TEMPERATURE-SENSITIVE MUTATIONS IN *DROSOPHILA MELANOGASTER* XII. THE GENETIC AND DEVELOPMENTAL EFFECTS OF DOMINANT LETHALS ON CHROMOSOME 3[1,2,3]

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Manuscript received October 2, 1972
Revised copy received November 16, 1972

ABSTRACT

Out of 25,000 EMS-treated third chromosomes examined, ten dominant temperature-sensitive (DTS) lethal mutations which are lethal when heterozygous at 29°C but survive at 22°C were recovered. Seven of the eight mutations mapped were tested for complementation; these mutants probably define eight loci. Only *DTS-2* survived in homozygous condition at 22°C; homozygous *DTS-2* females expressed a maternal effect on embryonic viability. Two of the mutant-bearing chromosomes, *DTS-1* and *DTS-6*, exhibited dominant phenotypes similar to those associated with *Minutes*. Each of the seven mutants examined exhibited a characteristic phenotype with respect to the time of death at 29°C and the temperature-sensitive period during development. Only *DTS-4* exhibited dominant lethality in triploid females.

DOMINANT mutations have been described for both higher organisms and micro-organisms at a gross morphological level. However, the distinction between dominance and recessiveness becomes less clear as one attempts to define them at the molecular level. BERNSTEIN and FISHER (1968) have attributed the molecular basis for dominance in micro-organisms to the polymerization of wild-type and mutant polypeptide monomers resulting in either the restoration of biological activity (if the wild-type is dominant) or the inactivation of the polymeric hybrid complex (if the mutant is dominant).

H. J. MULLER (1927) first attributed the marked increase in the mortality of developing embryos after treatment of Drosophila males with X-rays to the production of dominant lethal mutations in the sperm. Several other workers (e.g., PONTECORVO 1942; HALDANE and LEA 1947) have found similar results and in the majority of cases, cytological observations and genetical experimentation have suggested that dominant lethality results from abnormal chromosomal complements. Genetically-contrived dominant lethality resulting from the presence of deletions or duplications has been found to yield lethal phenotypes similar to the dominant lethal phenotypes obtained after X-irradiation (VON BORSTEL and REKEMEYER 1959).

[1] This work was supported by the National Research Council of Canada, grant A-1764 and the National Cancer Institute of Canada, grant 6051.

[2] Taken in part from material to be submitted by J.J.H. in partial fulfilment of requirements for the degree of Doctor of Philosophy in Genetics at the University of British Columbia.

[3] Part XI of this series is by AYLES *et al.*, Devel. Biol., in press.

The nature of dominant lethal mutations precludes their direct genetic analysis since successive generations cannot be propagated. However, the recovery of temperature-sensitive lethals in Drosophila (SUZUKI *et al.* 1967) suggested a possible means of screening for conditional mutants which would behave as dominant lethals only under restrictive conditions. Dominant temperature-sensitive lethals in *Drosophila melanogaster* have been recovered in chromosomes 2 and 3 which, in heterozygous condition, die at 29°C but are completely viable at 22°C (BAILLIE, SUZUKI and TARASOFF 1968; SUZUKI and PROCUNIER 1969; HOLDEN and SUZUKI 1968).

The present report discusses the genetic properties and basic developmental patterns of seven dominant temperature-sensitive lethals recovered on chromosome 3.

MATERIALS AND METHODS

Screening procedures: Within 48 hours of eclosion, males of isogenic Oregon-R and Samarkand stocks were collected and fed 0.025M ethyl methanesulfonate (EMS) dissolved in 1% sucrose for 24 hours (LEWIS and BACHER 1968). The mutagenic effectiveness of each treatment was assayed by crossing ten of the EMS-treated males to attached-X-bearing females at room temperature (RT, 22° ± 1°C) and scoring the F_1 sex ratio. Since the sex-linked lethal frequency reflects the frequency of autosomal lethals (WALLACE 1951), crosses to detect autosomal mutants were made only if the sex ratio (males/females) of offspring in the attached-X test was less than 0.50.

The complete screening protocols for the detection of dominant temperature-sensitive (*DTS*) lethal mutations on chromosome 3 are shown in Figure 1. This procedure is similar to the protocol used for the detection of *DTS*-lethals on chromosome 2 (SUZUKI and PROCUNIER 1969) with the substitution of chromosome 3 markers, *TM2/CxD, Sb Ser/Xa,* or *Gl/TM3.* These various

