*TEMPERATURE-SENSITIVE MUTATIONS IN DROSOPHILA MELANOGASTER, III. DOMINANT LETHALS AND SEMILETHALS ON CHROMOSOME 2**,†*

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Abstract.—Dominant temperature-sensitive lethal and semilethal (*DTS-L*) mutations induced by ethyl methanesulfonate have been recovered in chromosome 2 of *Drosophila melanogaster*. The 19 (0.39% of the chromosomes screened) characterized had greater than 65 per cent viability at 22° ± 2°C and less than 5 per cent at 29° ± 0.5°C. Fifteen of the *DTS-L*'s map genetically as point mutants, one appears to be a synthetic lethal, and the remaining three yielded sterile females. Ten different *DTS* mutants had a high survival in triploid females at 29°C. Eleven *DTS-L*'s were found to map in the same genetic region (*dp-b*). All behaved as recessive lethals at 22°C and failed to survive at 22°C when combined with mutants *3* and *12*. Crosses of the 11 mutants in all possible pairwise combinations yielded a circular complementation map with a side branch. The effective lethal phases and temperature-sensitive periods during development were determined for 15 of the mutants.

The phenomenon of dominant lethality induced by radiation,[1] chemical treatment,[2] or by "genetic contrivance"[3] has been extensively studied in higher organisms. These studies indicate that dominant lethality occurs very early in embryogenesis and may be the consequence of chromosome rearrangement or loss.[4] The recent demonstration that conditional lethal mutations of the temperature-sensitive (*ts*) class are readily induced in *Drosophila melanogaster*[5] prompted us to determine whether dominant *ts* lethals, which are point mutants, could be detected and characterized genetically.[6] The recovery of *DTS-L* mutations would provide a means of comparing the properties of dominant and recessive lethals. In addition, the recovery of dominant conditionally lethal or sterile mutants may provide a means of studying loci such as genetically redundant regions not previously detectable by recessive mutations.

Methods and Materials.—Screening technique: Males of isogenic Oregon-R and Samarkand[7] wild-type stocks were collected within 48 hr of eclosion and fed 0.025 *M* ethyl methanesulfonate (EMS) in 1% sucrose for 24 hr as described previously.[5] After each EMS feeding, ten of the males were crossed to attached-X-bearing females at room temperature (22° ± 2°C). Since previous studies[2] showed that the frequency of autosomal lethals can be deduced from sex-linked lethal frequencies, further crosses were made only if the sex ratio (♂/♀) in this cross was less than 0.50.

The complete screening protocol for the detection of *DTS* lethals on chromosome 2 is shown in Figure 1. 10–15 EMS-treated males were mated with 20–30 virgin *Cy/Pm* females. The matings were made in quarter-pint bottles at room temperature, and the parents discarded after 6 days. *Cy* and *Pm* are inversions of chromosome 2 associated with dominant phenotypes of curled wings and dark-brown variegated eyes, respectively. Both inversions are lethal in the homozygous condition. F_1 *Cy*/+* (where +* denotes a treated chromosome) males were individually mated in shell vials at room temperature with three virgin *Cy/Pm* females. After 4 days, the adults were transferred to fresh vials,

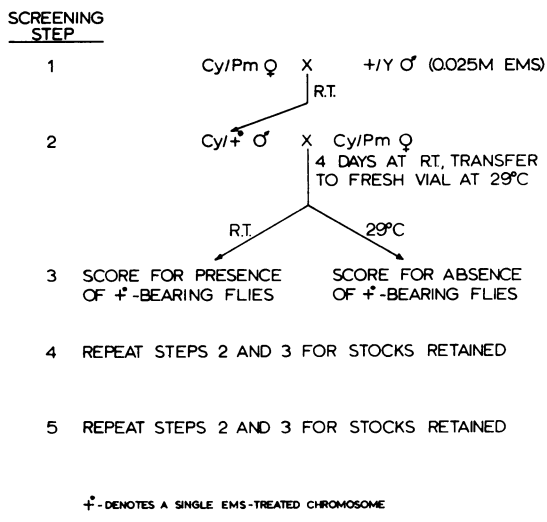

FIG. 1.—Screening protocol for the recovery of *DTS* lethal and semilethal mutations on chromosome 2.

