X-RAY INDUCED CHROMOSOMAL ABERRATIONS IN *ANOPHELES ALBIMANUS*

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ABSTRACT. Males of *Anopheles albimanus* were exposed to X-rays (2968 R or 5088 R), and chromosome aberrations in their progeny were identified cytologically. A total of 1121 treated sperm were assayed and 277 aberrations were observed as follows: 148 translocations, 67 pericentric inversions, and 62 paracentric inversions. No attempt was made to record deletions, duplications or other more minor changes in chromosome structure. More Y-linked translocations were obtained from males that were irradiated when they were 24–48 hr old than from males that were 0–24 hr old.

INTRODUCTION

Theoretically, compound chromosomes (Foster et al. 1972) and homozygous translocations (Serebrovsky 1940, Curtis 1968a) are two types of chromosomal aberrations that are promising for the construction of genetic control systems. The principal desirable attribute of these aberrations lies in the instability of a mixed population composed of a normal type and the aberration type. Matings between compound and normal types are usually sterile, and a translocation heterozygote produces 40–60% unbalanced gametes. Therefore, it should be possible to replace a natural population with normal chromosomes with a type bearing an aberration (Curtis 1968b). In effect, the aberration can be used to manipulate genetically a natural population and thereby change the noxious nature of a pest species.

Over the past few years, we have been conducting genetic studies on *Anopheles albimanus* Wiedemann for the purpose of eventually assembling a genetic control system for this important vector of human malaria. Previously, we reported the use of radiation for inducing numerous translocations and inversions, and also the assembly of 4 whole-arm translocations into "capture" strains for use in a cross scheme to make and detect compound chromosomes (Kaiser et al. 1982). Four (of 8 which were tested) of the whole-arm translocations were fully viable when homozygous (Kaiser et al. 1983). In the previous work, we isolated only one autosomal translocation with breakpoints close to the centromeres on the left arms, i.e. *T(2L;3L)* type, of both autosomes. None of the whole-arm translocations which were viable when homozygous were of the *T(2R;3L)* or *T(2L;3L)* types. Our primary goal is the synthesis of compound chromosomes by using a cross scheme similar to the one used for *Drosophila* (Holm 1976), and in this work we thought it would be advisable to test as many combinations of translocations in "capture" strains as possible. Therefore, the research described in this present paper was conducted specifically to induce translocations of *T(2L;3L)* and *T(2R;3L)* types and to evaluate whether any of these translocations were viable when homozygous. We also compared in a qualitative manner the relative efficiency of inducing translocations in the sperm of males of different ages and with two different doses of X-rays.

MATERIALS AND METHODS

Two stocks were used: WILD, is homozygous for the dominant marker *stripe* (st^+) the locus of which is located near the centromere on the right arm of chromosome 3; the other stock is homozygous for *white thorax* (st^w). The routine maintenance of these stocks followed established procedures (Kaiser et al. 1981).

Three different groups of WILD males were treated with X-rays. Males, which were either 0–24 hr (Group A) or 24–48 hr (Group B) old, were irradiated with a dose of 5088 R (90 KVP at 424 R/min). The other group (C) was 24–48 hr old and was treated with a dose of 2968 R. The irradiated males were crossed with st^w females. The F_1 adults were backcrossed to st^w, and the females were isolated in vials for oviposition. The fertility of each egg batch was observed, and batches with > 30% sterility, which indicated the presence of chromosomal damage, were retained and reared as families. Polytene chromosomes from salivary glands of 4th stage st^+ larvae were examined using a standard technique (Kaiser et al. 1982) and standard map (Keppler et al. 1973), to detect and categorize new aberrations. If a whole-arm reciprocal translocation was identified, st^+ individuals from that family were saved and crossed to st^w to found a new stock. No effort was made to record minor chromosome damage, e.g. deletions.

Autosomal translocations with breakpoints close to the centromeres were tested to determine their viability when homozygous. The translocated chromosomes were marked at the loci for *stripe* (st^+) and *ebony (eb)*, a semi-dominant trait on the left arm of chromosome 2

(Benedict et al. 1979), and these two markers were used in a scheme to assess the homoviability of the translocations. Each translocation was marked with st^+ and eb^+ (the wild type allele of *eb*), and we crossed the translocation heterozygotes to a stock marked with *st* (recessive allele of st^+) and *eb*. The fertility of each F_1 egg batch was measured, and the family was reared if the fertility corresponded to that expected of a translocation heterozygote. Salivary gland chromosomes from larvae with an st^+ *eb* phenotype were examined to verify the presence of the translocation in a family. For each translocation, mosquitoes which were heterozygous for the translocation and of the presumed genotype, st^+/st eb^+/eb were crossed, and the viability of the homozygote was based on the frequency of the st^+ eb^+ phenotype and a cytological examination of salivary gland chromosomes.