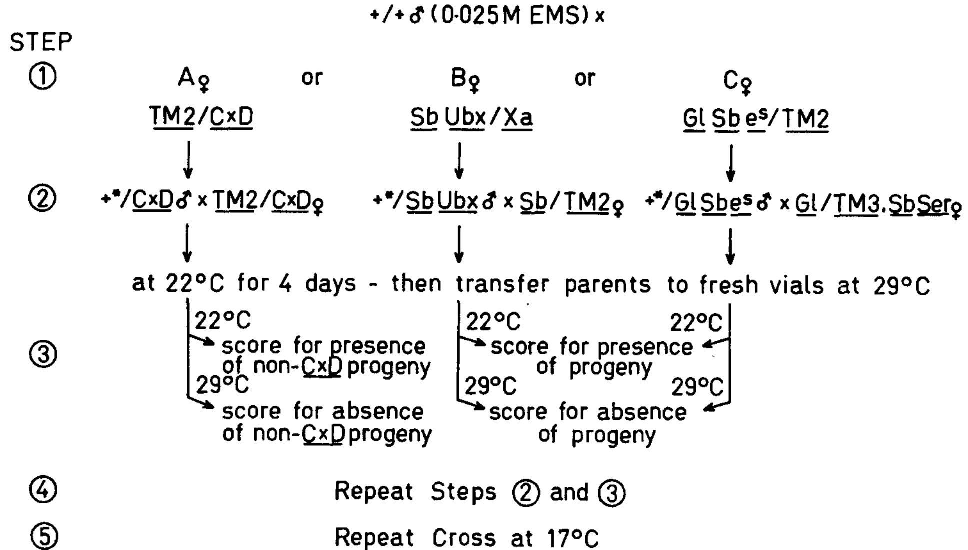

FIGURE 1.—Genetic crosses for the detection of dominant temperature-sensitive lethal mutations on chromosome 3. A, B and C utilize different balanced chromosomes.

balancers have dominant visible phenotypes and are lethal when homozygous (for a complete description of the mutants and chromosomes used, see LINDSLEY and GRELL 1968).

Several lines were maintained for each confirmed *DTS*-lethal and these were tested for lethality at 29°C every one or two generations. In addition, for each experiment, control crosses were made to test for the presence of the *DTS's*. For example, in the mapping crosses, *DTS/III-ple* and *DTS/steroca* males were testcrossed at both 22° and 29°C. In this way, any contamination or modification of the *DTS* effect (due to change in residual background) could be detected. Only lines showing 100% lethality at 29°C were retained.

The mutations recovered are designated as $L(3)X^{DTS}$ (where X is the specific number of the mutation). For this paper, we will refer to specific mutations by the abbreviation of *DTS-X*. Each of the confirmed *DTS*-lethals was also tested for viability and homozygosis at 17°C. Unexpectedly, four temperature-sensitive mutations which appeared to be lethal at 29°C only when individuals were heterozygous for *TM2* were recovered and confirmed. In crosses at 22°C, such heterozygotes survive. Flies heterozygous for one of these chromosomes and *CxD*, *TM3* or a wild-type chromosome are viable at 29°C. Studies on these four mutations will be discussed in a separate report.

Genetic localization of confirmed DTS-lethals: The genetic positions of the DTS-lethals were determined relative to the recessive markers carried by the "*III-ple*" ($ru\ h\ st\ p^p\ ss\ e^s$) and "*steroca*" ($st\ e^s\ ro\ ca$) stocks (LINDSLEY and GRELL 1968). Females heterozygous for each *DTS*-lethal and either *III-ple* or *steroca* were testcrossed at 22°C and 29°C. At least 40 vials containing single females and three males were set up for each *DTS*-lethal.

Viability of the DTS-lethals in triploids: In order to determine whether two doses of a wild-type allele are sufficient to overcome the lethal effect of a *DTS* mutation at 29°C, *DTS*-bearing males were mated to triploid females carrying an attached-X chromosome (homozygous for either $y^2\ w^a\ ec\ sc$ or $y\ w\ fa^{no}$) and the multiply-inverted rod X chromosome, *FM6* (marked with *y B*). This cross was: $\hat{XX}$, $y^2\ sc\ w^a\ ec$ or $y\ w\ fa^{no}/FM6,\ y^{31d}\ sc^8\ dm\ B;3A+$ ♀ × +/Y; *DTS/TM3, Sb Ser* ♂. The expression of *Sb* in triploids permitted the distinction of *DTS*-bearing from *TM3*-bearing progeny. The absence of *DTS*-bearing diploid progeny at the restrictive temperature confirmed the presence of the *DTS*-lethal in the male parent. The presumed triploid females recovered at 29°C were individually tested for the presence of the attached-X chromosome in order to confirm their triploid constitution.

Determination of the Effective Lethal Phase (LP) and Temperature-Sensitive Period (TSP) of the DTS-lethals; growth curves of the DTS-lethals at 22°C and 29°C: The effective lethal phase, that is, the developmental stage at which death occurs at 29°C, was determined for each of the DTS-lethal stocks. One hundred to two hundred pairs of *DTS*-bearing flies from each stock were placed in empty quarter-pint milk bottles inverted over petri plates containing standard Drosophila medium. Eggs were collected within one to two hour intervals. Duplicate cultures were simultaneously collected and maintained at 22°C and 29°C and inspected every twelve hours for developmental stages reached and the onset of death.

