weak and strong alleles reveal that defects are visible during early segmentation stages. In the region corresponding to tracheal pits 6–8, segmentation is disorganized (Figs. 5A, B). At the time of germ band shortening it is clear that one abdominal segment is missing and A4 appears wider than normal (compare wild type, Fig. 5C to *hopscotch*, Fig. 5D). In the *hopscotch* embryo shown in Fig. 5D no other defects are detectable; this embryo corresponds to a rescued *hopscotch* embryo. Some *hopscotch* embryos exhibit thoracic defects; as, for example, partial (Fig. 5E) and

TABLE 4
GERMLINE CLONE ANALYSIS OF *l(1)hop*

Mutation	Male	N	N_{unf}	Egg development (%)				
				f_{unh}	f_L	f_P	$f_A\delta$	$f_A\varphi$
C111	+/Y	995	103	91	9	3	0	1
	+/DpY	856	91	48	52	20	14	0.5
	Df/DpY	463	61	68	32	12	10	0
	+/Y;DpA/+	484	85	71	29	9	0	5
msv1	+/Y	780	79	74	26	11	0	5
	+/DpY	1012	143	21	79	40	11	6
	Df/DpY	655	81	56	44	19	15	0
	+/Y;DpA/+	410	62	28	72	31	2	19
VA85	+/Y	875	87	98	2	0	0	0
	Df/DpY	424	37	82	18	2	1	0

Note. Females possessing homozygous germline clones for *l(1)hop* alleles were crossed with four genetically different males: (1) wild-type (+/Y); (2) +/*Dp(1;Y)v*$^+$*Yy*$^+$ or *v*$^+$*Yy*$^+$*3* symbolized as +/*DpY*; (3) *Df(1)N71/Dp(1;Y)v*$^+$*Yy*$^+$*3* symbolized as *Df/DpY*; and (4) +/Y;*Dp(1; 2)v*^{65b} *or Dp(1; 3)* v^{74c}/+ symbolized as +/Y; *DpA*/+. Results are shown for three *hopscotch* mutations *l(1)hop*^{C111}, *l(1)hop*^{VA85}, and *l*(1)hop^{msvl}. The number of eggs analyzed (N), the number of unfertilized eggs (N_{unf}) and the percentages unhatched (f_{unh}), larvae (f_L), pupae (f_P) and adult males and females (f_A) are indicated.

