Cytogenetic Analysis of Chromosome Region 73AD of *Drosophila melanogaster*

John M. Belote,*,† F. Michael Hoffmann,‡ Michael McKeown,*,§ Regina L. Chorsky† and Bruce S. Baker*,[1]

** Department of Biology B-022, University of California at San Diego, La Jolla, California 92093, †Department of Biology, Biological Research Laboratories, Syracuse University, Syracuse, New York 13244, ‡McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706, and §Molecular Biology and Virology Laboratory, The Salk Institute, San Diego, California 92138*

Manuscript received September 19, 1989
Accepted for publication April 14, 1990

ABSTRACT

The 73AD salivary chromosome region of *Drosophila melanogaster* was subjected to mutational analysis in order to (1) generate a collection of chromosome breakpoints that would allow a correlation between the genetic, cytological and molecular maps of the region and (2) define the number and gross organization of complementation groups within this interval. Eighteen complementation groups were defined and mapped to the 73A2–73B7 region, which is comprised of 17 polytene bands. These complementation groups include the previously known *scarlet* (*st*), *transformer* (*tra*) and *Dominant temperature-sensitive lethal-5* (*DTS-5*) genes, as well as 13 new recessive lethal complementation groups and one male and female sterile locus. One of the newly identified lethal complementation groups corresponds to the molecularly identified *abl* locus, and another gene is defined by mutant alleles that exhibit an interaction with with the *abl* mutants. We also recovered several mutations in the 73C1–D1·2 interval, representing two lethal complementation groups, one new visible mutant, *plucked* (*plk*), and a previously known visible, *dark body* (*db*). There is no evidence of a complex of sex determination genes in the region near *tra*.

SALIVARY gland chromosome region 73AD of *Drosophila melanogaster* is the site of two genes that are the subjects of molecular genetic analyses in our laboratories: the Drosophila Abelson proto-oncogene homolog (*abl*), and *transformer* (*tra*), a gene controlling female sexual differentiation (STURTEVANT 1945; BAKER and RIDGE 1980). The *abl* locus was cloned by cross-hybridization to the Abelson murine leukemia virus *v-abl* oncogene sequence, and mapped by *in situ* hybridization to chromosome region 73B1·2 (HOFFMAN-FALK *et al.* 1983; HOFFMANN *et al.* 1983; HENKEMEYER *et al.* 1987). The *tra* gene was deletion mapped to salivary chromosome region 73A7–9 and cloned both by us (BELOTE *et al.* 1985; MCKEOWN *et al.* 1986; MCKEOWN, BELOTE and BAKER 1987) and by others (BUTLER *et al.* 1986) by chromosomal walks that isolated a contiguous region of about 250 kb. For the details of the molecular studies of this cloned region, see BUTLER *et al.* (1986); MCKEOWN, BELOTE and BAKER (1987); HENKEMEYER *et al.* (1987); and TEARLE *et al.* (1989).

In addition to *abl* and *tra*, three other previously identified mutants map at or near this region of the genome: the eye color mutant *scarlet* (*st*) (ASHBURNER *et al.* 1981; TEARLE *et al.* 1989), the dominant temperature-sensitive lethal, *DTS-5* (HOLDEN and SUZUKI 1973), and the body color mutant *dark body* (*db*) (CHOVNICK and TALSMA 1966).

In conjunction with our molecular analyses of the *abl* and *tra* genes, we undertook a cytogenetic characterization of the 73AD region where these genes reside. The major purposes of this mutational analysis were to (1) isolate mutations that correspond to the *abl* gene so that its function could be genetically analyzed; and (2) isolate chromosomal breakpoints in the region that could be detected cytologically and/or by Southern blot analysis and that would thus aid in the identification of the location of the *tra* gene within the cloned DNA of the region. By using X-rays as a mutagen we hoped to generate deficiencies of all or part of the 73AD region, isolate inversions or translocations with breakpoints in the 73AD interval, and recover new mutants at the *tra* and *abl* loci. Of special interest were alleles associated with inversion or translocation breakpoints that could be mapped both cytologically and molecularly. In addition to the X-ray mutagenesis experiment, we carried out a mutant hunt using the chemical mutagen, ethylmethane sulfonate (EMS). These combined studies have allowed us to identify 22 complementation groups within the 73AD interval, map them to specific chromosome regions, and correlate the molecular map of cloned DNA with the genetic map.

[1] Current address: Department of Biological Sciences, Stanford University, Stanford, California 94305.

MATERIALS AND METHODS

Drosophila culture: Flies were raised at 25° on a cornmeal-molasses-agar-yeast-propionic acid medium seeded with live yeast. For descriptions of the mutants used, see LINDSLEY and GRELL (1968), LINDSLEY and ZIMM (1986; 1987) or the indicated references.

X-ray mutagenesis: Adult males were collected from a *Ki roe p^p* isogenic third chromosome line and aged 2–7 days prior to irradiation. Groups of 25 flies were etherized, placed in gelatin capsules and subjected to an X-ray dose of 4000 r (Torrex 150 X-ray Inspection System, 120 kV, 5 mA, 0.5-mm plexiglass filter). They were then placed in quarter-pint bottles of media containing about 40 *st^1* or *st^{82c3} e* virgin females. After 3 days, the flies were transferred to fresh bottles for 3 additional days.

An initial attempt to isolate X-ray induced mutations in the 73AD region by irradiating the *st^1*-bearing chromosome was unsuccessful. There was an unusually high degree of embryonic lethality among the F_1 progeny relative to the progeny of similar crosses using nonirradiated parents. Among the surviving adults there was a very low frequency of newly induced *st^-* mutations (only 2 out of approximately 10,000 F_1 adults scored, $2/10{,}000 = 2.0 \times 10^{-4}$, were scarlet-eyed individuals). Among 2853 fertile test crosses (22.8% of the F_1 males were sterile), none showed any evidence of a newly induced mutation in the 73AD region. One explanation for these negative results is that the eggs produced by the *st^1* strain females are defective for some chromosome repair function such that X-ray-damaged paternal chromosomes were not effectively repaired in the zygote, leading to a high frequency of embryonic death, and a low frequency of X-ray-induced mutations among the F_1 progeny.

Use of a strain containing a *st^{82c3} e* third chromosome eliminated the problems encountered with the *st^1* stock. The viability of F_1 embryos was much improved, and the frequency of newly induced *st* mutants was higher ($19/40{,}000 = 4.8 \times 10^{-4}$). The F_1 scarlet individuals were crossed to *Df(3)st-81k17/TM3, Sb Ser p^p e* or *Df(3)st-81k17/TM6B, Hu Tb e ca* flies to confirm the presence of a newly induced *st* mutation or *st* region deficiency, and to make the irradiated chromosome heterozygous with a balancer chromosome, thus establishing a balanced stock. The non-scarlet F_1 males from the above crossing scheme were used to set up the second part of the mutant screen (see Figure 1a).

Approximately 40,000 flies were scored in the F_1, resulting in the isolation of 21 new *st* mutants. A subset of these X-ray-induced *st* mutations were deficiencies involving chromosome region 73A (for a detailed description of these *st* mutants, see TEARLE *et al.* 1989). Flies carrying the newly induced *st* alleles were mated to *Df(3L)st-81k172/TM3, Sb Ser p^p e* or *Df(3L)st-81k172/TM6B, Hu Tb e ca* flies, and the *Ki*-bearing chromosomes recovered and maintained over the balancer. In the second step of the mutant hunt, non-*st* F_1 males were singly crossed to two or three *Df(3L)st-81k172/TM3, Sb Ser p^p e* or *Df(3L)st-81k172/TM6B, Hu Tb e ca* females and the progeny from each vial scored for the presence of recessive mutants (*i.e.*, lethals, visibles or sex-transforming mutants) uncovered by the deficiency. Of 11,221 single F_1 male crosses, 8,431 yielded progeny that could be scored for the occurrence of new mutant alleles. If such mutants were evident, F_2 sibs carrying the *Ki roe p^p* chromosome over *TM3* or *TM6B* were used to establish mutant stocks.

