# TEMPERATURE-SENSITIVE MUTATIONS IN *DROSOPHILA MELANOGASTER*. XVII. HEAT- AND COLD-SENSITIVE LETHALS ON CHROMOSOME 3[1, 2]

S. ELAINE TASAKA AND DAVID T. SUZUKI

*Department of Zoology, The University of British Columbia, Vancouver, B.C., Canada*

Manuscript received December 18, 1972
Transmitted by G. LEFEVRE

ABSTRACT

Ethyl methanesulfonate-treated third chromosome of *Drosophila melanogaster* were tested for the presence of dominant and recessive temperature-sensitive lethal mutations at 17°, 22° and 29°C. Out of 1,176 chromosomes tested, no dominant ts lethals, 21 heat-sensitive, 22 cold-sensitive and 10 heat-cold-sensitive lethals were recovered. Heat-cold sensitivity was produced by a single mutation in all cases. Sixty-two percent of the ts lethals were fertile as homozygotes in both sexes. Surprisingly, 88% of the ts lethals mapped between *st* and *Sb*, a region straddling the centromere and estimated to comprise 12.9% of the genetic length and 55% of the physical length of chromosome 3. All but one of the heat- and cold-sensitive lethals complemented with each other at their respective restrictive temperatures.

TEMPERATURE-sensitive (ts) mutations are now being exploited to study a variety of genetic, developmental and neurological phenomena in *Drosophila melanogaster* (see SUZUKI 1970 for a review). Temperature sensitivity is not a property confined to mutations on certain chromosomes or to a limited number of sites within a chromosome (SUZUKI and PITERNICK 1972). Several different screening programs have yielded a variety of thermally-affected mutations.

Thus, heat-sensitive, recessive and dominant ts mutations have been analyzed on the *X* chromosome (SUZUKI *et al.* 1967; WRIGHT 1968; SUZUKI and PITERNICK 1973), *Y* chromosomes (AYLES *et al.* 1972), chromosome 2 (BAILLIE, SUZUKI and TARASOFF 1968; SUZUKI and PROCUNIER 1969) and chromosome 3 (FATTIG and RICKOLL 1972; HOLDEN and SUZUKI 1968, 1973). In addition, cold-sensitive recessive lethals on the *X* chromosome (MAYOH and SUZUKI 1972) and dominant lethals on the autosomes (ROSENBLUTH, EZELL and SUZUKI 1972) have been studied.

The third chromosome appears to differ from the second in terms of the frequency of dominant ts lethals recovered on it. More than 25,000 mutagenized third chromosomes were screened for dominant heat-sensitive lethals and only ten were found (HOLDEN and SUZUKI 1973), whereas 19 dominant heat-sensitive

[1] This research was supported by National Research Council of Canada grant A1764 and National Cancer Institute of Canada, contract 6051.

[2] Paper number XVI in this series is H. MAYOH and D. T. SUZUKI, Can. J. Genet. Cytol.: in press.

lethals were recovered from 4,857 second chromosomes (SUZUKI and PROCUNIER 1969). In a search for dominant cold-sensitive lethals, 23 were recovered out of 5,046 mutagenized second chromosomes, whereas none were found among 6,552 treated thirds (ROSENBLUTH, EZELL and SUZUKI 1972).

Comparisons between dominant and recessive, cold- and heat-sensitive mutations in terms of their induction frequencies and general properties have been difficult owing to the differences in screening conditions for each class of mutation. Consequently, we decided to analyze a set of mutagenized chromosomes simultaneously for heat- and cold-sensitive, dominant and recessive lethal mutations. Testing for viability and lethality was performed at 17°, 22° and 29°C so that a number of different classes of ts lethals could be expected. Chromosome 3 was chosen because of the pecularities already mentioned and possible comparisons with chromosome 2 mutations already reported (BAILLIE, SUZUKI and TARASOFF 1968; SUZUKI and PROCUNIER 1969; ROSENBLUTH, EZELL and SUZUKI 1972). This paper summarizes the properties of the mutations recovered, their genetic positions and functional relations.