In order to determine the temperature-sensitive period (TSP), the developmental interval during which the organism is irrevocably committed to death by the restrictive temperature, a series of reciprocal shift-up and shift-down experiments were performed (SUZUKI and PROCUNIER 1969; TARASOFF and SUZUKI 1970). Preliminary experiments were initiated with eggs zero to two hours old and a shift interval of twelve hours. More accurate determinations of the TSP were made by initiating experiments with cultures synchronized at discrete developmental stages —newly hatched/molted first, second or third instar larvae, late third instar larvae, or prepupae. During the latter stage, the larvae become immobilized, the anterior spiracles evert but the larval skin is still white. Prepupae were removed from the side of the dish by using a paint brush which had been moistened in a semi-liquid mixture of Drosophila medium and distilled water. Careful manipulation and transfer of the prepupae to the side of a vial resulted in complete survival. For shifts later than the prepupal stage, pupal development was determined by the appearance of specific adult appendages and pigmentation. The earliest time at which a shift-down fails to yield adults delineates the initiation of the TSP, whereas the first culture to yield adults in a shift-up marks the end of the TSP.

RESULTS

Ten confirmed *DTS*-lethal mutations were recovered from 25,000 chromosomes tested. This frequency of DTS-lethals (0.04%) contrasts sharply with the frequency of 0.34% for DTS-lethals on chromosome 2 (SUZUKI and PROCUNIER 1969). Three of the mutations were lost and of these, two (*DTS-9* and *DTS-10*) rendered females sterile at both 17°C and 22°C and therefore would not have been mappable. The other *DTS* (*DTS-8*) was lost after it was found to kill in the second instar and genetically localized to the left of *ss* (*spineless*—56.7). The present assignment of numbers to each mutation was made after the completion of the crossover experiments.

The relative viability of each of the *DTS*-lethals was determined by the ratio of the number of *DTS/A* (where *A* refers to either *TM3* or *Gl*) or *DTS/B* (where *B* refers to either *Gl Sb* e^s or *TM2*) offspring to the total number of progeny recovered from a cross of *DTS/TM3* (or *DTS/TM2*) males by *Gl/TM3* (or *Gl Sb* e^s*/TM2*) females. The values obtained for control crosses in which males heterozygous for *TM3* or *TM2* and either an Oregon-R or Samarkand chromosome were crossed to *Gl/TM3* or *Gl Sb* e^s*/TM2* females, respectively, were very close to the 0.67 ratio of +-bearing progeny to the total predicted by Mendelian expectations (Table 1). The viabilities of the *DTS*-lethals were determined relative to the observed control values for the wild-type stock from which the *DTS*'s were derived. All of the *DTS* mutations are completely lethal at the restrictive temperature and have a viability greater than 90% that of the wild type under permissive conditions.

TABLE 1

Relative viabilities of flies bearing + or L(3)X^{DTS} *chromosomes at different temperatures*
Cross A: + or *DTS/TM3* ♂ × *G1/TM3* ♀
Cross B: + or *DTS/TM2* ♂ × *G1 Sb* e^s*/TM2* ♀

			17°C			22°C			29°C		
	Stock of origin	Cross made	Total progeny	Progeny ratio*	Percent of wild type	Total progeny	Progeny ratio*	Percent of wild type	Total progeny	Progeny ratio*	Percent of wild type
Oregon-R		A	898	0.63	...	1846	0.67	...	3718	0.72	.
Oregon-R		B	396	0.65	...	846	0.70	...	969	0.65	.
Samarkand		A	927	0.64	...	1527	0.67	...	1608	0.72	.
Samarkand		B	532	0.66	...	1072	0.66	...	1063	0.68	.
DTS-1	Oregon-R	B	888	0.70	107.5	2389	0.62	92.5	858	...	.
DTS-2	Samarkand	A	174	0.65	101.6	1011	0.62	92.5	582	...	.
DTS-3	Samarkand	B	908	0.64	97.0	3449	0.66	100.0	496	...	0
DTS-3	Samarkand	A	...	...	...	473	0.71	106.0	890	...	0
DTS-4	Samarkand	B	992	0.63	95.5	1869	0.65	98.5	593	...	0
DTS-4	Samarkand	A	...	...	...	1419	0.66	98.5	319	...	0
DTS-5	Samarkand	B	688	0.62	93.9	950	0.63	95.5	723	...	0
DTS-5	Samarkand	A	...	...	...	285	0.60	89.6	261	...	0
DTS-6	Samarkand	B	159	0.65	98.5	985	0.65	98.5	1163	...	0
DTS-7	Samarkand	A	...	...	...	1369	0.64	95.5	427	...	0

* Progeny ratio: ratio of + or *DTS*-bearing progeny to total offspring.

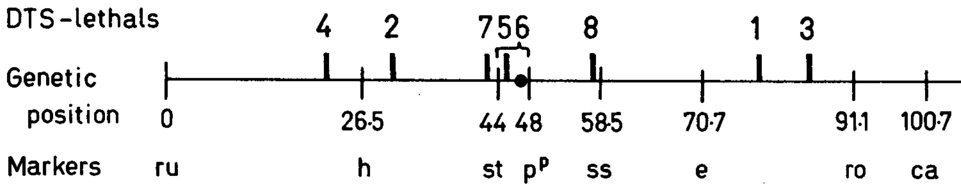

FIGURE 2.—Genetic positions of the *DTS*-lethal mutations on chromosome 3. ●—centromere.

