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Recessive, Sex-Linked, Cold-Sensitive Induced Mutation in *Drosophila melanogaster*

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ABSTRACT. This study was conducted to induce cold-sensitive mutations in the Basc stock of *Drosophila melanogaster* and to identify the effective lethal phase (LP) and the temperature-sensitive period (TSP) for the mutants. Ethyl methanesulfonate (EMS) was used as the mutagenic agent. Basc EMS-treated males were mated to virgin Canton-S females. Progeny were mated individually and tested for lethality at 18°C and for viability at 25°C. The stable stocks obtained were retested for lethality at 18°C. Two different sex-linked recessive cold-sensitive lethal mutants were obtained out of 884 tested F1 males. The LP and TSP for both stocks were determined and described. Due to the variability and instability of cold-sensitive stocks, care should be taken in using such stocks.

Cold-sensitive lethal mutations survive at high temperature and die at low temperature. These conditional lethal mutations have been isolated and analysed in several kinds of organisms, bacteria (Guthrie *et al.* 1969 and Nomura 1973), Fungi (Waldron and Roberts 1974), Neurospora (Schlitt and Russell 1974) and Drosophila (Hoge 1915, Villee 1943a, 1943b and 1944, Baillie *et al.* 1968 and Rosenbluth *et al.* 1970).

Cold-sensitive sex-linked lethals have been recovered and analysed in *Drosophila melanogaster.* In one study several cold-sensitive sex-linked lethals were recovered using EMS as a mutagenic agent (Rosenbluth *et al.* 1972). Mayoh and Suzuki (1973) have also reported the recovery of cold-sensitive recessive sex-linked lethals in *D. melanogaster* using EMS and indicated that 1.53 to 3.00 percent of all EMS-induced TS lethals were Cold-sensitive. Wright (1973) has also reported the recovery of seven cold-sensitive recessive sex-linked lethals in *D. melanogaster* using the same mutagen (EMS). Interestingly, no dominant

cold-sensitive sex-linked lethals have been isolated in all of these studies using EMS.

Materials and Methods

The screening procedure for the detection of recessive cold-sensitive lethal mutations on the X-chromosome is shown in Fig. 1. The mutants or the stocks obtained designated as cold-sensitive 1 (CS1) and cold-sensitive 2 (CS2).

Adult males (1-2 days old) from Basc stock were fed for 24 hr. on kimwipes saturated with 0.025 M EMS in Sterile 1% sucrose solution (Lewis and Bacher 1968). Twenty EMS-treated males were allowed to cross with 20 virgin Canton-S females in bottles containing standard Drosophila medium (Step 1). The cultures were maintained at 25°C.

The F_1 progeny were isolated and females were individually mated to their brothers in vials at 18°C (Step 2). At this temperature, 31 days were required for the emergence of F_2 progeny. The complete absence of Basc males in these cultures indicated lethality at 18°C and only those cultures were used for the following mating. Those F_2 cultures that contained Basc males were discarded.

The F_2 heterozygous Basc females were mated to their F_2 wild type brothers (Step 3) in vials at 25°C. After about 10 to 11 days of incubation, the cultures were screened for the presence of Basc males without etherizing the flies. Only F_3 cultures that contained Basc males were used. The presence of Basc males in these F_3 cultures grown at 25°C and the absence of Basc males in F_2 cultures grown at 18°C indicated that a cold-sensitive mutant has been isolated. The F_3 cultures demonstrated to carry cold-sensitive mutants were used to establish stable stocks (Step 4). These stable stocks then retested for lethality at 18°C (Step 5) and only those cultures that proved to carry cold-sensitive mutation allowed to produce large number of progeny and were used to detect the effective lethal phase (LP) and temperature sensitive period (TSP) (Tarasoff and Suzuki 1970).