## MATERIALS AND METHODS

The procedure for screening third chromosomes for the presence of ts lethals is shown in Figure 1. This method permitted the detection of mutations exhibiting dominant or recessive ts lethality or a recessive or dominant visible phenotype at any temperature. The mutagen, ethyl methanesulfonate (EMS), was administered as described by LEWIS and BACHER (1968). Detailed descriptions of the properties of the mutations and chromosome aberrations used can be found in LINDSLEY and GRELL (1968). Males isogenic for a third chromosome marked with *st* were used for mutagenizing. EMS-treated males were mated to virgin *Sb Ubx/Xa* females (10♂:20♀ per bottle) in quarter-pint milk bottles containing standard Drosophila medium. These cultures were raised at 22°C. The *st*/Sb Ubx* male progeny (where *st** denotes an EMS-treated chromosome) were individually mated at 22°C in vials to three *Gl Sb $e^s$/TM2* virgin females. Note that the paternally-inherited *Sb Ubx* chromosome is lethal in combination with either maternal chromosome so all surviving progeny carry the paternally-derived *st** chromosome. All *st*/TM2* females and males were separated and placed together into fresh vials (the females were not necessarily virgin and could have mated with either *st*/Gl Sb $e^s$* or *st*/TM2* males). This step (step 3, Figure 1) makes each EMS-treated *st* chromosome homozygous. These first vials in step 3 were kept at 22°C; after three days, the parents were transferred to fresh vials at 17°C; four days later, the parents were transferred to new vials at 29°C; three days later, the parents were discarded. Thus, each set of step 3 parents was mated at 17°, 22° and 29°C. Since developmental time at 17°C is greatly extended, scoring could only be done at that temperature 4 to 5 weeks after the culture was started. Thus, the cold cultures were scorable only long after the others were completed. Consequently, fresh cultures of the 22°C vials were established by repeating step 3 (Figure 1) before 17°C cultures were scored in order to ensure that a stock culture would be kept in the event the 17°C culture showed cold sensitivity. All vials were scored for the presence of *st*/st** progeny by etherizing the flies in each vial and recording the number of heterozygotes and homozygotes. Note that any dominant heat- or cold-sensitive lethal is readily detectable by the absence of *st**-bearing progeny at a restrictive temperature. At any one of the temperatures, reduced numbers of *st*/st** homozygotes relative to the number of heterozygotes suggested the induction of a ts lethal or semilethal mutation on the *st** chromosome. Any set of vials that indicated temperature sensitivity was classified as a putative temperature-sensitive stock and replicated from *st**-bearing flies in the 22°C culture vial. These stocks were then checked for the presence of the *Gl Sb $e^s$* chromosome and when necessary, *st*/TM2* virgin females were collected.

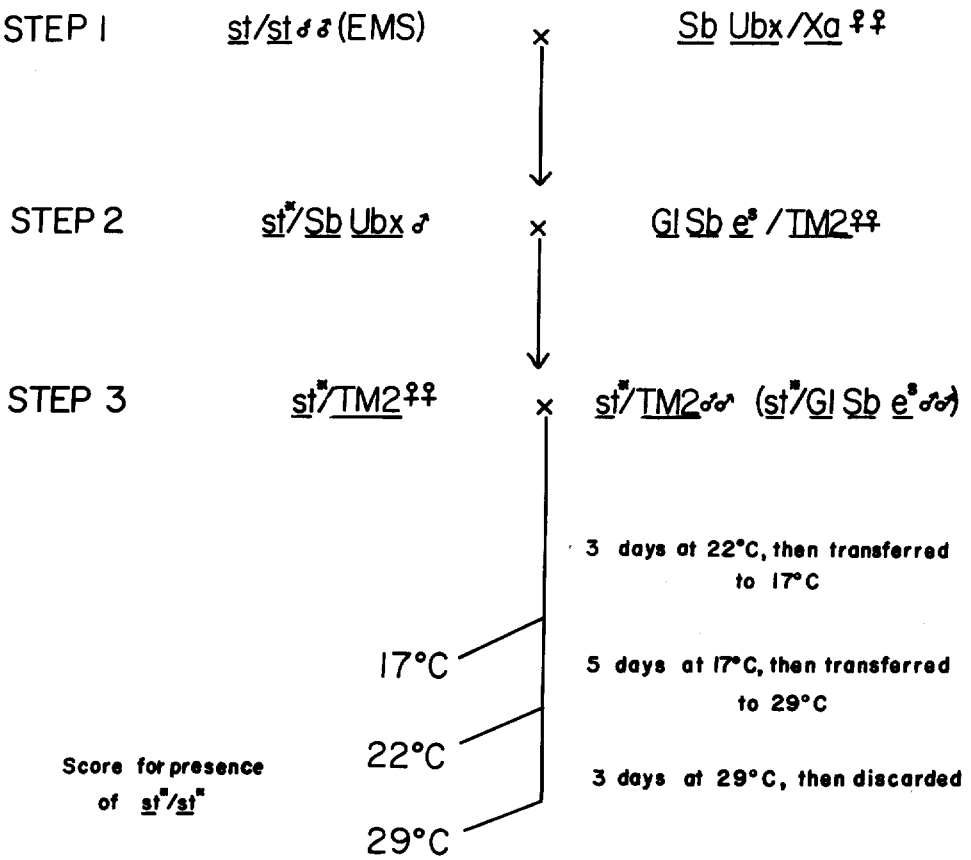


FIGURE 1.—Screening protocol for the detection of temperature-sensitive lethal mutations on chromosome 3.

Two separate mutagenesis experiments were carried out. In an early experiment, 1,008 chromosomes treated with 0.025M EMS were tested only at 17° and 22°C. In a second test, 1,176 chromosomes exposed to 0.0125M EMS were screened at all three temperatures following the protocol in Figure 1. Initially, as a control experiment, a non-isogenic *st* strain was tested using the procedure described following feeding only a sucrose solution.

Each putative ts lethal stock was retested several times before it was classified as a confirmed ts mutation. In each of the first and second retests of each stock, seven vials of 3 non-virgin females/vial were analyzed. Five of the seven vials contained heterozygous females and males and two contained homozygous females and males. These parents were first kept at 22°C for 3 days, then transferred to the appropriate non-permissive temperature. All progeny in each vial were scored for the number of heterozygous and homozygous survivors. The third retest involved only 4 vials per stock and in subsequent retests only 1 or 2 vials were analyzed. The progeny from those vials yielding maximum lethality at restrictive temperatures and viability at permissive temperatures were used to establish the stock.

