GENETIC SEXING STRAINS OF *ANOPHELES ALBIMANUS* WIEDEMANN[1, 2]

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ABSTRACT. Two new genetic-sexing strains of *Anopheles albimanus* Wiedemann are described and compared to previously existing strains. Each of the genetic-sexing systems was synthesized by organizing propoxur resistance, a T(Y;2R) translocation, and an In(2R) inversion in an aberration complex that pseudolinks resistance to the Y chromosome and suppresses recombination during meiosis in the male. A discussion of the genetic sexing of mosquitoes is included which details the prerequisites for developing a genetic sexing system, the essential steps for maintaining such a system, and genetic phenomena that could cause problems for the genetic sexing system.

Several recent publications have introduced the innovation of using specially constructed strains of insects for the preferential elimination of females by genetic methods (Sakai and Baker 1974; Whitten et al. 1975, Curtis et al. 1976, Curtis 1978, Seawright et al. 1978). These genetic sexing systems are very promising for reducing cost of production in the mass rearing of mosquitoes for use in the sterile male technique. The strain (dubbed MACHO) of *Anopheles albimanus* Wiedemann, described by Kaiser et al. (1978) was implemented successfully in a mass production plant in El Salvador (Bailey et al. 1980). Production of sterile males at that facility exceeded 1,000,000 per day compared to 170,000 per day when an inefficient mechanical system was used to sex a normal strain.

Kaiser et al. (1978) listed several genetic sexing strains of *An. albimanus*. The males of these strains were heterozygous for a male-linked translocation, an inversion, and *propoxur resistance;* the females carried no chromosome aberrations and were susceptible to propoxur. Preferential killing of the females was accomplished by treating the egg stage with propoxur. In the best of these strains, genetic recombination resulted in the production of 0.2% resistant females per generation. This small leakage of resistant females caused an eventual breakdown of the genetic sexing system through the increased buildup in frequency of resistant females and susceptible males over time. Therefore, screening for new inversions was continued in an effort to obtain an inversion that barely overlapped the pr^r locus and the chromosome breakpoint in the *T(Y;2R)6* stock. In this present paper, these latest efforts are summarized along with a discussion of genetic sexing applicable to mosquitoes.

METHODS AND MATERIALS

All handling and rearing procedures were similar to those outlined by Kaiser et al. (1978). Males, less than 24 hr old, of the *T(Y;2R)6* stock (described by Kaiser et al., 1979) were irradiated with 3180-3480R with either an X-ray machine (90KVP at 212 R/min.) or gamma rays (Cs^{137} at 2080 R/min). These irradiated

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males, which were heterozygous for *propoxur resistance (T(Y;2R)6 pr^r/pr^s)*, were crossed to females homozygous for propoxur susceptibility (*pr^s/pr^s*). The F_1 larvae were treated for 1 hr with 20 ppm of propoxur to eliminate the recombinant, susceptible males (usually 19%) and the surviving males were backcrossed to *pr^s/pr^s* females. The F_2 generation was reared in families, and the larvae were treated for 1 hr with 20 ppm of propoxur when they were in the 3rd or 4th stage. Survivors of the insecticidal treatment were reared to the adult stage and scored for sex, and those families consisting of at least 10 males and no females were crossed to *pr^s/pr^s* females.

If an F_2 family contained a single female, it was discarded. No attempt was made to record the numbers of males and females in the culled families. In a screening program of this sort, the labor and space requirements prohibited accurately counting and maintaining stocks of dubious value. The number of stocks carried into the F_3 was limited to families consisting of at least 10 males on the basis of 2 factors. The 1st involved the expected size of each family, which was affected by the fecundity of the parental females, the semi-sterility caused by the translocation, mortality caused by the insecticide treatment, and natural mortality. Females of *An. albimanus* usually lay 50–150 eggs, of which half were sterile, and of those that hatched half were susceptible to the propoxur treatment. Also, our rearing of the larvae was often only 50% effective. Therefore, we expected the size of families to range from 5 to 50 mosquitoes.

The 2nd factor was concerned with the probability of incorrectly (no inversion) keeping an F_2 family for further study in the subsequent generation. The probability (P) of incorrectly keeping a family was given by Mather (1957): $P = M^n$, where M is the expected frequency of males and n is the size of a family. Since an average of 19% recombinant, resistant female gametes are produced by males of the *T(Y;2R)6* stock (Kaiser et al. 1978), P $= (0.81)^{10}$ or 0.122 for a family size of 10. Therefore, by chance alone, 12.2% of the F_2 families composed of 10 mosquitoes would be all males. Since about 90% efficiency was desired in the screening system, the lower limit on family size was set at 10 mosquitoes.

