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## Genetic Method for the Preferential Elimination of Females of *Anopheles albimanus*

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Prior experience with drug treatment markedly attenuates the formation of conditioned taste aversions in rats (9). Our results are interesting in that most of the patients in this study had received many prior treatments that caused GI discomfort (the average number per child was 23). Furthermore, most of the patients were aware that the cause of their nausea and vomiting was their therapy. These factors, however, did not preclude the formation of long-lasting taste aversions, suggesting that humans have a strong propensity to form these aversions.

Further data were obtained through the use of questionnaires, the results of which support and extend these findings. Patients completed diet inventory forms during sessions 1 and 2 to provide information about their food preferences and usual diet, as well as specific foods eaten before drug treatments. These data (10) revealed that patients receiving GI toxic chemotherapy were significantly more likely to report avoiding or disliking a specific food eaten before a clinic visit than control (group 3) patients. Thus, learned taste aversions may occur not only to a novel food presented by the experimenter just prior to drug administration, but also to foods in the patients' diets which happen to be eaten up to several hours before treatment. This is consistent with the findings of Garb and Stunkard (11) that human subjects report the development of aversions to a wide variety of foods consumed coincidentally before a bout of illness.

The demonstration of taste aversions in children receiving chemotherapy treatments may prove to be of importance to physicians who administer treatments which induce nausea and vomiting. Such aversions may be one of the factors contributing to the anorexia and weight loss seen in patients with cancer. Additional work is needed to delineate the factors controlling the occurrence of these aversions in order to develop methods for minimizing or eliminating them.

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- The diagnoses of the patients in this study were: group 1: lymphoma (4), lymphosarcoma (1), acute lymphocytic leukemia (2), acute myelogenous leukemia (2), Wilms tumor (3), Ewings sarcoma (1), Hodgkins disease (1); group 2: lymphoma (3), acute lymphocytic leukemia (2), Wilms tumor (1), Ewings sarcoma (1), Hodgkins disease (1), osteogenic sarcoma (1), rhabdosarcoma (1), otiosarcoma (1), astrosarcoma (1); group 3: acute lymphocytic leukemia (10), idiopathic thrombocytopenia (3), astrocytoma (1), undifferentiated leukemia (1).
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- Patients in groups 1 and 2 received one of the following doses of chemotherapeutic agents known to cause significant GI discomfort: (numbers in parentheses indicate the number of patients in groups 1 and 2, respectively, receiving this dose), adriamycin, 20 to 60 mg/m<sup>2</sup> (2, 4); daunomycin, 45 to 60 mg/m<sup>2</sup> (0, 2); cyclophosphamide, 500 to 1200 mg/m<sup>2</sup> (5, 0); cytosine arabinoside, 100 to 150 mg/m<sup>2</sup> (1, 2); actinomycin D, 316 µg/m<sup>2</sup> (1, 0); nitrogen mustard, 6 mg/m<sup>2</sup> (0, 1); procarbazine, 100 mg/m<sup>2</sup> orally (1, 0); or a combination of GI toxic drugs: adriamycin, 20 to 40 mg/m<sup>2</sup>, plus cytosine arabinoside, 100 mg/m<sup>2</sup> (3, 1); 5 aza cytidine, 100 mg/m<sup>2</sup>, plus cytosine arabinoside, 75 mg/m<sup>2</sup> (1, 0); cyclophosphamide, 300 mg/m<sup>2</sup>, plus adriamycin, 60 mg/m<sup>2</sup> (0, 1); cyclophosphamide, 300 mg/m<sup>2</sup>, plus 5-fluorouracil, 225 mg/m<sup>2</sup> (0, 1). In addition to the GI toxic drugs, 9 of 14 patients in group 1 and 6 of 12 patients in group 2 received antiemetics; 6 of 14 patients in group 1 and 6 of 12 patients in group 2 received vincristine (1.5 mg/m<sup>2</sup>). Reports of nausea or emesis, or both, ranging from mild to severe occurred in 11 of 14 patients in group 1 and 8 of 12 patients in group 2. In group 3, 11 patients received vincristine (1.2 to 2.0 mg/m<sup>2</sup>), 4 patients received no drug treatment. There were no reports of nausea or emesis in this group.
- A significantly lower percentage of the ice cream consumed by group 1 (experimental) patients was Mapletoff as compared to the combined control groups (Mann-Whitney U test:  $P \leq .05$ ).
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- I thank M. J. Wallace, J. Hartmann, I. D. Bernstein, R. Chard, and W. A. Bleyer for invaluable assistance in the implementation of this study. Supported by the Diet, Nutrition, and Cancer Program of the National Cancer Institute (CP 65790-68). I also thank R. Bolles and S. Woods for their critical reading of the manuscript.

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## Genetic Method for the Preferential Elimination of Females of *Anopheles albimanus*

**Abstract.** Recent field experiments demonstrated the possibility of using the sterile male method for the control of *Anopheles albimanus* Wiedemann, the most important vector of human malaria in Central America. Until now there was no practical method for excluding females from the releases of sterile males. A genetic method was developed for the preferential elimination of females during any of the four life stages. This genetic sexing system utilizes propoxur (*o*-isopropoxyphenyl methylcarbamate) susceptibility as a recessive conditional lethal, a T(Y:2R) translocation, and an In(2R) inversion. The propoxur resistance allele (dominant) was linked to the Y chromosome via a radiation-induced translocation, and genetic recombination was suppressed by inversions. In one of the strains produced, 99.7 percent of the females are eliminated when treated with propoxur, without male loss.