The criteria for retention of a stock as a confirmed ts were arbitrarily designated as greater than 15% of the expected number of survivors at permissive temperatures and less than 4% under restrictive conditions. The percentages obtained are calculated against survival of heterozygotes where *st*/st** individuals are expected to be one-third of the total progeny.

Conditions in the retests were designed to retain those ts strains with maximum viability

TABLE 1

*Categories of recessive mutations expected in screening chromosome 3's for viability at 17°, 22° and 29°C*

| Category | | Viability of homozygotes at each temperature | | |
|---|---|---|---|---|
| | | 17°C | 22°C | 29°C |
| non-mutant | | + | + | + |
| non-ts lethal | | — | — | — |
| heat-sensitive | a | + | + | — |
| | b | + | — | — |
| cold-sensitive | a | — | + | + |
| | b | — | — | + |
| heat-cold-sensitive | | — | + | — |
| heat-cold dependent | | + | — | + |
| non-ts visible phenotype | | m* | m | m |

* m = mutant phenotype

differences between permissive and restrictive conditions. In all stocks yielding fertile homozygotes, the progeny of homozygotes were tested for survival at different temperatures, thereby eliminating competition with heterozygous sibs. In those stocks in which *st*/st** flies were sterile, the heterozygotes were tested in bottles as well as in vials, in order to rule out any possible effect of crowding on recovery of progeny. Five pairs of heterozygotes were mated per bottle at the restrictive temperature and the parents were removed after three days. Finally, each chromosome was maintained and tested in combination with the two different balancers, *TM2* and *TM3,Sb Ser*, thereby reducing an effect of genetic modifiers on survival.

In those cases where crosses of *st*/st** males and females failed to yield progeny, homozygous *st** males and females were crossed to *st*/TM3,Sb Ser* females and males, respectively. The results of these crosses indicate which sex is sterile.

Tests of each chromosome at 17°, 22° and 29°C could yield the different classes of mutations shown in Table 1. Recessive mutations in all but the heat-cold-dependent classes of mutations were recovered. Note that, in addition, dominant heat- or cold-sensitive lethal mutations were also detectable in progeny of the step 3 cross (Figure 1).

In order to define the genetic positions of the ts mutations, two marked chromosomes, one carrying the dominant markers *R* (*Roughened* 1.4), *D* (*Dichaete* 40.7) and *Sb* (*Stubble* 58.2) and the other *Sb*, *D1$^s$* (*Delta$^s$* 66.2) and *Dr* (*Drop* 99.2) were synthesized and balanced over *TM6* and *TM3*, respectively. These markers define genetic regions spanning the entire third chromosome and are shown in Figure 2. In addition to the dominant markers *st* on all ts chromosomes was also used as markers. Thus, the chromosome could be subdivided into 7 genetic regions of varying size, with the centromere (sfa) and its associated heterochromatin lying in region 4 between *st* and *Sb*. *TM3,Sb Ser/1(3)ET$^{ts}$* or *1(3)ET$^{ts}$/1(3)ET$^{ts}$* males (*1(3)ET$^{ts}$* represents the mutagenized chromosome carrying *st* and confirmed for ts lethality) of each ts stock were simultaneously crossed to *R D Sb/TM6* and *Sb D1$^s$ Dr/TM3* females (10♀:10♂/bottle) at 22°C. Virgin *1(3)ET$^{ts}$/R D Sb* and *1(3)ET$^{ts}$/Sb D1$^s$ Dr* females were then testcrossed to *1(3)ET$^{ts}$/TM3,Sb Ser* or *1(3)ET$^{ts}$/1(3)ET$^{ts}$* males (1♀:3♂/vial; 10♀:10♂/bottle) at re-

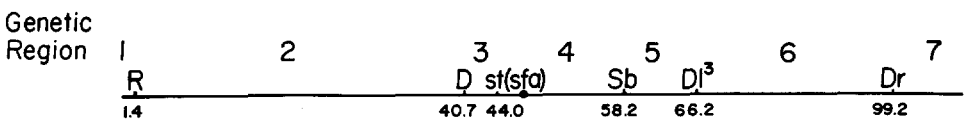


FIGURE 2.—Genetic markers used to locate ts mutations on chromosome 3.

strictive temperature. The parents were mated for 4 days at 22° and 29°C and 6 days at 17°C before being discarded. All non-*TM3,Sb Ser*-bearing progeny were then scored. The original isogenized chromosome 3 marked with *st* and several *1(3)ET*$^{ts}$ chromosomes were scored in a similar manner at the permissive temperatures in order to recover standard crossover values for comparison. Note that the *st–Sb* region is scored in both tests of the left and right arm markers.