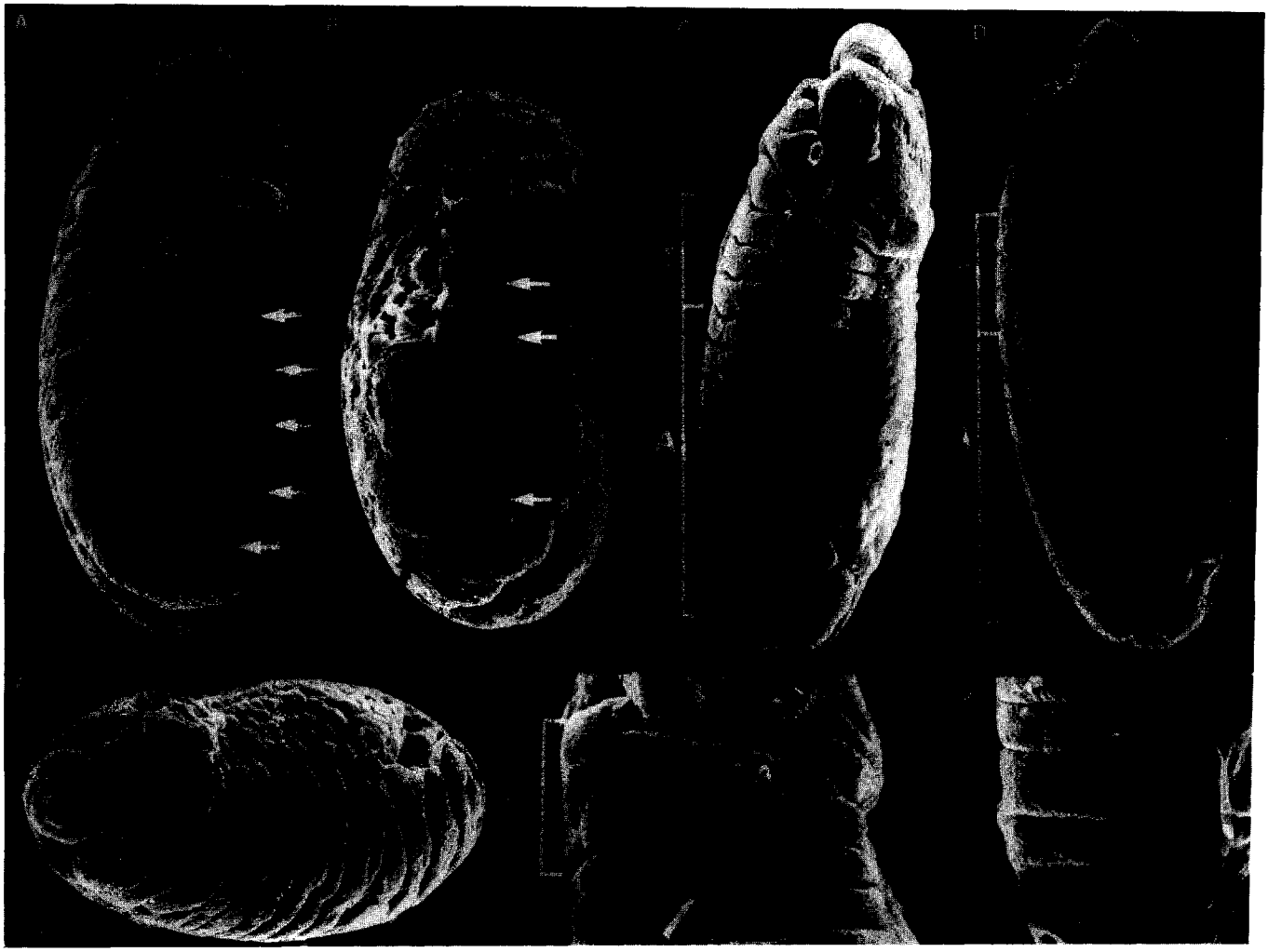

FIG. 5. Scanning electron micrographs (SEM) of *hopscotch* embryos. (A) is a 7-hr wild-type embryo showing complete germ band elongation. Tracheal pits are clearly visible (some indicated by arrows). In *hopscotch* embryos defects are first detectable at 7 hr (B, note the abnormal tracheal pits in the abdominal area, arrows). At 9 hr of development *hopscotch* embryos are missing one abdominal segment [compare wild type (C) to a *hopscotch* embryo (D); no other defects are detectable in this mutant embryo]. Abdominal defects sometimes cover parts of A4 or 5 (G, see text). Variable thoracic defects are also detectable, including partial (arrow in E) and complete (bracket in F) fusion of T1 and T2. Abbreviations: A, abdominal and T, thoracic regions.

complete (Fig. 5F) fusions of T1 and T2. Fusion of T2 and T3 is sometimes observed (result not shown). Head involution occurs normally in *hopscotch* embryos (Fig. 4). One third of the embryos examined at 9 to 10 hr show additional abdominal defects; such as localized defects associated with A4 (arrow in Fig. 7G).

Internal Structures of Hopscotch Embryos

Histological sections were prepared of embryos derived from germline clones of *hopscotch* to detect any major changes in the morphology of internal structures. No defects were detected prior to 9 hr of embryonic development (Figs. 6A and B). However, after 9 hr some *hopscotch* embryos showed localized cell death in the abdominal ganglia (Fig. 6D). At 15 hr (Fig. 6C) a large gap in the ventral ganglia is detected following shortening and condensation of the nervous system. To better identify the defects associated with the segmental ganglia, the ventral nerve cord was stained for acetylcholineesterase. As shown in Fig. 7 the ganglia corresponding to the abdominal 5 segment are missing. In some *hopscotch* embryo the A4 and A6 ganglia appear connected.

