GENETIC CHARACTERIZATION OF THE 87C REGION OF THE THIRD CHROMOSOME OF *DROSOPHILA MELANOGASTER**

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ABSTRACT

Ethyl methanesulphonate (EMS) was used to induce 39 lethal and 13 karmoisin mutations within *Df(3R)kar*^{3J}, a nine-band deficiency extending from 87C1 to 87C9 (inclusive). Five complementation groups (four lethal and one visible) were identified and cytologically mapped between 87C4–5 and 87C9, one complementation group per band, with the exception of complementation group A, which is localized to 87C4–5. These positions were determined using a set of overlapping deficiencies, each having at least one breakpoint in the 87C1–9 region. Mutations within a single complementation group have similar lethal phases or subvital phenotypes, consistent with the notion that each complementation group represents a single functional locus. No mutations localized to 87C1–C3. The inability to induce mutations in the 87C1 heat-shock puff locus is consistent with the current interpretation of a duplication of coding sequences at the 87A7 and 87C1 heat-shock puffs.

IN recent years, much attention has been directed towards elucidating the molecular organization and mechanisms of regulation of genes in higher eukaryotes. *Drosophila melanogaster* has proved to be a popular organism for investigating gene organization. The bands of the polytene salivary gland chromosomes have long been thought to reflect individual genetic units, and this speculation has been put on a firm basis by JUDD and co-workers who have shown that, for a small region of the *X* chromosome, each genetic complementation group can be mapped to single band (JUDD, CHEN and KAUFMAN 1972). Though exceptions have been found to this generalization, it seems likely that, in most cases, the correspondence between bands and complementation groups will hold (LEFEVRE 1974).

The relationship between the structural and genetic organization of the chromosome is, however, far from clear. This is apparent from the discrepancy between the estimates of "gene number" based upon the haploid DNA content of Drosophila, on the one hand, and genetic methods on the other.

In order to understand why Drosophila requires so much DNA and how this

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DNA is organized and expressed, a combination of detailed genetic and biochemical analyses of a region of the Drosophila genome is required. The interval from 87A to 87C on the third chromosome is well-suited to such a study as it includes two loci (87A7 and 87C1) whose activities can be induced by heat shock to form large "puffs" in polytene chromosomes (RITOSSA 1963; ASHBURNER 1970). It was suggested (TISSIÈRES, MITCHELL and TRACY 1974; MCKENZIE, HENIKOF and MESELSON 1975) that the 87C1 puff codes for the major 70,000d heat-shock-induced protein (hsp 70), and our initial aim was to identify the corresponding locus by genetic techniques. This was to be achieved by a genetic saturation analysis of a small region of chromosome *3R* including 87C1.

The results of this study are given in this paper. Although lethal and visible complementation groups can be assigned to each band in the 87C4–5 to 87C9 interval, no mutations mapped to the bands 87C1 to C3. In retrospect, this result is not surprising (at least with respect to 87C1) since, while this study was in progress, ISH-HOROWICZ, HOLDEN and GEHRING (1977) and ISH-HOROWICZ *et al.* (1979) have shown that the 87C1 hps 70-coding sequences are duplicated at 87A7.

MATERIALS AND METHODS

Drosophila cultures and stocks: Drosophila cultures were maintained on a standard yeast, cornmeal, sucrose and agar medium.

The standard mutant and balancer stocks used are described by LINDSLEY and GRELL (1968) and were obtained from the Drosophila Stock Center, California Institute of Technology, Pasadena. *In(3LR)TM3, ri p^p sep bx^{34e} e^s Sb Ser* is abbreviated as *TM3* throughout this paper. *Df(3R)kar^{D3}* was obtained from F. RITOSSA, and *l(3)m107*, *l(3)c4g*, *l(3)m114* and *Dp(2;3)ry+^{w70h}* were provided by W. GELBART.

The sources and cytological extents of the deficiencies used are summarized in Table 1.

TABLE 1

Cytogenetic data on the deficiencies used in these studies

Stock	Breakpoints Proximal	Distal	Visible loci included	Source
Df(3R)kar^{3J}	87B15–C1	87C9–D1,2	*kar*	ISH-HOROWICZ *et al.* (1977)
Df(3R)kar^{3Q}	87B2–4	87C9–D3,4	*kar*	ISH-HOROWICZ *et al.* (1977)
Df(3R)kar^{3l}	87C2,3	87D3,4	*kar*	SCHALET
Df(3R)kar^{D3}	86E16–18	87D3,4	*kar*	SCALENGHE & RITOSSA (1976)
Df(3R)T-10	86F2–4	87C6–7	—	ISH-HOROWICZ (unpublished)
Df(3R)T-32	86E2–4	87C6–7	—	ISH-HOROWICZ (unpublished)
Df(3R)kar^{Sz11}	87C7–8	87E5–6	*kar, ry*	GAUSZ *et al.*
Df(3R)kar^{Sz12}	87B1–2	87C8–9	*kar*	GAUSZ *et al.*
Df(3R)kar^{Sz21}	87C7	87C8–9	*kar*	GAUSZ *et al.*
Df(3R)kar^{Sz27}	87C7	87F1	*kar, ry*	GAUSZ *et al.*
Df(3R)kar^{Sz29}	87C3–4	87C9–D1,2	*kar*	GAUSZ *et al.*
Df(3R)kar^{Sz31}	87C6–7	87C9–D3,4	*kar*	GAUSZ *et al.*
Df(3R)kar^{Sz37}	87C5–6	87D14–E1	*kar, ry*	GAUSZ *et al.*

The isolation procedure of deficiencies will be given elsewhere (GAUSZ *et al.*, in preparation). Only one band is given if the breakpoint is at that band, two bands are named if the breakpoint is between the given bands, three bands indicate the limits of the breakpoints.

The isolation of deficiencies with breakpoints within 87C1–C9 will be included in a separate report (GAUSZ *et al.* in preparation).

Isolation of recessive lethal and visible mutations in the 87C1 to 87C9 region: Males (aged 24 to 48 hr), homozygous for *mwh e*, were fed 0.025 M ethyl methanesulphonate (EMS) for 24 hr according to the method of LEWIS and BACHER (1968), and mated *en masse* to *CxD/TM3* virgin females. F_1 *mwh e/CxD* males were collected and individually mated in vials to three *Df(3R)kar^{3J}/CxD* virgin females at 24° (Figure 1). Vials were inspected for the presence of non-*CxD* progeny (*i.e.*, *mwh e/Df(3R)kar^{3J}*). Any vial showing either a reduced number or complete absence of straight-winged progeny was retained as having a putative semilethal or lethal mutation in the 87C1–87C9 region. All straight-winged flies were also examined for obvious morphological or behavioral anomalies. Unfortunately, since the lethal/*CxD* males could not be distinguished from *Df(3R)kar^{3J}/CxD* males, ten *CxD*-bearing males from each vial were individually mated to *Df(3R)kar^{3J}/TM3* virgins and the lethal/*Df(3R)kar^{3J}* progeny rechecked. The lethal/*TM3* (ebony phenotype) flies were then used to establish stocks.

