TEMPERATURE-SENSITIVE MUTATIONS IN *DROSOPHILA MELANOGASTER*. IX. DOMINANT COLD-SENSITIVE LETHALS ON THE AUTOSOMES¹

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ABSTRACT

Ethyl methanesulfonate-treated autosomes were screened for the presence of dominant cold-sensitive (DCS) lethal mutations in *Drosophila melanogaster*. None was found among 6,552 treated and 168 untreated third chromosomes. Twenty-three DCS-L chromosomes which caused death at 17°C but survived at 22°C and 29°C were recovered from 5,046 mutagenized chromosome 2's. The DCS-L mutations all mapped around *dp* and appeared to be functionally allelic. Lethality of heterozygotes for most of the DCS-L's occurred over a prolonged interval from the embryonic through the larval instars. Prolonged incubation at 17°C did not demonstrate any maternal effect on zygotic survival.

DOMINANT and recessive conditional lethal mutations which cause death at high temperatures but permit survival at lower temperatures have now been extensively reported in *Drosophila melanogaster* (SUZUKI *et al.* 1967; BAILLIE, SUZUKI and TARASOFF 1968; SUZUKI and PROCUNIER 1969). The genetic properties of such "heat-sensitive" mutations conform to the expectations of missense changes which produce thermolabile products. That is, heat-sensitive lethals are not induced by a frame-shift mutagen and are most frequent among lethals induced by a mutagen causing single-base changes (SUZUKI *et al.* 1967). Moreover, chemically induced mutations map within a single region as points extensively throughout the chromosomes (SUZUKI, in preparation). The utility of temperature-sensitive (ts) mutations in a variety of genetic and developmental studies has been reviewed recently (SUZUKI 1970).

In bacteriophage T4, heat-sensitive lethals were found to map extensively throughout the viral chromosome (EDGAR and LIELAUSIS 1964). In contrast, "cold-sensitive" lethals, that is, mutations allowing survival at higher temperatures but producing death at low temperatures, are strikingly clustered within a limited number of phage cistrons (SCOTT 1968). In bacteria, a considerable proportion of cold-sensitive mutations has been found to disrupt the regulatory properties of allosteric proteins (O'DONOVAN and INGRAHAM 1965) or the self-assembly of ribosomes (GUTHRIE, NASHIMOTO and NOMURA 1969; TAI, KESSLER

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and INGRAHAM 1969). Cold sensitivity tested in heterozygotes of one ribosomal mutant was found to be dominant (GUTHRIE *et al.* 1969).

It was of interest, therefore, to determine: (1) whether dominant cold-sensitive lethal mutations could be recovered in *Drosophila*; (2) the genetic distribution of such lethals in comparison with dominant heat sensitives already known (HOLDEN and SUZUKI 1968; SUZUKI and PROCUNIER 1969); and (3) the developmental time of lethality at restrictive temperatures as a possible indication of the nature of the functional defect. This is a preliminary report on dominant autosomal cold-sensitive lethal mutations in *Drosophila*.

MATERIALS AND METHODS

Screening: Newly eclosed Oregon-R males were fed 0.025 M ethyl methanesulfonate (EMS) dissolved in 1% sucrose, or 1% sucrose (controls) for 2+ hr (LEWIS and BACHER, 1968). The entire screening protocol is shown in Figure 1. Treated second and third chromosomes were recovered in F_1 males at 22°C balanced over the multiply inverted chromosomes *Ins(2L+2R)Cy* (associated with a dominant phenotype of upcurved wings) or *In(2LR)Pm* (dominant dark brown eye color) on the second chromosomes. On the third chromosome, *TM-3* (enlarged halteres) or *CxD* (wings held out) was used as the balancer chromosome and the same pro-

FIGURE 1.—Screening protocol for the detection and recovery of dominant cold-sensitive lethal mutations on chromosome 2. +* = an EMS-treated chromosome 2.

cedure as in Figure 1 was followed. A complete description of the balancers and markers used can be found in LINDSLEY and GRELL (1968). Each F_1 male was individually mated to 3 virgin *Cy/Pm* or *TM-3/CxD* females in vials at 22°C and transferred to fresh vials at 17°C after 4 days.

