hempa selection of *A. aegypti* (George and Brown, 1967) where the percent sterility of the F_1 generation was 66%, F_4 generation 89%, F_5 generation 68% and the F_6 generation 91%.

The four-fold differences in susceptibility that existed between the selected and the unselected populations in the 9th and 11th generation were probably due to vigor tolerance. Figures 2 and 3 show the dosage-response lines of the selected and unselected populations, respectively. The F_{19} and F_{20} susceptibility levels (LC_{50}) for both populations reverted to approximately the same or became more susceptible than comparable F_1 generation population. Thus, these data indicate that resistance was not demonstrated in the selected population through 20 generations.

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INDUCTION OF HEAT-SENSITIVE LETHALS IN *CULEX TRITAENIORHYNCHUS* BY ETHYL METHANESULFONATE

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ABSTRACT. The virus vector mosquito, *Culex tritaeniorhynchus*, was treated with the mutagenic agent, ethyl methanesulfonate. Two temperature-sensitive conditional recessive sex-linked lethals and 22 morphological mutations were induced and recovered. By the use of these conditional lethals and appropriate genetic stocks, it is now possible to produce mosquitoes of only one sex (males) for a mass release program.

Conditional mutations have been useful in the analysis of development, genetic fine structure and gene action in a number of organisms (Foster and Suzuki, 1970; Suzuki, 1970; Loomis, 1969; Chovnick *et al.*, 1962). Moreover it has been suggested that these mutations, especially temperature-sensitive conditional lethals, may be employed in various ways in insect control programs (Knipling, 1960; LaChance and Knipling, 1962; Klassen *et al.*, 1970a, b; Sandler and Novitski, 1957; Curtis, 1968; Wehrhahn and Klassen, 1971; Whitten, 1971; Smith, 1971). Recessive heat-sensitive lethal factors has been isolated in the house fly, *Musca domestica*, by McDonald and Overland (1972).

In addition to the possibility of using temperature-sensitive conditional lethals themselves for control purposes, this class of lethals also can be used to produce adults of only one sex (males). In most field experiments involving the release of male insects, the separation of sexes can be difficult, expensive, time-consuming, and not completely effective. In the vector mosquito, *Culex tritaeniorhynchus*, a method for detecting temperature-sensitive lethals in the sex chromosomes is available (Sakai and Baker, 1972). In this species

and several other culicine mosquitoes sex appears to be determined as if by a single pair of alleles, *M* and *m*, for which males are heterozygous (*m/M*) and females homozygous (*m/m*). Thus, if a recessive, temperature-sensitive conditional lethal were induced on the *m*-bearing chromosome and recombination was suppressed in males between the *m* and *M*-bearing chromosomes, it would be possible to produce a strain in which females are homozygous for the recessive temperature-sensitive conditional lethal and males heterozygous.

Preliminary experiments suggest that for *C. tritaeniorhynchus* temperatures between 26° C to 32° C do not affect normal viability and routinely permit the rearing of a majority of eggs to adults. In most strains continuous temperatures above 32° C or below 26° C during the preimaginal stages usually result in reduced viability ratios (total adults: total eggs). The experiments discussed below were designed to recover heat-sensitive lethals (*hsl*), i.e., those mutations that allowed survival at 26° C (the permissive temperature) but caused death at 32° C (the restrictive temperature) and any other mutation induced by the mutagenic agent in the experiment.

MATERIALS AND METHODS. The protocol for detecting heat-sensitive dominant and recessive mutations given in Figure 1 has been adapted from a previously described method to detect and measure concealed variability in *C. tritaeniorhynchus* (Sakai and Baker, 1972). The mating scheme involves two dominant markers (Delta, *D*, and the male sex allele, *M*), a recessive marker (white eye, *w*) and a crossover suppressor, M^1 (a pericentric inversion on the *M*-bearing chromosome, *I(1):12*, Baker *et al.*, 1971). Little or no crossing over has been found in females of this species (Baker and Sakai, 1974).