EMS mutagenesis: Males from either a wild-type Canton S strain (2033 chromosomes screened), an isogenic *kar ry red* line (2200 chromosomes screened) or an isogenic *kar red e* line (3300 chromosomes screened) were fed EMS according to the method of LEWIS and BACHER (1968) and crossed to virgin females carrying either *TM3* or *TM6B*. Sons carrying a mutagenized third chromosomes over *TM3* or *TM6B* were crossed individually to *Df(3L)st-e5/TM6B* or *Df(3L)st4/TM6B* virgin females. Progeny from each vial were scored for the presence of newly induced lethal mutations uncovered by the deficiency (see Figure 1b for a diagram of this crossing scheme). The balanced heterozygote siblings from the test crosses were used to establish stocks of each putative recessive lethal. If the nonbalancer class survived, the flies were examined for visible or sex-transformation phenotypes. A subset (700) of the *kar red e* mutagenized chromosomes were tested for sterility phenotypes.

Cytological analysis: Flies carrying the chromosome to be examined were crossed to flies of a wild-type strain (Canton S) and the larvae reared at 18° in uncrowded well-yeasted vials. In most cases, the chromosome of interest was balanced over *TM6B, Hu Tb e ca*, so that the larvae carrying the mutant chromosome could be easily distinguished from those carrying the balancer by virtue of the dominant larval marker, *Tubby*, carried on *TM6B* (CRAYMER 1984). Salivary glands from late third instar larvae were dissected in 0.7% saline, fixed for 45 sec in 45% acetic acid, and transferred to a drop of lacto-acetic orcein on a coverslip. After 5–8 min, a microscope slide was applied and the glands squashed. Chromosome spreads were examined under phase contrast optics using a Zeiss Universal microscope.

Lethal phase determinations: In order to establish the effective lethal periods of lethal mutants, flies carrying the lethal allele of interest heterozygous over a balancer chromosome were crossed to wild-type flies (either Canton S or Oregon R), and virgin females heterozygous for the lethal mutant were collected. These females were then mated to *Df(3L)st-81k172/TM6B* males, eggs were collected, and hatching frequency was scored. In this way, embryonic lethal mutants, uncovered by the deficiency, result in a hatching frequency of about 75% (*i.e.*, one-fourth of the zygotes should be lethal/deficiency). Such outcrossing to wild-type strains greatly improved the hatching frequency in both experimental and control crosses, and more consistent results were obtained among the various trials. In addition, by testing the mutants as hemizygotes (*i.e.*, mutant/deficiency), it is likely that the observed lethal period reflects the phenotype of the mutant allele of interest, and is not the result of another third chromosome mutation lying outside the 73AD region. For each mutant tested, at least 300 eggs were examined. As controls, the unmutagenized isogenic third chromosome was substituted for the lethal alleles in the above crossing scheme.

While the above protocol was useful for identifying the lethal phases of embryonic lethals, a second series of crosses was carried out to better examine cases of larval and/or pupal lethality. Females carrying the lethal mutant balanced over *TM6B, Hu Tb e ca* were crossed to *Df(3L)st-81k17/TM6B, Hu Tb e ca* males. Eggs were collected, transferred to vials, and their fate monitored throughout development. Individuals carrying the mutant over the deficiency could be easily recognized in the larval and pupal stages on the basis of their being wild type for the dominant *Tubby* mutant carried on the balancer. Non-Tubby larvae and pupae were examined under the dissecting microscope for any obvious defects or abnormal phenotypes.

Complementation analyses: Mutant alleles were tested for complementation with the various deficiency chromosomes listed in Table 1, in order to map them to subregions of the 73AD interval. Mutants that mapped to the same

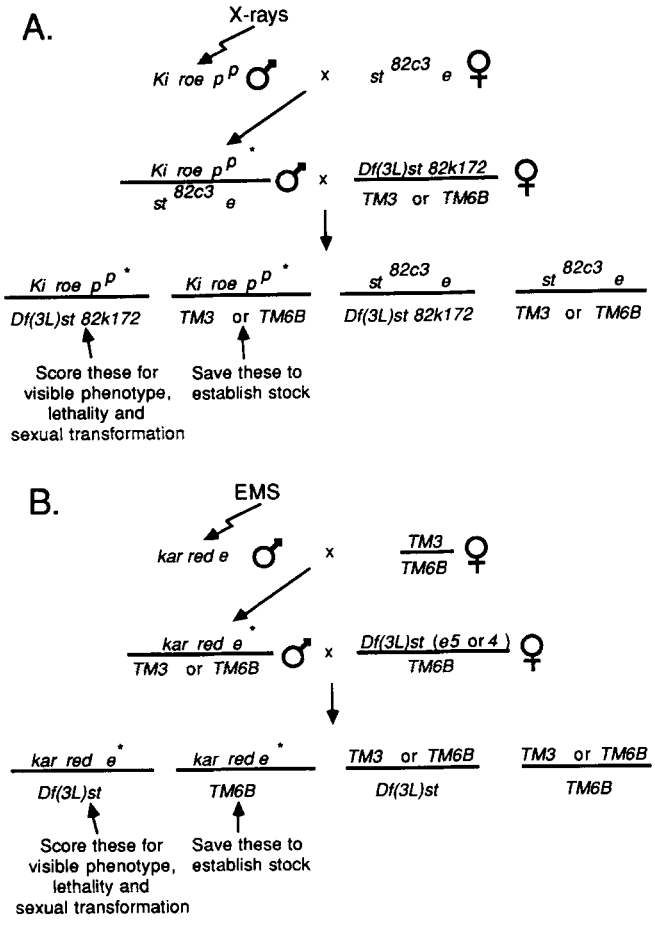

FIGURE 1.—Crossing schemes for mutant isolation. The crossing schemes described in MATERIALS AND METHODS are shown. (*) indicates a mutagen-treated chromosome. A) X-ray mutagenesis. B) EMS mutagenesis.

subregion were then tested for complementation in *trans*-heterozygous combinations. In pairwise combinations where the *trans*-heterozygotes survived, males and females were examined for any obvious visible phenotypes, and then mated to wild-type partners to assess their fertility.

Microscopic analysis of adult flies: The effects of the *plk* mutant on the adult bristle pattern was examined in flies that had been prepared according to the procedure of SZABAD (1978). After dissolving the soft tissues of the adult in 10% KOH, the cuticular structures were washed, dehydrated in propanol, mounted in Euparal between coverslips, and examined under bright field optics.

RESULTS

X-ray mutagenesis screen: The crossing scheme used to isolate X-ray-induced mutations in region 73AD is shown in Figure 1. Of 7920 non-scarlet males crossed to deficiency/balancer females, 1889 (23.9%) were sterile. The progeny from the remaining matings were scored for the presence or absence of the Kinked, nonbalancer class. If this class of progeny was present, then these flies were examined for any visible phenotype that might be the result of a newly induced recessive mutation in the 73AD region. The absence of this class indicates a mutant lesion (*e.g.*, point mutation or deletion) in region 73AD that exhibits a recessive lethal phenotype that is uncovered by the *Df(3)st-81k17* deficiency. From the 6031 crosses that yielded sufficient F_2 progeny to score in this way, 21 lethal mutants, and three visible mutants were recovered.