In the initial screening, the 17°C cultures were scored first for the absence of $+*/Cy$ or $+*/CxD$ flies ($+$ denotes the mutagenized chromosome) without etherization. Cultures retained by this quick scanning process were than etherized and the phenotypic classes noted. All progeny at both 17°C and 22°C were counted in each subsequent retest. Since the 22°C cultures developed so much faster than the 17°C replicates, in the initial screen when 17°C cultures yielded fewer than 5 $+$ -bearing offspring, the 22°C replicate cultures were simply checked for the presence of $+$ -bearing flies. If there were, the stocks were retained for retesting. Owing to the protracted development time at 17°C, such cultures were scored as soon after eclosion as possible (when a sufficient number had hatched to yield a minimum of twenty flies). This resulted in the inadvertent selection of mutants which were not lethal but which exhibited a delayed development.

In the second retest (Step 5, Figure 1), $+$ -bearing offspring in the 22°C cultures were maintained for stocks until the 17°C cultures had been scored thoroughly. The stocks retained at this step were classified either as putative Dominant Cold Sensitives (DCS) or Dominant Retarded. In all cases where the females heterozygous for the treated chromosome were fertile at 22°C, homozygotes failed to survive at 22°C. Thus stocks were kept balanced over inversions. In some cases, the heterozygous females were sterile or semisterile and the chromosomes were maintained by crossing males bearing the chromosome to *Cy/Pm* females.

The putative DCS stocks were subjected to a third retest as shown in Figure 1 (Step 6) and the stocks retained were classed as confirmed DCS lethals.

Genetic Position: All of the second chromosome confirmed DCS lethal mutations yielding fertile females were mapped genetically using the markers (followed by their symbols and genetic locations): *aristaless* (*al*, 0.1); *dumpy* (*dp*, 13.0); *black* (*b*, 48.5); *purple* (*pr*, 54.5); *cinnabar* (*cn*, 57.5); *curved* (*c*, 75.5); *brown* (*br*, 104.5). Note that the centromere lies between *pr* and *cn* at 55. The left and right parts of the chromosome were marked by two stocks, *al dp b pr* and *b cn c br*, respectively. DCS/*Cy* males were crossed to both marker stocks at 22°C and their female offspring testcrossed. Chromosomes of Oregon-R males were tested as controls. In the testcross, parents were mated for 1 to 2 days in vials at 22°C and then transferred to quarter pint bottles at 17°C (20 pairs per bottle). All progeny at 17°C were scored.

Developmental Analysis: The "effective lethal phase" (LP) of the mutants, that is, the time at which death occurred at the restrictive temperature, was determined for each lethal stock. Adult *Cy*/DCS flies of each stock which had recently eclosed at 22°C were mated at 17°C and 22°C. Eggs were collected during a 12-hr interval at 22°C and shifted immediately to 17°C. Adults placed at 17°C were conditioned for 3–4 days and their eggs were collected during a 12-hr interval at 17°C. The number of eggs was recorded at the time of removal of the parents and the cultures were maintained at 17°C and inspected periodically for egg hatching, larval instars present, pupation and eclosion.

The contribution of cytoplasmic ribosomal material to the egg in accordance to the maternal genotype is well known (BROWN and GURDON 1964). If, in fact, DCS lethality results from defective ribosomal assembly at restrictive temperatures, prolonged incubation of parental females at 17°C might be expected to induce progressive loss of egg hatchability. In order to test this, *Cy*/DCS females of each stock were crossed to Oregon-R males at 22°C and eggs collected in order to provide a normal hatch rate. The parents were then shifted to 17°C and successive collections of eggs were made at 3-day intervals over a 42-day period. After each collection, approximate numbers of eggs were recorded and the cultures incubated at 17°C for 35–40 days, at which time all adult progeny were counted in each culture.

