ISOLATION OF RECESSIVE THIRD-CHROMOSOME TEMPERATURE-SENSITIVE MUTANTS IN *DROSOPHILA MELANOGASTER*

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RECESSIVE temperature sensitive (*ts*) mutants have been recovered and used in analysis of the genetic organization and physiology of bacteriophages (EDGAR and LIELAUSIS 1964; EDGAR, DENHARDT and EPSTEIN 1964; FATTIG and LANNI 1965) and other microorganisms (HARTWELL 1967; EIDLIC and NEIDHARDT 1965; YOSHIKAWA 1970). Recently such conditional lethal mutations have been induced in *Drosophila melanogaster* (SUZUKI *et al.* 1967; BAILLIE, SUZUKI and TARASOFF 1968), in *Musca domestica* (McDONALD and OVERLAND 1972) and in *Habrobracon serinopae* (SMITH 1968). Ethylmethanesulfonate (EMS)-induced recessive *ts* mutants in *Drosophila* are extensively distributed throughout the X chromosome and map as point mutations (SUZUKI 1970), and numerous second-chromosome *ts* mutants have been recovered (BAILLIE, SUZUKI and TARASOFF 1968). In addition, a number of "cold-sensitive" conditional lethals and dominant temperature-sensitive (DTS) mutants have been found on the second and third chromosomes of *Drosophila* (SUZUKI 1970). We report here the isolation and partial characterization of recessive *ts* mutants located on the third chromosome of *Drosophila*.

MATERIALS AND METHODS

The mutagenesis procedure used was that of LEWIS and BACHER (1968). Oregon-R *Drosophila melanogaster* males were collected 0-48 hr after eclosion and placed in a one-quarter pint milk bottle containing a tissue saturated with a 0.025 M solution of EMS (Eastman Kodak Company) in a 1% sucrose solution. (In later experiments the concentration of EMS was reduced to 0.005 M). After 24 hr the flies were transferred to fresh culture bottles and mass mated with virgin females of the tester strain *Dr/TM3, Sb Ser*, then incubated at 22°C. (*Dr* = Drop, *Sb* = Stubble, *Ser* = Serrate, *TM3* = *In(3LR)TM3*; see LINDSLEY and GRELL 1968.) Parental flies were removed at the onset of pupation. Stubble, Serrate male F₁ flies were crossed individually in vials with virgin *Dr/TM3, Sb Ser* females. These F₁ pairwise crosses were incubated at 22°C and the parents removed at the onset of pupation. Stubble, Serrate progeny from each individual vial were collected, crossed, and incubated 3-4 days at 22°C (the "permissive" temperature). Parents were then transferred to a second vial which was incubated at 29°C (the "restrictive" temperature). This procedure produces flies homozygous for treated third chromosomes. Homozygous strains bearing ordinary recessive lethal mutations produce no wild-type progeny at either temperature, and nonlethal homozygotes produce wild-type progeny at both temperatures. Strains bearing recessive *ts* mutations produce phenotypically wild-type progeny at 22°C but not at 29°C in the above test. The isolation and testing procedure is shown in Figure 1.

Complementation tests were performed by making all possible pairwise crosses between the

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ISOLATION PROCEDURE

$$P_1: \frac{Dr}{TM3, Sb Ser} \text{ ♀♀} \times \frac{+}{+} \text{ ♂♂ (EMS-treated Oregon-R)}$$

$$F_1: \frac{+}{TM3, Sb Ser} \cdot \frac{+}{Dr} \text{ (discard)}$$

$$\downarrow$$

$$\frac{+}{TM3, Sb Ser} \text{ ♂♂} \times \frac{Dr}{TM3, Sb Ser} \text{ ♀♀ (pairwise)}$$

$$F_2: \frac{+}{TM3, Sb Ser} \cdot \frac{Dr}{TM3, Sb Ser} \cdot \frac{TM3, Sb Ser}{TM3, Sb Ser} \text{ (lethal)} \cdot \frac{+}{Dr}$$

self cross at 22° & 29°

$$F_3: \frac{+}{+} \cdot \frac{+}{TM3, Sb Ser} \cdot \frac{TM3, Sb Ser}{TM3, Sb Ser}$$

If nonlethal	22°	lives	lives	dies
	29°	lives	lives	dies
If recessive lethal	22°	dies	lives	dies
	29°	dies	lives	dies
If ts lethal	22°	lives	lives	dies
	29°	dies	lives	dies

FIGURE 1.—Isolation procedure for third chromosome *ts* mutants. The following gene symbols are used: *Dr*, Drop (eye); *TM3*, Third Multiple 3 (a crossover suppressor extending almost the entire length of chromosome 3); *Sb*, Stubble (bristles); *Ser*, Serrate (wings). The *TM3, Sb Ser* marked balancer chromosome prevents loss of induced mutations through crossover events and allows identification of individuals heterozygous for treated third chromosomes.

nine *ts* mutants which were fertile as homozygotes and incubating these crosses at 29°C. Survival of these heterozygotes to adulthood was scored as complementation.