The approximate genetic positions of the DTS-lethals are shown in Figure 2 and the data used in these determinations are summarized in Table 2. For both the wild-type and *DTS*-chromosomes, there was a large variation in the values obtained for the map distances between the markers of the *III-ple* and *steroca* chromosomes. It has been shown, however, that differences in genetic background can greatly affect crossover frequencies (SUZUKI 1962). Since the *DTS*-stocks contained X, 2 and 4 chromosomes carrying possible mutations and/or chromosomal abnormalities, there are no adequate control values with which the *DTS*'s can be compared. The *DTS*-lethals which could be mapped are seen to be distributed throughout the third chromosome in eight distinct positions, a pattern very different from the highly clustered distribution of mutants on chromosome 2 (SUZUKI and PROCUNIER 1969). In addition, the *Minute* phenotype associated with *DTS-1* could be seen to segregate with the lethal phenotype, suggesting that a single mutation was responsible for the two characteristics. *DTS-6* females are almost sterile at 22°C but shifting of eggs laid by *DTS-6/III-ple* females mated to *III-ple* males at 22° to 29°C permitted approximate localization of the mutation. However, too few progeny were obtained to determine whether the *Minute* phenotype was also associated with the locus determining lethality.

Although the genetic positions of the *DTS*-lethals were found to be distinct from one another, it was decided to carry out complementation tests in order to establish whether any of the mutations might be functionally related. Crosses were made to generate all possible heterozygotes for two different *DTS* mutations at 22°C. These studies revealed that all but one of the combinations survived under permissive conditions (Table 3). Trans-heterozygotes for *DTS-5* and *DTS-7* did not survive at either 17°C or 22°C, although these mutations probably are not allelic since *DTS-7* maps 1.7 units to the left of *st* (based on 132 recombinants between *h* and *st*) and *DTS-5* maps 0.3 units to the right of *st* (based on 67 recombinants between *st* and p^p). Although both mutations have lethal phases in the pupal stage, these are phenotypically distinguishable. In addition, the TSP for *DTS-7* extends from the late third instar through the first one-third of the pupal stage, whereas that for *DTS-5* ends abruptly with pupation (Figure 3).

The developmental properties of each *DTS*-lethal-bearing chromosome in homozygotes are shown in Table 4. *DTS/Inv* males were crossed to *DTS/Inv* (where *Inv* refers to either *TM2* or *TM3*) females and the rates of hatching of the eggs and of eclosion of their offspring were determined. Eggs from the latter crosses were collected at 22°C as described. First instar larvae, within two to six hours of hatching, were collected from plates and placed into vials. The extent

TABLE 2

Crossover results from testcrosses of +- and DTS-bearing females at 22°C and 29°C

Stock	Temp.	*ru* +/+ *h*	Percent*	*h* +/+ *st*	Percent	*st* +/+ p^p	Percent	p^p +/+ *ss*	Percent	*ss* +/+ e^s	Percent	e^s +/+ *ro*	Percent	*ro* +/+ *ca*	Percent	Number scored	Map position
Oregon-R	22°	467/316	22.3	400/335	20.9	76/69	4.1	184/215	11.3	201/298	14.2	....	...	....	...	3518	...
	29°	194/150	20.7	198/178	22.6	49/55	6.3	119/156	16.6	101/138	14.4	....	...	....	...	1661	...
	22°	....	...	....	...	....	...	....	...	....	...	250/229	23.2	104/104	10.1	2063	...
Samarkand	22°	354/238	24.7	313/197	21.2	49/33	3.4	141/140	11.7	128/215	14.3	....	...	....	...	2397	...
	29°	257/149	22.7	181/178	20.1	13/10	1.3	61/73	7.5	112/131	13.6	....	...	....	...	1789	...
	22°	....	...	....	...	....	...	....	...	....	...	210/264	23.1	109/106	13.0	3767	...
	29°	....	...	....	...	....	...	....	...	....	...	459/413	24.9	236/252	11.3	1904	...
DTS-1	22°	25/89	16.3	51/79	18.6	8/11	2.7	25/42	9.6	28/70	14.0	....	...	....	...	698	...
	29°	47/43	18.9	31/59	18.9	2/14	3.4	4/65	14.5	7/90	20.4	....	...	....	...	475	78
DTS-2	22°	168/173	20.4	233/136	22.1	52/29	4.9	118/105	13.4	106/118	13.4	....	...	....	...	1668	...
	29°	18/234	24.3	215/80	28.5	44/2	4.4	107/31	13.3	103/50	14.8	....	...	....	...	1026	33.4
DTS-3	22°	....	...	....	...	....	...	....	...	....	...	205/164	21.7	124/117	14.1	1704	...
	29°	....	...	....	...	....	...	....	...	....	...	63/131	23.4	78/0	9.4	830	84.5
DTS-4	22°	215/205	24.2	305/126	24.9	41/21	3.6	128/92	12.7	96/133	13.2	....	...	....	...	1734	...
	29°	17/209	23.5	291/4	26.8	18/2	2.1	105/27	13.7	124/35	16.5	....	...	....	...	961	24.5
DTS-5	22°	604/548	21.6	535/471	18.9	104/82	3.5	436/308	13.9	329/397	13.6	....	...	....	...	5336	...
	29°	36/361	17.0	5/595	25.6	62/5	2.9	303/8	13.3	341/17	15.2	....	...	....	...	2340	44.3
DTS-6	29°	0/30	27.5	0/22	20.2	0/0	< 1	12/0	11.0	18/2	18.3	....	...	....	...	109	st-p^p
DTS-7	22°	266/229	25.5	238/178	21.4	40/36	3.9	146/119	13.6	124/159	14.6	....	...	....	...	1944	...
	29°	11/121	23.8	13/118	23.6	23/3	4.7	72/5	13.9	72/8	14.4	....	...	....	...	554	42.3
DTS-8	29°	75/169	26.8	26/156	20.0	3/14	1.9	32/101	14.6	112/10	13.4	....	...	....	...	910	55.5