The tracheal system of *hopscotch* embryos can be visualized in whole cuticle mounts. In some *hopscotch* embryos the two dorsal lateral tracheal trunks are broken in the region corresponding to the abdominal defect. In other embryos these trunks appear connected normally.

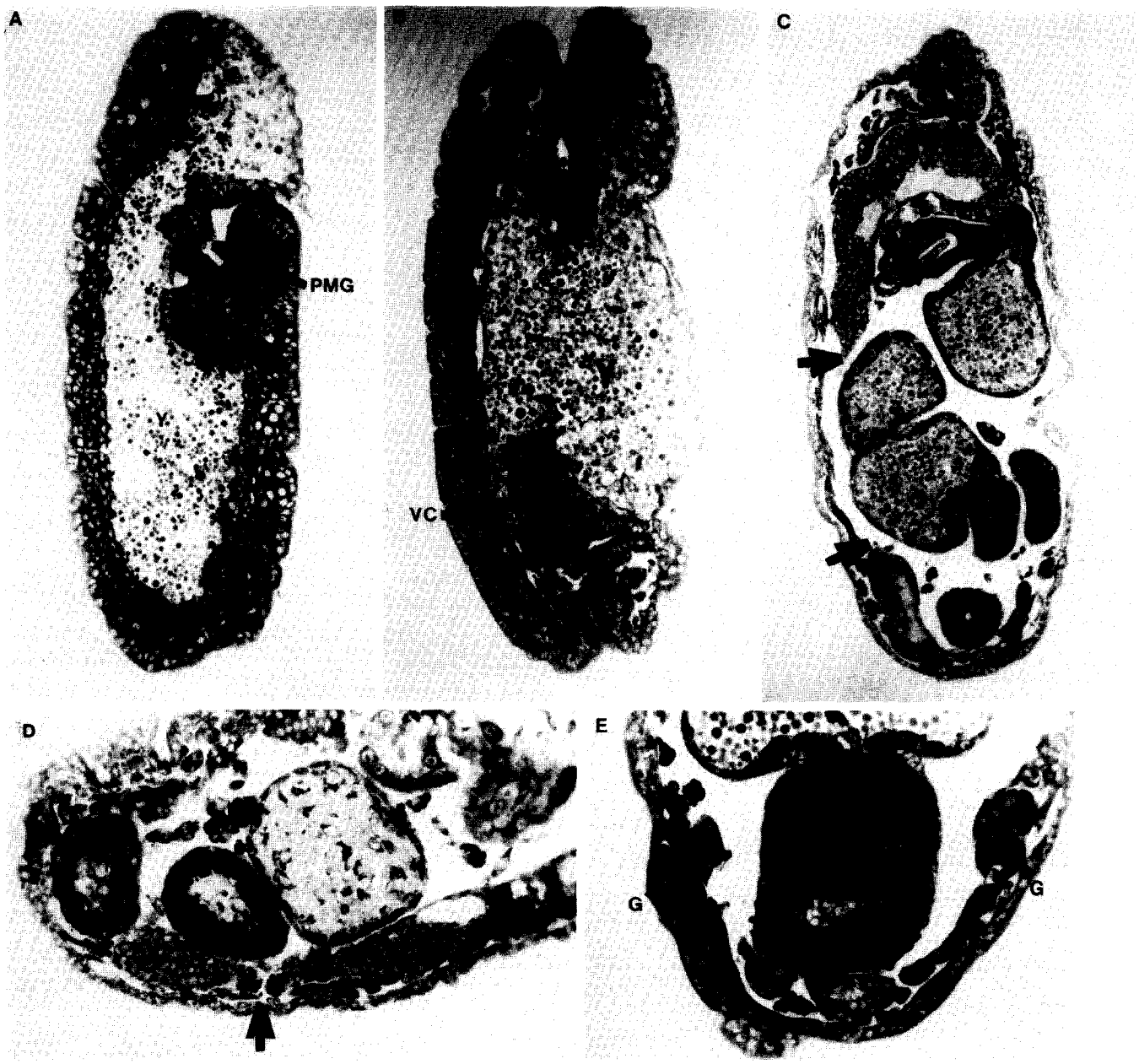

FIG. 6. Histological sections of *hopscotch* embryos. (A to C) are sagittal sections of *hopscotch* embryos at 7, 9, and 17 hr of development, respectively. No obvious morphological defects are detectable at the first two ages (A and B). However, a large gap in the nervous system is detectable at the later stage (arrows indicate anterior and posterior extent of the gap in C). Cell death in the nervous system is detectable already at 9 to 10 hr (see arrow in D). *Hopscotch* embryos possess morphologically normal gonads (E). Abbreviations: Y, yolk; PMG, posterior midgut invagination; G, gonad; VC, ventral nerve cord.

The Absence of Abdominal 5 Does not Cause Sterility

The gonad is thought to originate either from embryonic segments A4 or A5 (Lewis, 1978). Because the maternal effect of *l(1)hop* is localized to the A5 area of the embryo, it was of particular interest to determine if sectioned *hopscotch* embryos exhibited defects in the presumptive gonads. Gonads, however, were present and appeared normal (Fig. 6E).

All of the *l(1)hop*/+ adult progeny derived from germline clones show abdominal cuticle defects, but no other morphological defects are found. Externally, wild-type male and female abdomens differ in pigmentation pattern and number of segments (Ferris, 1950). Females possess seven, clearly distinguishable, abdominal segments each with a similar pigmentation pattern (Fig. 8A). The male abdomen shows six obvious segments with