which were incubated at 29°C ± 0.5°C. The 29°C culture vials were scored, without etherization, 12 days later for the absence of $Pm/+^*$ flies. Flies in the 29°C culture vials retained after this initial screening step were then etherized and scored for the presence of $+^*$-bearing individuals. Cultures from those males yielding fewer than three offspring carrying the $+^*$ chromosome at 29°C and more than five at room temperature were retained. Five to seven males carrying the treated chromosome from the room-temperature vial of each stock were individually crossed at room temperature to three virgin *Cy/Pm* females. These adults were then transferred to fresh vials at 29°C, and the offspring were scored at both temperatures for each vial. Vials in which fewer than 5% of the offspring at 29°C and more than 50% of the offspring at room temperature carried the $+^*$ chromosome were classified as putative *DTS* lethal or semilethal strains (henceforth referred to as *DTS-L*). Seven males from room-temperature vials that had the lowest survival of the $+^*$ chromosome at 29°C were retested, as in the initial screening, and the lines which continued to yield reduced survival values were retained as confirmed *DTS-L*'s. In addition, step 2 (Fig. 1) was also carried out for all putative *DTS-L* mutants at 17°C. All the confirmed second-chromosome *DTS-L* mutants were maintained at room temperature as heterozygotes with *Cy*.

Survival in triploids: To measure the degree of dominance of the mutants, the viabilities of ten different *DTS-L*-bearing chromosomes and one Oregon-R chromosome were scored in triploid females at 29°C. Each chromosome was tested in crosses made in ten quarter-pint bottles. In each bottle, 10 +/Y; *Cy/DTS-L* males were crossed to 20 triploid females that carried an attached-X chromosome homozygous for *y*, *w*, and fa^{no} and the multiply inverted rod X, *FM-6*, marked with *y* and *B*.[7] Thus, wild-type females were triploid, *B* females were diploid, and diploid males were *y B*. The presence of the *DTS-L* in the parent was confirmed by the absence of the non-*Cy* phenotype in the diploid offspring. The relative viability of the *DTS-L* in the diploid sibs provided a control for possible modifiers of lethality introduced from the triploid stock.

Genetic mapping: The *DTS* mutants were mapped genetically with the markers *al*, *dp*, *b*, *pr*, *c*, *px*, and *sp* (*all*),[7] which span almost the entire second chromosome. *Cy/DTS-L* males were crossed to *Cy/all* females at room temperature, and the F_1 *DTS-L/all* females were backcrossed in quarter-pint bottles at 29°C to *Cy/all* males; all progeny were scored.

Temperature effects: Eggs from each stock were collected by placing approximately 50 pairs of *DTS-L*-bearing flies for 1–2 hr in an empty quarter-pint milk bottle inverted over a Petri plate containing standard *Drosophila* medium and grape juice for a darkened background. The effective lethal phase was determined by inspecting 29°C cultures every 12 hr for the occurrence of death (Fig. 2). The temperature-sensitive period, the developmental stage during which incubation at 29°C results in death, was delineated by reciprocal shifts of different cultures of each stock from 29°C to room temperature (shift-down) and vice versa (shift-up) at successive 6-hr intervals for the first 24 hr and at 24-hr intervals thereafter. All tests were corrected for differences in developmental rates relative to room temperature.

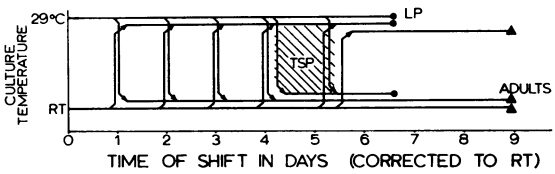

FIG. 2.—Protocol for shift experiments to determine the temperature-sensitive period and effective lethal phase of *DTS-L19*. *RT*, room temperature; *TSP*-temperature-sensitive period; *LP*, lethal phase.

Results.—Of 4857 F_1 males which were fertile at both 29°C and room temperature, 19 were shown to carry *DTS* lethals after step 3 (Fig. 1) in the screening procedure. Mutants 1 to 5 were induced in Oregon-R males and the rest in a Samarkand stock. In three of the *DTS-L* stocks, *Cy/DTS-L* females were found to be sterile and therefore intractable to simple genetic localization. In a cross of $Cy/+ \sigma^{\prime} \times Cy/Pm \varphi$, the expected ratio of +-bearing to the total number of offspring is 0.67 (since the *Cy/Cy* class is lethal) if the + chromosome had no detrimental effect in the heterozygote, a ratio closely approximated by the controls (Table 1*a*). The viability of each *DTS-L* has been estimated relative to the observed control values (Table 1*b*). Only two of the *DTS-L*'s had a relative viability less than 88 per cent at room temperature, and all had less than 5 per cent viability at the restrictive temperature, nine being complete lethals.