## RESULTS

Among 336 non-mutagenized chromosomes tested from the non-isogenic *st* stock, no ts lethals were recovered but 33 (10%) were scored as lethals. The existence of lethal mutations in the *st* stock, therefore, prompted us to isogenize a viable *st* chromosome and all EMS treatments were made with the isogenic line. In the test of 0.025M EMS-treated chromosomes, ten of the 1,008 chromosomes scored carried confirmed ts lethal mutations. In this test, 805 (79.8%) of the chromosomes behaved as non-ts lethals (i.e., did not survive as homozygotes at either temperature).

In the second test after 0.0125M EMS treatment, step 3 (Figure 1) was carried out at all three temperatures. A total of 1,176 chromosomes was tested, of which 942 (80.1%) were scored as non-ts lethals and 4.6% as confirmed ts lethals. The result from the two test series are shown in Table 2. It can be seen that cold- and heat-sensitive lethals which die only at the extreme temperature were much more frequent (1.7%) than those which die at both 29° and 22°C (0.2%) or 22° and 17°C (0.1%). 0.9% of the chromosomes were classed as heat- and cold-sensitive lethals but it must immediately be pointed out that this could be the consequence of the induction of both a cold- and a heat-sensitive lethal on one chromosome at a frequency of 0.002 if the two events are independent. Thus, we would not expect many of the heat- and cold-sensitive chromosomes to carry two lethals, but crossover tests can show how many mutant sites are involved. It should be noted that no mutations of the reciprocal type, that is, heat-cold-dependent, were recovered. Recessive mutations producing obvious visible pheno-

TABLE 2

*Number of chromosomes recovered in different classes after EMS treatment*

| Category | | Screen 1 0.025M EMS | | Screen 2 0.0125M EMS | |
|---|---|---|---|---|---|
| | | Number | Percentage | Number | Percentage |
| non-mutant | | 187 | 18.6 | 166 | 14.1 |
| steriles | | 6 | 0.6 | 5 | 0.4 |
| non-ts lethals | | 805 | 79.8 | 942 | 80.0 |
| heat-sensitive | a | | | 20 | 1.7 |
| | b | (1)* | | 1 | 0.1 |
| cold-sensitive | a | 8 | 0.8 | 20 | 1.7 |
| | b | | | 2 | 0.2 |
| heat-cold sensitive | | (1)* | | 10 | 0.9 |
| heat-cold-dependent | | | | 0 | 0 |
| visibles | | | | 10 | 0.9 |

* No frequency given because not all chromosomes were scored at high temperature.

types occurred among 0.9% of the treated chromosomes. One semi-dominant non-ts mutation which was viable when homozygous was recovered but no dominant ts lethals were detected. A considerable proportion of the lethals induced are expected to carry multiple lethal mutations. Undoubtedly many ts mutations were not observed because of the epistasis of a nonconditional lethal induced on the same chromosome.

Sixty-two percent (31/50) of the stocks could be maintained as fertile homozygotes at the permissive temperatures of the 19 stocks in which crosses between homozygotes were sterile, 8 were the result of male sterility, 3 of female sterility and 8 of sterility of both. Again, it should be emphasized that sterility resulting from homozygosis may not be the consequence of the mutation responsible for the ts lethality.

A total of fifty-eight chromosomes carrying induced ts and visible mutations were mapped by crossing over. A minimum of 150 progeny of the mapping cross were scored for any test and over three thousand were scored for several. An average of 1,145 progeny were scored per stock. Genetic mapping permits ready determination of a single or multiple mutation as the basis for each ts chromosome. Of the 58 mutant chromosomes tested, 9 were found to carry more than one mutation. Of the nine multiply mutant chromosomes, two heat- and two cold-sensitive chromosomes were found to carry two mutations each. One heat-cold-sensitive chromosome carried both a heat-cold-sensitive and a cold-sensitive lethal. In addition, three chromosomes which were scored as ts lethals each had a second site non-ts mutation expressing a visible mutant phenotype in survivors at permissive temperatures. Finally, one of the chromosomes retained for its visible phenotype was also found to have two mutations. Thus, of the chromosomes tested, a total of 58 ts lethal, 7 non-ts visible and 2 ts visible mutations were demonstrated.

Genetic localization of the heat-cold-sensitive mutations suggests that both heat and cold sensitivity are often properties of single loci. This is further supported by the finding that 6 of 17 lethals selected on the basis of cold sensitivity in bacteriophage λ were also heat-sensitive (Cox and Strack 1971). In our tests, the number of mutants scorable as cold-sensitive was 41 and as heat-sensitive was 33. Of these, ten are heat-cold-sensitive lethals.

In contrast with the report of Fattig and Rickoll (1972) that nine heat-sensitive lethals were distributed at a number of sites along the entire third chromosome, we find ts lethals to be highly clustered on the genetic map (Figure 3). Eighty-eight percent (52/59) of the ts mutations tested map in the *st-Sb* region which encompasses the centromere and proximal heterochromatin. No ts mutations map to the left of *st* and only seven fall to the right of *Sb* (Figure 3). In contrast to this, seven non-ts mutations with visible phenotypic effects map in a number of regions and two were located to the left of *st*.