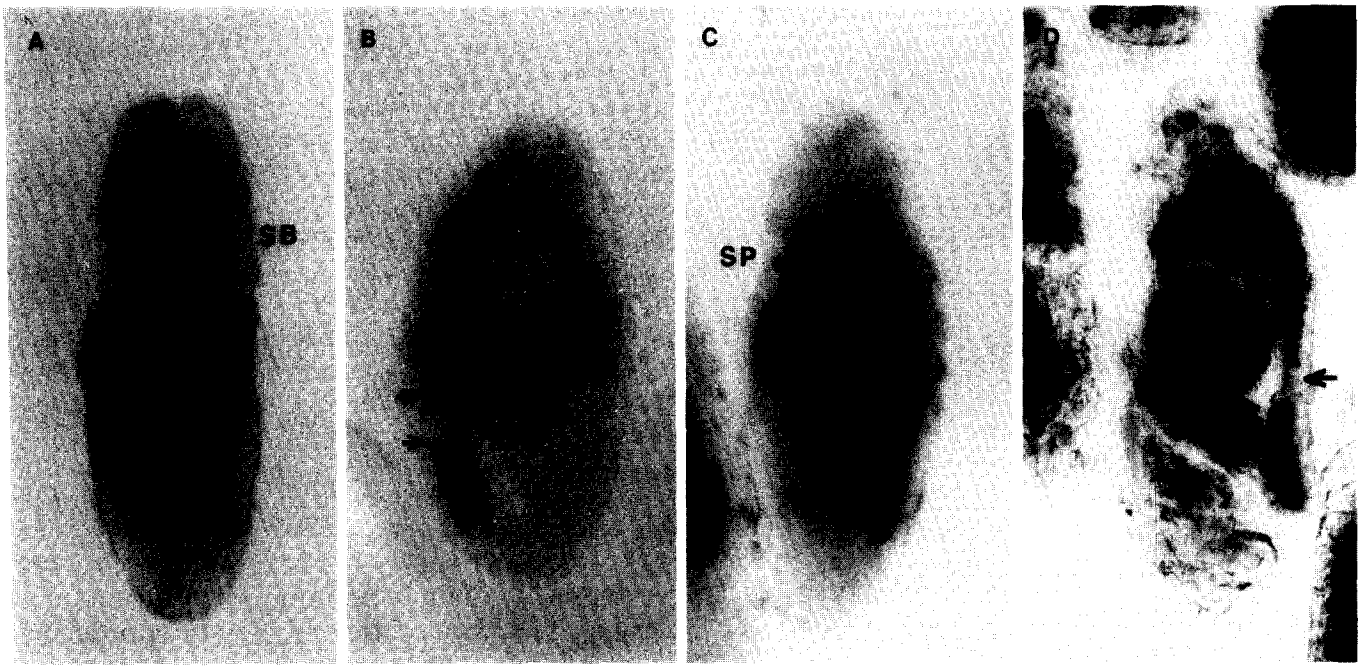

FIG. 7. Ventral cord of *hopscotch* embryos. Ventral views of wild-type (A) and *hopscotch* (B) 9- to 10-hr embryos stained for AChE. Note in (B) the three most posterior ganglia corresponding to A6, 7, and 8, and the gap between A4 and A6 (indicated by arrows). Lateral views of wild-type (C) and *hopscotch* (D) 13-hr embryos. The nervous system has condensed eliminating evidence of the segmental nature of the ventral cord; however, the gap in the mutant embryo is clearly evident. Abbreviations: supraesopheageal (Spg) and subesopheageal (Sbg).

the posterior two being darkly pigmented (Figure 8C). Adult females derived from germline clone are all missing one abdominal segment (Fig. 8B). Of these females, 60% are missing a single segment while the remaining 40% exhibit defects extending to adjacent segments (more than 200 females were examined). For example, one hemitergite is missing (this defect is usually associated with A4 (Fig. 8B). In males derived from germline clones only five segments are detectable (more than 150 males were examined) (Fig. 8D), two being darkly pigmented. In 66% of these cases only one segment is missing (Fig. 8D). Among the remaining 33% additional segment deficiencies, similar to those previously described for females, are observed. Succinodehydrogenase staining of germline clone-derived *l(1)hop/DpY* males was performed to detect the presence of the abdominal 5 male specific muscle (Lawrence and Johnston, 1984). As shown in Fig. 9 those males possess this segment specific muscle.