Cytological examination of the salivary chromosomes from F_3 larvae were used to confirm and map new inversions. Crossover data were also collected by treating the F_3 larvae with propoxur, and stocks with <3.0% resistant females were retained for further analysis.

A simple computer model was used to predict the deterioration of genetic sexing systems, of the type described by Kaiser et al. (1978), for anopheline mosquitoes by calculating the percentage of resistant females that would accumulate over time in stocks with crossover rates varying from 0.2 to 2.0%. No selective advantage was allowed for either *pr^s* or *pr^r*, and the population was moved through the model in discrete generations.

RESULTS AND DISCUSSION

A summary of the genetic sexing stocks of *An. albimanus*, including those reported previously by Kaiser et al. (1979), is given in Table 1. The stocks covered in part in this report are marked with an asterisk. All of these strains were obtained from irradiation of males of the *T(Y;2R)6* translocation. A total of 750 F_2 families were evaluated, and 30 (or 4.0%) families composed of males only were held for collection of crossover data and cytological analysis in the F_3 generation. Of these 30, only 8 actually had a large inversion on the Y-2R chromosome that resulted in reduced crossingover in the F_3 generation. However, in 2 of the 8 stocks, the inversions were not quite large enough to suppress the production of recombinant types below 4.0%; therefore, these 2 stocks were discarded.

As noted earlier, about 19% crossover types were produced in the *T(Y;2R)6* strain, and the probability (P) of obtaining

Table 1. List of strains of *Anopheles albimanus* assembled by using radiation for the purpose of preferential elimination of females with propoxur. Socks marked with an asterisk are discussed in the text.

Stock	Chromosome[a] breakpoints (Inversions)	Sterility (%)	Crossover[b] frequency (%)
In (2R) [T(Y;2R)3]1[c]	10C 13C	56	2.3±0.3
In (2R) [T(Y;2R)3]2[c]	11A, Y	46	0.2±0.3
In (2R) [T(Y;2R)6]1[c]	8B, 11A	49	2.0±0.3
In (2;2R) [T(Y;2R)4]1[c]	8B, 25B (*In2*) 9B, 12C (*In2R*)	65	2.1±0.5
**In (2R) [T(Y;2R)6]1*[c]	11A, Y	51	0.8±0.2
**In (2R) [T(Y;2R)6]2*[c]	11B, Y	51	0.8±0.2
**In (2R) [T(Y;2R)6]3*[c]	10C, 12C	52	2.4±0.4
**In (2R) [T(Y;2R)6]4*[c]	9B, Y	62	0.2±0.2
**In (2R) [T(Y;2R)6]5*	11A, Y	57	0.5±0.2
**In (2R) [T(Y;2R)6]6*	6A, Y	50	0.5±0.2

[a] According to the standard map published by Keppler et al. (1973).
[b] Crossover frequency corresponds to percentage resistant females.
[c] Strains were noted by Kaiser et al. (1979), but additional crossover data were included in present report.

a family of size n with no resistant females was given by: $P = (0.81)^n$. It follows that the probability (P_c) of making a correct judgment that a large inversion was induced is obtained by: $P_c = 1.0 - P$. The relationship between P_c and family size for *T(Y;2R)6* is summarized in Fig. 1. A similar plot can be made for any specific crossover frequency. In Table 2, a summary is given for the 30 families grouped according to size with the corresponding probability range for each interval. One of the F_2 families was composed of 31 males and no females, yet in the F_3 generation there were 20% resistant females in that stock. Similarly, this sort of situation was observed for F_2 families with 27, 25, 23, 22, 21, and 20 males. The probability of obtaining a family of 20 males without an inversion to suppress recombination is only 0.012, and this diminishes to 0.0015 for a family of 31 males. These observations indicate a need for more information on sex-distorter mechanisms and/or genes causing variable rates of recombination.

Of the stocks listed in Table 1, the last 2 have not been reported before, but the breakpoints (refer to the standard map of Keppler et al. 1973) and cytology of the other stocks were discussed by Kaiser et al. (1979). Photographs of *In(2R)[T(Y;2R)6]6* and *In(2R)[T(Y;2R)6]5* are shown in Fig. 2 (a and b).

In the former stock (Fig. 2a), a rather large inversion covers about 75% of 2R from the *T(Y;2R)6* breakpoint in Region 13 to Region 6 at the free end. Since the inversion must include *pr*[r], the 0.5% recombinant females probably arise from 2-strand double crossover events. Kaiser et al. (1979) measured crossingover be-

Table 2. Summary of 30 F_2 families that were suspected of bearing an inversion on 2R.