RESULTS

A total of 1121 irradiated sperm were assayed for translocations and inversions. The frequencies of F_1 progeny that had reduced fertility are shown in Table 1. For the three groups of irradiated males, more genetic damage was caused by the irradiation of Group B males with 5 KR. The percentages of F_2 egg batches with reduced fertility from Group A and Group B males irradiated with 5 KR were 21.1 and 34.2, respectively. In comparison, 11.2% of the F_2 egg batches from Group C, irradiated with 3 KR, had reduced fertility.

Table 1. Summary of genetic damage in F_1 progeny from irradiated males of *An. albimanus* (Group A—0–24 hr old, dose = 5088R; Group B—24–48 hr old, dose = 5088R; Group C—24–48 hr old, dose = 2968R).

	F_1 progeny tested		F_1 progeny with < 70% fertility	
Group	Percent egg hatch (N♀ × T♂)[a]	No.	No.	Percentage
A	12.9	336	71	21.1
B	23.0	527	180	34.2
C	23.7	258	29	11.2

[a] Samples of 200 eggs; N = normal, T = treated with radiation.

The salivary gland chromosomes of fourth-stage larvae of 280 families were examined for the presence of translocations and inversions, and 277 of these chromosomal aberrations (Table 2) were observed as follows: 148 reciprocal translocations (103 autosomal and 45 sex-linked); 67 pericentric inversions; 62 paracentric inversions. More than one aberration was observed in 50 F_2 families, and no aberration at all was recorded for 58 families. As shown in Table 2, there was a marked difference in the number of Y-linked translocations which were detected in groups A and B. Although the same dose of radiation was used on the parental groups, only one Y-linked translocation was recorded amongst the progeny of the younger males (Group A). At first glance, there also appears to be differences between the groups for the total number of aberrations, but actually more aberrations per F_2 family were recorded for groups A and C than for group B.

Table 2. Summary of chromosomal aberrations that were isolated from irradiated males of *An. albimanus*.

Type of aberration	Chromosomes involved	No. aberrations for indicated group of irradiated males		
		A	B	C
Autosomal translocation	2R/3R	8	16	5
	2R/3L	11	20	2
	2L/3R	2	15	2
	2L/3L	6	14	2
Male-linked translocation	Y/2R	1	8	0
	Y/2L	0	2	0
	Y/3R	0	3	0
	Y/3L	0	3	0
X-linked translocation	X/2R	2	7	1
	X/2L	2	1	1
	X/3R	1	6	2
	X/3L	2	3	0
Pericentric inversion	2	4	27	5
	3	3	20	8
Paracentric inversion	2R	7	6	1
	2L	3	8	3
	3R	2	15	0
	3L	3	10	2
	X	0	2	0

Six whole-arm heterozygous translocations were established in stocks (Table 3) and were tested for their viability when homozygous. None of the translocations were homoviable. Homozygotes of *T(2L;3R)5* were observed on the basis of the presence of the proper mutant phenotype (employed in the cross-scheme) and confirmation by examination of the polytene chromosomes. The homozygous larvae had an exceptionally slow development time, and they died during the pupal stage. For the other translocations, no homozygotes were detected.

DISCUSSION

The research described in this present paper is the second such experiment that we have

conducted for making whole-arm translocations in *An. albimanus*. In the previous work (Kaiser et al. 1982), 1669 sperm were tested and 175 chromosomal aberrations (10.5%) were recorded, and four (out of eight tested) homozygous translocations were established. As shown in Table 2, we were more successful in this later work, in that 277 aberrations were detected from 1121 irradiated sperm; however, none of the whole-arm translocations were viable when homozygous. Aberrations were recorded for 24.7% of the irradiated sperm in the second trial compared to 10.5% in the earlier work. Since the radiation dose and the experimental procedures were virtually the same, it is impossible to explain the seemingly significant difference. Comparisons of this type are dubious, at best, and it is with some skepticism that we view the large difference in the number of male-linked translocations obtained from Groups A and B males. There could be a real difference, which is related to the age of the male, but a replicated experiment is in order before any such conclusion or inference can be made.

On the basis of our prior experience, it was a surprise that none of the whole-arm translocations, reported here, were viable as homozygotes. There is one notable aspect that is different between our prior and present work. We reported previously that three out of four of the homozygous translocations were first paired in double heterozygous stocks which were assembled for the purpose of "capture" strains for compound chromosomes. Recently Asman et al. (1982) enhanced the vigor of homozygous translocation stocks of *Culex tarsalis* Coq. by combining weak translocations with similar breakpoints. In their "pseudohomozygotes" it is reasonable to expect that genetic recombination would rearrange the chromosomes so that genetic damage would be reduced. Our work would tend to substantiate this idea, but as noted above, it is very difficult to compare data from different experiments.