l(1)hop/+ female and *l(1)hop/DpY* male flies derived from *hopscotch* germline clones, are fertile in spite of the absence of one abdominal segment. For example, the fertility of more than 200 $l(1)hop^{msv1}/+$ and 150 $l(1)hop^{C111}/+$ female progeny, derived from germline clones, was examined and all were fertile. Similarly, single matings between 65 $l(1)hop^{msv1}/DpY$, 51 $l(1)hop^{C111}/DpY$, and 35 $l(1)hop^{HC257}/DpY$ germline clone-derived male progeny and two virgin females indicated that 52, 43, and 27 males were fertile, respectively. In these latter crosses, the high rate of sterility is related to poor viability of the mutant flies.

DISCUSSION

This paper describes the zygotic and maternal effect phenotypes of mutations at the *l(1)hopscotch* locus. Genetic tests indicate that the 17 *hopscotch* mutations can be classified into three groups: (1) class A amorphs (9 alleles) are indistinguishable from a deficiency of the *hopscotch* locus; (2) class B amorphs (7 alleles) complement to various extent the zygotic lethality when in *trans* with the hypomorphic allele $l(1)hop^{msv1}$; and (3) $l(1)hop^{msv1}$ a hypomorphic allele. The complementation pattern suggests that $l(1)hop^{GA32}$ and $l(1)hop^{M13}$ may represent weak alleles that can only be detected in the presence of $l(1)hop^{msv1}$. The full complementation observed between any of three alleles ($l(1)hop^{M4}$, $l(1)hop^{M38}$, $l(1)hop^{M75}$), and $l(1)hop^{msv1}$, and the antimorphic effect of $l(1)hop^{VA85}$ suggests that the *hopscotch* gene product forms part of a multimeric structure. The zygotic phenotype and the *trans*-allelic complementation tests indicate that the lack of $l(1)hop^{+}$ gene activity affects the divisions of all diploid cells. Furthermore, the adult