Crossover tests of the *st* control chromosome, as well as ts mutations at a permissive temperature, revealed greatest differences in values from standard crossover distances in the *R–D* segment, a very large crossover region (Table 3). Fluctuations in the other regions undoubtedly reflect the genetic heterogeneity

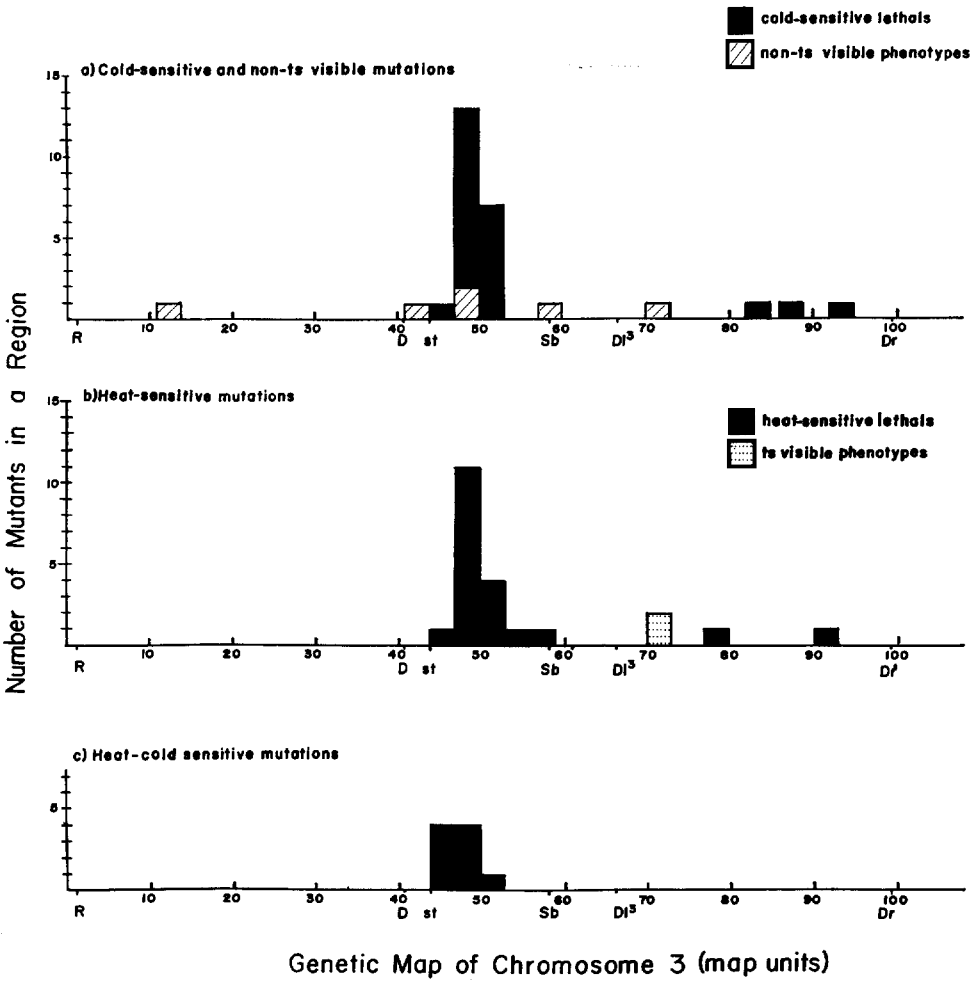


FIGURE 3.—The genetic distribution of different classes of ts lethal mutations.

TABLE 3

*Control crossover values at 22°C for regions spanning the left and right arm of chromosome 3*

| Stocks | Regions in left arm | | | No. of progeny | Regions in right arm | | | No. of progeny |
|---|---|---|---|---|---|---|---|---|
| | *R–D* | *D–st* | *st*–Sb* | | *st*–Sb* | *Sb–Dl³* | *Dl³–Dr* | |
| † | 39.3 | 3.3 | 14.2 | .... | 14.2 | 8.0 | 33.0 | .... |
| *st* | 32.6 | 9.0 | 17.3 | 10,042 | 21.4 | 8.6 | 30.2 | 11,745 |
| *1(3)ET9$^{hts}$* | 56.2 | 4.3 | 12.2 | 1,630 | 17.3 | 12.6 | 32.6 | 1,553 |
| *1(3)ET5$^{hts}$* | 47.4 | 3.6 | 17.2 | 1,458 | 17.1 | 7.3 | 34.2 | 1,725 |
| *1(3)ET26$^{cts}$* | 36.2 | 4.1 | 19.4 | 3,465 | 16.5 | 10.7 | 32.4 | 2,100 |
| *1(3)ET2$^{cts}$* | 44.4 | 2.2 | 13.0 | 1,118 | 16.3 | 10.8 | 34.8 | 1,349 |

* Centromere between *st–Sb*.
† LINDSLEY and GRELL (1968).

of the different crosses. Nevertheless, while unequivocal assignments of each mutation to a region could be made, the actual genetic position within the region could not be stated except in a relative way.

