A Genetic Analysis of the *rose-gespleten* Region (68C8-69B5) of *Drosophila melanogaster*

Anna Marie Hoogwerf,[1] Michael Akam[2] and David Roberts[3]

Genetics Laboratory, Biochemistry Department, Oxford University, Oxford OX1 3QU, England

Manuscript received March 23, 1987
Revised copy accepted December 7, 1987

ABSTRACT

We describe a genetic analysis of the region 68C8-69B5 defined by *Df(3L)vin-7*. We have induced 35 new lethal mutations in this region, which together with 20 existing lethal mutations, visible mutations, genes identified by protein products and one gene deduced from complementation data fall into 37 complementation groups in this 35-band interval. Using existing and newly induced deficiencies we have assigned these to 11 intervals defined by deficiency breakpoints. Those mutations which fell in the same breakpoint interval as the *Lsp-2* gene, which codes for the abundant larval serum protein 2, were the subject of detailed study. None was rescued by the active *Lsp-2* gene transformed on to chromosome *II* and we conclude that, as yet, we have no lethal mutations of *Lsp-2*.

WE report here the isolation and complementation mapping of lethal mutations in the region 68C8-69B5, on the left arm of chromosome *III* of *Drosophila melanogaster*, as defined by *Df(3L)vin-7*.

This project was initiated to provide a better map of the region of the *Lsp-2* gene, which encodes the second major protein component of the serum of late third instar larvae (ROBERTS, WOLFE and AKAM 1977), and in an attempt to isolate recessive lethal mutations of that gene. Our hope was to learn something of the function of this abundant protein from the phenotype of *Lsp-2* mutations.

In this we have failed. The results define five lethal complementation groups lying between the deficiency breakpoints that flank the *Lsp-2* locus, but we reject all of these as candidates for *Lsp-2* mutations. The mutations recovered do however define 32 lethal complementation groups, in addition to the 3 visible and 2 protein coding loci located within this 35-band region.

MATERIALS AND METHODS

Culture conditions: Stocks were maintained on standard yeast-cornmeal-agar medium in half-pint milk bottles at 25°. Nipagin was added as an inhibitor of moulds. For cytological studies and for the analysis of hemolymph proteins flies were reared on yeast-glucose-agar medium (10% yeast, 10% glucose and 3% agar autoclaved after mixing). The temperature-sensitive stocks were kept at 18° and transferred to 29° as necessary.

Stocks: The Oregon-R strain maintained in this laboratory for over 15 yr was used as the reference wild-type strain. Unless indicated otherwise the LSP-2 of all stocks was indistinguishable from that of the Oregon-R stock. Many of the stocks used in this work have been described by us previously AKAM (1977), AKAM, ROBERTS and WOLFE (1978) and AKAM *et al.* (1978). Additional stocks were obtained as follows: *Tp(3:3)Ubx*^{P20}/TM1 Me (Tp(3:3)68A-68E;89E), E. B. LEWIS, California Institute of Technology; *l(3)VII-5 p*^{P} *e*/*TM3(In(3LR) TM3 Sb Ser e)* A. SHEARN, Johns Hopkins University, Baltimore; and lethal mutants in the *Lsp-2* region (Table 1) from E. MEYEROWITZ, California Institute of Technology. The Ightham stocks (pronounced "item") were established from a gravid female caught in the village of Ightham in Kent (AKAM *et al.* 1978). The remaining stocks have been in this laboratory for many years and the phenotypes of all these mutants are described in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1985, 1986, 1987) with the exception of *vin* (ANXOLABEHERE and PERIQUET 1973).

One allele of every complementation group described here will be maintained by us for reference purposes for at least 12 months and will be deposited at the European Stock Centre in Umeå.

EMS mutagenesis: Males homozygous for the markers *vin* and *app*, which flank the *Lsp-2* locus and marked with *v* (which in the presence of *vin* gives a bright orange eye) were mutagenized by the method of LEWIS and BACHER (1968). Lethal mutations were recovered as shown in Figure 1.