* Percent of total progeny.

TABLE 3

Ratios of DTS-a/DTS-b *to total progeny from the cross* DTS-a/TM3 × DTS-b/TM3 *at 22°C*

DTS-a	*DTS-b*: *DTS-1*	*DTS-2*	*DTS-3*	*DTS-4*	*DTS-5*	*DTS-6*	*DTS-7*
DTS-1	0/197						
DTS-2	43/114	79/159					
DTS-3	119/331	31/92	0/375				
DTS-4	15/81	35/87	34/89	0/427			
DTS-5	30/85	50/125	33/111	57/141	0/306		
DTS-6	30/102	68/214	45/150	69/225	71/281	———	
DTS-7	51/140	76/198	62/181	77/217	0/596	55/182	6/327

of egg hatchability could be calculated from the ratio of larvae to eggs. The number of pupae and finally the number of adults eclosing provided an indication of the survival of the larvae collected. Since *TM2* homozygotes died as larvae and *TM3* homozygotes died during the egg stage, it was possible to determine the lethal phase of the *DTS* homozygotes from the above information. Only *DTS-2* survived in the homozygous condition, although failure of the other *DTS*-homozygotes to survive may have been caused by recessive lethals induced elsewhere on the chromosomes.

The viabilities of triploids carrying the *DTS*-lethals are shown in Table 5. Al-

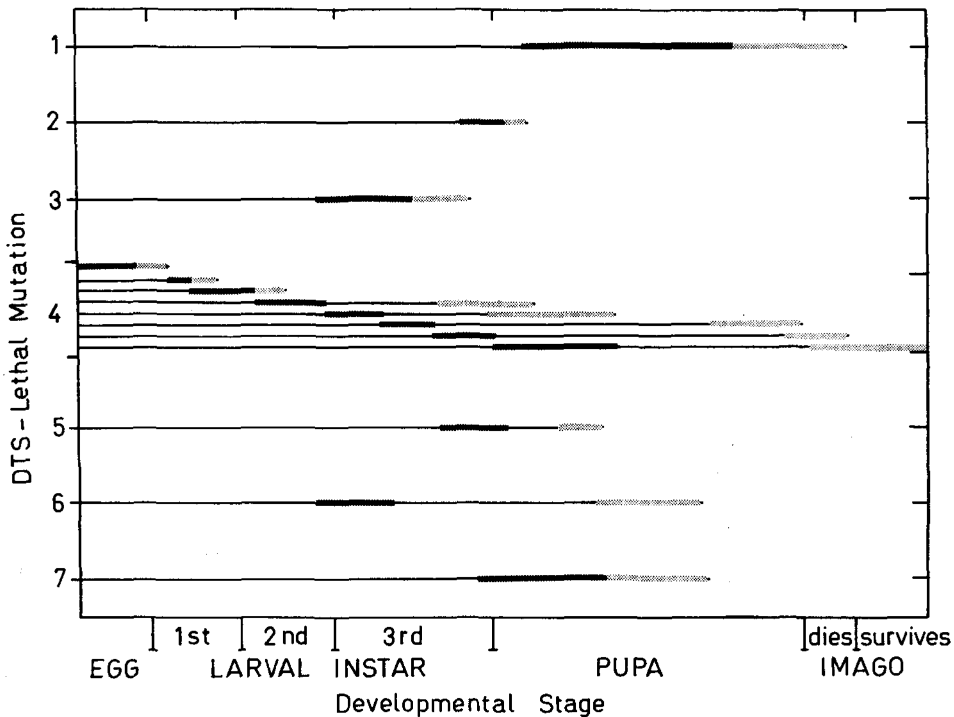

FIGURE 3.—The Effective Lethal Phase (LP) and temperature-sensitive period (TSP) of each *DTS*-lethal mutation. Crosshatch represents LP; heavy bar represents TSP.