Each mutation was mapped with the two test chromosomes spanning each arm. The ratio of reciprocal crossover products in the region in which the lethal was localized was used to calculate the distance of the mutation from the two flanking markers. By coincidence, most of the ts's fell within the region of the chromosome (*st-Sb*) which was included in both marker stocks. Thus, two values could be calculated for each ts in that region and in a sense, provide a measure of the effect of genetic heterogeneity on crossover values. In most cases, the position of the ts mapped in the two stocks agreed within three map units, a discrepancy that is not unusual for this particular region (HANNAH-ALAVA 1969). Three chromosomes that differed in the genetic position by more than three map units are being tested for the possibility of the presence of two separate but closely linked mutations. The *st-Sb* region was divided into five subregions of equal length and each ts was located by its relative position within the region.

The unexpected localization of the bulk of the ts lethals in the *st-Sb* region and the difficulty in assigning an unequivocal genetic position of any mutation owing to the variability in crossover values led to the disturbing possibility that a spontaneous ts mutation may have occurred in the isogenized *st* chromosome prior to mutagenesis. Consequently, all heat- and cold-sensitive lethals were crossed *inter se* in both reciprocal crosses. Of 33 heat-senstive lethals tested, only one pair failed to complement at 29°C and all of the 30 cold-sensitive lethals tested complemented at 17°C. Thus, complementation tests clearly show that the mutations are functionally distinct and support the extensive genetic distribution of sites in the *st-Sb* region.

## DISCUSSION

In tests for autosomal dominant cold-sensitive lethal mutations, ROSENBLUTH, EZELL and SUZUKI (1972) found none on 6,552 EMS-treated third chromosomes. We can now add the 2,184 chromosomes in the present experiments which did not carry such mutations, for a grand total of 8,736. Thus, if dominant cold-sensitives do occur on chromosome 3, they are exceedingly rare. Dominant heat-sensitive lethals do occur on chromosome 3 but are also rare (10/25,000—HOLDEN and SUZUKI 1968, 1973) so the failure to detect one in the 1,176 chromosomes tested at 29°C is not surprising.

Recessive ts lethal mutations on chromosome 3 have not proven as difficult to recover. Five percent (5%) of the mutagenized chromosomes carried a ts mutation. Of these, equal numbers of heat- and cold-sensitives were found. The frequency of heat-sensitive lethals (3.0%) is somewhat less than the values reported for the *X* (6.3%) and second (8.6%) chromosomes (SUZUKI *et al.* 1967; BAILLIE, SUZUKI and TARASOFF 1968). However, this value compares closely for those (4.4%) reported by FATTIG and RICKOLL (1972).

That recessive heat- and cold-sensitive (15–30%) lethals have been recovered on the *X* chromosome in different experiments (SUZUKI *et al.* 1967; MAYOH and

SUZUKI 1973) hints that heat-sensitives are more readily recovered than cold-sensitives. However, in our experiments the simultaneous testing of chromosomes at 17° and 29°C reveals that heat- and cold-sensitive lethals occur with equal frequency. Moreover, about a third of all heat- and cold-sensitive lethals are also cold- and heat-sensitive, respectively. The genetic studies strongly suggest that, in every case, heat-cold sensitivity is the property of a single mutation. Such mutations have been reported in Drosophila. BRIDGES and DOBZHANSKY (1933) found a heat-cold-sensitive mutation, *proboscipedia,* which produced oral lobes that were arista-like at 15°C and tarsus-like at 29°C. SCHARLOO (1961) has reported a heat-cold-sensitive lethal.

If the heat-cold-sensitive phenotype is indeed the consequence of one gene, the basis for it is of interest. Temperature optima for enzyme activity could be accentuated in a thermolabile protein (HOCHACHKA and SOMERO 1971). It will be of great interest to determine whether the temperature-sensitive periods of a heat-cold-sensitive lethal delineated at both high and low temperatures will coincide.

Genetic maps of heat-sensitive lethal mutations show that while they are not randomly distributed along the *X* chromosome (SUZUKI and PITERNICK 1973) or the T4 phage chromosome (EDGAR and LIELAUSIS 1964), they nevertheless map extensively throughout the entire chromosome. Cold-sensitive lethals, on the other hand, do not map as extensively on the *X*-chromosome of Drosophila (MAYOH and SUZUKI 1973) or the phage chromosomes of T4 (SCOTTI 1969) and λ (COX and STRACK 1971). Our genetic localizations of ts mutations are strikingly different from sex-linked mutations. FATTIG and RICKOLL (1972) reported nine mutants localized randomly throughout chromosome 3, whereas our map of each class of ts mutants (heat, cold and heat-cold) reveals a distinct clustering between *st* and *Sb* and a complete absence to the left of *st* (Figure 3). Moreover, since the left-most portion of the *st–Sb* region is to the left of the centromere, most mutants appear to map to the right of this point.

The seven visible mutations detected in these experiments were located along the entire chromosome (Figure 3) including the left arm. This tends to rule out a bias in the selection procedure which would preferentially prevent recovery of mutations in certain genetic segments. Moreover, the nonallelism indicated by complete complementation of all but one pair of ts mutations eliminates the recurrent detection of a preexisting mutation in the stock. We must conclude, then, that the genetic map of the ts mutations (Figure 3) is an accurate reflection of a random sample of ts mutations on chromosome 3. The difference between our data and those of FATTIG and RICKOLL (1972) could reflect an unusual stock difference in regional susceptibility to ts induction.