Three precautions were taken to ensure that the newly induced mutations were, in fact, within the 87C1–87C9 region. First, recombination of the *Df(3R)kar^{3J}* chromosome with a multiply marked, lethal-free third chromosome (*ru h th st cu sr e^{s} ca*) led to the replacement of most of the original *Df(3R)kar^{3J}*-bearing chromosome, eliminating most, if not all, other recessive lethal mutations that may have resulted from the original irradiation or had arisen subsequently (ISH-HOROWICZ, HOLDEN and GEHRING 1977). The *Df(3R)kar^{3J}* chromosome used in the isolation procedure was *ru h th st Df (3R)kar^{3J} sr e^{s} ca*. Second, all newly induced mutations were regarded as putative until they were shown to lie within the 87C1–87C9 region by expression of the lethal or visible phenotypes in *trans*-heterozygotes with other independently isolated deficiencies that span the 87C1–87C9 region, but extend further to the right or left of these bands. The final precaution was that all mutations should show pseudo-dominance with at least one of the deficiencies having one or two breakpoints within the 87C1–87C9 region. All mutations discussed in this report fulfill the above requirements and are thus considered to be within this short region of the third chromosome.

In a second screen, mutations in the region 86E/F to 87D1–D2, were isolated from mutagenized wild-type males (Oregon-R-369) using *Df(3R)kar^{D3}* (SCALENGHE and RITOSSA 1976). Some of the mutations obtained proved to be lethal with *Df(3R)kar^{3J}* and are included in this report. The screening procedure will be presented elsewhere.

Three point mutations, *l(3)m107*, *l(3)c4g* and *l(3)m117*, were presumed to be in the region of interest (DELAND 1971) and have been included in our studies.

Genetic complementation: Reciprocal pairwise complementation tests were made for all mutations that proved to be within the 87C1–87C9 region. For mutations isolated in the first

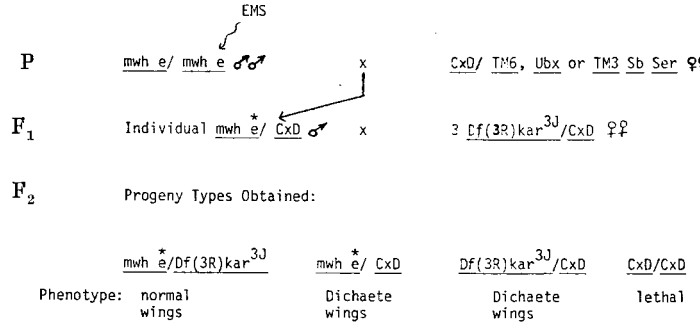

FIGURE 1.—The selection method for visible and lethal mutations within 87C1 to 9. Visible or obvious behavioral mutations are expressed in the *mwh e/Df(3R)kar^{3J}* progeny. The absence of such normal-winged progeny in the F_2 indicates the presence of a recessive lethal in the region deleted by *Df(3R)kar^{3J}*.

experiment, replicates of each cross (using single males and three virgin females) were maintained at 18°, 25° and 29° in order to test for temperature sensitivity of the *trans* heterozygotes. Since few of these crosses indicated temperature sensitivity of the *trans* heterozygotes, the *inter se* crosses for mutations isolated in the second screen were carried out only at 25°. Between 50 and 300 progeny were obtained for each cross. These were examined for viability and phenotypic abnormalities, and the fertility of the female *trans* heterozygotes was determined in the *SzA* and *SzD* complementation groups.

As a control, all the mutations were simultaneously tested with *Df(3R)kar^{3J}*.

Determination of the effective lethal phases: From each complementation group, representative alleles [those exhibiting complete lethality with *Df(3R)kar^{3J}*] were selected for lethal phase (LP) determinations. Males from each stock were mated to *Df(3R)kar^{3J}/TM3* virgin females for three days, after which time eggs were collected at two-hour intervals on small Petri dishes containing agar and placed at 25°. The hatched larvae were counted 24 to 48 hr later, and the percentage egg viability was calculated.

For determinations of larval and pupal lethality, eggs were collected as described above and, in groups of 50, were placed in vials containing standard medium. After eclosion, adults were counted, and the medium was removed and washed to determine the number of lethal larvae and pupae (including both prepupae and pharate adults). Lethal pupae were dissected to determine the genotype (if sufficiently developed) and the stage of lethality.

Controls for both LP studies were progeny from the cross *cu kar e^{s}/TM3* males mated to *Df(3R)kar^{3J}/TM3* virgin females.

Determination of the complementation group affecting the morphology of the Malpighian tubules: Embryos and first-instar larvae homozygous for *Df(3R)kar^{3J}* have transparent Malpighian tubules, whereas those of the wild-type are opaque (ISH-HOROWICZ, HOLDEN and GEHRING 1977). In order to determine whether this phenotype is uniquely associated with alleles from one of the complementation groups, eggs were collected on yeasted agar plates as for the LP determinations. The first-instar larvae were continuously collected after hatching, mounted in viscous fluorocarbon oil (VOLTALEF 3S, PLASTIMER, PARIS) and examined under the dissecting microscope for the Malpighian tubule defect. After all larvae had hatched, the unhatched eggs were examined similarly. The percentage of embryos and first-instar larvae having defective Malpighian tubules was then calculated.

Transplantation studies and determination of the autonomous nature of the Malpighian tubule defect: In order to test for autonomy of the Malpighian tubule defect, newly hatched, homozygous first-instar larvae were selected from two different mutant stocks [*l(3)SzA^{5}* and *l(3)SzA^{7}*], and the posterior halves were injected into the abdomens of wild-type females. The transplantation was carried out by the method of EPHRUSSI and BEADLE (1936). After nine days, the implants were recovered and analyzed morphologically. As controls *l(3)SzA^{5}/TM3* and *l(3)SzA^{7}/TM3* posterior halves of first-instar larvae were injected and analyzed similarly.

Salivary gland chromosome squash preparations: Larval salivary gland chromosome preparations were made to determine (1) the cytological extents of the various deficiencies used, (2) whether chromosomal abnormalities were associated with any of the EMS-induced mutations, and (3) whether there was any effect of the EMS-induced mutations or puffing at either 87A or 87C1 after heat shock. For (1) and (2), males from each stock were mated to wild-type virgin females, and for (3), the males were mated to virgin females of the genotype *In(3R)Na/TM3*. Prior to dissection, larvae from the latter cross were exposed to 37.5° for twenty min to induce the heat-shock puffs. *In(3R)Na* has one breakpoint proximal to the 87A puff (in 86F), while the distal breakpoint is at 96F11–97A5 (LINDSLEY and GRELL 1968). Thus, heat-shock puffs are displaced distally and, in heterozygotes with a mutant chromosome, the 87A and 87C1 puff can be examined simultaneously in both the mutant and the control *In(3R)Na* chromosome. Heat-shocks were also performed on lethal/+ larvae, and the chromosomes in which asynapsis occurred at the 87A and 87C region were examined for differences in chromosome morphology and degrees of puffing.

Salivary gland chromosome preparations were made as described by HOLDEN and ASHBURNER (1978).

Deletion mapping: A set of overlapping deletions with breakpoints in the 87C1–87C9 region were used to order the specific complementation groups and assign them to particular bands and/or interbands. The deletions used are listed in Table 1 and Figure 2.