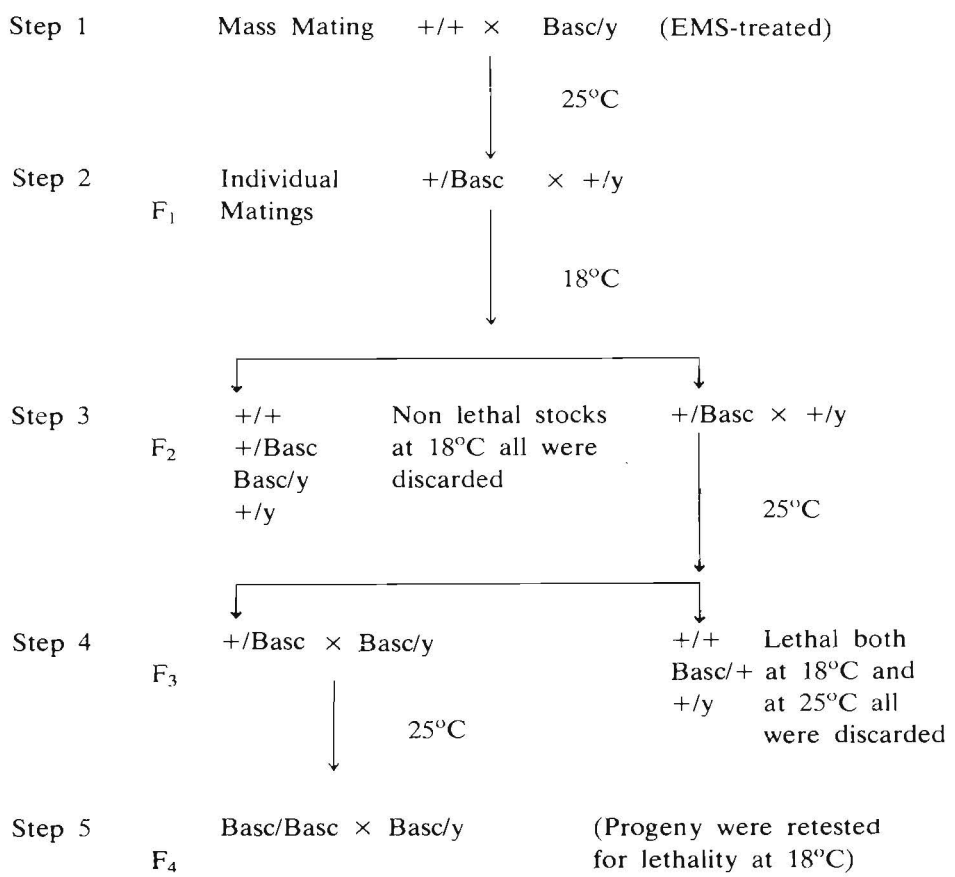

Fig. 1. The screening procedure for the detection and recovery of cold-sensitive, sex-linked, recessive lethal mutations.

Results

The study was started with 51 groups, each contained 20 Canton-S virgin females (step 1). Only 40 groups produced progeny. The results of the individual tests that were carried out at 18°C on 884 F_1 males (step 2) are shown in Table 1.

Progeny of each of the 86 potential CS lethal stock were tested for viability at 25°C. Flies were taken from the 86 cultures and used to establish 219 cultures. The characteristics of F_3 progeny which were grown at 25°C and tested for viability at this temperature are shown in Table 2.

Table 1. Characteristics of F_2 progeny which were grown and tested for lethality at 18°C

Possible lethal at 18°C	Non-lethal at 18°C	Sterile	Total
86	668	130	884

Table 2. Characteristics of F_3 progeny which were grown at 25°C and tested for viability at this temperature

Lethal at 18°C but viable at 25°C	Lethal at 18°C and 25°C	Sterile	Total
30	155	34	219

The 30 stocks (Table 2) tentatively identified as being lethal at 18°C but viable at 25°C were used to obtain stable stocks (Step 4) and then these stable stocks were retested for lethality at 18°C (Step 5). The characteristics of those 30 stocks which were retested for lethality at 18°C are shown in Table 3.

Table 3. Characteristics of those 30 stocks which were retested for lethality at 18°C

Lethal at 18°C but viable at 25°C	Non-lethal at 18°C and 25°C	Total
2	28	30

Tables 4 and 5 show the results obtained when CS_1 cultures were maintained at 18°C only or at 25°C only, respectively.

Table 4. Results obtained when CS_1 cultures were maintained at 18°C

Number of Collected eggs	Number of Hatched eggs at 18°C	Number of Unhatched eggs at 18°C	Hatchability %	Number which survived to 2nd larval instar
34	18	16	52.9	7
43	19	24	44.1	5

Table 5. Results obtained when CS_1 cultures were maintained at 25°C only

Number of Collected eggs	Number of Hatched eggs at 25°C	Number of Unhatched eggs at 25°C	Hatchability %	Adults emerged
62	44	18	71	28
74	58	16	78	49

From these results and the results obtained by shift experiments, it was found that the LP for CS_1 started early during embryonic stage and extended through the second larval instar stage and the TSP started in early embryonic stage also and ended no later than early first instar.