The 45 X-ray-induced mutants (21 *st* alleles, 21 non-*st* lethals, and 3 non-*st* visibles) were analyzed cytologically to see if they were associated with gross chromosomal rearrangements in the 73AD region. Of the 21 *st* mutants, 13 were associated with chromosome rearrangements (2 inversions, 9 deficiencies and 2 complex rearrangements) involving the 73A3·4 region (Table 1). A detailed description of the *st* mutants isolated in this screen is presented in TEARLE *et al.* (1989). Of the remaining 24 mutants (lethals and visibles), five were associated with chromosome breakpoints in the 73AD region, and the rest were cytologically normal. The results of the cytological analyses are given in Table 1 and Figure 2.

EMS mutagenesis screen: The chemical mutagen EMS is more efficient than X-rays at inducing mutations in Drosophila, but the vast majority of mutant lesions induced with EMS are not cytologically aberrant. Thus, an EMS mutagenesis screen is more useful than the X-ray screen described above for attempting to saturate a region with mutations, but it is not as useful for generating deficiencies and other chromosomal rearrangements that can be used to deletion map a region and to correlate the genetic map of a region with the cytological and molecular maps.

Since the genes of primary interest to us, *tra* and *abl*, are both located within the 73AB region, the EMS mutagenesis screen focused on that region, and not on the larger 73AD interval examined in the X-ray mutant hunt. The *Df(3L)st4* and *Df(3L)st-e5* deficiencies were used to screen for recessive lethals and visibles in 73AB. Males that carried an EMS-treated third chromosome over a balancer were singly mated to females carrying one of these deficiencies over *TM6B, Hu Tb e ca*. The progeny from approximately 7500 such matings were scored for recessive mutants uncovered by the deficiency (Figure lb). A total of 71 recessive lethals were isolated and retested, and balanced stocks were established. In addition, two new *tra* alleles were recovered in this screen. In some cases, the F_2 individuals that carried the mutagenized chromosome/deficiency were crossed to wild-type mates to test for the presence of a recessive sterile mutation uncovered by the deficiency. In this way, one mutant was discovered that behaved as a recessive male and female sterile mutant. Because of the low frequency of chromosome aberrations expected to be produced by EMS mutagenesis, most of the EMS-induced alleles were not examined cytologically.

Deletion mapping and complementation analyses:

TABLE 1

Chromosome rearrangements involving the 73AD region

Name	Cytology	Discoverers
Inversions:		
In(3LR)st a27	73A3,4; 87D13,14	BELOTE and MCKEOWN
In(3)st k21	73A3,4; centric heterochromatin	BELOTE and MCKEOWN
In(3L)plk	73D1,2; 75A1,2	BELOTE and MCKEOWN
In(3L) + Df(3L)st e5	60A - 72E5|73A2; 72F1,2|73C1,2 - 100F	BELOTE and MCKEOWN
In(3L) + Df(3L)std11	72D12,E1|73A10,11; 72E1,2|73D3	BELOTE and MCKEOWN
Translocations:	22A3,8; 73C5,D1	BELOTE and MCKEOWN
T(2;3)std9		
T(Y;2;3)std10	Y|54B10-15;44C2,4|54B10-15-60FY|73B1,2-100F 44C2,4|73B1,2-61A	BELOTE and MCKEOWN
T(2;3)std17	Centric heterochromatin; 73A1,2	BELOTE and MCKEOWN
DEFICIENCIES:		
Df(3L)st a20	73A3; 73A4 (not cytologically visible)	BELOTE and MCKEOWN
Df(3L)st b8	72D5,11; 74B2	BELOTE and MCKEOWN
Df(3L)st b11	72D10,11; 73D1,2	BELOTE and MCKEOWN
Df(3L)st e4	72D5,10; 73A5,8	BELOTE and MCKEOWN
In(3L) + Df(3L)st e5	60A-72E5|73A2; 72F1,2|73C1,2-100F	BELOTE and MCKEOWN
Df(3L)st fl3	72C1,2; 73A3,4	BELOTE and MCKEOWN
Df(3L)st g18	72E3; 74F4	BELOTE and MCKEOWN (1985)
Df(3L)st g24	72D1,2; 73A9,10	BELOTE and MCKEOWN
Df(3L)st j7	73A2,3; 73B1,2	BELOTE and MCKEOWN
Df(3L)st j11	72E2; 73E1,2	BELOTE and MCKEOWN
Df(3L)7P	73A1,2; 73A7,9	J. J. BONNER
Df(3L)tra	73A4; 74A1,2	B. S. BAKER
In(3L) + Df(3L)std11	72D12,E1|73A10,11; 72E1,2|73D3	BELOTE and MCKEOWN
Df(3L)st100.62	72F3,7; 73C1,2	ALEXANDER
Df(3L)E36	?; 73B1,2	BUTLER *et al.* (1986)
Df(3L)E34	73A1,2; 73B3	BUTLER *et al.* (1986)
Df(3L)st4	72D10; 73C1	ASHBURNER *et al.* (1981)
Df(3L)st81k17	73A1,2; 73D1,2	M. M. GREEN
Others:		
Dp(3;3)st⁺g18	72E1,2|74F4; 72E3|74F4; 72E2|75A1,2	BELOTE and MCKEOWN (1985)
Tp(1;3)st g182	1A-5B6,7|3L heterochromatin; 73B1,2|5B6,7-20F	BELOTE and MCKEOWN

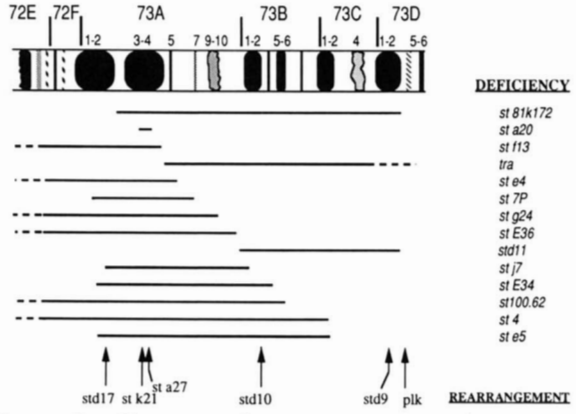

FIGURE 2.—Chromosomal rearrangements used to cytogenetically characterize the 73AD region. Lines represent chromosomal regions deleted in the mutants listed. Dotted lines indicate that the deficiency extends beyond the chromosome region shown. The arrows indicate the positions of rearrangement breakpoints for the inversions and translocations listed.

Figure 2 shows the chromosomal deficiencies, either induced in our X-ray mutagenesis or obtained from other sources, that were used to deletion map mutants within the 73AD region. Crosses were carried out in which mutant/balancer flies were mated to deficiency/balancer flies, and the phenotype of the mutant/deficiency progeny class scored. In this way, each of the new mutant alleles could be assigned to one of 14 subintervals of the 73A2-D2 region (Table 2 and Figure 3). Once the mutants had been deletion mapped in this way, *inter se* crosses were carried out to test each mutant for complementation with the other mutants that mapped to the same subinterval. These complementation tests allowed us to place all of the mutants into 21 complementation groups (17 in the 73A2–B8 region, and 4 within 73C1–D2). While it is unlikely that we achieved complete mutational saturation of the 73AD region, for most of the complementation groups more than one allele was identified.

We have mapped 17 complementation groups within 73AB, a region that is comprised of 17 polytene bands according to the map of BRIDGES (1941). Recently, one additional complementation group, *disabled*, has been identified by the isolation of mutants in the 73B region that interact with *abl* mutants (GERTLER *et al.* 1989).