RESULTS

Isolation of recessive lethal and visible mutations in the 87C1–87C9 region: In two separate screens for recessive lethal and visible mutations in the region 87C1–87C9, a total of 39 lethal and 13 karmoisin mutations were recovered. Using the smaller deficiency [*Df(3R)kar*^{3J}], 5726 chromosomes were screened and 20 lethal (0.350%) and 5 *kar* (0.087%) mutations were obtained. In the second experiment, using the larger deficiency [*Df(3R)kar*^{D3}], 19 lethal

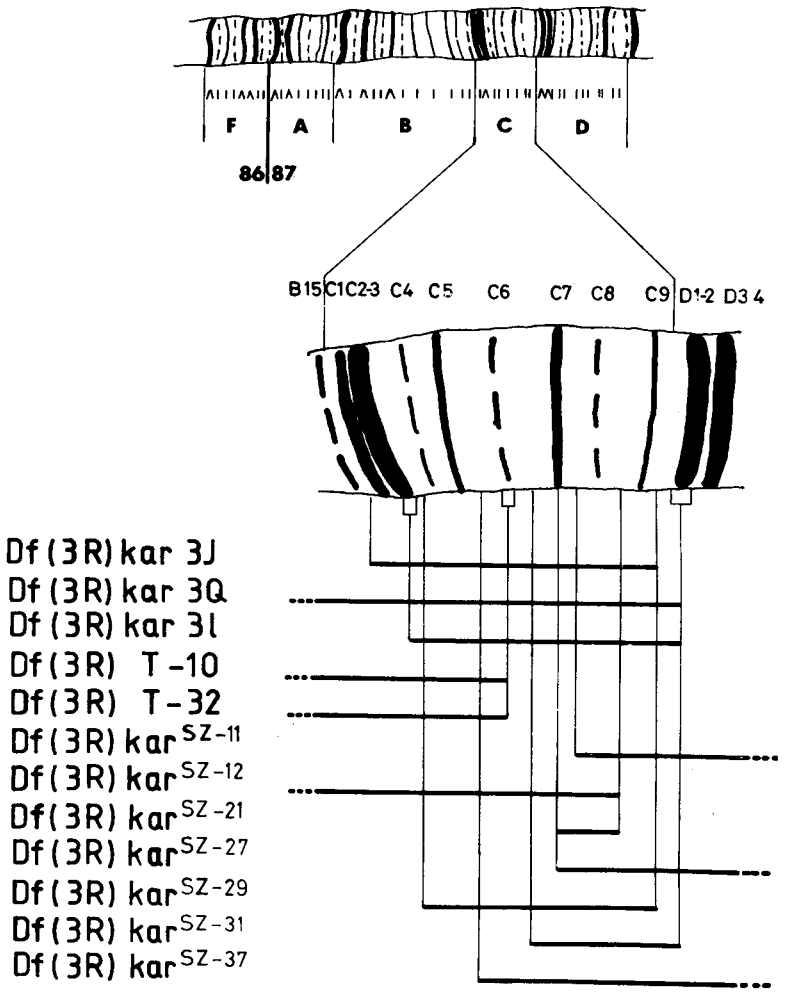

FIGURE 2.—Cytogenetic representation of the breakpoints within 87C of the deficiencies used in this study (listed in Table 1), based on the salivary chromosome map of BRIDGES (1935).

(0.229%) (within the 87C1–87C9 region) and eight *kar* (0.096%) mutations were recovered from 8303 chromosomes tested.

Determination of the complementation groups for the 87C1–87C9 mutations: The only fully penetrant, visible mutation (in the 87C1–87C9 region) recovered in these studies was karmoisin (*kar*). All 13 newly induced *kar* mutations proved to be allelic to the standard kar^1 and kar^2 alleles (kindly provided by W. GELBART). Although some of the newly recovered *kar* mutations are homozygous lethal, this lethality is due to recessive lethal mutations present elsewhere on the mutation-bearing chromosome since all *kar* alleles are completely viable with $Df(3R)kar^{3J}$ (as well as with other deficiencies in this region, listed in Table 1). In addition, all combinations of the eight different *kar* alleles isolated in the first screen are viable and have a wild-type eye color when heterozygous for other lethal mutations in the 87C1–87C9 region. On the basis of these results, none of the lethal mutations were small deficiencies extending into the *kar* locus, confirming cytological analysis of the mutations (see below).

The 42 recessive lethal mutations (including the three provided by W. GELBART) fall into four discrete complementation groups [*l(3)SzA*: 14 alleles; *l(3)SzB*: 6 alleles; *l(3)SzC*: 6 alleles; and *l(3)SzD*: 16 alleles] (Table 2). Mutations in Groups *SzB* and *SzC* are completely lethal with $Df(3R)kar^{3J}$, as homozygotes, and as *trans* heterozygotes with other members of the same complementation group, but are completely viable in combination with members of different complementation groups.

The complementation patterns for alleles in Groups *SzA* and *SzD* are far more

TABLE 2

Complementation groups in the 87C1 to 87C region

	Complementation groups				
	SzA	*SzB*	*SzC*	*kar*	*SzD*
Exp. 1	$l(3)SzA^1$		$l(3)SzC^1$	kar^{Sz1}	$l(3)SzD^1$
	$l(3)SzA^2$		$l(3)SzC^2$	kar^{Sz2}	$l(3)SzD^2$
	$l(3)SzA^{3*}$		$l(3)SzC^3$	kar^{Sz3}	$l(3)SzD^3$
	$l(3)SzA^4$			kar^{Sz4}	$l(3)SzD^4$
	$l(3)SzA^5$			kar^{Sz5}	$l(3)SzD^5$
	$l(3)SzA^6$				$l(3)SzD^6$
	$l(3)SzA^7$				$l(3)SzD^7$
	$l(3)SzA^8$				$l(3)SzD^8$
	$l(3)SzA^9$				
Exp. 2	$l(3)SzA^{10*}$	$l(3)SzB^1$	$l(3)SzC^4$	kar^{Sz6}	$l(3)SzD^9$
	$l(3)SzA^{11}$	$l(3)SzB^2$	$l(3)SzC^5$	kar^{Sz7}	$l(3)SzD^{10*}$
	$l(3)SzA^{12}$	$l(3)SzB^3$		kar^{Sz8}	$l(3)SzD^{11}$
	$l(3)SzA^{13}$	$l(3)SzB^4$		kar^{Sz9}	$l(3)SzD^{12}$
		$l(3)SzB^5$		kar^{Sz10}	$l(3)SzD^{13*}$
		$l(3)SzB^6$		kar^{Sz11}	$l(3)SzD^{14}$
				kar^{Sz12}	$l(3)SzD^{15*}$
				kar^{Sz13}	
Gelbart's mutants	*l(3)m107*		*l(3)c4g*		*l(3)m114*

* = alleles isolated as subvitals over $Df(3R)kar^{3J}$.

