GENETIC SEXING SYSTEM FOR THE PREFERENTIAL ELIMINATION OF FEMALES IN *CULEX QUINQUEFASCIATUS*

N. J. SHETTY[1]

Department of Zoology & Centre for Applied Genetics, Bangalore University, Bangalore India

ABSTRACT. A genetic sexing strain *T(1^M;2)1* of *Culex quinquefasciatus* was synthesized for the preferential elimination of females during the larval stage. Translocations were induced which linked the gene for resistance to malathion to the male-determining factor. Mitotic chromosomes were analyzed to determine the precise nature of the translocation.

INTRODUCTION

Culex quinquefasciatus Say is the most important carrier of Bancroftian filariasis in Asia, and the main urban nuisance mosquito. This species is found throughout the tropics and extends into the temperate regions of the northern and southern hemispheres (Mattingly 1951). Because of the serious problem of insecticide resistance in this species, alternative insect control methodologies based on genetic mechanisms are being explored. The success of genetic manipulation involving sterile-male releases can be enhanced by developing a method by which males can be easily separated from females during mass production. Since the females of this species are potential vectors, and cause biting nuisance, they should be eliminated during the early developmental stages by genetic methods. This will also help to lower the cost of mass production of males for release purposes. To achieve this objective, we have used a male-linked translocation and a malathion resistance gene to synthesize a genetic sexing strain of *Cx. quinquefasciatus*.

MATERIALS AND METHODS

The following strains were used in the experiment.

1. MANGALORE (MNG)—This strain is homozygous for malathion resistance and was originally collected from Mangalore, Karnataka, India during April 1980.

2. MADRAS (MDS)—This strain is homozygous for malathion susceptibility and was originally collected from Madras, Tamil Nadu, India during May 1978.

Our preliminary studies on the inheritance of malathion resistance in *Cx. quinquefasciatus* confirmed the observation of Tadano (1969) that malathion resistance is inherited as a partial dominant gene on linkage group 2.

For the induction of translocations, two-to-three-day-old males from MNG strain were exposed to 4,500 rads of gamma rays at the rate of 140 rads per minute from a ^{60}Co source at the Kidwai Memorial Institute of Oncology, Bangalore. The irradiated males were crossed to three-day-old, virgin MDS females. The resulting F_1 males were back crossed to MDS females, and after a blood meal, the females were individually isolated in vials for egg laying. The eggs were held for 72 hours to ensure complete hatching. The total number of eggs laid and the number of larvae from each female was recorded. Egg batches with greater than 35% sterility, indicating the possible presence of a reciprocal translocation, were exposed as early fourth instar larvae to 1.5 ppm malathion for 24 hours. The surviving larvae from each family were reared to the adult stage. Families showing only males were saved and were mated with MDS females for continuation of the line and further testing. Translocation breakpoints were ascertained from the mitotic chromosomes of pupal testes as described by French et al. (1962).

RESULTS AND DISCUSSION

Of the 119 backcross females that laid eggs, 87 families showing greater than 30% sterility were treated with malathion and the survivors were reared to adults. Twenty-two families showed a significant sex distortion favoring males but only one family, later designated as *T(1^M;2)1*, contained no females. This clearly showed that in the *T(1^M;2)1* line a translocation was induced which linked the male-determining locus to the malathion resistance gene. Examination of mitotic chromosomes from the testis of the *T(1^M;2)1* line confirmed a reciprocal translocation involving the shortest chromosome (sex chromosome) and one of the autosomes. The translocation break points are near the distal end of the metacentric chromosome 1 and about the middle of the larger arm of chromosome 2 (Fig. 1B). In *Cx. quinquefasciatus*, like other culicine mosquitoes, there is no heteromorphic pair of sex chromosomes. Sex is determined by a single pair of alleles, *M* and *m*, for

[1] Present address: Insects Affecting Man and Animals Research Laboratory, U.S.D.A., P. O. Box 14565, Gainesville, FL 32604