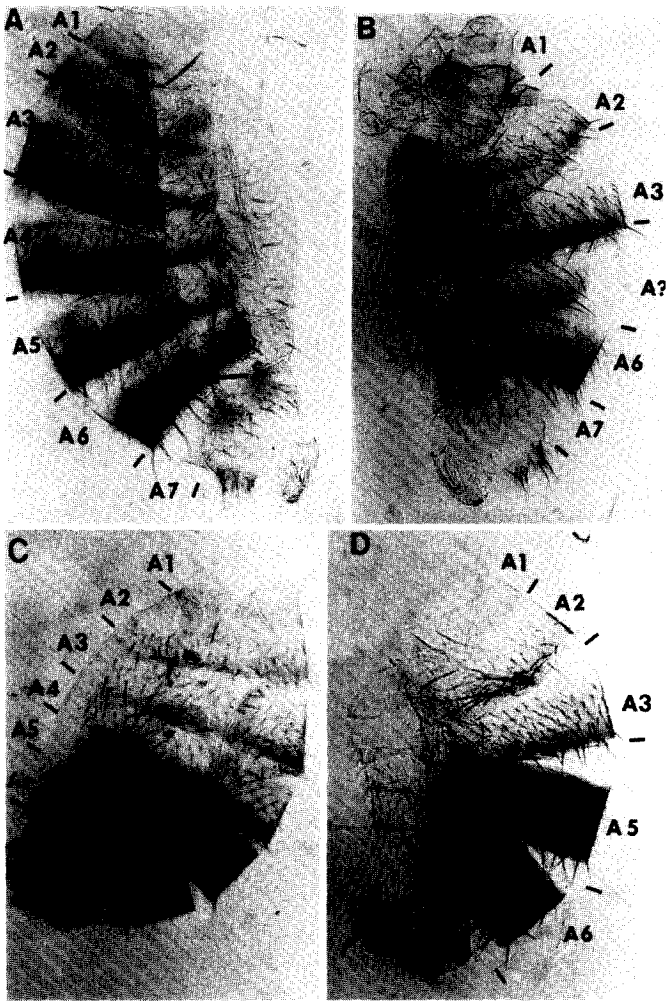

FIG. 8. Adult abdominal phenotype Cuticle phenotype of *l(1)hop/+* adult derived from a germline clone. The phenotypes of wild-type adult female (A) and male (C) flies are shown. *l(1)hop/+* females derived from germline clones are missing one abdominal segment (B). The identity of the segment in (B) between A3 and A6 is uncertain. *l(1)hop/DpY* adult males (D) derived from germline clones are missing A4. Note the presence of spermathecae (S) in females.

sterility observed in viable *hopscotch* flies homozygous for the hypomorphic allele or *trans*-heterozygous between the hypomorph and class B amorphs can be attributed to cell division defects of the follicle cells. Similarly, the male sterility phenotype of $l(1)hop^{msv1}/Y$ males (Dybas *et al.*, 1983) can be attributed to an effect on cell proliferation of non-germline-derived cells of the testis (i.e., the border cells).