The *st–Sb* region (14.2 map units) comprises 12.9% of the total genetic length of 110 map units of chromosome 3 (BRIDGES 1941a,b). By the use of the figures of BRIDGES (1935, 1937) and the cytological localizations of *st* (73A2–73B1) and *Sb* (89B4) as reported in LINDSLEY and GRELL (1968), it can be determined that there are 808 salivary gland chromosome bands between *st* and *Sb*. The entire third chromosome has approximately 2,062 bands. Thus, the *st–Sb* segment comprises 39% of the cytologically-detectable polytene chromomeres. In addition,

the heterochromatic areas are under-represented in polytene nuclei (RUDKIN 1964) so the *st–Sb* region actually encompasses a much larger proportion than 40% of the chromosome. Mitotic chromosomes suggest that heterochromatin comprises about 25% of the length of chromosome 3 (HANNAH 1951). Thus, salivary gland chromosomes are about ¾ of the length predicted by mitotic figures. The euchromatic segment would comprise 30% of a complete chromosome. This provides an estimate of 55% of the physical length of chromosome 3 falling between *st* and *Sb*. Thus, although this region undoubtedly is much larger than its genetic length would indicate, it would appear nevertheless that an excessive proportion of ts mutations are recovered there.

Despite the variation in crossover values observed in the mapping tests, we feel that the derived maps are reasonably accurate. LINDSLEY *et al.* (1972) reported a mutant, *Spl*, which was uncovered by a deletion for salivary chromosome bands 81F–82A. This deleted region lies immediately to the right of the centromere and corresponds to the genetic region between 47.7 and 47.8 (LINDSLEY and GRELL 1968). The mutation, *1(3)ET9$^{hts}$* which we localized genetically to 47.9, exhibits a phenotype similar to that described for *Spl*. Both show awkward leg movement and some melanization in the limbs and joints. We assume, therefore, that *Spl* and *1(3)ET9$^{hts}$* are allelic. The close agreement in the localizations of these two independently recovered mutants lends support to the validity of the map positions determined for the ts mutations.

Of all mutations in the *st–Sb* region which were tested for complementation, only one pair failed to complement. The two noncomplementing mutations map in the second subregion from the left (Figure 3). Both are heat-sensitive but whereas *1(3)ET9$^{hts}$* has a permissive temperature of 22°C, the other, *1(3)ET 23$^{hts}$*, has a permissive temperature of 17°C. Furthermore, the two mutants are distinguishable by differences in temperature threshold, effective lethal phases and sublethal phenotypes. *1(3)ET9$^{hts}$* has a lethal phase at 29°C in mid to late pupae but when the temperature is lowered to 25°C, the lethal phase occurs later. There is a certain amount of melanization in the legs of survivors at 25°–26°C and the legs are somewhat spastic. The lethal phase of *1(3)ET23$^{hts}$*, on the other hand, is very late, occurring at eclosion or in newly emerged adults. Melanization of hypoderm does not occur and locomotary movement is much more normal. Thus, the properties of the two mutants point to their independent origin.

In the screening for temperature-sensitive lethal mutations, a number of other kinds of mutants were also recovered. In the two experiments, eleven chromosomes were recovered which had a dominant phenotype of female sterility. These chromosomes allowed complete fertility of heterozygous males, but females were not fertile. While genetic studies of such mutations are limited, they may be of some interest in the study of female ovarian development. None were found to be ts.

Of all of the ts mutations generated, 62% could be maintained at permissive temperatures as homozygous stocks, a value similar to that (63%) reported by FATTIG and RICKOFF (1972). The value reported for the ts lethals on the *X* chromosome was 49% (SUZUKI and PITERNICK 1973). Male sterility could not

be the consequence of a *Y*-linked mutation since the $F_1$ male (Figure 1) carried a mutagenized paternal *Y* chromosome. Sterility may be the consequence of a second site mutation separate from the ts lethal. The high proportion of fertile homozygous lines allows the ready use of such mutations for developmental studies.

In addition to ts lethal mutations, two ts phenotypic mutants were recovered. Both were heat-sensitive and both mapped outside the *st–Sb* region. The mutant *1(3)ET16*$^{hts}$ mapped at 71.5 and had a normal phenotype at 22°C. At 29°C, the wings were dried out and wrinkled longitudinally. The other mutation is *1(3)ET18*$^{hts}$, which yielded normal adults at 22°C and both male and female flies about half the size of normal at 29°C. Weighing experiments corroborated the visual observation. Another mutation, *1(3)ET10*$^{hts}$, exhibits a phenotypic effect at 22°C which cannot be separated from the lethal by crossing over. While homozygotes are lethal at 29°C, the rare escapee exhibits an extreme disturbance of abdominal tergite patterns which is found on all homozygotes at 22°C.

The variety of ts mutations recovered in these experiments and their genetic and developmental properties augurs well for their use in future studies.

The many discussions, suggestions and interest of DR. TOM KAUFMAN greatly aided the carrying out and completion of this work. In addition, the help of DR. JEANETTE HOLDEN is gratefully acknowledged.

## LITERATURE CITED

AYLES, B., T. SANDERS, B. KIEFER and D. T. SUZUKI, 1973 Temperature-sensitive mutations in *Drosophila melanogaster*. XI. Male sterile mutants on the Y chromosome. Devel. Biol. (in press).