RESULTS

Screening: Out of 6,552 EMS-treated and 168 control third chromosomes, no confirmed dominant cold-sensitive lethals were recovered. On the other hand,

TABLE 1

Viabilities of different DCS-L stocks at different temperatures relative to Oregon-R control

Stock	<i>Cy</i> /DCS-L♂ × <i>Cy</i> / <i>Pm</i> ♀								<i>Cy</i> /DCS-L♂ × <i>Cy</i> /DCS-L♀			
	October, 1969				June, 1970				December, 1969		August, 1970	
	22°C		17°C		22°C		17°C		Average Number		Adults/Vial	
	Total	RVI	Total	RVI	Total	RVI	Total	RVI	29°C	22°C	17°C	17°C
Oregon-R	91	1.00	88	1.00	131	1.00	378	1.00	100	99	89	.
DCS-L1	84	0.93	40	0	182	0.89	163	0.01	27.5	63	0.3	0
2	58	0.46	31	0	160	0.65	92	0.02	24	19	0	0
3	301	0.51	302	0.01	92	0.37	175	0.02	..	..	..	..
4	110	0.55	49	0	120	0.76	122	0.02	50	49	0.7	2
5	61	0.51	54	0	97	0.45	112	0.04	30	41	0	0
6	59	0.45	45	0	163	0.61	147	0.02	38	37	0.5	1
7	91	0.75	27	0	224	1.04	169	0.70	64	63	1.3	>20
8	137	0.79	89	0.05	193	0.90	99	0.09	47	58	0	1
9	158	0.81	94	0	173	1.01	160	0.04	*	49	0	1
10	128	0.63	70	0	220	0.95	157	0.01	53	61	0	0
11	61	0.55	44	0.03	108	0.71	65	0.02	* 5.5	70	0	0
12	358	0.64	187	0	81	0.11	158	0.04	—	—	—	—
13	148	0.82	62	0.05	106	0.78	95	0	46	91	0	0
14	124	0.79	60	0.02	119	0.82	169	0.03	22	57	0	1
15	149	0.82	86	0.03	133	0.91	84	0.15	68	89	0	>8
16	140	0.82	64	0	130	0.89	63	0.02	63	83	0.3	1
17	148	0.81	77	0	190	0.84	144	0.02	55	74	0.3	0
18	76	0.88	31	0	197	0.84	175	0.04	38	69	3.8	>8
19	142	0.79	98	0	180	0.88	183	0	*12.3	89	2.8	3
20	136	0.98	65	0	215	0.93	132	0.07	57	76	3.8	1
21	155	0.81	91	0	166	0.95	158	0.02	64	49	5	1
22	161	0.72	92	0.02	201	0.87	153	0.07	76	71	8	>5
23	67	0.44	36	0	186	0.77	169	0.04	64	47	7	>20
D-11-75-4	90	0.81	130	0.94	124	1.05	85	0.63	10	32	30	>20

* Subsequent retests of these stocks revealed no significant differences between viabilities at 22°C and 29°C.

RVI: Relative viability index calculated as

$$\left(\frac{Cy/DCS-L + Pm/DCS-L}{Cy/Pm} \right) \left(\frac{Cy/Pm}{Cy/Ore-R + Pm/Ore-R} \right)$$

where each genotype represents the number of individuals of that class. Note that the numbers of *Cy/Pm* flies come from each separate lethal and control cross.

from 5,046 treated second chromosomes, 96 cultures (1.9%) were retained as possible mutants in the initial screen. Of these, 23 (0.46%) were kept as confirmed DCS-lethals (DCS-L), one mutant (D11-75-4) was kept as a possible DCS-male sterile, and 33 (0.66%) were kept as Dominant Retarded. Of the Dominant Retarded stocks, 14 caused female sterility at 22°C. Of 168 control second chromosomes, none was scored as a possible DCS lethal in the first screen.

The viabilities of flies bearing the DCS-L chromosomes were compared with non DCS-L-bearing siblings by crossing *Cy*/DCS-L males to *Cy*/*Pm* females and scoring their offspring (Table 1). In addition, *Cy*/DCS-L males were crossed to their *Cy*/DCS-L sisters and in every case, yielded only *Cy* offspring, thus indicating their recessive lethality at 22°C.

Females carrying mutants 3 and 12 were poorly fertile and therefore were maintained by crossing males to *Cy/Pm* females. Both these DCS-L's were eventually lost.

Initially, all 23 DCS-L's had a relative viability of less than 5% at 17°C and more than 43% at 22°C (Table 1). Over a 10-month period, the viabilities of most of the stocks at both temperatures increased somewhat, probably through the accumulation of modifiers. The viabilities of DCS-L's 7 and 23 increased considerably. All stocks retained their recessive lethality. The recessive lethality at 22°C could result from mutations other than the DCS-L elsewhere on the chromosome. DCS-L/*Cy* heterozygotes of all of the stocks survived at 29°C.