TABLE 3

Complementation matrix for mutations in complementation group SzA

Mutation	*SzA*[3]	*SzA*[10]	*SzA*[8]	*SzA*[11]	*SzA*[13]	*SzA*[1]	*SzA*[4]	*SzA*[9]	*SzA*[5]	*SzA*[2]	*SzA*[7]	*SzA*[12]	*SzA*[6]	*Df(3R)kar*[3J]
l(3)SzA[3]	—													
l(3)SzA[10]	+	—												
l(3)SzA[8]	4/136[a]	+[A]	—											
l(3)SzA[11]	+	10/91	+[A]	—										
l(3)SzA[13]	+[a]	12/119[A]	1/66	4/80[A]	—									
l(3)SzA[1]	+[a]	3/138[A]	5/88[A]	0/96	0/72	—								
l(3)SzA[4]	9/99[a]	5/110[SA]	0/145	0/121	0/74	0/142	—							
l(3)SzA[9]	8/99[a]	0/64	2/158[A]	0/78	0/73	0/134	0/141	—						
l(3)SzA[5]	1/90[a]	0/79	0/103	0/133	0/72	0/167	0/109	9/106[A*]	—					
l(3)SzA[2]	12/128[a]	0/283	0/108	0/83	0/104	0/145	0/164	0/90	0/91	—				
l(3)SzA[7]	0/88	0/106	0/129	0/120	0/79	0/186	0/110	0/95	0/198	0/139	—			
l(3)SzA[12]	0/81	0/100	0/64	0/104	0/77	0/78	0/64	0/121	0/112	0/86	0/74	—		
l(3)SzA[6]	0/191	0/75	0/85	0/109	0/93	0/130	0/134	0/105	0/129	0/137	0/118	0/106	—	
Df(3R)kar[3J]	16/242[a]	7/104[A]	0/95†	2/226[A]	0/133	0/88	0/120	0/131	0/116	0/95	0/132	0/100	0/104	—

The table shows number of heterozygotes/total number of flies. +: >50% expected yield of heterozygotes. (A): complementing *trans* heterozygotes with the "severe" phenotype. (a): *trans* heterozygotes with the "mild" phenotype. (S): female sterile. (*): failure to complement at 29° (0/77). (†): reduced number of *trans* heterozygotes at 29° (13/142) all of which have the "severe" phenotype (detailed description of the "mild" and "severe" phenotype is included in the text).

TABLE 4

Complementation matrix for mutations in complementation group SzD

Mutation	*SzD*^{13}	*SzD*^{15}	*SzD*^{10}	*SzD*^{9}	*SzD*^{14}	*SzD*^{1}	*SzD*^{4}	*SzD*^{8}	*SzD*^{3}	*SzD*^{7}	*SzD*^{2}	*SzD*^{6}	*SzD*^{11}	*SzD*^{5}	*SzD*^{12}	*Df(3R)kar*^{3J}
l(3)SzD^{13}	—															
l(3)SzD^{15}	+	—														
l(3)SzD^{10}	+	+	—													
l(3)SzD^{9}	+	+	+	—												
l(3)SzD^{14}	+	+	+^{SD}	6/123^{SD}	—											
l(3)SzD^{1}	+	+	+	7/88^{SD}	+	—										
l(3)SzD^{4}	+	+	+	+^{d}	+	+^{d}	—									
l(3)SzD^{8}	+	+	+^{Sd}	+^{S}	+	+^{d*}	0/113	—								
l(3)SzD^{3}	+	+	+	3/66^{SD}	0/101	0/139	0/80	0/169	—							
l(3)SzD^{7}	+	+	+	0/83	0/126	0/62	0/78	0/129	0/97	—						
l(3)SzD^{2}	+	+^{Sd}	+^{SD}	0/115	0/172	0/181	0/103	0/125	0/236	0/108	—					
l(3)SzD^{6}	+	+^{Sd}	+^{SD}	0/112	0/133	0/134	0/124	0/112	0/140	0/132	0/88	—				
l(3)SzD^{11}	+	+^{Sd}	+^{SD}	0/153	0/78	0/122	0/104	0/69	0/71	0/81	0/74	0/89	—			
l(3)SzD^{5}	+^{S}	+^{SD}	+^{SD}	0/112	0/150	0/135	0/155	0/285	0/117	0/121	0/94	0/98	0/76	—		
l(3)SzD^{12}	+^{S}	+^{SD}	+^{SD}	0/171	0/157	0/78	0/113	0/168	0/60	0/89	0/97	0/102	0/109	0/55	—	
Df(3R)kar^{3J}	+^{Sd}	+^{SD}	+^{SD}	0/252	0/241	0/155	0/129	0/155	0/164	0/159	0/105	0/128	0/226	0/145	0/190	—

The table is labelled similarly to Table 3, with the following differences: (d): "mild" phenotype of the *trans* heterozygotes. (D): "severe" phenotype of the *trans* heterozygotes. (*): fails to complement at 18° (0/62).

complex (Tables 3 and 4, respectively). In both of these complementation groups, there are both subvital and completely lethal alleles. *Trans* heterozygotes for two lethal alleles are generally lethal, but there are some combinations that are viable. In viable combinations, the *trans* heterozygotes are either phenotypically abnormal, female-sterile, or show both these characteristics. The surviving *trans* heterozygotes in the *SzA* and *SzD* groups have either a "severe" (designated by A and D, respectively, in Tables 3 and 4) or a somewhat "milder" (designated by a and d, respectively, in Tables 3 and 4) phenotype.

The characteristic phenotypes of *SzA trans* heterozygotes may be summarized as follows. The "A" or "severe" phenotype individuals have reduced or deformed wings, the adult cuticle is softer and more darkly pigmented than the wild type, the thorax is often slightly depressed and the flies die soon after eclosion. The "a" or "milder" phenotype is characterized by a slightly defective wing in which the distal part of the wing blade is bent upwards and slightly inwards. In the *SzD* complementation group, the "D" or "severe" phenotype is marked by wings diverging and being bent downwards, the posterior crossveins are absent, the scutellar bristles are reduced in size and there is incomplete abdominal segmentation. The "d" or "milder" phenotype is expressed incompletely, with the wings only slightly diverged.

In combination with $Df(3R)kar^{3J}$, the *SzA* subvital alleles have either the "A" phenotype (for example, $l(3)SzA^{10}$ and $l(3)SzA^{11}$ or the "a" phenotype, for example, $l(3)SzA^{8}$). Some of the subvital alleles are completely lethal with some of the lethal *SzA* alleles. For example, the subvital alleles $l(3)SzA^{10}$ and $l(3)SzA^{11}$ are completely lethal with the lethal alleles $l(3)SzA^{7}$ and $l(3)SzA^{12}$. For only two combinations of *SzA* alleles was there any indication of a temperature-sensitive effect on the viability of the *trans* heterozygotes. The $l(3)SzA^{5}/l(3)SzA^{9}$ individuals survive at 25° but not at 29°, and the $l(3)SzA^{8}/Df(3R)kar^{3J}$ individuals are lethal at 25°, but some survive at 29° (all of which have the "A" phenotype). In addition, animals heterozygous for either $l(3)SzA^{8}$ or *l(3)m107* and different third chromosomes tested (TM3 and MKRS) often have roof-like wings, indicating that these two Group *A* alleles have a weakly dominant phenotype.

The complementation map, based on the lethality of *l(3)SzA* alleles, is complex; some alleles (SzA^{6}, A^{7}, A^{12}) are completely noncomplementing, while SzA^{8} complements all alleles except SzA^{6}, A^{7} and A^{12}. The complementation results with SzA^{5}, A^{9} and A^{10} are such that a linear, or even a simple circular, map cannot be drawn.