The mode of action of *l(1)hop* during embryonic segmentation is complex. All 17 alleles in germline clones exhibit the same basic MEL phenotype in which three regions of the embryo appear affected. These defects are variable and sensitive to both the maternal and zygotic dosage of *hopscotch* activity. Introduction of a wild-type copy of the gene via the sperm can rescue all defects except those associated with A5. At the present time three possible modes of action of *hopscotch* gene product can be envisioned; (1) like the *torso* (Degelmann *et al.*, 1986) or *bicaudal* (Mohler and Wieschaus, 1985) loci, the wild-type hop^+ gene product may be required during oogenesis for establishing the segmental plan for the embryo; (2) like the *giant* (Petschek *et al.*, 1986) or *Krüppel* (Jäckle *et al.*, 1985) gene products, hop^+ may be required for the correct establishment of specific domains of the embryo; or (3) hop^+ may be segment specific and act in concert with homeotic loci to produce segmental identity. Further experiments, utilizing double mutant combinations (with mutations affecting segmental identity) and *in situ* hybridizations to embryos with molecular probes (e.g., Hafen *et al.*, 1984) will allow us to distinguish between these possibilities.

A puzzling observation is the presence of A5 in adult males derived from *hopscotch* germline clones. In the embryo it is clear that A5 is missing, based on analysis of the embryonic cuticle pattern and acetylcholinesterase staining of the ventral nerve cord. The presence of embryonic gonads in *hopscotch* embryos is consistant with the absence of A5, since recent observations on the phenotypes of *infra abdominal 4 (iab4)* and *iab5* mutations indicate that gonads originate from A4 (Karch *et al.*, 1985).

The progression from parasegments to segments (Martinez-Arias and Lawrence, 1985; Ingham *et al.*, 1985), provides a possible interpretation of this puzzling result. Martinez-Arias and Lawrence (1985) propose that initially parasegments form, composed of the posterior portion of one segment and the anterior portion of the adjacent segment. At the time of tracheal pit invagination, these parasegmental borders move posterior one half segment, producing the typical segmental character of the embryo (Ingham *et al.*, 1985). Our results are consistent with the absence of parasegment 10 (A4p/A5a) in the less extreme *hopscotch* embryonic phenotype. The removal of parasegment 10 results in the fusion of A4a and A5p within one segment, containing a A4 denticle belt at the anterior of the segment and the bare A5 cuticle at the posterior. Similarly, this A4a/A5p segment will contain neural and mesodermal components from two different abdominal segments. This compound segment may explain the unusual results seen both in the embryonic nervous system and in the adult abdomen. Thus, a normal embryonic gonad may form because of the presence of portions of A4 mesoderm. The presence of A4 and absence of A5 ganglia suggest that most of the ventral ganglia derive from the anterior compartments of segment. Finally, the abdominal 5-specific muscles, in rescued *l(1)hop/DpY* males derived from