Of the mutations retarding development time, 14 had to be maintained by crossing males to *Cy/Pm* females because the females carrying the chromosome were sterile or poorly viable. Progeny in six of these stocks had a high frequency of slender bristles and/or rough eyes, a phenotype characteristic of Minute mutations. Of the 19 retarded stocks in which females were fertile, two had adults with slender bristles or rough eyes. At 17°C, flies in the retarded stocks were delayed by 5 to 10 days while at 22°C, the delay was 1 to 3 days.

Genetic Mapping: The frequencies of reciprocal classes in the control crossover study at 17°C exhibited marked inequality reflecting reduced viability of the markers. This was most pronounced in all classes involving *dp*. Moreover, *dp* was poorly penetrant, especially in males, so female progeny only were used. Nevertheless, the map distances in controls were in good agreement with standard values, the distances obtained (followed by their values in LINDSLEY and GRELL 1968) being: *al-dp*, 16.2 (12.9); *dp-b*, 36.8 (35.5); *b-pr*, 5.5 (6.0); *b-cn*, 5.6 (9.0); *cn-c*, 17.7 (18.0); *c-bw*, 30.2 (29.0). While corrections could be made for the inviability of markers and for the leakiness of the DCS-L's, the data clearly permitted the ready localization of all the DCS mutations (Table 2). Surprisingly, all DCS-L mutations mapped near the *dp* locus, an area in which second chromosome dominant heat-sensitive lethals had been found to cluster (SUZUKI and PROCUNIER 1969). Besides a mutation in the *dp* region, DCS-L5 appeared to have a second mutation in the *cn* to *c* region. DCS-L7 and DCS-L23 were too leaky to localize.

Complementation Tests: Since all of the stocks had mutations located near *dp*, a complementation test at 22°C was carried out. Recessive lethality of the DCS-L-bearing chromosomes at 22°C could be the consequence of the DCS-L mutation itself or of recessive lethals elsewhere on the chromosome. However, the probability that different DCS-L chromosomes would carry the same second-site lethal is low; therefore, the failure of *trans* heterozygotes for two different DCS-L's to survive at 22°C would verify recessive lethality of the DCS mutations and their functional allelism.

Therefore, *Cy*/DCS-L flies of each stock were crossed to each other in all possible pairwise combinations at 22°C. In addition, each DCS-L stock was crossed to *Cy* flies carrying second chromosomes derived from Oregon-R and Samarkand wild-type stocks and the dominant heat-sensitive lethals 3 (located in the *dp* cluster) and 6 (between *b* and *pr*) (SUZUKI and PROCUNIER 1969). Three vials of 2 pairs of flies each were established for each cross. The percent non-*Cy* off-

TABLE 2
Female progeny in crossover test of different stocks at 17°C with the markers al dp b pr

Classes	Ore-R	1	2	4	5	6	8	9	10	11	13	14	15	16	17	18	19	20	21	22
<i>al dp b pr</i>	147	94	67	108	114	62	284	159	163	250	161	139	144	141	177	122	179	131	377	88
<i>+</i>	387	1	1	3	0	3	38	15	3	1	0	4	19	3	0	19	7	61	8	5
<i>+</i>	46	14	16	16	24	10	40	27	36	43	29	30	43	40	28	28	34	31	66	15
<i>al</i>	91	0	8	0	2	1	16	29	7	1	3	3	5	0	0	3	3	4	2	5
<i>+</i>	249	6	11	3	9	2	44	21	15	21	17	29	26	14	13	14	18	11	37	17
<i>al dp</i>	131	42	39	76	42	48	154	86	155	108	146	119	104	127	107	52	116	120	229	68
<i>+</i>	27	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	2	0	0
<i>al dp b</i>	20	10	6	19	10	13	28	19	23	20	21	30	26	23	33	15	20	26	42	12
<i>+</i>	31	0	5	1	0	1	8	13	6	5	6	6	3	2	6	4	8	1	21	2
<i>+</i>	6	1	1	1	1	0	2	1	4	2	4	1	6	4	4	0	0	2	8	2
<i>al</i>	7	0	1	0	1	0	0	6	0	0	0	0	0	0	0	0	0	0	0	2
<i>+</i>	3	1	1	2	2	3	7	1	0	1	2	3	2	5	3	1	3	4	4	0
<i>+</i>	1	0	0	0	1	0	5	0	0	1	1	0	0	0	2	2	0	2	2	0
<i>+</i>	2	0	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
<i>al dp</i>	0	1	3	2	3	3	5	3	2	2	2	4	2	1	3	1	3	1	3	2
<i>+</i>	5	1	0	0	1	2	0	5	0	0	1	0	7	1	0	2	0	1	5	4
Totals	1,153	171	159	231	210	148	637	385	414	454	394	368	387	361	376	263	391	397	805	222