In Table 3, the breakpoints are shown for the whole-arm translocations, all of which are not "perfect" whole-arm translocations. The breakpoints are close to the centromeres, and the distance between the chromosomal breaks and the centromeres, as seen in the polytene cells of the salivary glands, are expressed as a percentage of the total genome of *An. albimanus*. If these translocations were used in "capture" strains for "true" compound chromosomes, these percentages would correspond to the size of duplications and/or deletions that would be present in a zygote composed of a compound chromosome and an anueploid translocation. The size of most of these potential duplications and/or deletions, especially the latter, make their usefulness somewhat dubious in a scheme to isolate compound chromosomes.

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Table 3. List of translocations in *An. albimanus* which have breakpoints close to the centromeres.

	Breakpoints					
Translocation	Chromosome 2 (region)	Distance from centromere (μm)	Percentage of[a] polytene genome	Chromosome (region)	Distance from centromere (μm)	Percentage of polytene genome[a]
T(2R;3R)9	14E	17.4	3.54	33A	30.4	6.18
T(2R;3L)6	15A	12.0	2.43	38C	21.7	4.41
T(2L;3R)5	18A	23.9	4.86	35B	4.3	0.87
T(2L;3L)3	16B	4.3	0.87	37B	13.0	2.64
T(2L;3L)4	16A	7.8	1.58	37A	17.8	3.62
T(2L;3L)5	17B	11.3	2.29	38A	31.3	6.36

[a] Calculation was performed on the basis of a standard map (Keppler et al. 1973).

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A SURVEY FOR NATURAL POTENTIAL VECTORS OF *DIROFILARIA IMMITIS* IN VERO BEACH, FLORIDA[1]

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ABSTRACT. A survey to identify natural potential vectors of canine heartworm in the enzootic area of Vero Beach, Florida, revealed three species of wild-caught mosquitoes harboring presumed *Dirofilaria immitis* larvae. Two of these, *Aedes taeniorhynchus* and *Culex nigripalpus*, have not been previously described as vectors of this parasite, while the third, *Culex quinquefasciatus*, has been implicated in other studies.

INTRODUCTION

More than 30 years ago, Otto (1949) expressed his conviction that brackish water mosquitoes occurring along the eastern seaboard were important vectors of canine heartworm. Otto was aware of the heavy mortality suffered by one such species, *Aedes sollicitans* (Walker), when infected with *Dirofilaria immitis* (Leidy), the etiologic agent; his survey records nevertheless strongly suggested an association between salt marsh species and the geographic distribution of the disease. Nayar and Sauerman (1975) confirmed the occurrence of high mortality in infected *Ae. sollicitans* but at the same time demonstrated high experimental vector potential in another salt marsh species, *Ae. taeniorhynchus* (Wiedemann), which was capable of surviving and refeeding while heavily infected. It was the purpose of the survey reported herein to determine the natural potential vectors of canine heartworm in the enzootic region of Vero Beach, Florida, where substantial *Ae. taeniorhynchus* populations occur in association with an abundance of fresh water mosquito species.

MATERIALS AND METHODS

The basic strategy of epidemiological surveys to determine the natural potential vectors of *D. immitis* is to collect infected, wild mosquitoes from suspect areas. In this study, 3 residential yard sites in Vero Beach, Florida, were selected for the collection of indigenous mosquitoes. Site 1, in northeast Vero Beach, was most proximal (1.5 km) of the 3 sites to the Indian River salt marsh, while Site 2 was near the center of the city, about 2 km further west of the Indian River. Site 3 was southwest of the city, about 4 km from the river and considerably closer to the rural agricultural (citrus grove and cattle ranches) region west of the city. Each site was within a residential subdivision.

Mosquito populations were sampled using one CDC-type light trap and one lard-can, chick-baited trap (each with dry-ice adjuvant) suspended by wire from available vegetation, not more than a meter from the ground. Traps were set out from 1700 hr to 0800 hr twice weekly from April to August and yielded 210 samples (35 collections × 3 sites × 2 traps/site). In both traps, captured mosquitoes had access to 10% sucrose solution.

At the laboratory, dissections were initiated immediately after anesthetizing and sorting to species. Midguts lacking blood or blood-meal remnants were discarded, while a midgut with a blood meal was examined at 100X for the presence of microfilariae, which were removed via drawn capillary pipette to a droplet of 2% formalin and measured. Malpighian tubules lacking obvious pathology (swollen or cleared areas with reticular discontinuities) were subjected to a mild coverslip compression to reveal the presence of prelarvae or early sausage stages, both

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