The viable combinations of *SzD* subvital alleles with $Df(3R)kar^{3J}$ show either the "d" or the "D" phenotype and are female sterile. In contrast to the *SzA* subvital alleles, the *SzD* subvital alleles are never lethal with other *SzD* lethal alleles. However, certain combinations of lethal alleles produce viable progeny that are wild type in appearance, whereas other combinations have either the "d" or "D" phenotype. The only temperature-sensitive combination of Group *D* alleles was $l(3)SzD^{1}/l(3)SzD^{8}$, which survives at both 18° and 25°, but is lethal at 29°. A complementation map for *SzD* alleles is shown in Figure 3.

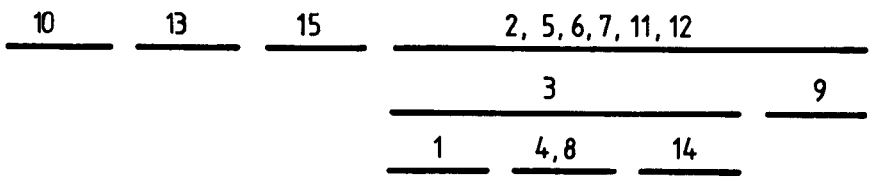

FIGURE 3.—Lethal complementation map of the *l(3)SzD* alleles.

Lethal phase (LP) determinations for the 87C1–87C9 mutations: From each complementation group, representative alleles [exhibiting complete lethality in combination with $Df(3R)kar^{3J}$ as well as with other lethal alleles from the same complementation group] were selected for LP determinations. Since the presence of other recessive lethal mutations outside the 87C1–87C9 region would interfere with the determination of the LP arising from a mutation within this region, males from each lethal stock were crossed to $Df(3R)kar^{3J}/TM3$ virgin females, and the viability of the lethal/$Df(3R)kar^{3J}$ embryos, larvae, pupae, and adults was calculated.

The results are summarized in Table 5 and indicate that, whereas the LPs for mutations in different complementation groups vary considerably, the LPs for different alleles from the same complementation group are generally very similar. Members of Group *SzB* are lethal during the prepupal stage; puparium formation is normal but head eversion never occurs. *l(3)SzC* alleles are larval lethals.

TABLE 5

Determination of the lethal phases of mutations in the 87C1 to 87C9 region

Compl. group	Allele	Embryonic viability: No. eggs laid	Embryonic viability: Percent lethal embryos	Postembryonic viability: No. eggs laid	Postembryonic viability: Percent adults closed	Postembryonic viability: Percent lethal pupae	Lethal phase
SzA	$l(3)SzA^2$	542	32.1	450	37.3	0	mainly larval
	$l(3)SzA^5$	344	40.9	400	44.8	0	egg-larval
	$l(3)SzA^7$	276	46.9	500	43.8	0	mainly egg
SzB	$l(3)SzB^2$	248	32.0	650	39.8	24.4	pupal
	$l(3)SzB^5$	317	33.4	400	41.3	21.8	pupal
SzC	$l(3)SzC^2$	101	22.7	400	42.3	0	larval
	$l(3)SzC^3$	305	29.5	350	40.8	0	larval
SzD	$l(3)SzD^6$	127	28.3	282	45.0	0	larval
	$l(3)SzD^7$	273	23.8	250	51.2	0	larval
	$l(3)SzD^8$	467	26.1	300	40.6	11.0	larval-pupal
Control	*cu kar* $e^8/TM3$	376	26.6	580	71.2	2.3	viable

All the tested alleles were crossed to $Df(3R)kar^{3J}/TM3$ virgins. Separate eggs collections were made for the determination of embryonic and postembryonic lethal phases except for A^2, A^5 and A^7. From all the crosses, 25% of the expected progeny are missing because *TM3/TM3* is an early embryonic lethal.

The LPs of *SzA* and *SzD* alleles tested are somewhat more variable than those for the *SzB* and *SzC* groups. One strong *SzA* allele [*l(3)SzA*^{7}] is predominantly lethal during the egg stage. This is surprising since homozygotes lacking the 87C1–87C9 region (*i.e.*, homozygous *Df(3R)kar*^{sJ}) die during the first larval instar (ISH-HOROWICZ, HOLDEN and GEHRING 1977). These results indicate that certain defective *SzA* gene products may be more detrimental than the loss of the product.

Two of the *SzD* alleles were shown to be larval lethals, while one allele has a polyphasic LP. For both the Group *SzA* and Group *SzD* alleles, considerable variability in the LPs is expected since some of the alleles are subvital, suggesting that some alleles have suffered only partial loss of the wild-type function.

Determination of the complementation group affecting Malpighian tubule morphology: The Malpighian tubules of wild-type embryos are opaque, tubular structures. ISH-HOROWICZ, HOLDEN and GEHRING (1977) reported that homozygous *Df(3R)kar*^{sJ} embryos and first-instar larvae have abnormal, transparent, Malpighian tubules. Since this phenotype was observed in 25% of the embryos in stock of other independently isolated deficiencies lacking the 87C1–87C9 region, it was reasonable to attribute the defective Malpighian tubule phenotype to a locus within the deleted region, rather than to a second mutation located elsewhere in the genome. Selected representatives of the five complementation groups were therefore examined for the presence of abnormal Malpighian tubules in embryos and first-instar larvae. In order to avoid homozygosis of undetected lethal mutations outside of the 87C1–C9 region (which may interfere with the expression of the mutation under study) and to determine specifically the locus that results in the formation of these defective organs, embryos and first-instar larvae of the genotype lethal/*Df(3R)kar*^{sJ} were examined.

The results indicate that only *SzA* alleles have transparent Malpighian tubules (Table 6), although not all alleles tested exhibit the same penetrance. For four of the mutations tested [*i.e.*,*l(3)SzA*^{2}, *A*^{4}, *A*^{5} and *A*^{7}], approximately 25% of all embryos from the cross had transparent Malpighian tubules (indicating 100% penetrance of the phenotype), whereas only a quarter of the *l(3)SzA*^{s}/*Df(3R)kar*^{sJ} embryos appeared mutant in this respect. Two other *SzA* alleles [*l(3)SzA*^{10} and *l(3)SzA*^{11}] showed an even lower penetrance and lower expressivity.

We also mapped the phenotype, using the deficiences. Thus *Df(3R)kar*^{Sz29}/*Df(3R)T-10* embryos, which lack 87C4 to C6 (*SzA* and *SzB* complementation groups) have transparent Malpighian tubules, whereas *Df(3R)kar*^{Sz37}/*Df(3R)T-10* embryos, which lack only 87C6 (*SzB*), have normal opaque tubules (Table 6).