TABLE 4

Determination of the effective lethal phase of homozygotes for the DTS-*lethals at 22°C*

Stock	Eggs	First instar larvae	Adults	Percent First instar larvae: eggs	Percent adults: First instar larvae	Percent adults: eggs	Homozygous *DTS*
Oregon-R/*TM3*	851	590	540	69.3	91.5	63.5	Viable
Samarkand/*TM3*	700	467	396	66.7	84.8	56.6	Viable
DTS-1/TM2	985	670	428	68.0	63.9	43.5	Egg lethal
DTS-2/TM3	624	468	437	75.0	93.4	70.0	Viable
DTS-3/TM2	1586	519	436	32.7	84.0	27.5	Egg lethal
DTS-4/TM3	1092	590	535	54.0	90.7	49.0	Egg lethal
DTS-5/TM2	409	363	146	88.8	40.2	35.7	1st instar lethal
DTS-5/TM3	877	555	358	63.3	64.5	40.8	1st instar lethal
DTS-7/TM3	415	285	179	68.7	62.8	43.1	1st instar lethal

though the yields per vial were low, tests at 22°C of the triploid progeny recovered at 29°C proved the 3N genotype of these females. It can be seen that *DTS-4* has a very reduced viability even in triploid females at 29°C, whereas the other *DTS*-lethals appear to survive at a rate comparable to their sibling non-*DTS*-bearing triploids and triploids bearing a wild-type third chromosome derived from an Oregon-R or Samarkand stock.

The LP's and TSP's delineated for each of the *DTS*-lethals studied are summarized in Figure 3. *DTS-1*, which is lethal at 29°C during the late pupal to early adult stages, has a TSP extending for about 100 hours prior to death. The large spread in the TSP for this and other *DTS*-lethals may be attributable to

TABLE 5

Progeny from the cross $\hat{XX}$, y^2 sc w^a ec *or* y w fa^{no}/FM6, y^{31d} sc^8 dm B;3A + ♀ × +/Y; 3*/TM3, Sb Ser ♂ *at 22°C and 29°C*
(where 3* represents a chromosome 3 carrying either a wild-type or *DTS*-lethal allele)

	3N♀				2N♀				2N♂			
	22°		29°		22°		29°		22°		29°	
	*3**	*TM3*	*3**	*TM3*	*3**	*TM3*	*3**	*TM3*	*3**	*TM3*	*3**	*TM3*
Oregon-R	27	23	45	37	44	48	65	52	39	29	40	21
Samarkand	41	30	39	15	57	45	40	32	42	31	22	23
DTS-1	..	..	13	20	..	..	2	29	..	..	—	14
DTS-2	..	..	3	6	..	..	—	14	..	..	—	5
DTS-3	..	..	23	26	..	..	—	18	..	..	—	29
DTS-4	44	35	1	28	49	46	—	26	..	..	—	17
	..	..	1	9	..	..	—	14	..	..	—	9
DTS-5	..	..	20	13	..	..	—	16	..	..	—	9
	..	..	17	14	..	..	—	16	..	..	—	7
DTS-6	..	..	2	7	..	..	—	10	..	..	—	6
DTS-7	42	35	33	27	64	37	1	26	40	27	(3)†	8
	..	..	10	13	..	..	1	19	..	..	—	9

† These males had a characteristic phenotype of rare "escaper" ♂♂ from this stock which are recovered at 29°—very heavy wing venation, abnormal leg segmentation, rough eyes.

asynchrony in the cultures. In terms of developmental stages, the TSP coincides with the interval following eversion of the cephalic complex until the fully-developed imago is formed.

The LP of *DTS-2* heterozygotes occurs during the early stages of pupation, as indicated by the successful eversion of the leg and wing discs but failure of the cephalic complex to evert. Although this phenotype resembles that of the mutation *l(2)crc* (FRISTROM 1965), individuals heterozygous for both *l(2)crc* and *DTS-2* were viable at 22°C, indicating an absence of a synergistic effect between the two mutations. The LP is preceded by a TSP persisting for about 15 hours. In crosses of *DTS-2/TM3* flies, the TSP's for lethality of the homozygotes and heterozygotes are identical. However, in the homozygous *DTS-2* stock, only 0%–5% of the egggs laid at 29°C hatch. Those 'escapes' (HADORN 1961) that do hatch at the restrictive temperature continue to develop until pupation, at which time death occurs. These observations suggest an embryonic 'boundary' for lethality which is overcome by the presence of a wild-type allele in the parents. Therefore, crosses were carried out to determine the nature of the parental influence on embryonic lethality. *DTS-2/DTS-2* males were crossed to +/+ females at 29°C, and of 1786 eggs collected, 1554 hatched and 1321 died as pupae. On the other hand, of 2316 eggs collected from the reciprocal cross of *DTS-2/DTS-2* females by +/+ males, only 52 hatched, thereby indicating that the embryonic lethality of heterozygotes as well as of homozygotes is maternally determined. Moreover, preincubation of *DTS-2/DTS-2* females at 29°C for 6 hours prior to egg collection results in complete egg lethality, suggesting that the maternally deposited factor for viability is thermolabile.