Complementation: All lethal mutations were balanced over TM3 and crossed to deficiencies of the *Lsp-2* region (Table 2), which were also balanced over *TM3*. The absence of Sb^+ flies in more than 100 progeny was taken as the criterion for the failure to complement. By this technique mutations were placed between deficiency breakpoints. Where two lethals occurred between the same two deficiency breakpoints they were crossed *inter se*. The absence of Sb^+ flies in more than 100 progeny was again taken as the criterion for the failure to complement.

Nomenclature of mutations: The region of 3L which we study here is flanked distally by *rs* (rose) and proximally by *gs* (gespleten). Following the precedent of JUDD, SHEN

[1] Present address: Department of Medical Genetics, Churchill Hospital, Headington, Oxford, England.
[2] Present address: Genetics Department, University of Cambridge, Downing Street, Cambridge CB2 3EH, England.
[3] To whom reprint requests should be sent.

TABLE 1

Genes and their alleles within the 67F-69B5 region

Gene	Allele	Mutagen	Chromosome	Comments[a]	Gene	Allele	Mutagen	Chromosome	Comments[a]
rsg3	*l(3)V4-3*	*EMS*	*red e*	1	*rsg21*	*l(3)1B*	EMS	*vin app*	
	l(3)BI^{D}	*EMS*	*vin app*		*rsg22**	*l(3)69*	EMS	*vin app*	
rsg4	*l(3)V7-1*	*EMS*	*rs^{2}*	1		*l(3)113*	EMS	*vin app*	
rsg5	*l(3)154*	*EMS*	*vin app*			*l(3)112-226*	EMS	*Oregon*	2
*rsg6**	*l(3)113-178*	*EMS*	*Oregon*	2	*rsg23**	*l(3)101*	EMS	*vin app*	
	l(3)113-253	*EMS*	*Oregon*	2		*l(3)21-2*	EMS	*vin app*	
	l(3)90	*EMS*	*vin app*			*l(3)112-25*	EMS	*Oregon*	2
	l(3)27-2	*EMS*	*vin app*			*l(3)112-139*	EMS	*Oregon*	2
rsg7	*l(3)112-204*	*EMS*	*Oregon*	2		*l(3)112-143*	EMS	*Oregon*	2
	l(3)112-10	*EMS*	*Oregon*	2		*l(3)112-186*	EMS	*Oregon*	2
	l(3)113-441	EMS	*Oregon*	2		*l(3)D^{11}*	EMS	*vin app*	
rsg8	*l(3)1-4*	EMS	*vin app*		*rsg24*	*l(3)145*	EMS	*vin app*	
rsg9	*l(3)12-5B*	EMS	*vin app*		*rsg25*	*l(3)58-2*	EMS	*vin app*	
rsg10	*l(3)146*	EMS	*vin app*		*rsg26*	*l(3)163*	EMS	*vin app*	
	l(3)155	EMS	*vin app*		*rsg27*	*l(3)62-2*	EMS	*vin app*	
*rsg11**	*l(3)70-2*	EMS	*vin app*		*rsg28*	*l(3)N24*	EMS	*vin app*	
	l(3)183	EMS	*vin app*		*rsg29*	*l(3)21b*	EMS	*vin app*	
	l(3)107-43	EMS	*Oregon*	2	*rsg30*		EMS	*vin app*	5
	l(3)V4-4	EMS	*red*	1	*rsg31*	*l(3)141*	EMS	*vin app*	
rsg12	*l(3)107*	EMS	*vin app*			*l(3)74*	EMS	*vin app*	
rsg13	*l(3)106-19*	EMS	*Oregon*	2	*rsg32*	*l(3)12-5*	EMS	*vin app*	
rsg14	*l(3)5*	EMS	*vin app*		*rsg33*	*l(3)47*	EMS	*vin app*	
rsg15	*l(3)141A*	EMS	*vin app*		*rsg34*	*l(3)13*	EMS	*vin app*	
	l(3)137	EMS	*vin app*		*Df(3L)vin-8*		EMS	*vin app*	
rsg16	*l(3)16-2*	EMS	*vin app*		*Df(3L)vin-9*		EMS	*vin app*	
rsg17	*l(3)P20*	Spontaneous		3	*Df(3)vin-10*	*l(3)139*	EMS	*vin app*	
*rsg18**	*l(3)V11-5*	ICR-170	*mwh e*	4	*Df(3L)vin-1*	*l(3)112*	EMS	*vin app*	
	l(3)V4-1	EMS	*red e*	1	*rt*				
	(3)V4-5^{ts}	EMS	*red*	1	*vin*				
	l(3)FII	EMS	*vin app*		*app*				
rsg19	*l(3)49*	EMS	*vin app*		*LSP-2*				
rsg20	*l(3)igh*	Spontaneous	*Ightham*		*Est-6*				
	l(3)BI^{P}	EMS	*vin app*						