Transplantation studies and determination of the autonomous nature of the Malpighian tubule defect: Posterior halves of newly hatched *l(3)SzA*^{5}/*Df(3R)kar*^{sJ} and *l(3)SzA*^{7}/*Df(3R)kar*^{sJ} first-instar larvae were injected into wild-type female hosts in order to determine the effect of a wild-type environment on the development of the mutant tissues. After nine days of *in vivo* culture, the Malpighian tubules had retained their transparent nature, whereas

TABLE 6

Determination of the complementation group showing the Malpighian tubule defect

Compl. group	Mutation	No. eggs laid	Embryos		Larvae		% of abnormal Malpighian tubules
			Wild type	Abnormal	Wild type	Abnormal	
SzA	*l(3)SzA²*	542	137	37	288	80	21.6
	l(3)SzA³	118	31	6	79	2	6.8
	l(3)SzA⁴	287	72	21	144	50	24.7
	l(3)SzA⁵	344	95	46	162	41	25.3
	l(3)SzA⁷	274	74	56	134	12	25.6
	l(3)SzA¹⁰	155	43	0	112	0	0
	l(3)SzA¹¹	202	53	0	149	0	0
SzB	*l(3)SzB²*	183	48	0	135	0	0
SzC	*l(3)SzC¹*	194	53	0	141	0	0
	l(3)SzC³	352	95	0	257	0	0
kar	*kar^{Sz-4}*	213	58	0	155	0	0
SzD	*l(3)SzD⁸*	108	33	0	75	0	0
	l(3)SzD¹⁰	498	124	0	374	0	0
	l(3)SzD¹³	347	91	0	256	0	0
	Df(3R)kar^{Sz29}	156	42	2	74	38	25.6
	Df(3R)kar^{Sz37}	208	57	0	151	0	0
Control	±	374	12	0	362	0	0

All mutants were crossed to *Df(3R)kar^{3J}/TM3* virgins except *Df(3R)kar^{Sz29}* and *Df(3R)kar^{Sz37}*, which were crossed to *Df(3R)T-10/TM3*.

implants of *l(3)SzA⁵/TM3* and *l(3)SzA⁷/TM3* half-embryos were still opaque. Moreover, the wild-type Malpighian tubules had become bright yellow, whereas the mutant tubules remained colorless. Although the implants from the heterozygous mutant embryos grew substantially during the culture period, those from the "hemizygous" [*i.e.*, *l(3)SzA/Df(3R)kar^{3J}*] embryos grew very poorly and had not progressed past the first-instar stage.

Deletion mapping and assignment of complementation groups to specific bands: In addition to the previously isolated deficiencies, new X-ray-induced deletions with at least one breakpoint in the 87C1–87C9 region were used to assign the five complementation groups to specific bands within this segment of the chromosome (Figure 2).

The results for representative alleles in each complementation group are summarized in Table 7. Since all of the EMS-induced mutations were lethal or karmoisin (in the case of *kar* alleles) with deficiency *Df(3R)kar^{Sz29}*, which has a proximal breakpoint between bands 87C3 and 87C4–5, we can assign all five complementation groups to the interval 87C4–87C9. The order of the complementation groups, based on their unambiguous complementation pattern with the other deficiencies is *l(3)SzA*, *l(3)SzB*, *l(3)SzC*, *kar* and *l(3)SzD* (Table 7). Furthermore, the groups can also be assigned to specific bands (or bands plus adjacent interbands) as follows: *l(3)SzA*, 87C4–5;*l(3)SzB*, 87C6; *l(3)SzC*, 87C7; *kar* 87C8; and *l(3)SzD*, 87C9. In cytological preparations from normal

TABLE 7

Deletion mapping of mutations in the 87C1 to 87C9 region

	Mutations checked				
	Group *A* *l(3)SzA*^{6},*l(3)SzA*^{7}	Group *B* *l(3)SzB*^{2},*l(3)SzB*^{5}	Group *C* *l(3)SzC*^{2},*l(3)SzC*^{5}	*kar* *kar*^{Sz1},*kar*^{Sz4}	Group *D* *l(3)SzD*^{7},*l(3)SzD*^{11}
Df(3R)kar^{3J}	—	—	—	—	—
Df(3R)kar^{3Q}	—	—	—	—	—
Df(3R)kar^{3l}	—	—	—	—	—
Df(3R)T-10	—	—	+	+	+
Df(3R)T-32	—	—	+	+	+
Df(3R)kar^{Sz11}	+	+	+	—	—
Df(3R)kar^{Sz12}	—	—	—	—	+
Df(3R)kar^{Sz21}	+	+	—	—	+
Df(3R)kar^{Sz27}	+	+	—	—	—
Df(3R)kar^{Sz29}	—	—	—	—	—
Df(3R)kar^{Sz31}	+	+	—	—	—
Df(3R)kar^{Sz37}	+	—	—	—	—
Assignment to band:	C4–5	C6	C7	C8	C9

Representative mutant alleles from each complementation group were crossed to the different deficiencies [*l(3)TM3* × *Df(3R)kar*^{x}/*TM3*]. — = noncomplementation, + = complementation.

larvae, we have been unable to resolve band 87C4, described by BRIDGES (1935). Preparations from larvae that are recovering from heat shock sometimes reveal a faint band that may be 87C4 (H. GYURKOVICS, unpublished observation), but it is unclear whether 87C4 and C5 are, in fact, two distinct bands. *Inter se* crosses between the different deficiencies (data not presented) were viable, with the exception of those combinations that overlapped a band to which a lethal complementation group was assigned. Those combinations that overlapped only for 87C8 were viable, fertile and *kar*, indicating that this locus is not essential for viability or fertility.

It is to be noted that no mutations were isolated in the region 87C1–87C3. Previous data (ISH-HOROWICZ, HOLDEN and GEHRING 1977) showed that *Df(3R)kar*^{3l} retains 87C1 and the associated heat-induced puff, whereas *Df(3R)kar*^{3J} lacks both this band and the puff. Thus, mutations in the 87C1 locus should be expressed in *trans* heterozygotes with *Df(3R)kar*^{3J}, but not with *Df(3R)kar*^{3l}. That no such mutations were found is consistent with the observation by W. GELBART (personal communication) that flies lacking 87C1 are viable.

Cytology of the 87C1–87C9 mutations: All of the mutations isolated in the first experiment were examined for chromosomal abnormalities in nonheat-shocked preparations and for the presence of the 87A7 and 87C1 puffs after heat shock. None of the mutations discussed in this report had any cytologically detectable aberrations and, in all cases, both 87A7 and 87C1 attained a wild-type puff size after exposure to 37.5°. These results do not, however, exclude the possibility that very small, cytologically undectable deletions were induced.

DISCUSSION

The purposes of this study were to saturate the 87C region and, in particular, to recover mutations at the heat-induced 87C1 locus. Since previous studies have indicated that 80 to 90% of chromosomal bands are associated with lethal-mutable loci (JUDD, CHEN and KAUFMAN 1972; HOCHMAN 1973), the recovery of lethal mutations at the 87C1 locus appeared hopeful.

