A GENETIC SEXING STRAIN OF *ANOPHELES QUADRIMACULATUS*, SPECIES A

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ABSTRACT. A genetic sexing strain of a mosquito, *Anopheles quadrimaculatus*, Species A, was synthesized for the preferential elimination of females during the egg stage. Malathion susceptibility was used as a conditional lethal, and the dominant malathion-resistance allele was linked to the Y chromosome via a radiation-induced reciprocal translocation involving the terminal end of the right arm of chromosome 3 and the Y chromosome. Examination of mitotic chromosomes and salivary polytene chromosomes revealed the precise nature of the translocation. Genetic leakage, through recombination, in the strain was very low (0.02%).

INTRODUCTION

Anopheles quadrimaculatus Say is a major biting nuisance throughout the southeastern United States and was formerly the principal malaria vector in this region. It is also an efficient host of the dog heartworm, *Dirofilaria immitis* (Leidy) (Lewandowski et al. 1980). However, as this mosquito has become highly resistant to several insecticides such as DDT, lindane and malathion (Roberts et al. 1984), it has become a good candidate for alternative control strategies, e.g., genetic control. In a successful field experiment with *Anopheles albimanus* Wiedemann, Lofgren et al. (1974) demonstrated that the sterile male technique (SIT) could be used to control an anopheline mosquito. The obvious need for sound and economical mass production methods for the SIT has prompted studies on the assembly of genetic sexing strains in many mosquitoes (Seawright et al. 1980, Robinson 1986).

It is readily appreciated that genetic sexing not only is advantageous in terms of saving space, cost of rearing, and of packing and distribution, but it also is of utmost importance in the use of SIT for haematophagous disease vectors of which females are responsible for disease transmission.

Hybridization experiments involving natural populations indicated the presence of a sibling species complex in *Anopheles quadrimaculatus*. Cytological analysis of the hybrids, revealed diagnostic X chromosomes and fixed autosomal inversions for differentiating Species A and Species B (unpublished data). In the present paper, a genetic sexing strain of *An. quadrimaculatus* Species A, utilizing a dominant malathion-resistant gene and a male linked translocation, is reported.

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MATERIALS AND METHODS

Two stocks of *An. quadrimaculatus* (Species A) were used to synthesize a genetic sexing strain: 1) RED BARN (RB) is a malathion resistant strain originally collected near Stuttgart, Arkansas in 1983. The mode of inheritance of resistance has been studied and is known to be under the control of a single, dominant, autosomal gene (unpublished data); 2) ORLANDO (ORL) strain is susceptible to malathion and has been maintained in laboratory culture for about 40 years.

Since male anophelines are heterogametic for sex determining chromosomes, a translocation between the Y chromosome and the autosomal linkage group containing the gene responsible for the malathion resistance provides the basis for a sexing system.

To induce a male-linked translocation adult RB males (less than 24 hr old) were exposed to 7 kR gamma rays ($^{Cs}137$ at 1,721 R/min). These irradiated males were crossed to ORLANDO females and the resulting F_1 males were backcrossed to ORLANDO females. The crossing scheme to detect the Y-linked translocation was similar to that of Kaiser et al. (1978). For the detection of male-linked translocations, 4th instar larvae of backcross families were treated with aqueous solutions of 400 ppm of malathion for 10 minutes. Linkage between sex and malathion resistance was taken as an indication that a reciprocal translocation had been induced. Mitotic chromosomes from adult testes and salivary gland polytene chromosomes were prepared as described by Mitchell et al. (1984) and used for cytological confirmation of suspected translocations.

The minimum concentration of malathion that could discriminate between resistant and susceptible genotypes in the egg stage was determined by treating groups of eggs less than 18 hr old with a series of malathion concentrations for 24 hr. Eggs were thoroughly rinsed with deionized water immediately after the 24-hr treatment. Mortality was determined about 2 days after the treatment by counting hatched

eggs. Larvae that survived the treatment were reared for confirmation of their genotype.

RESULTS

Twenty nine F_2 families showing at least 30% sterility were saved from 95 F_1 males. Seven of these families showed a significant sex distortion favoring males, but only one family, later designated as *T(Y;3R)1*, had no females. Males of this family were backcrossed to ORLANDO females for 12 generations (Table 1). The average sterility was 36.3%, which indicated one chromosomal aberration. From 10,000 larvae exposed to the insecticide only one adult female was produced, but as she died soon after emergence, it was impossible to determine if she was actually a resistant, recombinant type. However, since there is such a large difference between the heterozygous and homozygous susceptible types, this female was assumed to be a recombinant type. We do not know how many susceptible, recombinant males were produced, but again we assume the chance is not greater than for the resistant female.