None of the flies carrying *DTS-L* mutations in the homozygous condition survived at room temperature, a result which could be due to recessive lethals elsewhere in the chromosome.

The results of the tests of *DTS-L* viability at 29°C in triploid females are shown in Table 2. Although yields from each bottle were low, it is evident that the viability of each mutant was enhanced considerably in the presence of two doses of the wild-type alleles. This is unlike the findings with yeast, in which the frequencies of dominant lethals were the same in diploids and triploids.[8] Viabilities of all but one of the *DTS-L*'s tested were greater than 60 per cent in triploid females.

Recombination tests permitted assignment of each *DTS-L* mutation to a particular genetic region (except for *DTS-L8* which appears to be a synthetic lethal comprised of at least three components). However, poor viability of crossover progeny carrying several of the *all* markers at 29°C precluded an accurate localization of the mutants within a region, and the indicated positions therefore are very crudely estimated. Crosses between *DTS-L* mutations that mapped in different regions yielded transheterozygotes at room temperature, thus showing that, within a diploid nucleus, the presence of two *DTS-L*'s *per se* is not lethal at room temperature. *DTS-L6* and *DTS-L19* mapped in the *b–pr* and *pr–c*

TABLE 1. *Viabilities of the dominant temperature-sensitive lethal and semilethal mutations relative to the control values.*

		Ratio of ±-Bearing to Total Offspring					
		17°C		Room Temperature		29°C	
		Ratio	No.	Ratio	No.	Ratio	No.
(a) *Control*							
	Oregon-R	0.67	447	0.67	493	0.64	385
	Samarkand	0.65	209	0.62	238	0.68	177

			Viability Relative to Wild Type (%)					
			17°C		Room Temperature		29°C	
DTS-L	Stock of origin	Genetic position	(%)	(No.)	(%)	(No.)	(%)	(No.)
(b) *Experimental*								
1	Oregon-R	*c-px*	90	344	73	93	1	104
2	"	*dp-b*	97	509	100	353	0	138
3	"	*dp-b*	98	433	94	159	0	151
4	"	*dp-b*	101	527	93	156	4	103
5	"	*dp-b*	101	427	97	465	0	180
6	Samarkand	*b-pr*	105	493	96	702	0	156
7	"	*dp-b*	90	187	105	447	2	65
8	"	Synthetic	93	266	88	311	1	107
9	"	♀ sterile	67	364	93	683	0	156
10	"	*dp-b*	94	464	107	781	0	140
11	"	*dp-b*	96	529	100	690	2	161
12	"	*dp-b*	95	548	103	1,723	1	244
13	"	*dp-b*	97	535	107	1,750	0	276
14	"	*dp-b*	93	390	101	850	0	114
15	"	*c-px*	74	392	68	575	2	130
16	"	*dp-b*	100	477	104	527	1	86
18	"	♀ sterile	95	565	110	453	2	149
19	"	*pr-c*	98	624	107	605	3	149
20	"	♀ sterile	89	293	105	711	0	169

regions, respectively. *DTS-L1* and *DTS-L15* both appeared to be located just to the left of *px* and, when crossed at room temperature, failed to produce heterozygotes (i.e., they did not complement); therefore they are functionally allelic. This result suggests that, in spite of the differences in viability of recombinant classes, the relative genetic positions determined by crossing-over are reasonable.

Unexpectedly, 11 of the 15 mutants tested were closely linked to *dp* in the *dp–b* region. At room temperature, mutants *DTS-L3* and *DTS-L12* did not complement with each other or any other mutant in the genetic cluster, thereby showing that they are all functionally allelic and behave as recessive lethals at the permissive temperature. The fact that mutants in the cluster were induced in both Oregon-R and Samarkand strains makes it unlikely that their recovery reflects an aberrant regional susceptibility peculiar to a special second chromosome of one stock.

To determine whether any mutants within the cluster complement, all possible pairwise crosses of the mutants were made at 22° C. An average of 292 flies was scored for each complementation test. Where complementation did occur, the frequency of heterozygotes for the two mutants varied from 4 to 55 per cent of the expected number, assuming complete complementation. The different patterns of complementation can be represented in a circular complementation map which

TABLE 2. *Ratio of DTS-L- to Cy-bearing offspring of the cross: +/Y; Cy/DTS-L♂ × C(1)RM, y w fa^no/FM-6, y B; 3A ♀ at 29°C.*