The lethal phase of *DTS-3* occurs during the third larval instar. At 29°C, this stage may be prolonged for some fourteen days before death finally ensues. As these individuals age, fat deposits become depleted and the salivary glands degenerate. This suggests that the larvae prepare for metamorphosis since histolysis of the larval salivary glands normally takes place immediately prior to and during this transition stage. By using cultures synchronized at the first larval instar, it was possible to establish a TSP extending for about fifty hours beginning just prior to the second to third instar molt and ending about midway through the third instar stage.

Inspection of cultures of *DTS-4* heterozygotes indicates that the LP for cultures initiated at 29°C occurs during the egg and very early first instar stages. Eggs collected immediately after the parents are shifted to the restrictive temperature yield about 15% hatch. The larvae which do emerge never assume the elongated shape of normal first instar larvae, but rather remain the approximate size and shape of eggs. After twelve to sixteen hours, these larvae cease movement and death follows. When parents are preconditioned at 29°C for twenty-four hours and eggs are then collected, no hatching occurs, although some embryonic development may be detectable. The shifts-up reveal the sensitivity of the mutant to the high temperature throughout the embryonic and larval stages until midway through the pupal stage.

At 29°C, *DTS-5* heterozygotes die as pupae which phenotypically resemble the

mutant *halfway* (LINDSLEY and GRELL 1968). Within the pupal case, the anterior portions of the adult are fully differentiated but the abdominal region fails to develop and histolyzes. The TSP begins during the third larval instar and ends with puparium formation—a period lasting approximately 24 hours.

The lethal phase for *DTS-6* also occurs during the pupal stage. At 22°C the developmental time of *DTS-6* heterozygotes is longer than that of the wild type by four to six days. Although the shift experiments did not allow a precise delineation of the TSP for this mutation, it would appear that the sensitive interval occurs during the larval period.

When flies carrying *DTS-7* are maintained at 29°C, death occurs in the puparium. Dead late pupae (those which appear to have completed the development of the imago) as well as early pupae in which extensive histolysis has occurred, appear to be the expression of the LP of *DTS-7*. The TSP for this mutant immediately precedes the lethal phase, as indicated in Figure 3.

The extent of development of some of the *DTS*-lethals was examined at different temperatures (Table 6). For each *DTS*-lethal, a set of ten vials, each containing one pair of adults heterozygous for the *DTS*- lethal, was placed at 22°, 25°, 26°, 27°, 28° and 29°C. After four days, the parents in each set were removed. Cultures were periodically examined for the developmental stages reached and the time of death of the *DTS*-lethals at each temperature by the method described previously (TARASOFF and SUZUKI 1970). For *DTS-1* and *DTS-2*, complete viability was attained for cultures raised at 26°C or lower, whereas *DTS*'s 5 and 7 were viable even at 27°C. In addition, the LP's of *DTS*'s 5 and *6* were similar at 28° and 29°C, but those of *DTS*'s *1*, *2* and *3* differed with the temperature—with further development occurring at the lower temperatures.

DISCUSSION

The isolation, in *Drosophila melanogaster*, of a class of dominant mutations

TABLE 6

The effective lethal phases of DTS-*lethal mutations in heterozygotes at different temperatures*

DTS number	24°	25°	26°	27°	28°	29°
1	+*	+	+	+ extreme Minute phenotype	late pupa, early adult	late pupa
2 heterozygous parents	+	+	semi-viable, adult lethal	late pupa	*crc*-like	*crc*-like
2 homozygous parents	+	+	pupa	larva	egg	egg
3	+	+	+	late pupa, pseudopupa	pseudopupa	third instar
5	+	+	+	+	*halfway*-like late pupa	*halfway*-like late pupa
7	+	+	+	+	histolytic & late pupa	histolytic & late pupa

* + denotes viability.

which is conditionally lethal has permitted their genetic and developmental analyses. Studies on the nature of dominant lethals had previously been restricted to the production of gross chromosomal abnormalities by irradiation (MULLER 1927), by chemicals (VON BORSTEL and REKEMEYER 1959) and by genetic manipulation (NOVITSKI 1951). Death of the individuals carrying the dominant lethal thereby prevented further characterization of that mutation. Consequently, "dominant lethality" has been a descriptive category, probably encompassing a wide variety of genetic and chromosomal changes. SUZUKI and PROCUNIER (1969) and the present report indicate that a low proportion of mutations induced by EMS may also behave as dominant lethals. Although *EMS* is known to produce a preponderance of missense mutations in phages (KRIEG 1963) and few chromosomal rearrangements in Drosophila (KIM and SNYDER 1968), EMS-induced deletions have been recovered (WILLIAMSON 1970; E. B. LEWIS, unpublished). Although some temperature sensitivity could be the consequence of EMS-induced deletions (FRISTROM 1970), cytological and genetic studies indicate that flies heterozygous for deletions of extensive portions of the genome are viable (LEFEVRE and GREEN 1971; LINDSLEY *et al.* 1972), whereas even small homozygous deficiencies are cell lethals (DEMEREC 1934). Moreover, the viability of *DTS-2* as a homozygote is indicative that this mutant is not a deficiency. Finally, the genetic properties of sex-linked EMS-induced ts-lethals support a missense basis for the mutation (SUZUKI *et al.* 1967; SUZUKI 1970; and SUZUKI and PITERNICK manuscript submitted for publication). That is, ts mutations are much more frequent among EMS-induced mutations than those induced by mitomycin-C or γ-rays; all EMS-induced ts-lethals map as point mutations, whereas rearrangements have been found among EMS-induced non-ts-lethals and γ-ray-induced ts-lethals. In addition, a ts mutation of *vermilion* which affects the activity of tryptophan pyrrolase has been recovered (CAMFIELD and SUZUKI 1972).