Point mutations were initially isolated using the relatively small deficiency *Df(3R)kar*^{sJ}, which deletes 87C1 to 87C9. Four lethal and one visible complementation groups were distinguished by *inter se* crosses of the 39 newly induced lethal mutations (plus three previously reported lethal mutations) and the 13 *kar* alleles. Although *trans* heterozygotes for either *l(3)SzB* or *l(3)SzC* alleles were completely lethal, the complementation behavior within Groups *SzA* and *SzD* resembles the intracistronic complementation found at the rudimentary, dopa decarboxylase (*Ddc*) and α-methyldopa-hypersensitive (*amd*) loci (CARLSON 1971; FALK 1976; SPARROW and WRIGHT 1974; WRIGHT, BEWLEY and SHERALD 1976). For all these loci, we found that the degree of complementation varied considerably between pairs of alleles as judged by the differing severity of phenotype. A similar complementation pattern was observed for *l(3)SzA* and *l(3)SzD* alleles, the phenotype of *trans* heterozygotes varying from apparently wild type to female sterility, to weak and poorly viable *trans* heterozygotes with clearly defined abnormalities. This variable complementation pattern is understandable in terms of partial restoration of gene function (*i.e.*, through a multimeric protein), although the possibility of an interaction between distinct genes that are transcribed as a polycistronic HnRNA cannot be completely eliminated. The temperature-sensitive complementation within the *SzA* and *SzD* groups may reflect either the temperature sensitivity of polypeptides products or differential requirements for the active gene products at different growth rates. WRIGHT, BEWLEY and SHERALD (1976) and CLARK, VENKATARAMAN and HODGETTS (1978) found that the severity of the phenotype of *trans* heterozygotes of complementing alleles at the *Ddc* locus was related to the degree of restitution of enzyme activity.

Our assignment of the large number of mutations to Group *A* is further supported by their similar lethal phenotypes. The variation in their lethal phase and the incomplete penetrance of the Malpighian tubule phenotype probably occurs because the "weak" alleles [*e.g.*, *l(3)SzA*^{18}] show only partial loss of gene activity.

The developmental studies on the mutants provides some indication of a possible function for one of the loci in the 87C4–87C9 region. The examination of the Malphighian tubules in embryos of the genotype *l(3)SzA/Df(3R)kar*^{sJ} clearly indicated that this group of mutations affect the morphology of this organ. It has been shown (WESSING and EICHELBERG 1975; HEVERT 1975) that the anterior segment of the Malpighian tubules normally stores the primary urine in the form of crystalline concrements of uric acid, calcium phosphate and mucopolysaccharide lamellae. The concrements play an important role in the process of osmoregulation and they are either absent or severely reduced in numbers in

the severe *SzA* mutations. Transplantation studies using *l(3)SzA*^{5} or *l(3)SzA*^{7}/*Df(3R)kar*^{3J} posterior halves of embryos showed that the larval tissues grow poorly, if at all, compared to *l(3)SzA*^{5} or *l(3)SzA*^{7}/*TM3* half-embryos even when the implants are provided with a wild-type environment. Thus, the *SzA*$^{+}$ gene product is required for growth of most cell types. A detailed ultrastructural and histochemical analysis of these mutants may contribute to the understanding of the regulation excretion and provide clues as to the biochemical basis of the mutant phenotype.

Deletion mapping permitted the ordering of the different complementation groups and their assignment to specific bands (or bands plus adjacent interbands). *l(3)SzA* is associated with band(s) 87C4–5, the *l(3)SzB* mutations with 87C6, *l(3)SzC* alleles with 87C7, *kar* with 87C8, and the *l(3)SzD* alleles with 87C9. Using our deficiencies, VOELKER (personal communication) has localized the structural locus for malic enzyme to the next doublet 87D1–2 and has demonstrated that it is distinct from the *SzD* locus. Although we have already recovered many alleles at these loci, we cannot exclude the possibility that other as yet undiscovered complementation groups could map to these same bands. Our studies are, however, in general agreement with similar studies carried out for different regions of the Drosophila genome (HOCHMAN 1973; JUDD, CHEN and KAUFMAN 1972; SHANNON *et al.* 1972; WOODRUFF and ASHBURNER 1979).

Despite careful screening procedures, no lethal, semilethal, obvious visible or behavioral mutations were localized to band 87C1–3. The different breakpoint of *Df(3R)kar*^{3J} and *Df(3R)kar*^{3l} (Table 1) would have enabled us to map mutations associated with 87C1. In fact, all the mutations recovered failed to complement *Df(3R)kar*^{Szz9}, which is deleted for 87C4 to C9, but retains 87C1–3. Since no effect of the 87C1 locus on heat-induced polypeptide synthesis could be demonstrated (ISH-HOROWICZ, HOLDEN and GEHRING 1977), our failure to recover mutations at 87C1 is perhaps not surprising. Results of studies from several different laboratories (ISH-HOROWICZ *et al.* 1979; SPRADLING, PARDUE and PENMAN 1977; HENIKOFF and MESELSON 1977; SCHEDL *et al.* 1978; LIVAK *et al.* 1978; CRAIG, MCCARTHY and WADSWORTH 1979) now indicate that the 87A7 and 87C1 bands share coding sequences for hsp 70. Since elimination of one or the other of these loci does not affect the pattern of heat-induced polypeptide synthesis, it is perhaps not unexpected that one cannot recover point mutations in these bands that result in lethality of the individuals. LIS, PRESTIDGE and HOGNESS (1978) have described heat-shock-induced sequences that are unique to 87C1 and not found at 87A7. However, these sequences are repeated within the 87C1 band and it is not surprising that they are not lethal-mutable. Moreover, it is unclear whether their transcripts are messenger RNAs.

GELBART (personal communication) has constructed flies of the genotype *Df(3R)kar*^{3J}/*Df(3R)kar*^{3J}; *Dp(2;3)ry*$^{+w70h}$ that completely lack 87C1. These flies are viable, but female sterile. ISH-HOROWICZ (unpublished observations) has found that the genotype *Df(3R)kar*^{3l}/*Df(3R)kar*^{3J}; *Dp(2;3)ry*$^{+w70h}$ is also female sterile, but that *Df(3R)kar*^{Szz9}/*Df(3R)kar*^{3J}; *Dp(2;3)ry*$^{+w70h}$ is female fertile. *Dp(2;3)ry*$^{+w70h}$ is a duplication of 87C2–3 to 88C, broken within 87C2–3,

although the amount of 87C2–3 material that is duplicated is uncertain (H. GYURKOVICS, unpublished observations). If the duplication were to include functional 87C2–3 material, the only difference between the sterile *Df(3R)kar*^{sl}/*Df(3R)kar*^{sJ}; *Dp(2;3)ry*$^{+w70h}$ and fertile *Df(3R)kar*^{Sz29}/*Df(3R)kar*^{sJ}; *Dp(2;3)ry*$^{+w70h}$ genotypes is in the dosage (1 and 2, respectively) of 87C2–3. As heterozygous deficiencies for this region (*e.g.*, *Df(3R)kar*^{sJ}/+) are not female sterile, hemizygosity for 87C2–3 does not cause female sterility. It is more likely that a functional 87C2–3 is not included within the duplication and that the female sterility is associated, not with 87C1, but with some part of 87C2–3. A corollary is that, like 87C1, 87C2–3 is dispensable for viability, a view confirmed by our inability to recover lethal mutations that map to these bands. Since flies deficient for 87C1 also lack a functional 87C2–3, we cannot say whether a fertility function is also associated with 87C1.

The question remains, however, as to the feasibility of isolating point mutations at the 87A7 and 87C1 heat-induced loci. Further to the studies described here, we have also screened more than 8,000 EMS-treated chromosomes for mutations lethal with *Df(3R)kar*^{D3} and localizing to the 87A7 heat-induced band. None of the 125 induced lethals map to the 87A7 heat shock locus (GAUSZ *et al.*, unpublished observations). It is clear that the 87C1 locus is dispensable, indicating either that the product plays no essential role in development (with the possible exception of oogenesis) or that the function can be supplied by the 87A7 locus. It may still be feasible to isolate mutations at the 87A7 locus by selecting lethals in flies lacking the 87C1 locus. A further complication arises from the observations that the DNA sequences coding for hsp 70 are repeated at each of the two loci (ARTAVANIS-TSAKONAS *et al.* 1979, CRAIG, MCCARTHY and WADSWORTH 1979; ISH-HOROWICZ *et al.*, in preparation). If strains of flies with a single hsp 70 coding sequence 87A7 can be isolated, it may yet be possible to recover mutations at 87A7.