[a] 1, AKAM *et al.* (1978); 2, E. M. MEYEROWITZ (unpublished data); 3, Deduced by the failure of the breakpoint of *Tp(3)P20* to complement *Df(3L)vin-4* but to complement *Df(3L)vin-3* and all the lethal complementation groups between the proximal breakpoints of *Df(3L)vin-3* and *Df(3L)vin-4*; (*Tp(3)P20* from E. B. LEWIS, (unpublished data); 4, A. SHEARN, (unpublished data); 5, Deduced by the failure of *Df(3L)vin-11* to complement interval 18 and yet complement both of the complementation groups within it.

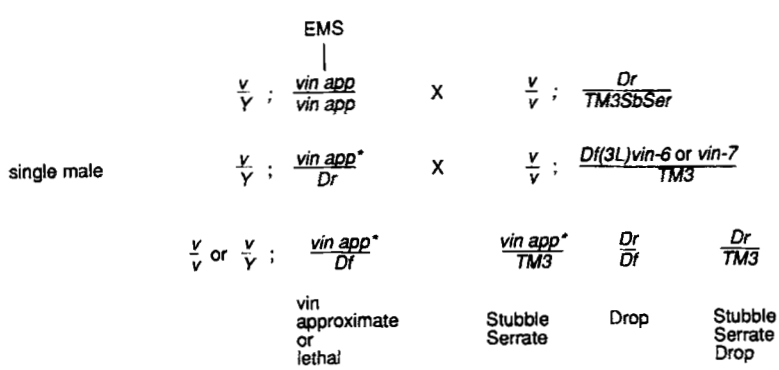

FIGURE 1.—Selection of chromosomes carrying induced lethal mutations uncovered by *Df(3L)vin 6* or *vin 7*. Male flies were treated with EMS and crossed as shown above. If the *vin app* class is missing in the F_2 generation then a balanced stock is established from the *Sb Ser* flies. Nineteen single lethal mutations and one deficiency were recovered from 1697 mutagenized chromosomes tested over *Df(3L)vin-7* (30 bands). Seventeen single lethal mutations, one double mutation and one deficiency were recovered from 2929 mutagenized chromosomes tested over *Df(3L)vin-6* (27 bands).

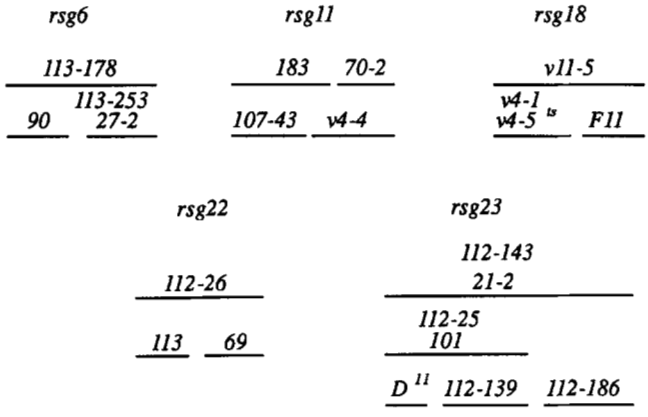

FIGURE 2.—Complementation maps of the complex loci marked with an asterisk in Table 1.

and KAUFMAN (1972) we have called the region RSG. The complementation groups within this region have been named *l(3)rsg1*, *l(3)rsg2* etc. Mutations within each complementation group retain their trivial names (see Table 1). A mutation which fails to complement two mutations which themselves complement one another presents a problem. Such a mutation may be a small deficiency, or the mutations may all be of a single gene and show intragenic complementation. Where there is no other evidence for a deficiency we have placed all the mutations in a single complementation group and given a single locus name. These complex loci are indicated by asterisks in Table 1 and the maps for these five complex complementation groups are presented in Figure 2.