TABLE 2

Alleles and lethal phases of 73AD loci

Rearrangement	Locus	Allele	Mutagen	Lethal phase[a]	Comment
	l(3)73Aa	1	X-ray	P,PA	
		2-9	X-ray		Aa[6] associated with *T(2;3)std 17*
		10-15	EMS		
Df(3L)st a20, left end					
	l(3)73Ab	1	EMS	P,PA	Order relative to *st* not known
		2-8	EMS		
	st	241	X-ray		One new allele associated with *In(3LR)st a 27*, and one associated with *In(3LR)st k21*
		a24	X-ray		
		a31	X-ray		
		b22	X-ray		*st* mapped to region around position 0 on molecular map
		g202	X-ray		
		g22	X-ray		
		k29	X-ray		
		l15	X-ray		
Df(3L)st fl3, right end					*Df(3L)tra/Df(3L)st e4* viable and fertile, no obvious phenotype
Df(3L)tra, left end					
Df(3L)st a20, right end					
	l(3)73Ac	1	X-ray	L	
		2-5	EMS		
	l(3)73Ad	1	X-ray	L	
	l(3)73Ae	1	X-ray	L	
	l(3)73Af	1	EMS	L	
		2-4	EMS		
	l(3)73Ag	1	EMS	P,PA	
Df(3L)st 7P, right end					
	l(3)73Ah	1	EMS	LL,P	Left of *tra* by transformation
	tra	v1,v2	EMS		
Df(3L)st E52, right end					
	mfs(3)73A		EMS		*Df(3L)st E52* complements male Fertility defect
	l(3)73Ai	1[b]	EMS	L,PA(asDTS)	aka *DTS-5*
		2,3	EMS	L	Right of *mfs(3)73A* by transformation
Df(3L)st g24, right end					
	l(3)73Aj	1	X-ray	E	Associated with a small deletion
Df(3L)st E36, right end					Break point in *abl*
Df(3L)std 11, left end					Break point in *abl*
Df(3L)st j7, right end					Break point in *abl*
	abl	1	EMS	PA	
		2	EMS	PA	Some adults
		3	EMS	PA	Some adults
	l(3)73Bb	1	X-ray	E	Associated with *T(Y;2;3)std 10*
		2-4	EMS		right of *abl*
Df(3L)st E34, right end					
	l(3)73Bc	1	X-ray	E	
		2	EMS		weak allele, Bc^2/Bc^3 survives; Bc^2/Bc^1 survives but eclosion delayed by 3 days
		3	EMS		
	dab[c]	m2	EMS	E,L	Localized by transformation,
		m29	EMS	E,L	Right of *l(3)73Bc*
Df(3L)st 100.62, right end					Break point in *dab*
	l(3)73Bd	1-3			
Df(3L)st 4					
	l(3)73Ca	1	X-ray		Associated with *T(2;3)std 9*
	l(3)73CD	1	X-ray	PA	
		2	X-ray	PA	Some adults with incomplete tergites
		3	X-ray	PA	
	db	1[d]		PA	Many female adults
		e25	X-ray	PA	Many female adults
		L17	X-ray	PA	Many female adults
	plk	1	X-ray		Associated with *In(3L)plk*
Df(3L)st 81k172					

[a] E = embryonic, L = larval, LL = late larval, P = pupul, PA = pharate adult.

[b] *l(3)73Ai* was originally discovered by Holden and Suzuki (1973) on the basis of its dominant temperature sensitive lethal phenotype (DTS). This allele was tested over deficiency (larval lethality) and as a dominant over wild type at the restrictive temperature (pharate adult lethality).

[c] The lethal phase of *dab* was determined by GERTLER *et al.* (1989). Since *dab* alleles were induced on a chromosome mutant for *abl* (*Df(3L)st j7*) the lethal phase determination for *dab* was done in flies that carried *Df(3L)st j7,dab* and *Df(3L)std 11* (both *abl* and *dab* partially or completely deleted). *abl* wild-type function was supplied by an abl^+ transposon.

[d] db^1 isolated by CHOVNICK and TALSMA (1966).

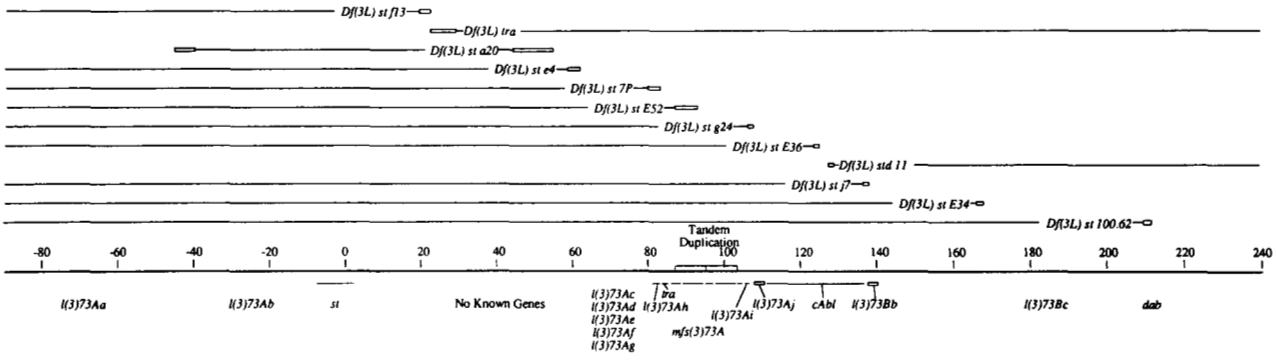

FIGURE 3.—Summary of genetic and molecular informaion about region 73AB. The extent of deletions within regions 73A and 73B is indicated at the top of the figure. Lines indicate the material that is missing and boxes indicate uncertainties with regard to the positions of the end points of deletions. The molecular characterization of these deletions is described elsewhere (BUTLER *et al.* 1986; MCKEOWN, BELOTE and BAKER 1987; HENKEMEYER *et al.* 1987; F. B. GERTLER and F. M. HOFFMANN, unpublished observations). Coordinates are as described in MCKEOWN, BELOTE and BAKER (1987), and begin from the left hand end of *In(3L)st-a27*. The centromere proximal region is to the right, in standard genetic order. Loci are positioned relative to known complementation information as described in Table 2. For genes for which transcriptional information has been published, the extent of the transcription units is indicated by thin lines below the coordinate line. Small boxes below the coordinate line identify sites of rearrangements associated with alleles of certain loci, specifically *l(3)73Aj* and *l(3)73Bb*. The loci *l(3)73Ac* through *l(3)73Ag* have not ben ordered relative to each other. Although *st* has been placed on the molecular map, the position of *l(3)73Ab* relative to it is not not known. Information regarding molecular positions of different loci has been presented elsewhere (BUTLER *et al.* 1986; MCKEOWN, BELOTE and BAKER 1987; HENKEMEYER *et al.* 1987; BELOTE *et al.* 1989; TEARLE *et al.* 1989; K. SAVILLE and J. BELOTE, unpublished observations; F. B. GERTLER and F. M. HOFFMANN, unpublished observations).