spring in each cross are presented in Table 3. If the level of 6% is arbitrarily designated as an indication of complementation, then Samarkand and DTS-L6 complement with each of the others. Oregon-R complemented with all except

TABLE 3

Percent non-Cy offspring from crosses of Cy males and females bearing different chromosomes

	Wild type						DTS-L																		DCS-L									
	OR	Sam	3	6	1	2	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22	23									
Oregon-R	37	30	32	33	24	10	14	3	13	22	29	24	14	15	13	13	22	18	17	19	12	30	15	21	19									
Samarkand		28	23	19	30	25	26	17	21	28	28	31	26	26	28	21	27	24	28	34	31	33	20	28	17									
DTS-L 3			0	26	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1	0	0	0	0	0	11									
6				0	24	24	24	30	27	25	15	30	22	32	30	34	27	32	21	30	23	17	33	23	27									
DCS-L 1					1	2	12	1	0	19	30	0	0	0	0	13	20	1	1	23	1	21	1	25	14									
2						0	4	2	0	18	18	4	0	1	2	8	3	4	6	1	18	22	4	0	16									
4							0	0	4	18	1	23	18	14	18	0	0	12	10	1	14	0	12	0	11									
5								1	1	14	4	0	0	1	1	1	2	0	1	6	0	8	0	3	4									
6									0	15	17	0	0	0	0	5	11	0	0	12	0	13	0	14	1									
7										0	29	18	26	6	16	21	26	24	11	25	14	36	16	16	17									
8											0	35	25	17	19	0	0	27	25	0	25	0	17	0	8									
9												0	0	0	0	25	33	0	0	30	0	29	0	32	8									
10													0	0	0	14	23	0	0	21	0	24	0	25	9									
11														0	0	13	22	0	0	17	0	21	0	24	5									
13															0	1	20	0	0	18	0	24	0	23	2									
14																0	3	12	16	0	20	0	14	0	6									
15																	0	24	19	0	25	0	18	0	8									
16																		0	0	17	0	28	0	23	2									
17																			0	13	0	25	0	21	1									
18																				0	18	0	14	1	10									
19																					0	29	2	30	10									
20																						0	20	1	11									
21																							0	18	1									
22																								0	1									
23																									0									

DCS-L5 but at a lower level. DTS-L3 did not complement with any except Oregon-R, Samarkand, DTS-L6 and DCS-L23. Using 6% as a cut-off level and omitting DCS-L's 5, 7 and 23, the data yield a complementation pattern of 9 groups that define 6 complons (Figure 2).

Developmental Analysis: All of the DCS lethals were immune to 17°C as adults. A summary of the data on development is presented in Table 4. In order to facilitate comparisons, all of the data are expressed as percentages of each stage detected relative to the original number of eggs. Each culture initially contained a minimum of 100 eggs. Since all *Cy* homozygotes die in the egg stage, maximum egg hatches would be 75%. For each larval instar, the maximum number of individuals observed at that stage at the time of a single observation was scored. The total numbers of pupae and adults were also scored. Egg hatches could be readily determined in 17°C cultures, but at 22°C, rapid growth and asynchrony of development obscured the counts. Consequently, hatch rates were estimated by the sum of the maximum number of first and second instar larvae observed simultaneously. The DCS mutations permitted considerable survival at 22°C. Since both *Cy* and DCS homozygotes were found to die at 22°C, complete survival of

FIGURE 2.—Complementation map of different DCS-L's on chromosome 2. Survival of more than 18% of the expected number of *trans* heterozygotes was classified as complementation.