To determine the developmental period of temperature sensitive lethality, females of each *ts* strain were allowed to lay eggs for 24 hr in medium vials at either the permissive or restrictive temperature. For each mutant strain, several culture vials started in this way were shifted daily from the permissive to the restrictive temperature, or *vice versa*. The earliest time when progeny cease to be produced in shift-down experiments is defined as the beginning of the temperature-sensitive period, and the earliest appearance of progeny in shift-up experiments is defined as its end.

RESULTS AND DISCUSSION

Of 235 chromosomes tested from males treated with 0.025 M EMS, 185 were found to bear ordinary recessive lethals, 39 were scored as nonlethals, and 11 were designated *ts* lethals. Six of the latter were fertile as homozygotes. Of 251 chromosomes tested from males treated with 0.005 M EMS, 137 were classed as recessive lethals, 104 as nonlethals, and 10 as *ts* lethals, seven of which were fertile as homozygotes. Despite the fact that the recessive lethality decreased appreciably with the decrease in mutagen concentration, the yield of *ts* mutants was not enhanced. We interpret this to indicate that at the higher mutagen concentration primary *ts* mutants fail to be recovered due to the induction of secondary ordinary lethal mutational events on the same chromosome.

Nine of the 13 fertile *ts* mutants have been maintained in stock culture and used in experiments. With one possible exception, all complement each other at the restrictive temperature, and preliminary mapping data (not reported here) indicate that they are scattered widely over the third chromosome.

Results of the "shift up-shift down" experiments for several of the mutants are presented in Figure 2. The variation in onset and duration of temperature-sensitive periods during development suggests that the mutants recovered affect diverse developmental processes, and is in agreement with similar observations in the house fly (McDONALD and OVERLAND 1972). The stage at which death actually occurs varies in our mutants and may occur some time after the functional developmental lesion occurs. For example, *ts* 7, whose developmental sensitive period ends during the third instar larval stage, continues development at 29°C to the mid-late pupal stage, at which time death occurs. In "leaky" *ts* mutants, (*ts* 106) death occurs so late that most flies which fail to survive at 29°C actually die in the act of eclosion, although some die as pupae prior to any indication of eclosion. Collectively, the mutants reported herein represent all classes of developmental lethals, including types which die at 29°C as eggs, larvae, and pupae.

The physiological attributes, complementation relationships, map distribution, and developmental lethality of the *ts* mutants analyzed in this study parallel the general properties of *ts* mutants located on other *Drosophila* chromosomes (SUZUKI 1970), those in bacteriophage T4 (EPSTEIN *et al.* 1963) and T5 (FATTIG and LANNI 1965), and such mutants in the housefly (McDONALD and OVERLAND 1972).

SMITH (1971) has suggested that temperature sensitive mutants released into

TEMPERATURE SENSITIVE PERIODS

FIGURE 2.—Temperature sensitive period of seven *ts* mutants. The solid lines indicate approximate duration of the periods of temperature-sensitive lethality during development, and have been adjusted for differing time-rates of development at 22°C and 29°C.

natural insect populations may be useful in biological control of pests and disease vectors. Presumably, such release would be accomplished during “permissive” seasons and the propagated conditional lethality expressed during a following “restrictive” season. Our results indicate that an understanding of the developmental effects of such lesions may be important in determining the effectiveness of such a control program. Mutants with abbreviated temperature-sensitive periods, such as our *ts5*, may complete development in significant numbers during a restrictive season due to diurnal fluctuations in temperature, while those such as *ts4* exhibit extended periods of susceptibility to the lethal effect of high temperature, and are thus less likely to escape selection.

We have extended the work reported above through a modified recovery procedure and recently have isolated 26 additional third chromosome *ts* mutants with conditional developmental lesions and several putative conditionally sterile adult *ts* mutants.

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