Family size (n)	Probability of no inversion in stock	Number families	
		With inversion	Without inversion
10–15	0.122 –.043	3	13
16–20	0.034 –.015	2	3
21–25	0.012 –.0052	1	4
26–31	0.0042–.0015	2	2

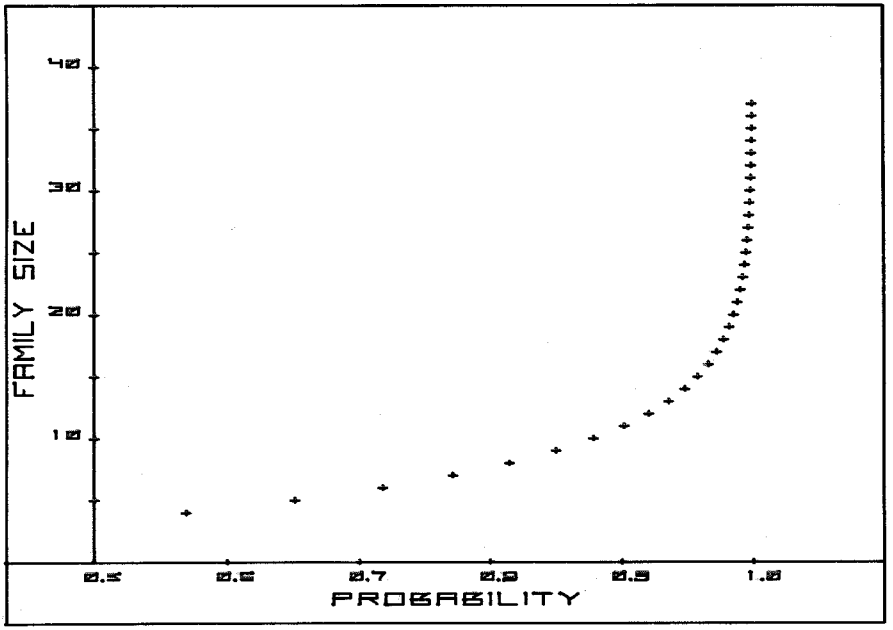

Fig. 1.—Relationship between family and the probability of correctly identifying an induced inversion on the Y-2R chromosome of the *T(Y;2R)6* translocation stock. Consult the text for a description of the screening system employed for detection of an inversion.

tween 6 *T(Y;2R)* translocations and pr^r and showed that a linear relationship exists between the frequency of crossingover and physical distance on the polytene 2R in *An. albimanus*. On the basis of their estimates of map distances of 19 and 8 units between pr^r and Regions 13 and 6, respectively, the expected frequency of double crossingover is 0.0152. Resistant females are produced by one-half of the double events; all two-strand events and one-half of the three-strand type switch the pr^r gene to the normal 2R inherited by the daughters of the males carrying the chromosome aberrations. The other three-strand type produces susceptible males that are double recombinants, and all of the gametes from 4-strand, double exchanges are aneuploid. Hence, the expected frequency of resistant females that arise from double crossingover is 0.0076 (or 0.76%), which is fairly close to the observed value of 0.0055 ($\chi^2 = 0.822$; P = 0.3 – 0.4).

In the latter stock (Fig. 2b) a small paracentric inversion is present and covers a segment of 2R from Region 13C, which is the *T(Y;2R)6* breakpoint, to Region 11A. Kaiser et al. (1979) estimated that pr^r is located in Region 9; therefore, the inversion covers 2/3 of the distance between the T(Y;2R)6 breakpoint and the pr^r locus, but crossingover has been reduced from 19.0 to 0.5%. This observation is similar to the reduction in crossingover in the *In(2R)[T(Y;2R)3]2* stock in which the inversion covers 76% of the distance between pr^r and the *T(Y;2R)3* break and crossingover is reduced from 27.0 to 0.2%. Moreover, the