In conclusion, the genetic analysis of the 87C1–87C9 region is consistent with the view that the 87C1 locus does not uniquely code for an indispensable gene product. A locus required for female fertility may be localized to the 87C2–3 doublet. Single complementation groups have been assigned to each band in the 87C4–5 to 87C9 region, supporting the notion that each band (or band plus adjacent interband) represents a single functional unit.

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LITERATURE CITED

ARTAVANIS-TSAKONAS, S., P. SCHEDL, M.-E. MIRAULT, L. MORAN and J. LIS, 1979 Genes for the 70,000 dalton heat shock protein in two cloned *Drosophila melanogaster* DNA segments. Cell **17**: 9–18.

ASHBURNER, M., 1970 Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. Chromosoma **31**: 336–376.

BRIDGES, C. B., 1935 Salivary chromosome maps. With a key to the banding of the chromosomes of *Drosophila melanogaster*. J. Heredity **26:** 60–64.

CARLSON, P., 1971 A genetic analysis of the *rudimentary* locus of *Drosophila melanogaster*. Genet. Res. **17:** 53–81.

CLARK, W. C., P. S. PASS, B. VENKATARAMAN and R. B. HODGETTS, 1978 Dopa decarboxylase from *Drosophila melanogaster*: Purification, characterisation and analysis of mutants. Mol. Gen. Genet. **162:** 287–297.

CRAIG, E. A., B. J. MCCARTHY and S. C. WADSWORTH, 1979 Sequence organization of two recombinant plasmids containing genes for the major heat shock-induced protein of *Drosophila melanogaster*. Cell **16:** 575–588.

DELAND, M., 1971 Genetic studies of a micro region of chromosome IIIR of *Drosophila melanogaster*. Ph.D. dissertation, University of Connecticut, Storrs, Connecticut.

EPHRUSSI, B. and G. W. BEADLE, 1936 A technique of transplantation for *Drosophila*. Amer. Naturalist **70:** 218–225.

FALK, D., 1976 Pyrimidine auxotrophy and the complementation map of the rudimentary locus of *Drosophila melanogaster*. Mol. Gen. Genet. **148:** 1–8.

HENIKOFF, S. and M. MESELSON, 1977 Transcription at two heat shock loci in *Drosophila*. Cell **12:** 441–451.

HEVERT, F., 1975 Physiologische mechanismen bei der naernbildung durch Malpigische Gefaesse. Fortschr. Zool. **23:** 173–192.

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

HOLDEN, J. J. and M. ASHBURNER, 1978 Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. IX. The salivary and prothoracic gland chromosomes of a dominant temperature sensitive lethal of *D. melanogaster*. Chromosoma (Berl.) **68:** 205–227.

ISH-HOROWICZ, D., J. J. HOLDEN and W. J. GEHRING, 1977 Deletions of two heat-activated loci in *Drosophila melanogaster*. Cell **12:** 643–652.

ISH-HOROWICZ, D., S. M. PINCHIN, J. GAUSZ, H. GYURKOVICS, G. BENCZE, M. GOLDSCHMIDT-CLERMONT and J. J. HOLDEN, 1979 Deletion mapping of two *Drosophila melanogaster* loci that code for the 70,000d heat-induced protein. Cell **17:** 556–571.

JUDD, B. N., M. W. CHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the *X*-chromosome of *Drosophila melanogaster*. Genetics **71:** 139–156.

LEFEVRE, G. JR., 1974 The relationship between genes and polytene chromosome bands. Ann. Rev. Genet. **8:** 51–62.

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

LINDSLEY, D. L. and E. N. GRELL, 1968 *Genetic variations of* Drosophila melanogaster. Carnegie Inst., Wash. Publ. No. 627.

LIS, J. T., L. PRESTIDGE, D. S. HOGNESS, 1978 A novel arrangement of tandemly repeated genes at a major heat shock site in *Drosophila melanogaster*. Cell **14:** 901–919.

LIVAK, K. J., R. FREUND, M. SCHWEBER, P, WENSINK, P. MESELSON, 1978 Sequence organization and transcription at two heat shock loci in *Drosophila*. Proc. Natl. Acad. Sci. U.S. **75:** 5613–5618.

MCKENZIE, S. L., S. HENIKOFF and M. MESELSON, 1975 Localization of RNA from heat-induced polysomes at puff sites in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **72:** 1117–1121.

RITOSSA, F. M., 1963 New puffs induced by temperature shock, DNP and salicylate in salivary chromosomes of *Drosophila melanogaster*. Drosophila Inform. Serv. **37:** 122–123.

SCALENGHE, F. and F. RITOSSA, 1976 Controllo dell'attivita genica in *Drosophila*. "Il puff al locus ebony e la glutamina sintetasi I". Atti della Accademia Nazionale dei Lincei, Ser. **8**: 441–528.

SCHEDL, P., S. ARTAVANIS-TSAKONAS, R. STEWARD, W. J. GEHRING, M.-E. MIRAULT, M. GOLDSCHMIDT-CLERMONT, L. MORAN and A. TISSIÈRES, 1978 Two hybrid plasmids with *Drosophila melanogaster* DNA sequences complementary to mRNA coding for the major heat shock protein. Cell **14**: 921–929.

SHANNON, M. P., T. C. KAUFMAN, M. W. SHEN and B. H. JUDD, 1972 Lethality patterns and morphology of selected lethal and semi-lethal mutations in the zeste-white region of *Drosophila melanogaster*. Genetics **72**: 615–638.

SPARROW, J. C. and T. R. F. WRIGHT, 1974 The selection for mutants in *Drosophila melanogaster* hypersensitive to *α-methyl* dopa, a dopadecarboxylase inhibitor. Mol. Gen. Genet. **130**: 127–141.

SPRADLING, A., M. L. PARDUE and S. PENMAN, 1977 Messenger RNA in heat shocked *Drosophila* cells. J. Molec. Biol. **109**: 559–587.

TISSIÈRES, A., H. K. MITCHELL and U. M. TRACEY, 1974 Protein synthesis in salivary glands of *Drosophila melanogaster*: Relation to chromosome puffs. J. Molec. Biol. **84**: 389–398.

WESSING, B. and B. EICHELBERG, 1975 Ultrastructural aspects of transport and accumulation of substances in the Malpighian tubules. Fortschw. Zool. **23**: 148–172.

WOODRUFF, R. C. and M. ASHBURNER, 1979 The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. II. Lethal mutations in the region. Genetics **92**: 133–149.

WRIGHT, T. R. F., G. C. BEWLEY and A. F. SHERALD, 1976 The genetics of dopa decaboxylase in *Drosophila melanogaster*. II. Isolation and characterization of dopa-decarboxylase-deficient mutants and their relationship to the α-methyl-dopa-hypersensitive mutants. Genetics **84**: 287–310.

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