The normal karyotype of this mosquito consists of two pairs of metacentric autosomes and a pair of heteromorphic sex chromosomes (Kitzmiller and French 1961). The Y chromosome is less than half the size of the X chromosome and in metaphase resembles a dot (Fig. 1A). Examination of mitotic chromosomes from the testes of *T(Y;3R)1* confirmed a reciprocal translocation involving the Y chromosome and one of two autosomes (Fig. 1B). It is difficult to determine from the mitotic metaphase preparation which of the two autosomes is involved, because the translocated piece is too small. However, it was the right arm of chromosome 3 since the malathion resistance was observed to be linked with stripe (st^+), the locus for which was reported on 3R (Mitchell and Seawright 1984). The precise nature of the translocation was revealed through cytological examination of salivary gland polytene chromosomes (Fig. 2). The translocation break point is located in region 23C, adjacent to a small paracentric inversion covering region 23C through 24A of 3R (unpublished data).

Table 1. Results of rearing *T(Y;3R)1* strain of *Anopheles quadrimaculatus*, Species A, treated as 4th instar larvae with 400 ppm of malathion for 1 hour.

Generation	Mean % sterility	No. of females	No. of males	% leakage
F_2	48	0	11	0
F_3–F_{12}	36.3 ± 4.9	1	4,327	0.02

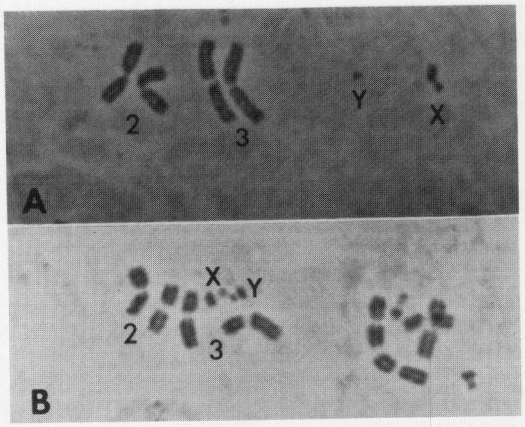

Fig. 1. Karyotypes from adult testes: of *Anopheles quadrimaculatus*, Species A: A—Normal, B—*T(Y;3R)1*.

Preliminary tests using 400 ppm of malathion had shown that susceptible eggs could be killed in 8 hours. We fixed the treatment time for 24 hr to reduce the insecticide concentration for the convenience and safety of personnel. A 24-hr treatment was used on an operational scale for the mass production of sterile males of *An. albimanus* (Bailey et al. 1980). Table 2 shows that corrected mortality of eggs of ORLANDO and *T(Y;3R)1* strain were 93.4% and 51.8%, respectively, at 100 ppm. There was no difference in mortality of the eggs treated with 200 to 400 ppm of malathion solution, which killed almost all susceptible ORLANDO eggs. The insecticide treatment seemed to stimulate a few susceptible eggs to hatch so that even at 800 ppm the level of unhatched eggs never reached 100%, but none of the hatched susceptibles developed to the 2nd instar. The *T(Y;3R)1* strain when outcrossed to ORLANDO females has a sex ratio slightly favoring females (female:male = 1.2:1) (Table 2). This seems to be the reason why the *T(Y;3R)1* strain showed 60.9 − 67.6% corrected mortality when treated with higher concentrations of malathion other than 100 ppm. Rearing the treated *T(Y;3R)1* strain produced only one female from the egg batches treated with 100 ppm. The female died before we could examine the ovarian polytene chromosomes. Whether it was a recombinant, resistant type or a susceptible type mosquito that survived the relatively low malathion concentration is unknown, but the frequency of undesirable types produced is low. However, we think that a malathion concentration of 200 ppm is more appropriate for use as a diagnostic concentration.

