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AN IMPROVED SECOND CHROMOSOME BALANCER IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Dominant temperature sensitive lethals (DTSLs) and semilethals were induced by EMS on the SM5 balancer of *Drosophila melanogaster* at the rate of 0.0025. SM5 DTSL4 is a fully penetrant line with 100% lethality at the nonpermissive temperature of 28°C. At the permissive temperature (19°C) it shows normal fertility and survival. DTSL4 is basically a pupal lethal but exerts strong paternal influence over embryonic and larval development. DTSL4 does not alter the recombination suppression properties of SM5. Individuals homozygous for second chromosome loci can now be generated with ease from SM5 DTSL4/+ parents at 28°C.

Key words: Dominant temperature sensitive lethal (DTSL), balancer chromosome, *Drosophila*.

The balancer (Bal) chromosomes in *Drosophila* are specially designed chromosomes carrying the following structural features and associated properties: (a) multiple paracentric inversions spanning the entire length of the chromosome thus preventing recovery of viable recombinant gametes from the inversion heterozygote females; (b) a dominant marker which is lethal when homozygous, thus preventing the survival of balancer homozygotes; and (c) several recessive markers which can be used to detect rare viable recombinant progeny of the inversion heterozygote mothers. Bal/+ heterozygotes on selfing produce Bal/+ and +/+ progeny in a 2:1 ratio (Bal/Bal individuals being lethal at a given developmental stage that is specific to each Bal). Thus balancers are extremely useful in generating genetically identical homozygous individuals. In spite of numerous advantages, the balancer heterozygotes inevitably produced on selfing are a severe disadvantage in several instances. For example if thousands of independently mutagenized homozygous progeny lines need to be generated from the pair-mated heterozygotes, the physical task of eliminating 2/3 of the Bal/+ progeny from each vial is extremely time consuming. A viable alternative would be to create a balancer carrying a conditionally expressed dominant lethal effect so that Bal/+ flies could be selectively eliminated under nonpermissive conditions.

Dominant temperature sensitive lethals (DTSLs) are reported on the second and third chromosome balancers of *Drosophila melanogaster* [1, 2] wherein Bal DTSL individuals are designed to survive at 19°C (permissive temperature) and die at 28°C (nonpermissive temperature). There are four DTSLs on second chromosome balancers Cy O and Pm [1]. Three of them (Cy O DTS 486, Cy O DTS 513 and Pm DTS 18) are female sterile at the restrictive temperature [1, 3] and are consequently of low utility. The only useful DTSL on chromosome 2 is Cy O DTS 100. DTSLs are rare on chromosome 3 [4], however, one DTSL has been transferred on to the third chromosome balancer TM5 [2].

Among all second chromosome balancers, Second Multiple 5 (SM5) is most effective [3]. Paucity of useful DTSLs on chromosome 2 balancers in general and SM5 in particular motivated me to induce a DTSL on SM5. The other reason for inducing a DTSL locally was that receiving DTSL stocks in Delhi's hot tropical climate in viable condition from other stock centers has been a serious problem.

Reported here is the induction of a DTSL on the second chromosome balancer SM5 which selectively kills the SM5 flies at 28°C but allows their survival at 19°C without affecting its ability to suppress recombination.

MATERIALS AND METHODS

Media and culture conditions. The flies were reared at 19, 25 or 28°C on standard fly food made of yeast, agar, maize flour and unrefined sugar.

Genetic stocks. The wild type Canton-S stock was isogenised before the start of the experiment. Second Multiple 5 is a second chromosome balancer with 15 overlapping paracentric inversions and transpositions along its entire length and carrying the genetic markers Curly (Cy), aristaless (al^2), lightoid (lt^v), cinnabar (cn^2) and speck (sp^2). Curly is a dominant wing marker but lethal in homozygous state. al dp b c px sp and cn are markers on the second chromosome. All markers and chromosomes other than DTSL 4 have been described previously [5].