Tables 6 and 7 show the results obtined when CS_2 cultures were maintained at 18°C only or at 25°C only, respectively.

Table 6. Results obtained when CS_2 cultures were maintained at 18°C

Number of Collected eggs	Number of Hatched eggs at 18°C	Number of Unhatched eggs at 18°C	Hatchability %	Number of those which died during larval stage
75	37	38	49.3	34
50	19	31	38.0	18

Table 7. Results obtained when CS_2 cultures were maintained at 25°C only

Number of Collected eggs	Number of Hatched eggs at 25°C	Number of Unhatched eggs at 25°C	Hatchability %	Adults emerged
34	32	2	94.0	21
96	55	41	57.3	43

From the results obtained in Tables 6 and 7 and from the results of shift experiments, it was found that the LP for CS_2 started during embryonic stage and

extended through third instar stage and the TSP for this stock started at embryonic stage and extended through early third larval instar stages.

Discussion

The results of this study showed that cold-sensitive recessive, sex-linked lethal mutations can be readily isolated in *melanogaster*. Previous studies also indicated that these kind of mutations can be readily detected in *D. melanogaster* with different rates (Rosenbluth *et al.* 1972, Mayoh and Suzuki 1973 and Wright 1973). The differences in the rates of the mutants obtained from these different studies are interesting.

Thirty stocks were retained as potential cold-sensitive lethals in the first trial of this study, but only two of these 30 were found to be cold-sensitive lethals. Similar observations have been made by other studies (Mayoh and Suzuki 1973 and Wright 1973). These changes in viability may reflect an instability which is the characteristics of mutations induced by EMS and has been attributed to mosaicism (Epler 1966 and Mayoh and Suzuki 1973). Other causes of variability of cold-sensitive stocks as suggested by Mayoh and Suzuki (1973) may result from changes in the genetic background or inherent instability of cold-sensitive mutants. The changes from 30 possible cold-sensitive mutants to only two cold-sensitive lethal mutants in this study may be due to the instability of cold-sensitive stock and not due to an inefficiency in the screening procedure use. Since similar observations have been made.

The LP and TSP for both lethal stocks were determined and described in the results. For LP, there is similarity between the result of this study and the results of some other studies (Holden and Suzuki 1973 and Mayoh and Suzuki 1973) that there is a prolonged period before death finally ensued. In spite of these observations, no explanation has been given to this phenomenon. But this type of phenomenon may indicate the formation of a thermolabile structural element which is necessary for continuous development and viability.

For TSP, the results of this study are consistent with the results of TSPs of many other studies (Tarasoff and Suzuki 1970, Holden and Suzuki 1973, Mayoh and Suzuki 1973 and Kaufman and Suzuki 1974) that, the TSP begin in one stage and extends through other developmental stage(s).

The stocks obtained in this study may be useful in such applications as virginizing methods, selective elimination of heterozygotes and fine structure studies.

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استحداث طفرات حساسة للبرودة متنحيه ومرتبطة بالجنس في حشرة *Drosophila melanogaster*

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تهدف هذه الدراسة إلى إستحداث طفرات في Basc Stock لحشرة الدروسفيلا ميلانوجاستر (ذبابة الخل) فقد تم التعرف على المرحلة المميتة الفعالة Effective (LP) Lethal Phase والفترة الحساسة لدرجة الحرارة Temperature - Sensitive Period (TSP) لهذه الطفرات المستحدثة. استخدمت المادة Ethyl Methanesulfonate (EMS) كمادة محدثة للطفرات. ولهذا الغرض لُقِّحت ذكور معاملة بهذه المادة بإناث عذراء عادية من Cantan - S. ثم تُرك النسل الناتج عن هذا التزاوج ليتزاوج فيما بينه بصورة إنفرادية واختبر عند درجة حرارة ١٨° مئوية للموت (العتبة المميتة) وعند درجة حرارة ٢٥° مئوية لاختبار حيويته. وبتتبع الأجيال التالية عند درجتي الحرارة السابقة كما هو موضح في التقرير أمكن الحصول على طفرتين مميتتين متنحيتين ومرتبطتين بالجنس من ٨٨٤ ذكر من الجيل الأول المختبر. كما أمكن تحديد ووصف كل من المرحلة المميتة الفعالة (LP) والفترة الحساسة لدرجة الحرارة (TPS) لهاتين الطفرتين. إن تباين وعدم استقرار الطفرات الحساسة للبرودة والمستحدثة بواسطة EMS يقتضي توخي الاحتياط عند استعمال هذا النوع من الطفرات في دراسات أخرى.