Mutant phenotypes: The following is a description of mutant phenotypes for the genes that we have mapped to the 73AD region. For lethal alleles, we describe the effective lethal phase of the hemizygous mutant condition (*i.e.*, mutant/deficiency) (Table 2). This avoids potential complications resulting from homozygosing additional newly induced mutations lying outside the 73AD region and allows a description of the strongest available loss-of-function phenotype for each locus. It should be noted that in most cases the mutants described here have not been mapped by recombinational analysis to confirm that they lie within the 73AD region. It is conceivable that some of the mutant phenotypes we describe are the result of mutations lying outside of the region but that interact in a dominant fashion with deficiencies of a specific locus within the 73AD interval.

l(3)73Aa: All of the alleles of this complementation group are recessive lethals, with hemizygous individuals dying in the late larval to late pupal stages. Some mutant flies survive to the pharate adult stage. Mutant alleles of this complementation group were recovered much more frequently than those of any of the other complementation group in the 73AD region, suggesting that this gene might be quite large. This gene maps to polytene band 73A2–3 by deficiency mapping analysis. One mutant allele, $l(3)73Aa^{6}$, is associated with a translocation, *T(2;3)std17*, with a breakpoint in 73A1·2.

l(3)73Ab: All alleles of this complementation group are recessive lethals with a lethal period in the late larval to late pupal periods. One allele of this gene, $l(3)73Ab^{4}$, has been shown to interact with the sex-transforming mutants *tra* and *tra-2* (D. ANDREW, personal communication). Specifically, diplo-*X* individuals simultaneously heterozygous for *tra-2*/+ and *tra*/+, a genotype that normally leads to wild-type development, frequently develop as intersexes if they are also heterozygous for the $l(3)73Ab^{4}$ allele. In addition, a deficiency which uncovers *l(3)73Ab* and *tra*, when heterozygous with wild type, leads to intersexual development of some *XX;tra-2*/+ individuals. The fact that such an interaction with the *tra-2*/+;*tra*/+ genotype is also seen with several mutants specifically affecting sexual differentiation (*e.g.*, Sxl^{F}, *dsx*, *ix*) has led to the suggestion that it can be used to identify other previously unknown sex differentiation mutants (BELOTE *et al.* 1985). However, since some genes that have no apparent direct role in sex differentiation (*e.g.*, *Minute* loci) also show this interaction, it cannot be used as the sole diagnostic test for sex differentiation mutants. In the case of *l(3)73Ab*, there is no independent evidence that it plays a direct role in sexual differentiation.

This gene lies proximal to *l(3)73Aa*, in polytene band region 73A3·4, since the deletion mutation, *Df(3L)st-a20*, removes both the *scarlet* and *l(3)73Ab* genes but does not remove $l(3)73Aa^{+}$. It has not been determined if this gene lies proximal or distal to *scarlet*.

scarlet: The wild-type product of the scarlet gene is required for the biosynthesis of the brown ommochrome eye pigment, xanthommatin. The exact biochemical role of this gene's product is not known. Mutant alleles at *st* are recessive and result in a bright red eye color phenotype. This gene lies in polytene

band 73A3·4. For a more detailed discussion of this gene, see TEARLE *et al.* (1989).

l(3)73Ac: This is a recessive lethal complementation group with a lethal period during the larval stages. Five alleles of this gene were isolated, one X-ray induced and four EMS induced. This gene lies at least 60 kb proximal to *st* and *l(3)73Ab* since the *Df(3L)st-e4* deficiency does not remove the wild-type allele of *l(3)73Ac*.

l(3)73Ad: Only one mutant allele of this gene, an X-ray-induced mutation, is extant, and it has a lethal period during the larval stages.

l(3)73Ae: The only mutant allele of this complementation group is X-ray induced and has an early larval lethal period.

l(3)73Af: There are four EMS-induced alleles that identify this complementation group. The one allele tested for lethal period ($l(3)73Af^1$) has an early larval lethal period.

l(3)73Ag: The only mutant allele of this complementation group is EMS induced and has a late pupal lethal period. The pupae frequently develop to the pharate adult stage and exhibit poorly developed, or histolyzed, abdomens. By deletion mapping, this gene lies between the *Df(3L)st-e4* and the *Df(3L)st 7P* breakpoints, since $l(3)Ag^1/Df(3L)st\text{-}e4$ flies are normal and $l(3)Ag^1/Df(3L)st\ 7P$ flies fail to survive. However, there appears to be a lethal interaction between the $l(3)Ag^1$-bearing chromosome and alleles of the *l(3)73Ai* complementation group, which lies well to the right of the *Df(3L)st 7P* breakpoint (see below). For example, *trans*-heterozygotes carrying the $l(3)Ag^1$ mutant over any of the three extant mutant alleles of *l(3)Ai* fail to survive beyond the late pupal period, even though each of the *l(3)Ai* mutant alleles is viable over the *Df(3L)st 7P* deficiency. The phenotype of the dead pupae is similar to the phenotype of *l(3)73Ag* homozygotes. One explanation for this lethality of *trans*-heterozygotes is that there are two independent mutants, one at *l(3)73Ag* and one at *l(3)73Ai*, present on the *l(3)73Ai*-bearing chromosome. Alternatively, it could be due to a lethal interaction between the $l(3)73Ag^1$ mutant allele and the loss of the *l(3)73Ai* wild-type function. Our data do not distinguish between these two possibilities.

The order of the *l(3)73Ac*, *l(3)73Ad*, *l(3)73Ae*, *l(3)73Af* and *l(3)73Ag* genes with respect to each other is not known, but they are all proximal to *st* and lie in the polytene band region 73A5 to 73A9. Molecular analysis of the breakpoints that delimit this region shows that all five of these genes lie within a ~20-kb region (MCKEOWN, BELOTE and BAKER 1987).

l(3)73Ah: The only mutant allele of this gene is EMS induced and is a recessive late pupal lethal mutant. Flies hemizygous for this mutant frequently develop to the pharate adult stage and exhibit a phenotype similar to that of $l(3)73Ag^1$ (poorly developed or degenerated abdomens). Molecular studies have shown that this gene lies just distal to the *transformer* gene and that the 3′ end of *l(3)73Ah* actually overlaps the 3′ end of the *tra* gene (MCKEOWN, BELOTE and BAKER 1987). A 3.8-kb restriction fragment carrying both this gene and *tra* rescues the mutant phenotype for both genes (K. SAVILLE and J. BELOTE, unpublished observations). There does not appear to be a functional relationship between *tra* and *l(3)73Ah* despite their close proximity (MCKEOWN, BELOTE and BAKER 1987). A deletion mutant of *tra* which removes the extreme 3′ noncoding region of *l(3)73Ah* retains wild-type *l(3)73Ah* function and produces wild-type or near wild-type size *l(3)73Ah* mRNA (M. MCKEOWN and J. M. BELOTE, unpublished observation).

transformer: This is a sex-transforming mutant that when hemizygous or homozygous transforms chromosomally female individuals (*i.e.*, *XX*) into phenotypically male flies with respect to all aspects of their somatic development (STURTEVANT 1945; BAKER and RIDGE 1980). The *tra* mutant has no apparent effect in chromosomally male flies (*i.e.*, *XY*). This is a previously identified gene that was known to map in the 73AD region. In our screen we isolated two EMS alleles of *tra* but failed to recover any X-ray-induced alleles. Both of the EMS-induced *tra* alleles behave as loss-of-function alleles. For further details of the molecular genetics of this locus see BUTLER *et al.*, 1986; MCKEOWN, BELOTE and BAKER 1987; MCKEOWN, BELOTE and BOGGS 1988; BELOTE *et al.* 1989; SOSNOWSKI, BELOTE and MCKEOWN 1989.

mfs(3)73A: The only mutant that defines this complementation group is an EMS-induced allele that has a recessive sterile phenotype in both males and females. Males hemizygous for this mutation produce mature-looking, but nonmotile sperm. Hemizygous females mate and store motile sperm, but they do not lay any eggs. Their ovaries do contain some well-developed öocytes; however, there are not as many as in wild type. From these phenotypes, it is not readily apparent what the the primary defect is in this mutant that leads to sterility in both sexes.

l(3)73Ai: The original allele defining this complementation group was discovered by HOLDEN and SUZUKI (1973) and exhibits a dominant temperature-sensitive lethal phenotype. This allele, originally named *DTS-5* and now called $l(3)73Ai^1$, kills heterozygous individuals during the late pupal stage when the cultures are maintained at 29°. At ambient temperatures of 25° or lower, the heterozygotes are wild type in phenotype. One characteristic abnormality of the $l(3)73Ai^1/+$ dead pharate adults is poorly developed or degenerated abdominal segments, similar to $l(3)73Ag^1$ and $l(3)73Ah^1$ hemizygotes and $l(3)73Ag^1/l(3)73Ai^-$ *trans*-heterozygotes described above. The

anterior portions of the dead pharate adults appear normal. This allele also behaves as a nonconditional recessive lethal mutant with a lethal period during the early larval stages.

