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Genetic sex separation in *Anopheles arabiensis* and the production of sterile hybrids

C. F. CURTIS [1]

Abstract

The gene for dieldrin resistance has been artificially male-linked so that females can be selectively killed with dieldrin. By intercrossing different sibling species of the Anopheles gambiae *complex, batches consisting of sterile males only can be reared. This seems to have potential for use in genetic control operations.*

Mosquitos of the *Anopheles gambiae* complex are the main vectors of malaria and filariasis in Africa and are therefore responsible for the transmission of more disease of man than any other insect. Within this complex, *A. arabiensis* (=*A. gambiae* species B) [a] is particularly difficult to control by conventional house spraying because of its tendency to rest out of doors (*1*). Therefore, consideration of genetic control is especially worthwhile for this species. A prerequisite for a genetic control operation is a method of eliminating females from batches of males being prepared for release so that those released do not add to the biting population. A genetic sexing system, designated R70, was produced in *A. gambiae sensu stricto* (=*A. gambiae* species A) (*2*). It is based on translocation of the semidominant autosomal gene for dieldrin resistance onto the Y chromosome so that when R70 males are crossed and backcrossed to homozygous dieldrin-susceptible females, the male progeny are heterozygous and the females are homozygous susceptible. Such females can be killed at the first-instar larval stage by a 24-h treatment with dieldrin at a concentration of 0.02 mg/litre leaving the males unharmed. I now report the production of a similar sexing system in *A. arabiensis*.

Materials, methods, and results

A stock of *A. arabiensis* was available from the Thiès area of Senegal, where it is the predominant species of the *A. gambiae* complex (*3*). It is dieldrin resistant and was chosen as one of the parents of the proposed system. For the other parent, dieldrin-susceptible *A. arabiensis* were required. Such a stock was not available from Senegal and, in fact, there have been difficulties in this laboratory in maintaining stocks of dieldrin-susceptible *A. arabiensis*. A few susceptible individuals were available from Mauritius and, although they could not be maintained as a stock, three males were successfully artificially mated to females of a well established predominantly dieldrin-resistant laboratory stock of Nigerian origin (KCD). The progeny were mated among themselves for two generations and mated females were then isolated for egg laying. Samples of ten first-instar larvae from each female were tested with dieldrin at a concentration of 0.02 mg/litre and in two cases all ten died, indicating homozygosity for susceptibility. The sibs of these were reared, rechecked for susceptibility at the adult stage, and pooled to yield a susceptible homozygote stock, which was designated SB. This stock was reared in the laboratory, though its egg production was rather low.

Adult males of the Thiès stock were irradiated with gamma rays at 40 Gy [a] and mated to SB females. The male progeny were backcrossed to SB females, which were isolated for egg laying. Among the egg batches laid by 18 females, five gave 38%–65% hatch, which suggested the presence of induced translocations. When the progenies from these were tested with dieldrin, four were found to include a proportion of resistant females and they were discarded. However, when the fifth family (designated T 15) was tested on 0.4% dieldrin papers for 1 h (a treatment that kills susceptible homozygotes but not heterozygotes), all 25 males survived and all 13 females died. The males were backcrossed to SB females and the progeny were reared without dieldrin treatment and mated among themselves to establish T 15 as a self-perpetuating stock. It has an egg hatchability of only about 45%, which is assumed to be due to the presence of a male-linked

[1] Research Fellow, Ross Institute, London School of Hygiene and Tropical Medicine, London WC1E 7HT, England.

[a] The Commission on Zoological Nomenclature has accepted the earlier described name of *A. arabiensis* Patton for *A. gambiae* species B.

[a] 1 Gray = 100 rad.

translocation. Crosses of T 15 males to homozygous dieldrin-susceptible females of *A. gambiae* s.s. (16cSS stock) were made. Exposure of the adult progeny to 0.4% dieldrin papers yielded 41 live males and 35 dead females. Treatment at the first-instar stage with dieldrin at 0.02 mg/litre[a] yielded 160 surviving males and no females. Thus any crossing over between the dieldrin locus and the translocation is sufficiently infrequent for practical purposes. Males that survived these treatments and that were species hybrids were mated to normal females; no larvae hatched from 1333 eggs laid. Dissection of the males confirmed that this sterility was due to early interruption of spermatogenesis typical of the hybrids from an *A. gambiae* s.s. female × *A. arabiensis* male cross (*4*). For comparison, R70 males were crossed to SB females. The testes of the males from this species cross, with *A. arabiensis* as the female parent, had spermatogenesis interrupted at various stages, with some spermatids and a few spermatozoa, which conforms with the results obtained by Davidson et al. (*4*). When the males were mated to normal females there was 1.3% hatch from 1778 eggs laid.

Discussion

It is thus possible to rear batches of mosquitos consisting only of sterile males. Before release experiments with such males are considered, it would be necessary to introduce into the parental stocks the genome of wild populations from the area where the releases were planned. Even if this were done, it is questionable whether F_1 species hybrids would be able to compete for mates in the field, in view of the fact that *A. arabiensis* and *A. gambiae* s.s. have behavioural isolating mechanisms that almost completely prevent cross mating in the field (*5*), though not in the laboratory. Fraccaro et al. (*6*) have shown that in the *A. maculipennis* complex, a species difference in mating behaviour is under the control of the Y chromosome. If the same were true in the *A. gambiae* complex, male hybrids with T 15 male parents would have mating behaviour of the *A. arabiensis* type. To increase the chances of obtaining sterile males with *A. arabiensis* mating behaviour, crosses have been carried out that allow the production of hybrids with autosomes very largely of *A. arabiensis* origin. By the use of the sex-linked marker white eye (*7*), it has been arranged that the X chromosomes of the male hybrids are of *A. gambiae* s.s. origin, which is sufficient to ensure that they are sterile.

[a] Subsequent work has shown that with *A. arabiensis* dieldrin at 0.01 mg/litre is preferable for the selective killing of susceptible homozygotes without any killing of heterozygotes.

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