Screening for DTSLs on the SM5 chromosome. The mating and screening protocol used for inducing a DTSL on the SM5 balancer is shown in Fig. 1. The mutagenized SM5 males (reared on 0.03 M EMS for 24 h) were mass mated to Canton-S virgin females and the F_1 was raised at 19°C. All Cy F_1 males would have a set of paternal chromosomes (X; II; III possibly bearing a mutation). In all 3,293 F_1 SM5*/+ males (where * is an EMS treated chromosome) were individually mated to +/+ virgin females at 19°C for 7 days. The parents were then transferred to fresh vials at 28°C for another 7 days and discarded. This is referred to as 'Cross II' in figure 1 and later in the text. The 19 and 28°C vial pairs were numbered

identically. The F_2 progeny of each pair of vials was scored for SM5 flies. Vials in which fewer than 10% of the progeny at 28°C and more than 50% of the progeny carried the SM5 chromosome were retained as putative DTSLs. Progeny from putative lines were crossed once more (Cross III, Fig. 1) and retested at 19 and 28°C for temperature sensitive lethality. The SM5/+ flies from the 19° vials of the retained lines were multiplied and maintained at 19°C.

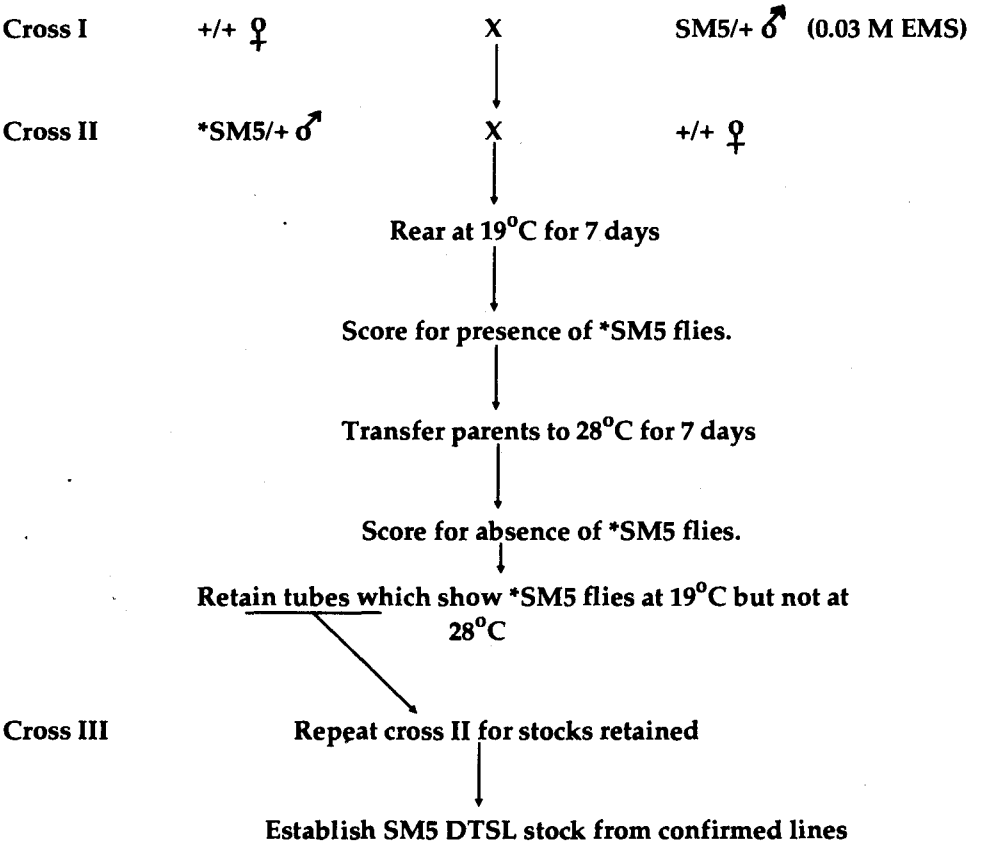

Fig. 1. Mating and screening protocol for recovering DTSLs on the SM5 balancer in *Drosophila*.