Two other alleles of this gene were isolated in our screens. Both were EMS induced and exhibit the non-temperature-sensitive recessive lethal phenotype similar to *l(3)73Ai*^{1}. Neither of these alleles has a dominant phenotype. These mutants have early larval lethal periods.

Both *mfs(3)73A* and *l(3)73Ai* map proximal to *tra* by approximately 20 kb. Molecular studies indicate that they represent two adjacent transcription units covering less than 4 kb (McKeown, Belote and Baker 1987; K. Saville and J. Belote, unpublished observations).

l(3)73Aj: There are three alleles representing this complementation group, one X-ray induced and two EMS induced. These mutants are recessive, with an effective lethal period during the embryonic stage. The X-ray-induced allele, *l(3)73Aj*^{1}, has been shown to be associated with a small deletion just distal to the *abl* transcription unit (Henkemeyer *et al.* 1987).

Abelson gene: This gene, abbreviated *abl*, was originally identified as a sequence that cross-hybridizes to the mammalian *c-abl* oncogene (Hoffman-Falk *et al.* 1983). The transcription unit containing the *c-abl*-like sequences has been shown to correspond to a gene, *abl*, defined by three late pupal lethal alleles (Henkemeyer *et al.* 1987). These mutants kill hemizygotes in the pharate adult stage. Adult escapers are sometimes observed, and these flies exhibit reduced longevity, reduced fecundity and roughened patterns of eye facets. This gene has been shown by *in situ* hybridization techniques and by the overlap of deficiencies *Df(3L)st-7* and *Df(3L)std11* to reside in polytene band 73B1·2.

l(3)73Bb: An X-ray-induced mutant allele belonging to this complementation group is a translocation, *T(Y;2;3)std10*, with a breakpoint in polytene band 73B1·2 (Figure 2 and Table 1). This breakpoint has been mapped molecularly to a position just proximal to the *abl* transcription unit (Henkemeyer *et al.* 1987). Hemizygotes for this mutant die in the embryonic or early larval stages. There are three other EMS-induced alleles of this complementation group.

l(3)73Bc: There are three alleles of this gene, one X-ray induced and two EMS induced. The X-ray-induced mutant exhibits a recessive embryonic lethal phenotype. The EMS mutations show evidence of interallelic complementation. *l(3)73Bc*^{2} complements *l(3)73Bc*^{3} and, when heterozygous with *l(3)73Bc*^{1}, delays eclosion of adult flies by 3 days.

disabled: This gene, abbreviated *dab*, was identified through its dominant interaction of mutant alleles with mutations in the *abl* gene (Gertler *et al.* 1989). *dab* mutations produce an early larval lethality associated with subtle defects in the axon bundles of the embryonic central nervous system. Animals doubly mutant for *abl* and *dab* do not develop axon bundles in the embryonic central nervous system and die as embryos. DNA containing *l(3)73Bb*, *l(3)73Bc* and *dab* transcription units, and crossing the breakpoint of *Df(3L)st-100.62* has been cloned and is being characterized (F. B. Gertler and F. M. Hoffmann, unpublished observations). We do not understand why the only *dab* alleles to be recovered were isolated in screens for mutations that interact with *abl*, and that no *dab* alleles were isolated in the screens for recessive lethals described here.

l(3)73Bd: There are three EMS-induced alleles in this lethal complementation group.

l(3)73Ca: This complementation group is defined by a single X-ray-induced allele that has a translocation breakpoint in salivary chromosome band 73C5-D1·2 (*i.e.*, the *l(3)73Ca*^{1} allele is associated with *T(2:3)std9*, Figure 2 and Table 1).

l(3)73CD: This complementation group is defined by three X-ray-induced alleles with late pupal lethal periods. One of these alleles, *l(3)73CD*^{2}, gives a significant number of adult escapers that frequently exhibit incomplete tergite differentiation.

dark body: The original mutant allele that defines this complementation group is a spontaneous mutant, *db*^{1}, discovered by Chovnick and Talsma (1966). Females homozygous for this mutant have a darkened cuticle similar to, but somewhat lighter than, the mutant *ebony*, and exhibit slightly reduced viability. Such flies sometimes have curled or blistered wings, and their fecundity is reduced. Homozygous males do not survive to the adult stage. Most of these males die as pharate adults with darkened cuticles. Both of these phenotypes of *db*^{1} map to the same region of the chromosome, 73C1 to 73D1·2, as determined by deficiency mapping. Two additional alleles of *db* were induced in our X-ray mutagenesis, *db*^{e25} and *db*^{L17}, and both of these have phenotypes similar to *db*^{1}. This observation strongly suggests that the two phenotypes, darkened cuticle and male-specific lethality, are due to lesions in a single gene, and are not the result of separate, closely linked mutations.

plucked: The single mutant allele of this gene is X-ray induced and is associated with an inversion breakpoint in chromosome band 73D1·2. Hemizygous *plk/Df* flies have many fewer bristles than wild type, and this is especially noticeable over the abdominal segments (Table 3 and Figure 4a). For example, in mutant flies the average number of bristles on the abdominal tergites is only about 20–65% of the wild-type number, depending on which segment is considered. The abdominal bristles that do form in *plk/DF* flies tend to be situated in the lateral and posterior regions

TABLE 3

Effect of the *plucked* mutant on bristle number in *Ki*/+ flies

Region of the fly	Sex	Mean bristle number in *plk*⁺ *Ki/Df*	(n)	Mean bristle number in *plk Ki /Df*	(n)	% of wild type
Abdominal tergites						
1st	Male	97.8 ± 10.7	5	22.2 ± 6.1	5	22.7
2nd	Male	87.2 ± 14.9	5	57.4 ± 4.3	5	65.8
3rd	Male	87.0 ± 14.9	5	49.8 ± 7.9	5	57.2
4th	Male	83.4 ± 9.2	5	37.8 ± 5.4	5	45.3
5th	Male	90.8 ± 13.8	5	43.0 ± 10.2	5	47.4
6th	Male	85.6 ± 15.4	5	48.4 ± 11.1	5	56.7
1st	Female	112.4 ± 19.2	5	17.3 ± 3.4	5	15.0
2nd	Female	97.6 ± 12.5	5	51.8 ± 11.7	5	53.1
3rd	Female	106.0 ± 12.1	5	39.8 ± 17.4	5	36.8
4th	Female	99.4 ± 17.7	5	33.8 ± 5.6	5	34.0
5th	Female	101.4 ± 13.7	5	31.0 ± 9.3	5	30.6
6th	Female	110.6 ± 8.0	5	33.8 ± 9.8	5	30.4
7th	Female	48.8 ± 8.7	5	12.8 ± 3.8	5	26.2
Femur	Female	101.4 ± 12.3	5	71.4 ± 8.0	5	70.4
Occipital	Female	38.3 ± 5.3	6	22.0 ± 2.0	6	57.4
Triple row (wing)	Female	82.7 ± 6.7	7	19.7 ± 2.1	7	23.8