During an experiment conducted in El Salvador, Lofgren *et al.* (1) demonstrated the possibility of using the sterile male method for the control of *Anopheles albimanus*, an important vector of human malaria in Central America. In view of the widespread occurrence of insecticide resistance in this species, the development of this alternate control method is most desirable and appropriate at this time. The success of the sterile male method relies on the efficient distribution of inundative releases of competitive, sterile males into the natural habitat of the target species. Therefore, a sound system must be available for the mass production of sterile males, but since the females of anopheline species are potential malaria vectors, they should be excluded from the releases. Also, if the females could be eliminated during the egg or early larval stages, the mass production of males could be conducted less expensively.

Since currently available methods for elimination of *A. albimanus* females in a mass production process are only 85 to 95 percent effective (unpublished data), we undertook the development of a genetic method for the preferential killing of females. We now describe the synthesis of a female-killing system for use in a mass production facility.

The genetic sexing scheme utilizes propoxur (*o*-isopropoxyphenyl methylcarbamate) susceptibility ( $pr^s$ ) as a recessive conditional lethal, a T(Y:2R) translocation, and an In(2R) inversion. The locus for propoxur resistance ( $pr^r$ ), a dominant trait, is on the right arm of chromosome 2, and this allele was linked to the Y chromosome via a radiation-induced translocation. Adult homozygous resistant males ( $pr^r/pr^r$ ), less than 24 hours old, were irradiated with 1700 R (dose rate 212 R/min) from an x-ray machine operated at 90 kV (peak). These irradiated males were crossed to suscep-

Table 1. Results of rearing T(Y:2R) translocation strains treated as larvae with 20 parts of propoxur per million (data collected over five generations).

Strain	Sterility (%)	Number of males	Number of females	Percent crossing over
T(Y:2R)2	34	425	127	23
T(Y:2R)3	52	759	276	27
T(Y:2R)4	48	1471	130	8
T(Y:2R)5	49	2669	176	6
T(Y:2R)6	52	793	190	19
T(Y:2R)7	57	762	142	16

Table 2. Results of rearing T(Y:2R) translocation-In(2R) inversion strains treated with propoxur.

Strain	Sterility (%)	Number of males	Number of females	Percent crossing over
In(2R)[T(Y:2R)3]1	56	2372	55	2.3
In(2R)[T(Y:2R)3]2	46	2945	6	0.20
In(2R)[T(Y:2R)6]1	49	1975	41	2.0
In(2R)[T(Y:2R)4:T(2R:3R)]1	65	807	17	2.1

tible females ( $pr^s/pr^s$ ), and the resultant  $F_1$  males were backcrossed to susceptible females. The backcross progeny from families that showed less than 60 percent egg hatch were single-family cultured (2) and treated in the larval stage with an exposure (30 minutes in 20 parts of propoxur per million) that killed the homozygotes ( $pr^s/pr^s$ ), but not the heterozygotes ( $pr^s/pr^r$ ). Discriminating treatments are available for separating the two genotypes during the egg, larval, pupal, or adult stages (unpublished data). If an appropriate translocation were present in an  $F_1$  male, then a distortion in sex ratio would be evident among his progeny. Chromosomal aberrations detected in this manner were confirmed by examination of salivary gland chromosomes with an established technique (3). A total of 1200 sperm were tested, and six T(Y:2R) translocations were isolated (Table 1).

Since genetic recombination occurs in both sexes of *A. albimanus*, some heterozygous resistant females ( $pr^r/pr^s$ ) are produced each generation. For the stocks we studied, the recombination frequencies ranged from 0.06 to 0.27. The male-linked translocations proved to

be holandric, a fact strongly suggesting that the crossing over is restricted to the autosome.

To suppress the incidence of recombination, we irradiated males of these translocation stocks to induce an inversion. Several inversions were isolated, and the strains showing the least number of recombinant, resistant females are shown in Table 2. For clarification, the strain designation In(2R)-[T(Y:2R)3]2 identifies the second inversion induced from the T(Y:2R)3 translocation strain. The inversion covers part of the Y-2R chromosome that resulted from the original irradiation and includes the centromere of the Y chromosome.

Of the four strains shown in Table 2, In(2R)[T(Y:2R)3]2 is the most appropriate for the purpose of a mass production system because it has the maximum level of fertility (54 percent) and the minimum recombination frequency (0.0016). It is of interest to note that the original T(Y:2R)3 strain usually produces 27 percent recombinant females.

In(2R)[T(Y:2R)3]2 has been mass cultured for several generations and has proved to be quite suitable for that type

of production. Exposure of this strain to propoxur eliminates 99.7 percent of the females. This elimination can be done in the egg stage or in subsequent stages of the life cycle, depending upon the need. Furthermore, since three pairs of chromosomes are found in this species it is a relatively simple matter to incorporate wild genome into the strain.

The genetic sexing system described herein is similar to those developed for *Lucilia cuprina* (4) and *Anopheles gambiae* (5). A "male-producing" strain was also developed in *Musca domestica* (6) by using a temperature-sensitive lethal and a male-determining autosome.

For those species of insect pests that can be controlled by the release of sterile males, the use of a genetic sexing system can be of great benefit, especially in the curtailment of rearing costs. However, cost is not the only consideration. The sterile male method has been considered unsuitable for some species where sexual separation is difficult because the females cause extensive damage or are potential disease vectors. In some cases, the treatment used to sterilize males is ineffective on the females. The development and implementation of genetic sexing systems could possibly increase the number of species for which the sterile male method can be used.

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7. We are indebted to C. F. Curtis and G. Davidson for kindly supplying the propoxur-resistant strain of *A. albimanus*. The research reported here was conducted in part with contract funds transferred from the Medical Research and Development Command, Office of the Surgeon General, U.S. Army.

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