DISCUSSION

Prezygotic techniques including meiotic drive genes and autosomal sex determining factors can be very efficient in developing a suitable

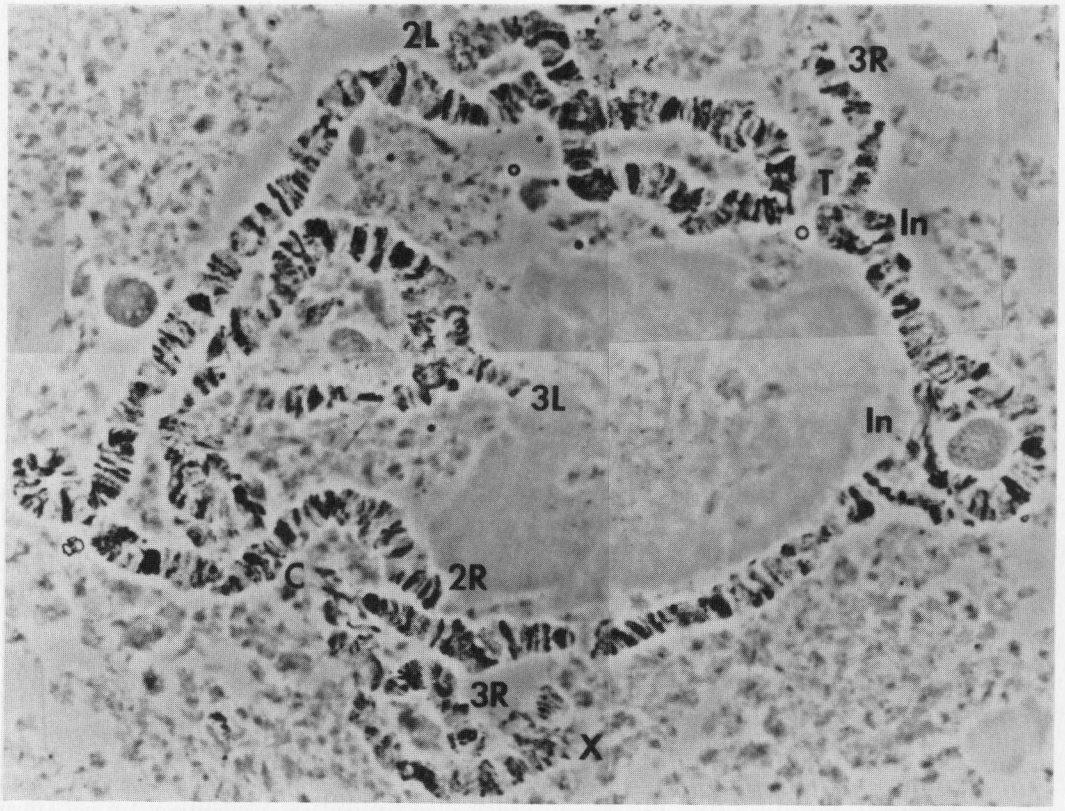

Fig. 2. Salivary gland polytene chromosomes from *T(Y;3R)1* male of *Anopheles quadrimaculatus*, Species A. The terminal end of chromosome 3R is broken off and connected to the chromocenter.

Table 2. Effects of malathion treatment on the corrected mortality of eggs and number of adults produced from the treated eggs.

Malathion concentration (ppm)	Egg mortality (%)		No. of adults *T(Y;3R)1*	
	ORLANDO	*T(Y;3R)1*	Male	Female
Untreated	6.0	36.1	688	839
100	93.4	51.8	791	1
200	99.0	62.4	968	0
400	98.4	60.9	686	0
800	99.9	67.6	637	0

genetic sexing strain. However, they are hard to exploit and have been developed only in *Musca domestica* Linn. and *Aedes aegypti* (Linn.) (Wood and Busch-Peterson 1982). There have been a number of reports on genetic sexing strains in mosquitoes (Curtis 1978, Baker et al. 1981, Robinson 1986) utilizing postzygotic techniques including heat-sensitive lethals and insecticide resistance. While employing heat-sensitive lethals requires basic information on mutant markers and cytogenetics, the use of insecticide resistance genes is more convenient because generally insecticide resistance is monofactorial and dominant or semidominant and can be found in field populations. Using propoxur resistance as a conditional lethal, Kaiser et al. (1978) used a sex-linked translocation inversion complex to reduce recombination to a minimum. This approach seems good for any candidate insect. Although we did not deliberately try to use a paracentric inversion on chromosome 3R, cytological examination of the salivary chromosomes in *T(Y;3R)1* shows that a naturally-occurring inversion in Region 23 was present. Although the inversion does not appear to cover the entire region between the malathion resistance gene locus and the translocation break point, the very low (0.02%) genetic recombination (Table 1) indicates the resistance gene is either in the inversion or the translocation is very close to the break point. The *T(Y;3R)1* stain is potentially a suitable sexing strain because of its very low recombination ratio and feasibility of discriminating sexes at the egg stage. The relatively low sterility can also be a minor advantage in mass rearing.

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