Embryonic, larval and pupal lethality were estimated by transferring synchronously laid eggs in batches of 100 to food vials. Unhatched eggs, dead larvae and pupae were counted at the end of 2, 5 and 8 days, respectively.

Survival of Cy (SM5) and Cy^+ (nonSM5) progenies was scored in reciprocal matings between 20 pairs of +/+ and SM5/+ flies at 19, 25 and 28°C.

RESULTS AND DISCUSSION

Out of the 3293 cross II vial pairs that were set up, 3100 were fertile and were scored at both temperatures. Out of the 13 lines retained at the end of cross II, 8 were retained at the end of cross III. The SM5/+ flies carrying these 8 putative DTSLs were intercrossed and frequencies of SM5/+ flies estimated (Table 1). Intermating of SM5/+ flies is expected to produce 2/3 SM5/+ progeny. The χ^2 test showed that, with the exception of line No. 5, the ratio of SM5/+ flies at 19°C did not deviate drastically from the expected value in any of the DTSLs. At 28°C the frequency of SM5/+ flies varied from 0 to 25%, deviating significantly from the expected value in each case. Only line 4 showed complete penetrance of dominant temperature sensitive lethality with 0% survival at 28°C and 69% at 19°C. Four lines had less than 9%, and three lines showed 12, 17 and 25% survival of SM5 DTSL/+ at 28°C. The 'conventional' viability limits set for defining DTSLs at nonpermissive and permissive temperatures are ≤ 0.1 and ≥ 0.5, respectively. DTSLs (defined by this criterion) were induced in 5/3100 (0.16%) of the treated SM5 chromosomes, the remaining 3/3100 being relegated to the DTS semilethal category. EMS induced DTSLs on chromosome 2 have been reported at the rate of 0.17% in Canton-S [4], 0.19% in Cy O and 0.16% in Pm backgrounds [1]. It needs to be pointed out that the frequency of 0.16% in the present and other [1] reports does not in any way reflect the rate at which loci on chromosome 2 are capable of mutating to a DTSL state. It is likely an overestimate because true frequencies of DTSL loci can be arrived at only after inter se complementation is conducted. In the case of SM5 and Cy O DTSLs complementation tests cannot be done because of intrinsic recessive lethality associated with the SM5/SM5 and CyO genotypes.

Table 1. Frequency of SM5 DTSL/+ flies among the selfed progeny of eight putative SM5 DTSL4/+ lines of *Drosophila*

DTSL line No.	19°C		28°C	
	total no. of flies	frequency of SM5 DTSL4/ + flies	total no. of flies	frequency of SM5 DTSL4/+ flies
1	632	0.60	572	0.08
2	580	0.55	599	0.17
3	594	0.64	572	0.12
4	515	0.69	426	0.00
5	402	0.39	348	0.06
6	432	0.64	441	0.08
7	378	0.59	353	0.07
8	654	0.71	780	0.25

Observations were also recorded on SM5 DTSL4 to assess its lethal phase, fertility and viability at various temperatures. Table 2 shows the proportion of two possible genotypes among the offspring of 20 pairs of parents of the direct (SM5 DTSL4/+ ♀ X +/+ ♂) and reciprocal (+/+ ♀ X SM5 DTSL4/+ ♂) crosses at 19, 25 and 28°C. The survival frequency of SM5 DTSL4/+ flies reduced with increase in temperature, reaching 0% at 28°C in both

Table 2. Frequency of SM5 DTSL4/+ flies among direct and reciprocal crosses between SM5 DTSL4/+ and +/+ parents in *Drosophila*

Female parent	Temperature °C	Total progeny size	SM5 DTSL4/+ ♀	SM5 DTSL4/+ ♂	+/+ ♀	+/+ ♂	Frequency of SM5 DTSL4/+ progeny
SM5 DTSL4/+	19	484	128	120	128	108	0.51
SM5 DTSL4/+	25	685	141	126	224	194	0.38*
SM5 DTSL4/+	28	438	0	0	220	218	0.0*
+/+	19	280	26	18	154	82	0.15*
+/+	25	523	10	18	270	225	0.05*
+/+	28	462	0	0	226	236	0.0*