Where one mutation fails to complement two others which are themselves separated by a deficiency breakpoint, the most reasonable explanation is that they represent small deficiencies thus: *l(3)139* and *l(3)112* are defined as deficiencies by this criterion.

The *rsg* region overlaps that studied by CROSBY and MEYEROWITZ (1986). The visible mutants *rt* and *vin* are included in both studies and allow the orientation of this complementation map. Twelve lethal mutations in the region were provided by E. MEYEROWITZ. These were selected over *Df(3L)vin-5* or *Df(3L)vin-66* but not reported by CROSBY and MEYEROWITZ (1986) as they all fell proximal to their region of interest but all twelve were uncovered by *Df(3L)vin-7* and are discussed here. One additional mutation in the *rsg* region was identified among samples of third chromosome lethal mutations which had previously been selected as having a lethal phase in the late third instar or pupal periods (A. SHEARN, unpublished data). *l(3)P20* is a lethal mutation associated with the breakpoint of $Tp(3:3)Ubx^{P20}$ in 68E3. The remaining mutation, *l(3)igh* was identified when isolating chromosomes from the wild type stock Ightham to establish LSP-2 fast variant lines (AKAM, ROBERTS and WOLFE 1978). One lethal complementation group (*rsg 30*) was deduced by the failure of a deficiency to complement with a breakpoint interval and yet complement both of the complementation groups within it (see Table 1). In addition to these lethal mutations, three visible mutations (*rt, vin, app*) and two loci defined by electrophoretic variants (*Est-6* and *Lsp-2*) also map in the *rsg* region between 68C8 and 69B4-5 (Table 1).

RESULTS

All of these mutations were placed between deficiency breakpoints by analyzing their pattern of complementation with a series of deficiencies (Figure 3). Mutations which fell into an interval between two adjacent deficiency breakpoints were crossed *inter se* in order to study the complementation relationships within these intervals. In addition a full complementation matrix was set up to look for any complexities in the complementation patterns between mutants of different breakpoint intervals. Several anomalies were discovered.

Two of the mutations induced on the *vin app* chromosomes turned out to be large deficiencies: *Df(3L)vin-8* (with breakpoints at 68C2-3-68F3-6) and *Df(3)vin-9* (68C5-6-69A5-B1) (Figure 4).

The lethal chromosome *l(3)BI* showed an apparently illogical complementation pattern which could be resolved only by assuming two mutations, either generated independently or as the product of an inversion. Further analysis showed that this chromosome is cytologically normal and that it carries two point mutations called $l(3)BI^D$ (distal *rsg3*) and $l(3)BI^P$ (proximal *rsg20*).

TABLE 2

Description of deficiencies used in this study

Deficiency	Breakpoints		
	AKAM *et al.* (1978)	CROSBY and MEYEROWITZ (1986)	Others
Df(3L)vin-2	67F2-2; 68D6		
Df(3L)vin-3	68C8-11; 68E3-4	68C5-6; 68E3-4	
Df(3L)vin-4	68A8-9; 68E3-4	68B1-3; 68F3-6	
Df(3L)vin-5	68A3; 69A1-2	68A2-3; 69A1-3	
Df(3L)vin-6	68C8-11; 69A4-5	68C8-11; 69A4-5	
Df(3L)vin-7	68C8-11; 69B4-5	68C8-11; 69B4-5	
Df(3L)vin-8			68C2-3; 68F3-6[a]
Df(3L)vin-9			68C5-6; 69A5-B1[a]
Df(3L)vin-66	68A3; 68D3	68A2-3; 68D3	
Df(3L)P20			68A; 68E[b]

[a] This paper.
[b] E. B. LEWIS (personal communication) and LINDSLEY and ZIMM (1987).