Three to four day-old males from lethal-free strains are treated with ethyl methanesulfonate (EMS), a chemical known to produce temperature-sensitive mutations in other organisms. The mutagen was added to a 1% sucrose solution which was then placed on cotton wicks (Lewis and Bacher, 1968). The males had the opportunity for 24 hours of tarsal contact as well as feeding on the mutagen. In the first experiments, three concentrations, .024 M, .012 M, and .005 M EMS were used. The .024 M and .012 M EMS treatments resulted in high sterility in the F_1 generations and therefore .005 M EMS was used for all subsequent experiments.

The treated males were then allowed to mate with virgin tester females, $\frac{w\ m\ D}{+m+}$ (Sakai and Baker, 1972). The adults were kept in cages in the insectaries where the temperature ranged from 28° C to 30° C. The eggs were collected within 12 hours of oviposition, and the larvae and pupae were reared in 26° C incubators. The $+D$ females were selected from the F_1 progeny and crossed with tester w*D* males. These males carry a crossover suppressor on the *M* chromosome, M^1. From the progeny of this mating (F_2), the $+D$ females and the $++$ males were selected and crossed. Four phenotypes are expected among the progeny of this cross: $++$ ♀, $+D$ ♀, $++$ ♂ and w*D* ♂. If a recessive lethal was induced on the tested chromosome (+m+), no $++$ ♀ would be found among the progeny of the above cross. If the lethality was detected at 26° C, the lethal is considered a non-heat-sensitive lethal. Moreover, if a recessive visible mutation is present on the tested chromosome, all $++$ ♀ ♀ will show the character. As we were attempting to isolate *hsl* mutants, only those lines which produced $++$ ♀ ♀ at 26°C were tested in the next generation by crossing the F_3 $+D$ ♀ ♀ with their $++$ brothers. For each chromosome tested, a minimum of two families was reared at 26° C and three families at 32° C. The absence or near absence of $++$ ♀ ♀ reared at 32° C and the presence of $++$ ♀ ♀ at 26° C for each line would indicate the presence of a recessive heat-sensitive lethal. Dominant heat-sensitive mutations can be detected by the absences of $++$ ♀ ♀, $+D$ ♀ ♀ and $++$ ♂ ♂ (only w*D* ♂ ♂