BAILLIE, D., D. T. SUZUKI and M. TARASOFF, 1968 Temperature-sensitive mutations in *Drosophila melanogaster*. II. Frequency among second chromosome recessive lethals induced by ethyl methanesulfonate. Can. J. Genet. Cytol. **10**: 412–420.

BRIDGES, C. B., 1935 Salivary chromosome maps. J. Heredity **26**: 60–64. ——, 1937 Correspondences between linkage map and salivary chromosome structure, as illustrated in the tip of chromosome 2R of Drosophila melanogaster. Cytologia, Fujii Jubilee Vol.: 745–755.

BRIDGES, C. B. and T. DOBZHANSKY, 1933 The mutant "proboscipedia" in *Drosophila melanogaster*—a case of hereditary homöosis. Arch. Entwicklungsmech. Organ. **127**: 575–590.

BRIDGES, P. N., 1941a A revised map of the left limb of the third chromosome of *Drosophila melanogaster*. J. Heredity **32**: 64–65. ——, 1941b A revision of the salivary gland 3R-chromosome map of *Drosophila melanogaster*. J. Heredity **32**: 299–300.

COX, J. H. and H. B. STRACK, 1971 Cold-sensitive mutants of bacteriophage λ. Genetics **67**: 5–17.

EDGAR, R. S. and I. LIELAUSIS, 1964 Temperature-sensitive mutants of bacteriophage T4D: their isolation and genetic characterization. Genetics **49**: 649–662.

FATTIG, W. D. and W. L. RICKOLL, 1972 Isolation of recessive third chromosome temperature-sensitive mutants in *Drosophila melanogaster*. Genetics **71**: 309–313.

HANNAH, A., 1951 Localization and function of heterochromatin in Drosophila. Adv. Genetics **4**: 87–125.

HANNAH-ALAVA, A., 1969 Localization of *Pc* and *Scx*. Drosophila Inform. Serv. **44**: 75.

HOCHACHKA, P. W. and G. N. SOMERO, 1971 Biochemical adaptation to the environment. Fish Physiol. **6**: 99–156.

HOLDEN, J. and D. T. SUZUKI, 1968 Dominant temperature-sensitive (DTS) lethal mutations on chromosome 3 of *Drosophila melanogaster*. Genetics **60**: 188–189 (Abstr.) ——, 1973 Temperature-sensitive mutations in *Drosophila melanogaster*. XII. The genetic and developmental effects of dominant lethals on chromosome 3. Genetics **73**: 445–458.

LEWIS, E. B. and F. BACHER, 1968 Method of feeding ethyl methanesulfonate (EMS) to Drosophila males. Drosophila Inform. Serv. **43**: 193.

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

LINDSLEY, D. L., L. SANDLER, B. S. B. BAKER, A. T. C. CARPENTER, R. E. DENELL, J. C. HALL, P. A. JACOBS, G. L. G. MIKLOS, B. K. DAVIS, R. C. GETHMANN, R. W. HARDY, A. HESSLER, S. M. MILLER, H. NOZAWA, D. M. PARRY, M. GOULD-SOMERO, 1972 Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71**: 157–184.

MAYOH, H. and D. T. SUZUKI, 1973 Temperature-sensitive mutations in *Drosophila melanogaster*. XVI. The genetic properties of sex-linked recessive cold-sensitive mutants. Can. J. Genet. Cytol. (in press).

ROSENBLUTH, R., D. EZELL and D. T. SUZUKI, 1972 Temperature-sensitive mutations in *Drosophila melanogaster*. IX. Dominant cold-sensitive lethals on the autosomes. Genetics **70**: 75–88.

RUDKIN, G. T., 1964 The structure and function of heterochromatin. Proc. XI Intern. Congr. Genet. **2**: 359–374.

SCHARLOO, W., 1961 Temperature and mutant expression. Experientia **17**: 121–122.

SUZUKI, D. T., 1970 Temperature-sensitive mutations in *Drosophila melanogaster*. Science **170**: 695–706.

SUZUKI, D. T. and L. K. PITERNICK, 1973 Temperature-sensitive mutations in *Drosophila melanogaster*. XVIII. Genetic positions and viability indices of sex-linked recessive lethals. Submitted for publication.

SUZUKI, D. T., L. K. PITERNICK, S. HAYASHI, M. TARASOFF, D. BAILLIE and U. ERASMUS, 1967 Temperature-sensitive mutations in *Drosophila melanogaster*. I. Relative frequencies among γ-ray and chemically induced sex-linked recessive lethals and semilethals. Proc. Natl. Acad. Sci. U.S. **57**: 907–912.

SUZUKI, D. T. and D. PROCUNIER, 1969 Temperature-sensitive mutations in *Drosophila melanogaster*. III. Dominant lethals and semilethals on chromosome 2. Proc. Natl. Acad. Sci. U.S. **62**: 369–376.

WRIGHT, T. R. F., 1968 The phenogenetics of temperature-sensitive alleles of *lethal myosperoid* in Drosophila. Proc. XII Intern. Congr. Genetics **1**: 141 (Abstr.)