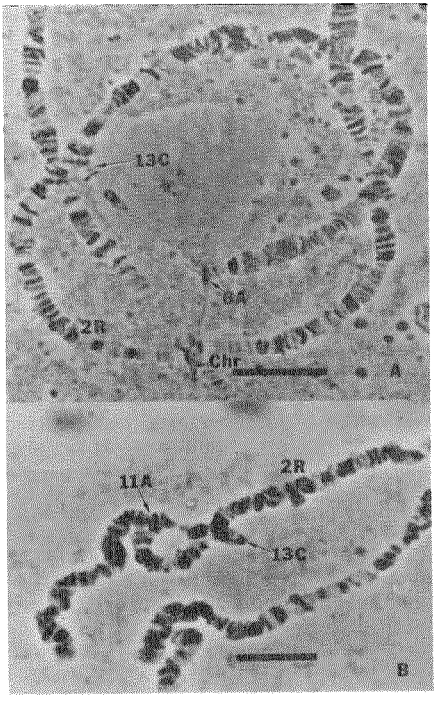

Fig. 2.—Polytene chromosome complements for 2 genetic sexing strains. A. *In (2R)[T(Y;2R)6]6* and B. *In (2R)[T(Y;2R)6]5*.

breaks on 2R for both these inversions are located in Region 11, which possibly indicates a low rate of crossingover between Regions 9 and 11.

Both of the strains produce slightly more recombinant, resistant females (0.5 compared with 0.2%) than the *In(2R)[T(Y;2R)3]2* and *In(2R)[T(Y;2R)6]4* stocks. Since both stocks are vigorous, and the inherent partial sterility caused by the chromosome aberrations is within tolerable limits, they could be used in a mass production system for sterile males.

The theoretical deterioration of genetic sexing stocks, e.g., those in Table 1 for anopheline species, is shown in Table 3. The generation time for *An. albimanus* in the laboratory is about 14 days which means that approximately 13 generations are produced in half of a year. After 1 year, there would be >3.0% resistant females in a strain with 0.2% crossingover. Models of this sort are useful for showing in a general way what type of replacement cycle is necessary for a strain in a mass production system. For example, during the conduct of a pilot release study of the use of sterile males for the control of *An. albimanus*, the upper limit of females (malaria vectors) in each daily release was set at 25,000 (or 2.5%). Generally, with a restriction of 2.5% females in releases, only stocks with 0.006 or less crossingover would be acceptable because of the periodical purge of the brood stock being used in mass production. By the time a stock is culled there are 4 types of males (*T In* pr^r/pr^s, *T In* pr^s/pr^s, *T In* pr^s/pr^r, and *T In* pr^r/pr^r) and 3 types of females (pr^s/pr^s, pr^s/pr^r, and pr^r/pr^r) present in the system. The strain must therefore be reconstituted by starting with *T In* pr^r/pr^s males and pr^s/pr^s females, and for obvious reasons this type of handling should be minimized in a mass production facility.

DISCUSSION

In addition to *An. albimanus*, genetic sexing systems have been developed for 3 other species of mosquitoes, viz. *Anopheles gambiae* Giles (Curtis et al. 1976), *Anopheles arabiensis* Patton (Curtis 1978), and *Culex tritaeniorhynchus* Giles (Sakai and Baker 1974). The stocks of the *An. gambiae* complex were established with dieldrin susceptibility as the conditional lethal, but the system for *Cx. tritaeniorhynchus* used an EMS-induced, heat-sensitive mutant. Genetic sexing systems can probably be developed for any species of mosquito for which satisfactory colonization procedures are available.

Knowledge concerning the mode of sex determination and a suitable conditional lethal are essential for proper planning during the synthesis of a genetic sexing

Table 3. Theoretical deterioration of genetic sexing systems with varying frequencies of crossingover. Consult the text for the assumptions used in calculating the percentage resistant females each generation.

Generation	Percentage resistant females in releases (in colony) at indicated crossover rates				
	0.002	0.006	0.010	0.014	0.018
5	0.71 (0.35)	2.11 (1.05)	3.49 (1.72)	4.84 (2.37)	6.18 (3.01)
10	1.36 (0.67)	4.02 (1.97)	6.58 (3.19)	9.06 (4.34)	11.45 (5.42)
15	2.01 (0.99)	5.88 (2.86)	9.54 (4.55)	13.01 (6.09)	16.29 (7.49)
20	2.66 (1.31)	7.68 (3.70)	12.36 (5.80)	16.71 (7.66)	20.74 (9.29)
25	3.29 (1.62)	9.44 (4.50)	15.04 (6.96)	20.16 (9.06)	24.83 (10.86)

system. More flexibility can be exercised in the type of approach if an extensive background of information is available on the formal genetics and cytogenetics of a species. Since chromosome aberrations are usually necessary, a profile of the response of a species to the effects of radiation is very helpful.