of the tergites. The bristles also exhibit an abnormal morphology; they are frequently split and shortened relative to those in their heterozygous sibs. It should be noted that the phenotype of *plk/DF* has been examined only in flies that are also heterozygous for the closely linked *Kinked* mutant, which also affects bristle morphology. It is thus possible that the effect of *plk* on bristle number and/or morphology involves an interaction with the *Ki* mutation. One region of the fly in which the plucked phenotype is very conspicuous is at the wing margins, where the mutant flies have only about a fourth as many triple row bristles as in wild type (Figure 4, b and c). Bristle number is also reduced over other regions of the fly, although in some body parts the difference between mutant and wild type is not particularly striking (Table 3). In addition to the bristle phenotype, the *In(3L)plk/Df* hemizygotes have an abnormal wing venation phenotype (*i.e.*, they have incomplete posterior crossveins, Figure 4, b and c). Moreover, males hemizygous for *plucked* frequently exhibit patches of pigmentation on the anterior regions of their fourth tergites, unlike wild-type males which are normally unpigmented in this portion of their cuticle. Many of the mutant males also have disorganized sex comb bristles. Females that carry the *plk* mutant chromosome over a deficiency for this region are sterile, due to a maternal effect lethal phenotype that kills progeny in the late embryo to early larval stages.

The relative order of the *plucked*, *dark body* and *l(3)73CD* genes is not known, but they all are proximal to the *Df(3L)st4* deletion breakpoints (*i.e.*, to the right of 73C1), and are at or distal to the breakpoint of *Df(3L)st-81k172* at 73D1·2.

DISCUSSION

In this study, two different mutagenesis experiments were carried out. One of these utilized X-rays as the mutagenic agent and was aimed primarily at generating chromosome rearrangements with breaks in the 73AD region. The other mutant screen, using EMS, was designed to saturate the 73AB region with mutations in order to define the number, organization and phenotypes of the complementation groups that surround *tra* and *abl*. One limitation of this approach is that any genes whose mutant phenotypes are not easily scorable (*e.g.*, behavioral changes and changes in nonessential enzyme activities) are likely to be missed. Another complication arises if a gene in the region of interest exists in the genome in more than one functional copy. In that case, it would be necessary to simultaneously mutate both copies of the gene for the mutant phenotype to appear, and thus identify the complementation group. In the present study, our molecular analysis of the region flanking *tra* has revealed a duplication of approximately 8 kb that contains one or possibly two duplicated transcription units (McKeown, Belote and Baker 1987). Thus, if the duplicated transcription units are functionally equivalent or interchangeable, then we probably have failed to isolate mutants that identify the genetic functions associated with these. No other repeated genes have been identified in the cloned portions of the 73AB region (McKeown, Belote and Baker 1987).

If one assumes that all genes in a particular region are equally mutable, then an equation which approximates the Poisson distribution ($n_0 = n_1^2/2n_2$, where n_0 is the number of complementation groups for which no mutant alleles have been found, n_1 is the

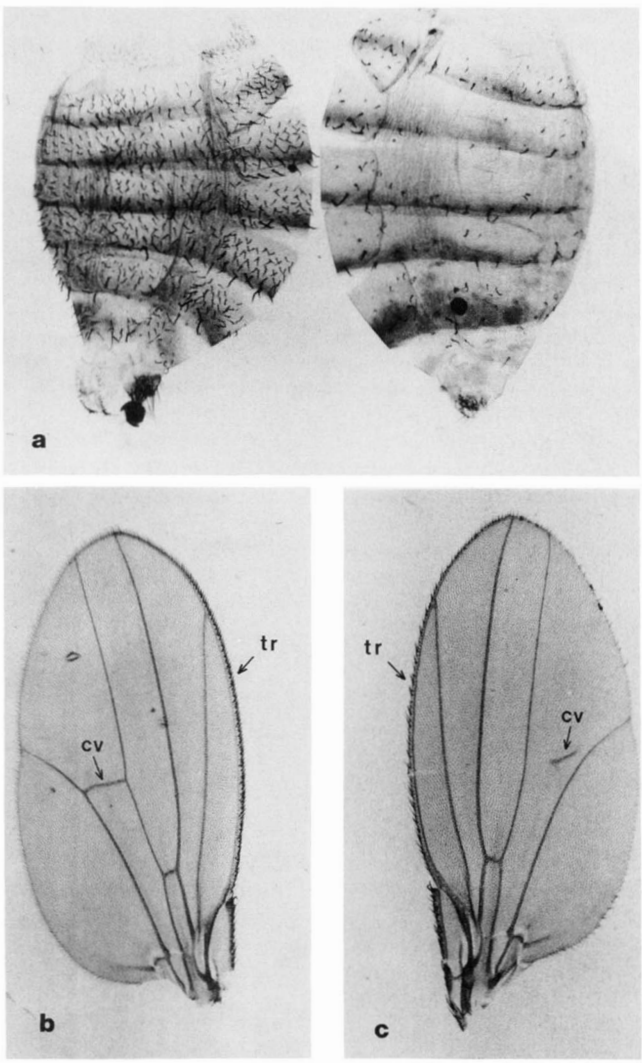

FIGURE 4.—Phenotype of *plk*. *In(3L)plk, plk Ki roe* p^p*/Df(3L)st-81k172* individuals were examined as described in MATERIALS AND METHODS a) Mounted abdomens from plk^+ (left) or *plk* (right) adults. The number of bristles is severely reduced in *plk* individuals. Note that both flies are heterozygous for *Kinked* which causes the bristles to be shorter than normal but which has no major effect on bristle number. b and c) Wings from plk^+ (b) and *plk* (c). cv, posterior crossvein; tr, triple row bristle. Note the incomplete crossvein and the reduced number of triple row bristles in the mutant.

number with only one mutant allele, and n_2 is the number of complementation groups with two or more mutant alleles) can be used to estimate the number of loci in that region (LEICHT and BONNER 1988). Using this calculation, we estimate that there are one or two as yet undiscovered complementation groups in the 73A2–73B7 interval that have mutant phenotypes that could have been recognized in our mutant screens. If the data from the EMS- and X-ray-induced mutants are considered separately, the above relation still predicts approximately the same number (15–22) of mutable loci as the prediction based on the pooled data. The region from 73B7 to 73D was not examined by EMS mutagenesis and is therefore likely to contain a number of unidentified loci. The fact that we recovered at least two alleles for each of the five genes that we knew beforehand mapped to the region [*i.e.*, *st, tra, abl, l(3)Ai* (formerly called *DTS-5*)] supports the notion that there are not a large number of undiscovered complementation groups in this region that can mutate to an easily scorable phenotype.

For region 73A2–73B7, for which the mutagenesis was more extensive than in the larger 73AD region, we were able to define 18 complementation groups. According to BRIDGES' (1941) cytological map of this region, there are 17 polytene bands in this interval. Thus, like several other regions of the Drosophila genome that have been subjected to saturation mutagenesis, this region shows a good correlation between the number of genes, as defined by mutationally identifiable complementation groups, and the number of chromomeres in the salivary gland chromosomes (LEFEVRE 1974; SPIERER 1984). It should be noted, however, that this correlation is not perfect for all of the regions that have been analyzed, and the relationship between genes and bands is still unclear (WRIGHT *et al.* 1981; LEFEVRE and WATKINS 1986).