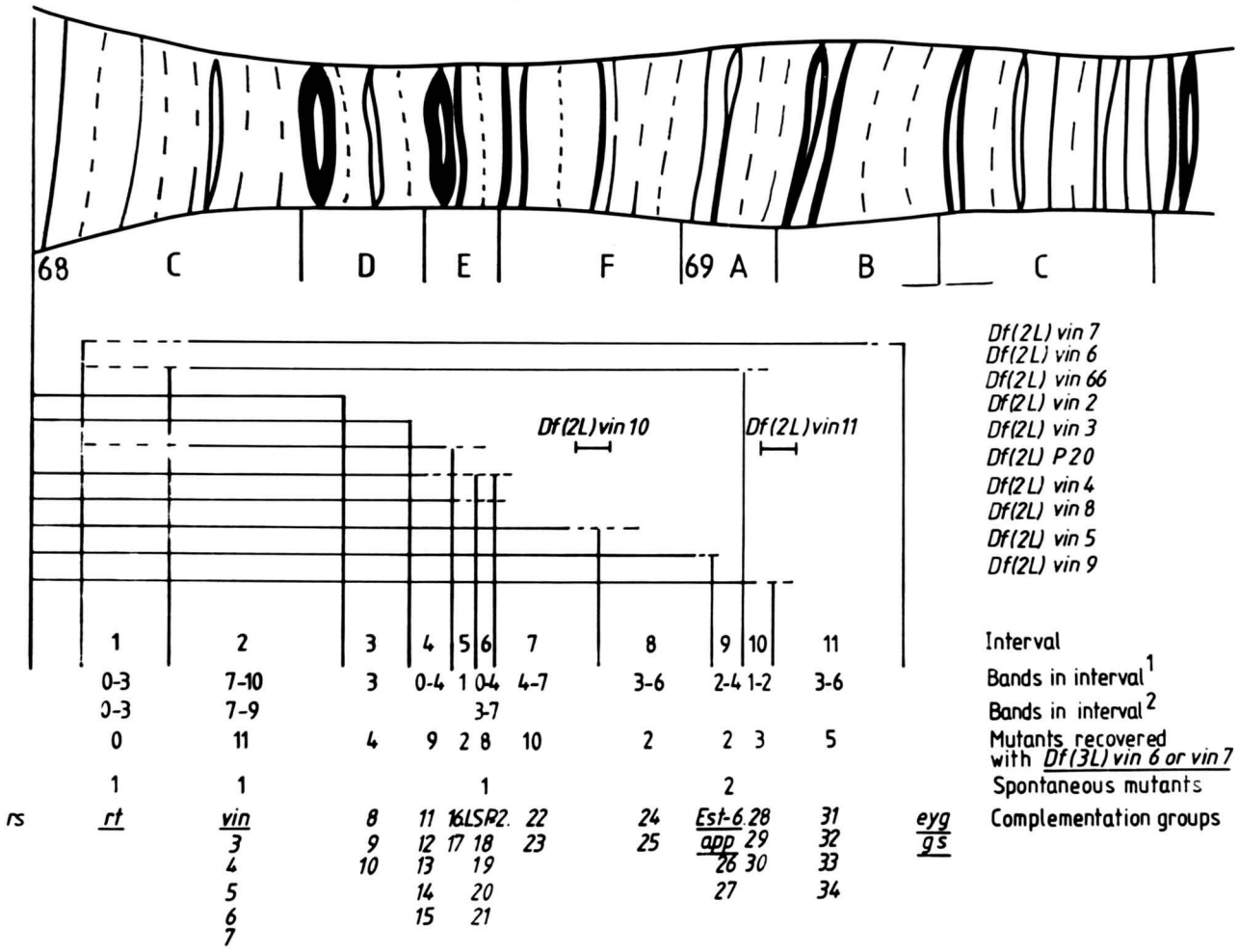

FIGURE 3.—The *rose-gespleten* (*rsg*) region around 68C on the left arm of chromosome *3*. The region is divided into intervals 1–11 by the breakpoints of deficiencies. The extent of each of the cytologically visible deficiencies defining these intervals is shown in the top half of the figure. [1]AKAM *et al.* (1978); [2]CROSBY and MEYEROWITZ (1986).