Stock	Triploid ♀ Ratio	Triploid ♀ (No.)	Diploid ♀ Ratio	Diploid ♀ (No.)	Diploid ♂ Ratio	Diploid ♂ (No.)
Oregon-R	1.0	28	1.55	56	1.44	44
DTS-L2	0.62	42	0.0	42	0.0	17
DTS-L3	0.66	78	0.06	100	0.02	42
DTS-L4	0.67	70	0.05	69	0.0	38
DTS-L5	1.0	49	0.0	45	0.0	38
DTS-L6	0.86	82	0.0	65	0.0	26
DTS-L10	0.29	27	0.1	45	0.0	19
DTS-L11	1.0	56	0.1	57	0.08	14
DTS-L12	0.90	38	0.02	43	0.0	28
DTS-L13	0.6	40	0.0	58	0.06	35
DTS-L14	1.0	45	0.0	47	0.04	25

has a tail (Fig. 3). Preliminary tests show that some heterozygotes for two different alleles in the cluster survive at 17° ± 0.5°C, therefore permitting us to determine whether they are separable by recombination.

Females carrying *DTS* mutants *9*, *18*, and *20* were completely sterile at room temperature and therefore prevented any genetic mapping. Males and females heterozygous for *DTS-L20* at room temperature showed abnormal chitin development on the dorsal surface of the abdomen and had a lethal phase in the late pupal stage at 29°C. If the cluster of 11 mutants and the *DTS-L1*, *DTS-L15* pair represent two different loci, and if the female steriles are in different genes, these data indicate that *DTS* lethals at different loci are induced in about 0.17 per cent of the treated second chromosomes.

Results of the studies on the effective lethal phases and temperature-sensitive periods of the mutants are summarized in Figure 4. *DTS-L19* has a lethal phase in the late pupal stage, after formation of eye pigment and wings (after 210 hr) and a TSP at 144–192 hr. Mutants *1* and *15* have a temperature-sensitive period at 115–120 hours, just prior to puparium formation, and a lethal phase at 120–144 hours. *DTS-L19* flies have a temperature-sensitive period at 125–150 hours and a lethal phase at the time of eclosion (190–210 hours)—and die in the process of emergence from the pupa cases.

The functional relatedness of the 11 clustered *DTS*-lethals is further supported by the similar pattern of the lethal phase and temperature-sensitive period. All have a temperature-sensitive period 18–24 hr after the culture is established; and the lethal phase of all but mutants *7*, *11*, and *16* occurs in the third larval instar between 75 and 90 hours. The lethal phase of *DTS-L11* and *16* is initiated just after the others in the cluster, but 10–40 per cent of the larvae manage to form puparia before death. Flies carrying *DTS-L7* die in the egg stage. These differences in lethal phase suggest that the mutants are not identical changes within the locus.

Discussion.—This report demonstrates that conditional lethality provides a means for the detection and maintenance of dominant lethal mutations for genetic analysis. The estimated frequency with which *DTS-L*'s in different loci are induced (0.17%) differs from the recessive *ts* lethal frequency by more than an order of magnitude,[5] but these mutants are sufficiently common to

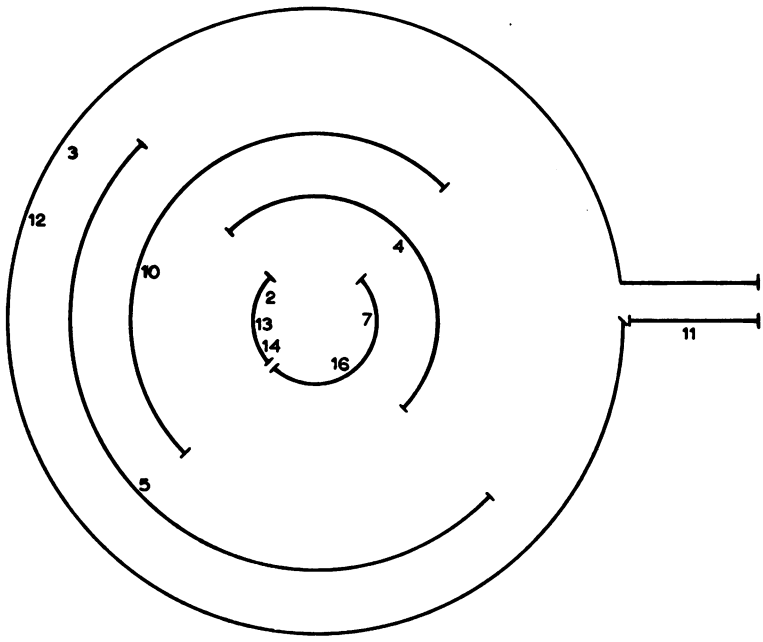

FIG. 3.—Complementation map of the mutants mapping genetically between *dp* and *b*.