DCS/*Cy* flies would yield an adult to egg ratio of 0.5. This ratio was greater than 0.2 in all but three stocks and greater than 0.3 in all but 5 (Table 4). In contrast, at 17°C, most of the stocks showed a greatly reduced viability of heterozygotes, regardless of whether the eggs were collected at 22°C or 17°C. The effective lethal phase for mutant 23 appeared to occur just prior to eclosion; most of the heterozygotes formed normal pupae and died during the emergence from the pupal case. In all other stocks, the lethal phases appeared to be prolonged, the cultures showing progressively fewer survivors at later stages. With the exception of mutant D11-75-4, the frequency of hatching from the egg was greater than 0.2 and the most extensive reduction in survivors occurred during the second and third larval instar stages.

At 22°C, the developmental rate of the DCS stocks was approximately the same as that of the +/*Cy* control, whereas at 17°C, development of the mutants was considerably retarded.

TABLE 4

Percentage of DCS-bearing flies surviving at different developmental stages

	Larval instars									Pupae†			Adults†		
	First			Second			Third								
	a*	b	c	a	b	c	a	b	c	a	b	c	a	b	c
1	48	30	..	39	8	..	23	1	..	35	1	..	33	0	..
2	33	15	43	20	8	8	40	1	0	34	1	0	33	0	0
4	60	38	34	20	9	5	50	3	1	67	6	0	65	3	0
5	36	43	35	18	3	5	30	0	0	43	0	0	26	0	0
6	36	40	23	31	2	6	43	1	1	29	1	1	28	0	1
7	30	20	25	18	11	8	33	5	4	35	8	6	37	2	6
8	35	36	38	20	19	20	40	2	1	22	2	1	21	0	0
9	35	33	43	25	11	9	25	5	3	40	2	3	40	2	3
10	57	44	35	40	7	13	41	1	1	38	0	0	36	0	0
11	42	37	22	37	7	6	36	2	0	37	2	0	36	1	0
13	47	47	30	40	3	5	33	0	0	50	0	0	61	0	0
14	47	44	40	43	13	14	44	3	0	15	3	0	16	2	0
15	75	45	31	65	12	12	50	11	7	63	8	6	65	8	4
16	43	48	57	34	13	8	33	2	9	62	0	0	56	0	0
17	44	46	50	25	5	11	32	1	0	38	0	0	36	0	0
18	15	27	24	13	9	7	15	6	2	18	8	1	9	3	0
19	52	58	57	35	15	9	37	1	2	61	3	1	61	2	0
20	32	29	20	10	8	8	25	26	3	30	18	2	30	9	0
21	38	35	32	31	4	14	33	2	2	56	1	1	60	1	1
22	45	61	51	13	24	14	37	10	2	57	12	3	56	7	3
23	41	25	39	29	11	13	35	43	32	54	44	43	30	8	17
+/ <i>Cy</i>	75	12	69	56	34	67	43	40	64	67	64	70	65	62	67

* Values calculated by number of larvae/number of eggs.

† Percentage of eggs reaching pupal and adult stages.

a Cultures left at 22°C.

b Eggs collected at 22°C and shifted to 17°C.

c Eggs collected and retained at 17°C.

Mutant D11-75-4 is noteworthy in that males which developed at 22°C were weak and produced progeny only after a week or more. D11-75-4 males that developed at 17°C were almost entirely sterile.

All but one stock of DCS/*Cy* females mated to wild-type males continued to produce developmentally competent eggs (i.e., hatching and eclosing) over a 42-day period at 17°C. Plates from stock number 4 became overgrown with micro-organisms after yielding eggs and progeny for at least 23 days at 17°C.