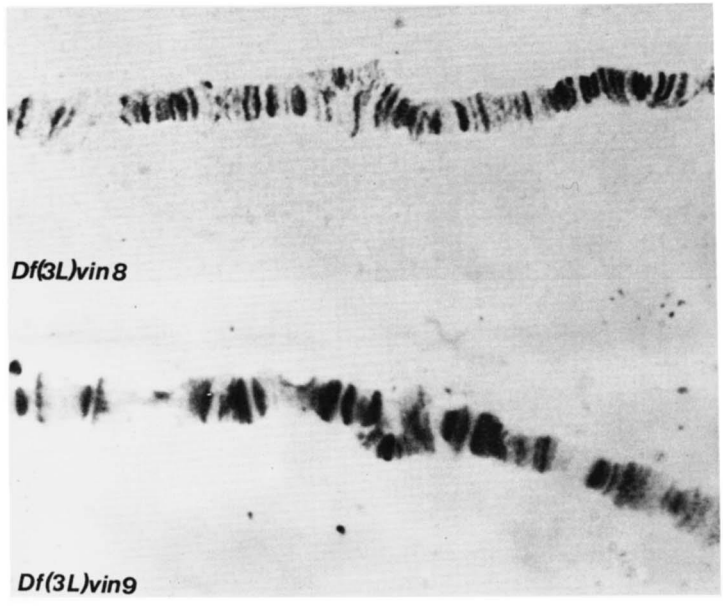

FIGURE 4.—Two new large deficiencies in the 68E region: *Df(3L)vin 8* and *Df(3L)vin 9*.

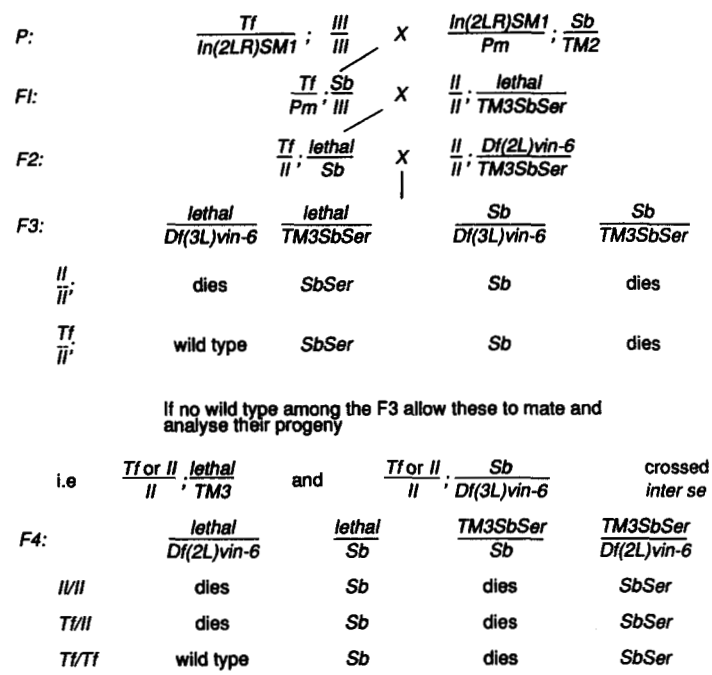

FIGURE 5.—Breeding scheme used to study the possible rescue of lethal mutants by the second chromosome carrying an active *Lsp-2* gene (Tf). The male parent is always given first. *l/Df(3L)vin 6* progeny of the F_2 generation normally die. If however the lethal were due to a mutation of the *Lsp-2* gene then it would be rescued by the active transformed gene on the second chromosome. It may be that the single dose of active, transformed *Lsp-2* produces insufficient LSP-2 (less than 50% of wild type) to effect a rescue but the homozygous *Tf* progeny of the F_3 generation will produce greater than 50% LSP-2 which is known to be sufficient to rescue the *Lsp-2* deficiency.

Two other lethal chromosomes have been designated deficiencies *viz. Df(3L)vin-10* and *Df(3L)vin-11*, because they fail to complement mutations which complement each other and which fall on either side of a deficiency breakpoint. These chromosomes are cytologically normal in the *rsg* region.

Five complementation groups show complex behaviour which we attribute to interallelic complementation (Figure 2).