warrant extensive screening for their detection. Mortimer[9] estimated that the proportion of recessive to dominant lethals induced by X rays in yeast was 15:1, and Hartwell[10] found that 4 out of 400 *ts* mutants detected in haploid yeast cells behaved as *DTS-L*'s in diploid lines. Our demonstration of the recessive lethality of *DTS-L*'s at room temperature suggests that a number of *DTS-L*'s would be lost by initial screening in haploids. The ready genetic localization of 15/16 *DTS-L*'s in *Drosophila* shows that *DTS* lethality is not the consequence of a number of different detrimental mutations whose cumulative effect on viability is temperature-dependent; nor are they gross chromosomal rearrangements. Indeed, it has been suggested that a proportion of X-ray-induced dominant lethals does not result from chromosome aberrations.[3]

Thus far, determinations of temperature-sensitive period and lethal phase of the *DTS* mutants under restrictive temperatures suggest that they affect development in a manner similar to recessive non-*ts* and *ts* lethals.[11, 12] The effects of *DTS-L9*, *18*, and *20*, which probably represent different loci, may be of considerable interest. While all three behave as *DTS*-lethals and therefore must be required for viability in both sexes, they show sexual dimorphism in gross phenotype (i.e., fertility) under permissive conditions. The striking sexual dimorphism in expression of the autosomal mutant *tra*,[13] and in the temperature senstivity of a sex-linked recessive *ts* lethal,[12] makes it of interest to determine whether the *DTS-L*'s which produce sterile females at room temperature fall into a similar class.

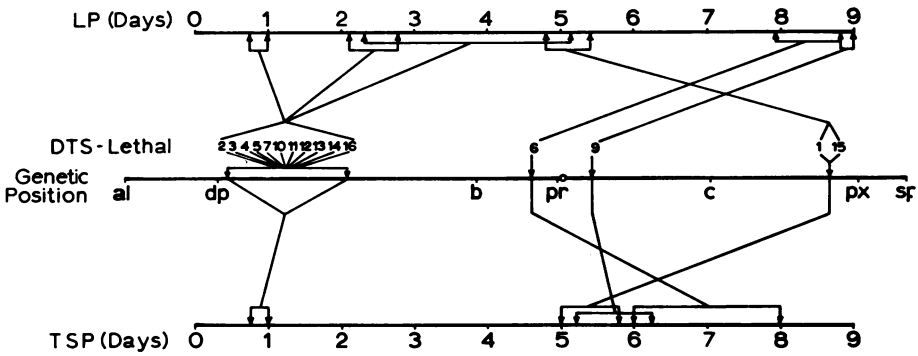

FIG. 4.—Genetic map and the temperature-sensitive period and lethal phase of each *DTS* lethal. The developmental times at room temperature are indicated.

The demonstration that 11 of the 19 *DTS-L*'s occupied a single genetic region and were functionally allelic was not anticipated. The induction of mutants in the cluster in completely different genetic backgrounds indicates that such mutants might be expected in any genetic background. Moreover, the differences in viability indices, in complementation patterns, and in the lethal phase of the mutants suggest that they are not homoalleles and therefore ought to be separable by recombination, since heterozygotes for some alleles in the cluster do survive at 17°C. It is noteworthy that the cluster is closely linked to the site of *dp*, a locus known to be highly susceptible to mutagenesis by a number of agents.[14] We suggest, therefore, that the recovery of a number of alleles within a single locus is the consequence of a high sensitivity of the genetic region to EMS–which may not be unrelated to the known mutagenic susceptibility of the *dumpy* locus.

The recovery of dominant *ts* lethal mutations will permit an objective comparison of genetic and developmental properties of dominant and recessive mutations. Of greater importance, however, is the possibility offered for the detection of mutants in loci not previously amenable to experimental analysis (e.g., redundant regions with functions necessary for viability or fertility). The detection and analysis of recessive sex-linked and autosomal *ts* lethals,[5] "cold sensitive" lethals,[15] *DTS*-lethals, and Y-linked *ts* male fertility mutants[16] suggests that temperature sensitivity may be found for any class of loci amenable to detection. This property may be of considerable worth in a variety of analyses in higher organisms.

We wish to thank Misses Rachel Pratt, Jeanette Holden, and Jill Cameron for their excellent technical assistance in the isolation of some of the mutants studied.

Abbreviations used: *DTS-L*, dominant temperature-sensitive lethal; *ts*, temperature-sensitive; EMS, ethyl methanesulfonate.

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