*Deviation from expected frequency 0.5 significant at 5% level.

crosses. Survival at 25°C (intermediate to permissive and nonpermissive temperature) was significantly lower than at 19°C. The proportion of SM5 DTSL4/+ flies was as much as 4 times in the direct cross (44.5%) than in the reciprocal cross (10%) although the total progeny sizes analysed was similar in the two crosses. The SM5 DTSL4/+ genotype had a strong selective paternal influence on the survival of SM5 DTSL4/+ progeny. It was noted that at 19 and 25°C the female:male ratio was higher (1.13:1) than at 28°C (0.93:1).

The estimates of embryonic, larval and pupal lethality in the direct and reciprocal cross are presented in Table 3. The pattern of stage-specific lethality was markedly different in the two crosses. Total lethality (over all development stages) in the direct and reciprocal

Table 3. Embryonic, larval and pupal lethality in the progeny of direct and reciprocal crosses between *Drosophila* strains SM5 DTSL4/+ and +/+ at 28°C

Female parent	Total No. of eggs	Unhatched eggs (%)	Dead larvae (%)	Unenclosed pupae (%)	No. of adults SM5	No. of adults DSTL/+
SM5 DTSL4/+	1340	199 (14.85)	(3.43)	1095 (47.30)	o	4
+/+	1200	800 (66.66)	(22.0)	136 (4.75)	o	o

crosses was 65.6 and 93.4%, respectively. It exceeded the value of 50% lethality, expected on account of DTSL4, by 15.6% in the direct cross and by 43.4% in the reciprocal cross. Embryonic, larval and pupal lethality in the direct cross was 14.86, 3.43 and 47.3% respectively. Similarly embryonic, larval and pupal lethality in the reciprocal cross was 66.6,

22.0 and 4.7%, respectively. It is difficult to assign a phenocritical period to DTSL4 based on these results. However, in the direct cross 47.3% (a figure close to 50%) individuals died at pupal stage. Thus, DSTL4 appears to be a pupal lethal. The high levels of embryonic and larval lethality in the reciprocal cross confirm the observation (Table 2) that DTSL4 has a dominant paternal influence with respect to embryonic and larval survival.

The largest cluster of DTSLs on chromosome 2 (the 1(2)25AD complex) has an embryonic temperature sensitive lethal phase and maps near dp (2–13.0) locus [5]. DTS 513 (presumably a member of this complex) is an embryonic lethal [6], female sterile and exhibits a characteristic short egg phenotype at 28°C [3]. The DTSL4 is neither an embryonic lethal nor a female sterile with a small egg phenotype. Given these results DTSL4 does not seem to be a member of the 1(2)25 AD gene complex.

The al Cy lt cn sp DTSL4/+ females were backcrossed to al dp b cn (cross A) and al dp b c px sp (cross B) at 22°C to test if induction of DTSL4 on the SM5 balancer had affected its property of preventing cross-overs. 1094 SM5 DTSL4/al dp b cn and 981 al dp b cn/+ flies were scored in cross A and 514 SM5 DTSL4/al dp b c px sp and 168 al dp b c px sp/+ flies were scored in cross B. No recombinant classes were observed in the progenies. This suggests that the SM5 DTSL4 balancer has retained its ability to prevent recombination along chromosome 2. Classical studies demonstrated that dominant lethals are due to gross chromosomal rearrangements and deletions, affecting fertility and survival of individuals [7–9]. SM5 DTSL4/+ exhibit normal fertility and survival at the permissive temperature. Genetic damage based on chromosomal aberrations is not expected to have temperature dependent conditional manifestation. These observations suggest that major chromosomal aberrations are not the likely cause of dominant lethality in DTSL4. SM5 DTSL4 is a new and improved chromosome 2 balancer and can be used to generate adults homozygous for chromosome 2 providing yet another tool for chromosome engineering in *Drosophila melanogaster*.

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