X-irradiation-induced and genetically-contrived dominant lethality resulting from duplications and deletions invariably results in early embryonic death (VON BORSTEL and REKEMEYER 1959). Our studies, on the other hand, yielded dominant lethals which permitted considerable development beyond the embryonic to the larval and pupal stages. Although the relative rarity of *DTS*-lethals requires large screening programs for their recovery, loci which are extensively duplicated may only be genetically detectable among *DTS* mutations. The continued lethality of *DTS-4* in triploids at 29°C shows that a mutation can be dominant over at least two wild-type alleles.

The possible molecular basis for dominant lethality merits brief mention, even though our experiments do not permit any definitive interpretation. Genetically-controlled cellular functions which tolerate little deviation from wild-type levels of product might be detectable only by the recovery of *DTS* mutations. Similarly, mutations which would create a high level of cellular "noise", such as defects in polymerases or in the translational apparatus, could be dominant lethals. Indeed, GUTHRIE, NASHIMOTO and NOMURA (1969) found that cold-sensitive lethal mutations in *E. coli* which affected ribosome assembly were dominant. Such defects might be expected to show a continuous temperature sensitivity sim-

ilar to that of *DTS-4*. In addition, genes controlling regulatory molecules, such as repressors, have been found to have dominant alleles (SADLER and NOVICK 1965). The aggregation of defective polypeptides in enzymic or structural polymers can result in loss of biological activity of the complex in spite of wild-type subunits (GAREN and GAREN 1963; BERNSTEIN and FISHER 1968). A distinction among these alternatives will be possible only upon detection of the primary lesion imposed by the *DTS*-lethals.

The discrepancy in recovery frequency of *DTS*-lethals on chromosomes 2 (0.34%) and 3 (0.04%) becomes less dramatic upon inspection of the genetic distributions of the mutations. A high frequency of dominant lethals of both heat- and cold-sensitive types on chromosome 2 occur in a "hot spot" within a small genetic segment to the right of *dp* (SUZUKI and PROCUNIER 1969; ROSENBLUTH, EZELL and SUZUKI 1972). *DTS*-lethals on chromosome 3, on the other hand, were distributed throughout the chromosome. In terms of different sites, the frequency on chromosome 2 is 0.16%.

Two mutants, *DTS-1* and *DTS-6*, are worth special mention. Heterozygotes for either mutation at 22°C are phenotypically identical to the *Minute* class of mutations; i.e., the developmental period is retarded and bristles on the thorax are very short and slender. Furthermore, *DTS-1* enhanced the dominant effect of *sc* and resulted in synthetic semi-lethality with *Dl* (see LINDSLEY and GRELL 1968, for a description of *Minutes*). Both of these effects are characteristic of *Minute* mutations. K. C. ATWOOD (cf LINDSLEY and GRELL 1968) has suggested that *Minutes* may represent mutations in sites of tRNA synthesis. Support for this proposal has resulted from the demonstration by *in situ* tRNA hybridization to salivary gland chromosomes that hybridization does appear to occur near bands known to be associated with *Minutes* (STEFFENSON and WIMBER 1971). Recently, SMITH *et al.* (1970) showed that ts mutations could occur in tRNA genes. The pupal TSP of *DTS-1* provides an intriguing incentive for determining whether the locus does, in fact, produce a thermolabile tRNA. This is currently under investigation.

The utility of ts mutations in analyses of development has been reviewed recently (SUZUKI 1970). The recovery of *DTS*-lethals provides another tool in the extensive array available for chromosome mechanics in Drosophila. Thus, translocations between the Y-chromosome and an egg lethal *DTS* allow the early elimination of male zygotes. WRIGHT (1970) has utilized *DTS*-lethals to select specific classes of heterozygotes. In cultures of mammalian cells, the recovery of ts-lethals may include *DTS*-lethals that could allow analyses of cell cycles *in vitro* (THOMPSON *et al.* 1970). Finally, the selection of *DTS*-lethals with continuous sensitivity to high or low temperatures encountered in the wild might be a useful genetic tool for pest control.

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