The mutant phenotype of *tra* is formally similar to that of homeotic loci in that one normal developmental pathway is inappropriately substituted for another. A number of the genes controlling segment identity and number are known to be grouped together in complexes such as the Bithorax Complex (LEWIS 1978) and the Antennapedia Complex (KAUFMAN, LEWIS and WAKIMOTO 1980). As such, it was at least a formal possibility that there might be a complex of genes controlling sexual differentiation in the region of *tra*. The data presented in this paper argue strongly against this hypothesis. The six genes to the left of *tra* and the seven genes to the right have no sex-specific phenotypes. The only genes in our screens, other than *tra*, that show sex-specific differences are *l(3)73Ab*, the sex-specific phenotype of which is revealed only as a result of its interaction with mutants for both *tra* and *tra-2*, and *db*, a gene that appears to be necessary in both sexes with males being more sensitive to loss of gene function than females. The lack of a sex determination gene complex near *tra* is similar to what has been observed for both *Sex lethal* (NICKLAS and CLINE 1983) and *doublesex* (B. S. BAKER, T. HAZELRIGG and G. HOFF, submitted for publication).

This research was supported by grants from the National Institutes of Health to J.M.B., F.M.H., M.M., and B.S.B., as well as grants from the National Science Foundation to B.S.B., and from the American Cancer Society to F.M.H. During part of this work, M.M. was a Helen Hay Whitney Foundation Postdoctoral Fellow. M.M. is currently a Pew Foundation Scholar in the Biomedical Sciences at The Salk Institute, and part of his work there was supported by Cancer Center Core Grant CA-14195.

LITERATURE CITED

ASHBURNER, M., P. ANGEL, C. DETWILER, J. FAITHFULL, D. GUBB, G. HARRINGTON, T. LITTLEWOOD, S. TSUBOTA, V. VELISSARIOU and V. WALKER, 1981 New mutants. Drosophila Inform. Serv. **56:** 186–191.

BAKER, B. S., and K. RIDGE, 1980 Sex and the single cell: on the action of major loci affecting sex determination in *Drosophila melanogaster*. Genetics **94:** 383–423.

BELOTE, J. M., and M. MCKEOWN, 1985 Post-replicative repair of an X-ray damaged chromosome following fertilization in *Drosophila melanogaster*. Drosophila Inform. Serv. **61:** 33–34.

BELOTE, J. M., M. MCKEOWN, D. J. ANDREW, T. N. SCOTT, M. F. WOLFNER and B. S. BAKER, 1985 Control of sexual differentiation in *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **50:** 605–614.

BELOTE, J. M., M. MCKEOWN, R. T. BOGGS, R. OHKAWA and B. A. SOSNOWSKI, 1989 The molecular genetics of *transformer*, a genetic switch controlling sex differentiation in *Drosophila*. Dev. Genet. **10:** 143–154.

BRIDGES, P. N., 1941 A revised map of the left limb of the third chromosome of *Drosophila melanogaster*. J. Hered. **32:** 299–300.

BUTLER, B., V. PIRROTTA, I. IRMINGER-FINGER and R. NÖTHIGER, 1986 The sex-determining gene *tra* of *Drosophila*: molecular cloning and transformation studies. EMBO J. **5:** 3607–3613.

CHOVNICK, A., and J. TALSMA, 1966 New mutant: *dark body*. Drosophila Inform. Serv. *41:* 58.

CRAYMER, L., 1984 New mutants. Drosophila Inform. Serv. **60:** 234.

GERTLER, F. B., R. L. BENNETT, M. J. CLARK and F. M. HOFFMANN, 1989 Drosophila *abl* tyrosine kinase in embryonic CNS axons: a role in axonogenesis is revealed through dosage-sensitive interactions with *disabled*. Cell **58:** 103–113.

HENKEMEYER, M. J., F. B. GERTLER, W. GOODMAN and F. M. HOFFMANN, 1987 The *Drosophila* Abelson proto-oncogene homolog: identification of mutant alleles that have pleiotropic effects late in development. Cell **51:** 821–828.

HOFFMAN-FALK, H., P. EINAT, B.-Z. SHILO, and F. M. HOFFMANN, 1983 *Drosophila melanogaster* DNA clones homologous to vertebrate oncogenes: evidence for a common ancestor to the *src* and *abl* cellular genes. Cell **32:** 589–598.

HOFFMANN, F. M., L. D. FRESCO, H. HOFFMAN-FALK and B.-Z. SHILO, 1983 Nucleotide sequences of the Drosophila *src* and *abl* homologs: conservation and variability in the *src* family oncogenes. Cell **35:** 393–401.

HOLDEN, J. J. A., and D. T. SUZUKI, 1973 Temperature sensitive mutations in *Drosophila melanogaster*. XII. The genetic and developmental effects of dominant lethals on chromosome 3. Genetics **73:** 445–458.

KAUFMAN, T. C., R. LEWIS and B. WAKIMOTO, 1980 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homoeotic gene complex in polytene chromosome interval 84A,B. Genetics **94:** 115–133.

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

LEFEVRE, G., and W. WATKINS, 1986 The question of the total gene number in *Drosophila melanogaster*. Genetics **113:** 869–895.

LEICHT, B. G., and J. J. BONNER, 1988 Genetic analysis of chromosomal region 67A–D of *Drosophila melanogaster*. Genetics **119:** 579–593.

LEWIS,E. B., 1978 A gene complex controlling segmentation in *Drosophila melanogaster*. Nature **276:** 565–570.

LEWIS. E. B., and Bacher, 1968 Method for feeding ethyl methane sulfonate (EMS) to Drosophila males. Drosophila Inform. Serv. **43:** 193.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*. Part 2: lethals; maps. Drosophila Inform. Serv. **64.**

LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*. Part 3: rearrangements. Drosophila Inform. Serv. **65.**

MCKEOWN, M., J. M. BELOTE and B. S. BAKER, 1987 A molecular analysis of *transformer*, a gene in *Drosophila melanogaster* that controls female differentiation. Cell **48:** 489–499.

MCKEOWN, M., J. M. BELOTE, and R. T. BOGGS, 1988 Ectopic expression of the female *transformer* gene product leads to female differentiation of chromosomally male *Drosophila*. Cell **53:** 887 -895.

MCKEOWN, M., J. M. BELOTE, D. J. ANDREW, T. N. SCOTT, M. F. WOLFNER and S. S. BAKER, 1986 Molecular genetics of sex determination in Drosophila, pp. 3–17 in *Gametogenesis and the Early Embryo* (Society for Developmental Biology Symposium 44), edited by J. G. GALL. Alan R. Liss, New York.

NICKLAS, J. A., and T. W. CLINE, 1983 Vital genes that flank *Sex-lethal*, an X-linked sex-determining gene of *Drosophila melanogaster*. Genetics **103:** 617–631.

SOSNOWSKI, B. A., J. M. BELOTE and M. MCKEOWN, 1989 Sex-specific alternative splicing of RNA from the *transformer* gene results from sequence-dependent splice site blockage. Cell **58:** 449–459.

SPIERER, P., 1984 A molecular approach to chromosome organization. Dev. Genet. **4:** 333–339.

STURTEVANT, A. H., 1945 A gene in *Drosophila melanogaster* that transforms females into males. Genetics **30:** 297–299.

SZABAD, J., 1978 Quick preparation of *Drosophila* for microscopic analysis. Drosophila Inform.Serv. **53:** 215.

TEARLE, R. G., J. M. BELOTE, M. MCKEOWN, B. S. BAKER and A. J. HOWELLS, 1989 Cloning and characterization of the *scarlet* gene of *Drosophila melanogaster*. Genetics **122:** 595–606.

WRIGHT, T. R. F., W. BEERMANN, J. L. MARSH, C. P. BISHOP, R. STEWARD, B. C. BLACK, A. D. TOMSETT and E. Y. WRIGHT, 1981 The genetics of dopa decarboxylase in *Drosophila melanogaster*. IV. The genetics and cytology of the 37B1O-37D1 region. Chromosoma **83:** 45–58.

Communicating editor: R. E. DENELL