Further analysis of lethal mutations within the LSP-2 interval: Deficiency mapping of electrophoretic variants indicates that the structural gene for LSP-2 maps to interval 6 of Figure 3. We initially suspected that of the lethal mutations within this region, *l(3)igh* was a likely candidate for an *Lsp-2* mutation, as this chromosome encodes a fast electrophoretic allele of *Lsp-2*.

However, *l(3)igh* appears not to be an allele of the *Lsp-2* locus. The *l(3)igh* mutation is completely recessive, and should therefore be rescued by a single wild type copy of the *Lsp-2* gene. Two active insertions (2B6 and 2A1) of a wild-type *Lsp-2* gene have been obtained on chromosome 2 by P element mediated transformation (J.-A. LEPESANT, H. BENES and T. JOWETT, unpublished results). These transformants were recognized by the presence of wild-type LSP-2 after the transformation of a stock homozygous for *Lsp-2*^{slow}. The homozygous transformants survive and produce at least 60% of the wild-type LSP-2. We know that flies with 50% wild-type LSP-2 (heterozygous deficiencies) survive. Both transformed chromosomes failed to rescue (Figure 5) the recessive lethal phenotype of any of the lethal mutations mapping to the interval 14 (Figure 3) either in the heterozygous (one dose of transformed LSP-2) or homozygous (two doses) conditions. We conclude therefore, that none of these is an *Lsp-2* mutation.

DISCUSSION

This is the second analysis carried out by this laboratory on the region surrounding one of the larval serum protein genes. These results are similar to those presented by us for the LSP-1 β region (ROBERTS *et al.* 1985) and to those reported for other small regions of the *Drosophila* genome (JUDD, SHEN and KAUFFMAN 1972; HILLIKER *et al.* 1980; WOODRUFF and ASHBURNER 1979a, b; WRIGHT *et al.* 1981; NIKLAS and CLINE 1983; KOTARSKI, PICKERT and MACINTYRE 1983). This study deals with the region 68C8-69B4-5 which is uncovered by a set of deletions which we reported earlier (AKAM *et al.* 1978). This region overlaps at its distal end with that studied in similar detail by CROSBY and MEYEROWITZ (1986) 68A3-68C11 and by CAMPBELL, HILLIKER and PHILLIPS (1986) 68A2-C1.

Within the 35 band region uncovered by *Df(3L)vin-7* we identify a total of 32 lethal complementation groups, plus three genes with visible alleles, *rt, vin* and *app* and two genes recognized by electrophoretic

variants of their proteins namely *Lsp-2* and *Est-6*. This total of 37 complementation groups in an interval of 35 bands is comparable with the one locus per band relationship found in most of the saturation mutagensis studies mentioned above. Although considerable doubt has been expressed as to how valid this relationship really is (LEFEVRE and WATKINS 1986).

One aim of this study was to induce a lethal mutation in the *Lsp-2* gene which, by analysis of the mutant phenotype, might give an insight into the function of this abundant protein. Four lethal complementation groups, represented by eight lethal mutations, lie in the same breakpoint interval as the *Lsp-2* gene. Because none is rescued by the transformed *Lsp-2* gene we conclude that none is likely to be a lethal mutation affecting LSP-2.

We are still uncertain whether or not an *Lsp-2* null mutation would result in a lethal phenotype. The possibility that the *Lsp-2* gene cannot mutate to lethality is supported by the observation (D. B. ROBERTS, unpublished results) that flies completely lacking the major and related serum protein LSP-1 are viable.

On the other hand, while spontaneously occurring null alleles of all three *Lsp-1* genes have been found (D. B. ROBERTS, unpublished results) no spontaneously occuring null allele of *Lsp-2* has been found, suggesting that such alleles might be lethal.

We are not yet able to conclude whether or not the *Lsp-2* gene is necessary for the viability of *Drosophila*.

We wish to thank JEAN MATTHEWS for making the fly medium. We also thank GEOFF RICHARDS and LYNN CROSBY for their willing discussion over the assignment of the deficiency breakpoints. A.M.H. was supported by Research Scholarship from the Medical Research Council and the work was supported by a grant to D.B.R. from the Science and Engineering Research Council.

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Communicating editor: A. CHOVNICK