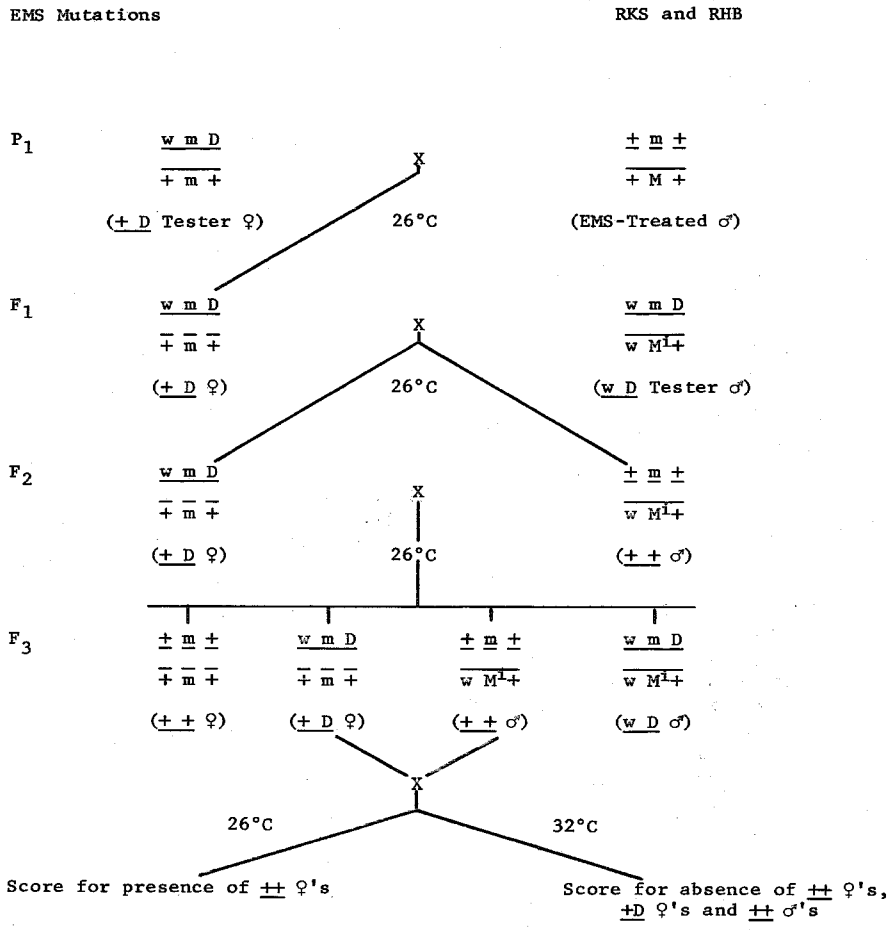

FIG. 1. Diagrammatic representation of the mating scheme used to recover recessive and dominant heat-sensitive mutations on the *m*-bearing chromosome (w=white eye, *m*, *M*=sex alleles, M^1=cross-over suppressor on *M*-bearing chromosome, and *D*=Delta). The broken line represents the EMS-treated chromosome.

would be present). The controls were males from the same stock which were fed with the 1% sucrose solution only.

RESULTS AND DISCUSSION. Preliminary experiments have shown some promising results: among the 712 treated chromosomes which have been tested, two appear to contain recessive, temperature-sensitive conditional lethals. Tables 1 and 2 summarize the data.

A homozygous female line has been established from one of these strains (*hsl*–20) by crossing the ++ females to the ++ males in the F_4 generation of the scheme in Figure 1. Data for the line are also summarized in Table 2. The proposed genotypes of the parents are $\frac{hsl\text{–}20\ m}{hsl\text{–}20\ m}$(♀) and $\frac{hsl\text{–}20\ m}{+M^1}$(♂). This strain can be maintained indefinitely with no culling, since an inversion is present that suppresses recombination. The females homozygous for *hsl* survive at 26°C but die at 32° C. Since the *hsl* mutation is recessive and the males are obligate heterozygotes for *hsl*, they survive at both temperatures. Therefore, in a control program, the *hsl* strain can be used to produce only male offspring: large numbers of mosquitoes can be reared at 26° C to maintain the strain. In the generation for male releases, the mosquitoes can be reared at 32° C to produce only males. Experiments are now in progress to produce additional temperature-sensitive lethals. For the temperature-sensitive mutations, the sensitive period during the life cycle is being determined by shifting mosquitoes from the permissive temperature to the restrictive temperature and vice versa at different successive intervals after the eggs are laid. Once the sensitive interval of the life cycle is precisely delineated, in order to mass produce adults of one sex (males), the mosquito cultures need to be subjected to the restrictive temperature for only the critical sensitive interval.

In addition to the conditional temperature-sensitive lethals, 22 mutations were also detected and isolated. Further experiments with these mutants are in progress and will be reported elsewhere.

TABLE 1. Summary of experiments to induce temperature-sensitive lethals with EMS.

Treatment	No. of chromosomes tested	No. lethal at 26° C	% lethal at 26° C	No. lethal at 32° C but not at 26° C	% lethal at 32° C but not at 26° C
EMS	712	444	62.4	2	0.3
Controls	35	0	0	0	0

TABLE 2. Summary of temperature experiments.

	26° C				32° C			
	♀		♂		♀		♂	
Strain	++	+D	++	wD	++	+D	++	wD
hsl–20*	712	1502	1459	1386	11	1390	1396	1291
hsl–20**	528	....	780	....	0	....	219	....
hsl–25*	380	506	477	489	1	418	376	442

* $\frac{hsl+m+}{+w\ m\ D}$ ♀ x $\frac{hsl+m+}{+w\ M^1+}$ ♂.

** $\frac{hsl+m+}{hsl+m+}$ ♀ x $\frac{hsl+m+}{+w\ M^1+}$ ♂.

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