For all species of mosquitoes that have been studied, the males have either heteromorphic (X-Y) sex chromosomes or are heterozygous for a single locus on homomorphic sex chromosomes. If the male is heterogametic, the use of recessive, conditional lethal traits is facilitated in a system. A strain can be constructed so that the males are heterozygous for the lethal, but the females are homozygous and die when the condition is applied. In the selection of a suitable conditional lethal, the approach depends on the amount of basic knowledge available concerning the genetics and cytogenetics of the species. Naturally occurring conditional traits, e.g., pesticide resistance, cold or heat tolerance, etc., are common in many species, especially those that are distributed over a wide range or have been routinely subjected to insecticidal sprays. In the absence of an extensive genetic background, this type of natural lethal trait is the most available source. *Propoxur resistance* in *An. albimanus* and *dieldrin resistance* in *An. gambiae* are good examples of naturally-occurring conditional traits.

If a sizable body of genetics information is available, the conditional lethal trait can be induced and detected by using an efficient screening system, e.g., the methods employed by Sakai and Baker (1974). The key word in the last statement is "efficient," because conditional lethals can be induced and detected without extensive data on mutant markers and cytological information, but the process would be very laborious without mutant markers and specific chromosome inversions. Whether one selects induced or natural conditional traits, the monofactorial lethal should be recessive and the response of the heterozygote and homozygote should not overlap, i.e., the discriminating dose of the condition (whether it is a pesticide, heat, cold, etc.) should kill only the homozygous recessive. Some conditional traits will allow a considerable amount of flexibility in selection of the life stage to be killed. The use of propoxur in the genetic sexing of *An. albimanus* is a good example of complete freedom; this compound could be used to kill the homozygous susceptible in any of the 4 life stages. Curtis et al. (1976) found that dieldrin was ineffective as an egg treatment, and consequently they had to treat the 1st stage larvae. Some induced heat-sensitive lethals are sensitive over a rather short interval, so care should be taken in the design of a screening procedure to ensure that the lethal condition is effective in killing the eggs or 1st stage larvae. If only late stage larvae, pupae, or adults are affected by the lethal condition, the economic advantage of rearing and handling only the males is lost.

One of the most frequent concerns of

entomologists who have a limited background in genetics involves the stability of a genetic sexing system. This is a very legitimate concern as can be seen in the "leakage" of resistant females in the stocks listed in Table 1. The imperfections imposed by crossingover results in a progressive deterioration of these stocks. If genetic recombination were the only influence on a strain, definite plans could be made for culling the colony at necessary intervals. However, there are other problems that could arise, both genetic and otherwise.

Care must be taken to prevent contamination of a strain containing males bearing an aberration with normal males. Males of the stocks listed in Table 1 are partially sterile, and thus they suffer a distinct disadvantage in competing against normal males. Therefore, the introduction of a single normal male into one of these strains could have serious consequences. As noted by Bailey et al. (1980), maintenance of a normal strain in the same mass production plant with a genetic sexing system is unwise.

Alternate resistance (or tolerance) mechanisms can be present or evolve in the females in a genetic sexing strain. Diligence in using large samples in evaluating a strain is important, because mechanisms of very low frequency (<1.0%) could eventually cause a significant problem (see Bailey et al. 1980) in a mass rearing plant where millions of mosquitoes are produced each week. In a massive colony of millions of mosquitoes, the evolution of undesirable traits directly or accidentally can occur rapidly in response to selection pressure.

There are also several genetic phenomena that could lead to the deterioration of a strain. Sex determination is not always simple and straightforward, for there are mechanisms, occurring at low frequencies, that can affect sex. Baker and Sakai (1976) recently reported a sex-determining locus on an autosome in *Cx. tritaeniorhynchus*. Similar loci could cause trouble in a genetic sexing system, especially if they were temperature dependent such as the *intersex* trait in *Aedes aegypti* (L.) (Craig and Hickey 1967). Sex-determining alleles that are only expressed when opposite a "sensitive" allele may also be a problem. There are a number of possibilities or combinations of infrequent mechanisms that could be of great importance in a sexing system. From work with *Drosophila* spp., we know that nondisjunction results in the production of unusual types, e.g., XO males that are usually sterile. For mosquitoes, almost nothing is known about dosage compensation and the sex of mosquitoes of unusual sex chromosome complements.

Depending on the type and size of an aberration, some of the aneuploid crossover types could be viable despite small duplications and deficiencies. The use of an inversion to stabilize a strain by controlling recombination may not be completely reliable if some of the aneuploid types are viable. Again, emphasis should be placed on the selection that is inherent in a huge colony under uniform conditions.

In view of the many unknown phenomena that can cause problems, a good quality control system is required to monitor a genetic sexing system. The components (conditional lethal and chromosome aberrations) used in the synthesis of a strain will dictate the nature and selection of quality control procedures.

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