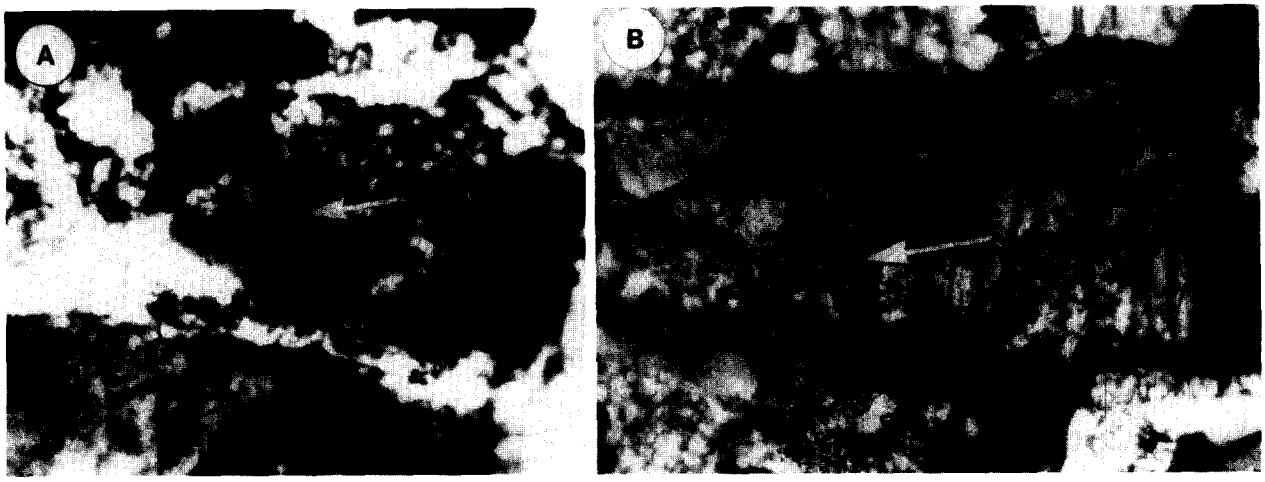

FIG. 9. Abdominal 5 male specific muscle. 70 *l(1)hop*^{msv1}/*DpY* males derived from homozygous germline clones were stained for succinodehydrogenase activity. Positive identification of the male muscle was observed in 42 cases. Although, usually they were well formed (A), occasionally they appeared underdeveloped (B).

germline clones, may actually be dependent upon the posterior portion of the 5th segment. The pattern of expression of genes such as *fushi-tarazu* (Hafen *et al.*, 1984; Degelmann *et al.*, 1986) will assist in understanding the effect of *hop* on embryonic segmentaton.

The MEL phenotype of *l(1)hop* addresses the problem of localized maternal information in the egg. Genetic data and ligation experiments support models in which maternally organized global gradients set up the coordinates and polarity of the egg. These gradients are interpreted during zygotic development. This is in contrast with the model derived from pole cell determination in which germ cell formation is under the control of localized determinants (Illmensee and Mahowald, 1974). In this paper we report the identification of a MEL phenotype which disrupts structures in two specific regions of the embryo. To date, a set of MEL loci (*fs(1)151, fs(1)1502,* torso-like loci) has been identified with localized MEL effects (see review by Mahowald and Hardy, 1985). In addition, a set of zygotic lethal loci, *l(1)polehole* (Perrimon *et al.*, 1985a), and *l(1)ultraspiracle* (Perrimon *et al.*, 1985b) display specific MEL phenotypes. These loci suggest that some maternal gene products may be (1) localized in specific regions of the cytoplasm of the egg, or, alternatively (2) widely distributed, but selectively utilized by subsets of embryonic cells. Our characterization of *l(1)hop* is another example of a zygotic lethal locus with a specific MEL phenotype. We know that many more such loci are present in the *Drosophila* genome (Perrimon, unpublished). It will certainly be important to identify and characterize more of these loci to further understand the complex maternal control of segmentation.

Another complexity found in our study of *l(1)hop* is that the maternal defect appears localized, whereas the late zygotic lethal defect has a more general effect. It is difficult to understand the relationship between these two effects if they are a consequence of the same function of one gene product. There are a number of other possible mechanisms to explain these two phenotypes. For example, it is possible that these pleiotropic effects are attributable to different functions of the same gene product. Or, differential utilization of one gene product at different devlopmental times, where the presence of different molecular environments may result in different effects. Finally, it is possible that a single product from this locus serves a regulatory function required for the modification of other gene products. Modification of a localized embryonic gene product could be responsible for the early, specific MEL phenotype. Likewise, modification of a late gene product, required in all diploid cells, could be responsible for the late, global phenotype. This last hypothesis is supported by a mutation at the *c21R* locus (Cheney *et al.*, 1984) where a thermosensitive allele, *1(3)c21R*^{RW630}, is viable and fertile at the permissive temperature (20°C) but shows a larval-pupal lethal phase at the restrictive temperature (27°C). These larvae exhibit a small disc phenotype. When females are shifted from 20 to 27°C they become progressively sterile. Utilizing two-dimensional gel electrophoresis these authors have shown that three proteins exhibit different mobilities following the temperature shift suggesting that the wild type *c21R* gene product codes for a protein-modifying enzyme. Finally, it is possible that the pleiotropic effects of *hopscotch* are associated with the presence of multiple transcripts showing stage specificity (cf. *Ultra-*

bithorax, Bender *et al.*, 1983). Future molecular characterization of *hopscotch* should clarify its mode of action.

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