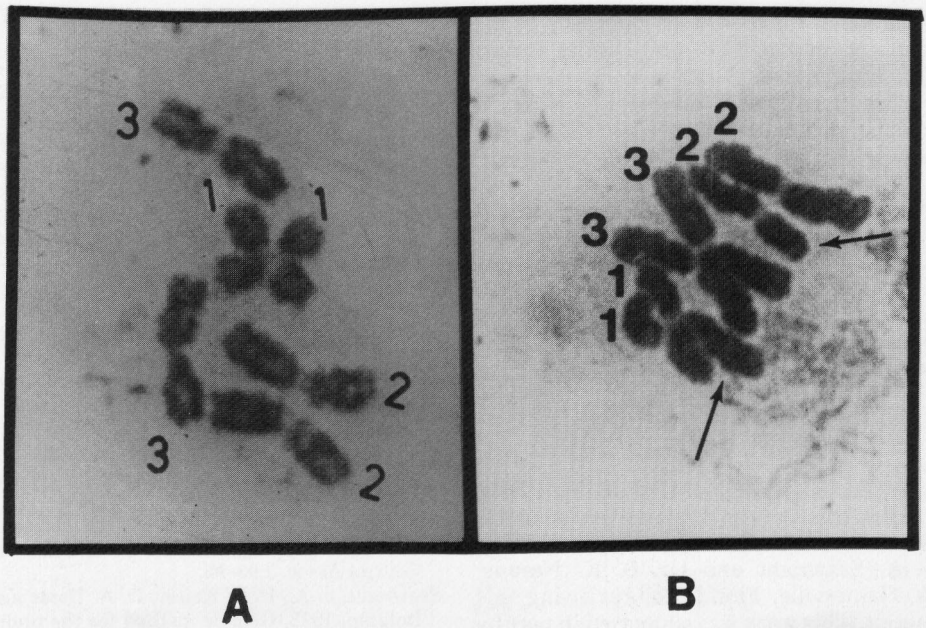

Fig. 1. Metaphase chromosomes from pupal testis. A. Normal, B. *T(1^{M};2)1*. Arrows point to exchanged segments.

which males are heterozygous, M/m and females are homozygous, *m m* (Gilchrist and Haldane 1947). The three pairs of chromosomes (2n = 6) have been correlated with their linkage groups, the *M* and *m* alleles have been assigned to the smallest pair of chromosomes (Dennhoffer 1972, Bhalla et al. 1974). LG 1, LG 11 and LG 111 corresponded to chromosome 1 (shortest), 2 (largest) and 3 (intermediate) as described by Bhalla et al. (1974).

The *T(1^{M};2)1* males when crossed with susceptible females showed 48.8% sterility. A total of 23,392 eggs were collected and 11,968 early fourth instar larvae were exposed to 1.5 ppm malathion for 24 hours; only one female was recovered. This female was isolated and released into a cage containing males from the susceptible strain, but she died immediately after the blood meal. Hence, it was not possible to confirm the supposition that this female was a resistant, recombinant type. No other females were recovered in the subsequent 10 generations of tests with the *T(1^{M};2)1* stock. Thus, the line showed only 0.02% crossing over between sex and malathion resistance, assuming that the single female was a true recombinant.

Genetic sexing systems have been reported in several species of mosquitoes. These include *An. gambiae* Giles *ss* (Curtis et al. 1976), *An. arabiensis* Patton (Curtis 1978), *An. albimanus* Wiedemann (Seawright et al. 1978), *Culex tritaeniorhynchus* Giles (Baker et al. 1978), *An. culicifacies* Giles (Baker et al. 1981), *An. stephensi* Liston (Robinson 1986) and *An. quadrimaculatus* Say, Species A (Kim et al. 1987).

The *T(1^{M};2)1* strain is a suitable sexing strain because of the very low rate of recombination between sex and the resistance gene and high vigor. The *T(1^{M};2)1* strain males showed higher mating competitiveness than the normal laboratory males and field collected males in the laboratory cages (unpublished data). Competition tests of the *T(1^{M};2)1* strain males against field collected males for field collected females in large field cages are scheduled for the future. This strain can be mass reared because of its low sterility.

The mechanical separation of sexes in the pupal stage of *Cx. quinquefasciatus* by using a grid has been developed (Sharma 1985). This method is based on the difference in size between male and female pupae. Mosquito rearing must be done under carefully controlled conditions to maximize the size difference of male and female pupae. Under certain conditions, such as overcrowding and feeding, there may be an overlap in the size of the pupae causing difficulties in separation of sexes based on size alone. The genetic sexing system is independent of external variables in the rearing conditions, and thus is advantageous over the former method. Though the present genetic sexing method involves the elimination of females at the 4th instar larva stage, a discriminating dose of malathion could easily be adjusted to eliminate females at the beginning of larval life.

Chromosomal translocations can easily be induced in *Cx. quinquefasciatus*. During earlier work, 30 different translocation stocks were isolated. These included 12 male-linked (TM), 8 autosomal simple reciprocal translocations and 10 male-linked double translocation heterozygotes (Shetty 1982, 1986). Of these, one sex-linked double translocation heterozygote, MPL-K (1; 2; 3), showed higher mating competitiveness than the normal males both in the laboratory and field cages (Shetty 1984, 1986). Thus, this line may well be suitable for mass rearing and field release for the suppression of natural populations of *Cx. quinquefasciatus*.

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