DISCUSSION

No striking properties were found that distinguish the dominant cold-sensitive lethals as a class from the dominant heat sensitives. The frequency of recovered DCS lethals was low, as it had been for the DTS-L's, (less than 0.5%) when compared with that of the sex-linked recessive heat sensitives (where the 0.025M EMS-induced *ts* mutation frequency was *ca.* 10%; SUZUKI *et al.* 1967). The recent recovery of autosomal recessive *ts* lethals (E. TASAKA and K. NEWCOMBE,

unpublished) will permit a better comparison of genetic properties of dominant and recessive autosomal *ts* mutations. The failure to detect DCS mutations on chromosome 3 in contrast to their recovery on chromosome 2 resembles the disparate frequencies with which dominant heat-sensitives were induced on the two chromosomes: 48 DTS on 17,000 second chromosomes tested and only 8 DTS on 26,000 third chromosomes (SUZUKI 1970). The finding that all DCS and most of the DTS lethals reside within a restricted segment of chromosome 2, shows that this frequency difference is due to an apparent "hot spot" and that along the rest of chromosome 2, dominant temperature-sensitive mutations are also rare.

The failure of second chromosomes from Oregon-R to complement with DCS chromosomes at as high a level as did those from Samarkand and DTS-L6 (Table 3) suggests that some mutations may have been induced on chromosomes carrying pre-existing lethals. The recessive lethalties of the DCS chromosomes in certain combinations (Figure 2) could be accounted for by this and therefore by themselves, cannot be taken as proof of functional relatedness of the DCS lethals. However, the failure of DTS-L3 to complement with any of the DCS-L's while complementing well with Oregon-R (Table 3) does suggest that all the dominant temperature-sensitive lethals in the *dp* region are functionally related and make this "hot spot" particularly interesting.

Circular complementation maps have been extensively documented in microorganisms. However, the complementation maps for both DTS and DCS lethals are circularized owing to single alleles (mutant 14 for the DCS mutations) which could be double mutants. Genetic recombination between the different alleles within the locus should resolve this question. The recent synthesis of overlapping deletions spanning most of the autosomes (SEATTLE-LA JOLLA DROSOPHILA LABORATORIES 1971) provides a simple means for the accurate cytological and genetic localization of the DCS-L mutations. It will be of considerable interest to correlate the cytological, genetic and complementation data on the nature of the DCS cluster.

The survival of DCS lethals at 29°C and of DTS lethals at 17°C shows that DCS and DTS lethals represent distinct classes of defects rather than mutations which permit viability only within a narrow temperature optimum. This does not necessarily imply two or more separate cistrons since heat- and cold-sensitive alleles can be recovered within a single cistron. The genetic distribution of the heat- and cold-sensitive mutations within this area should indicate the size of the region and their relationship to the complementation map (M. J. SCHEWE, unpublished, has already recovered recombinants from *trans* heterozygotes for different DTS-L's).

The relative rarity of dominant *ts* lethals warrants mention. Dominant point mutations which are lethal may result in either the disruption in function of polymeric units, or in the loss of an active gene product for which two gene doses are required. Dominants of the latter type are unlikely since the survival of heterozygotes for numerous deletions (LINDSLEY and GRELL 1968) shows that cells must have extensive compensatory mechanisms for the loss of an entire dose of the gene segment. We conclude that the low frequency of dominant tempera-

ture-sensitive lethal mutations reflects either a relatively small number of loci capable of mutating to a dominant allele or the low probability of affecting a gene product at a site which would interfere with the activity of other wild-type products at the restrictive temperature but not at the permissive one. Possible non-ts dominants involved in polymeric structures might be recovered if the proportion of defective polypeptides was reduced by selecting in triploids.

Cold-sensitive lethality is a phenotype which efficiently enriches for defects in ribosome assembly in *E. coli* (GUTHRIE *et al.* 1969). Two tests for ribosomal defects showed that selection for autosomal DCS lethals is not an effective method for detecting such mutations in *Drosophila*. Comparison of ribosomal proteins from wild type and all DCS stocks by electrophoresis revealed no differences (M. GOULD-SOMERO, unpublished). Further tests for intermediate ribosomal subunits are in progress. Maternally derived ribosomes function in newly fertilized eggs (BROWN and GURDON 1964). However, our studies showed that prolonged maternal exposure to the restrictive temperature did not result in cytoplasmically induced zygotic mortality. It is possible, however, that ribosomes produced at different times during development are not all similar.

An unforeseen result of the screening at 17°C was the recovery of developmentally retarded mutants that were recessive lethals and some of which had slender bristles and rough eyes, which are characteristic of Minute mutations. This occurred because retardation was much more noticeable at 17°C than at 22°C and suggests that screening for Minutes might best be carried out at low temperatures.

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