MATING COMPETITIVENESS OF CHEMOSTERILIZED MALES OF A GENETIC SEXING STRAIN OF *ANOPHELES ALBIMANUS* IN LABORATORY AND FIELD TESTS[1, 2]

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ABSTRACT. Field tests were conducted to assay the mating competitiveness of the genetic sexing strain (MACHO) developed for *Anopheles albimanus* Wiedemann. The Amayo River, which is located in the coastal plain of El Salvador, was used as the release area. Chemosterilized MACHO males, heterozygous for a Y:autosome translocation and an inversion, were released along with males and females of a newly colonized wild-type strain. The competitiveness (c) of the MACHO males averaged 0.785 for the field releases, which indicates these males are capable of inducing high levels of sterility in indigenous field populations. Also, when MACHO males (sterile and nonsterile) were compared with wild-type males and colony males in laboratory cages containing wild-type females, they were competitive. Neither the sterilization process nor the chromosomal aberrations inherent in the sexing strain impeded the mating vigor of the MACHO males.

INTRODUCTION

Lofgren et al. (1974) obtained suppression of a natural population of *Anopheles albimanus* Wiedemann, the primary vector of malaria in Central America, by using inundative releases of chemosterilized males at Lake Apastepeque, El Salvador. This experiment stimulated the initiation of a larger sterile-male release covering ca. 130 km^2 of the coastal plain in El Salvador, which necessitated improvements in methodology for mass production procedures (Dame et al. 1978, Bailey et al. 1978, Bailey et al. 1979b). One of the more significant developments was the synthesis of a genetic sexing system for *An albimanus* described by Seawright et al. (1978). The development and advantages of the sexing system were outlined by Kaiser et al. (1978).

The genetic sexing system, In(2R)-[T(Y;2R)3]3 (designated MACHO), was synthesized by using gamma radiation to link the *propoxur resistance* (*pr*^{r}) allele to the Y chromosome via a reciprocal translocation. Then, the strain was re-irradiated to induce an inversion which would suppress genetic recombinants. The resulting MACHO strain, which consists of resistant males and susceptible females, can be treated with propoxur during any of the 4 life-stages, thereby allowing the preferential elimination of the females. Utilization of MACHO has enhanced the mass production of *An. albimanus* in El Salvador by quadrupling the number of males reared and sterilized for release on the coastal plain (Bailey et al. 1979a).

The success or failure of a sterile male technique program, which utilizes a postmating genetic mechanism, depends on the ability of the release male to mate competitively in the field. There has been some skepticism concerning the release of MACHO males because they are heterozygous for a translocation and an inversion, are chemosterilized, and are adapted to laboratory maintenance. Therefore, competitive mating tests were conducted in the field in El Salvador to ascertain the sexual vigor of males of the MACHO strain. Similar competitive

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mating tests were also conducted in laboratory cages.

MATERIALS AND METHODS

STRAINS. Three strains of *An. albimanus* were used in the competitive mating studies: (1) SANTA TECLA—a wild-type strain started from females collected in District 13 of the coastal plain of El Salvador in 1975; (2) MACHO—a strain that is heterozygous for a Y-autosome translocation and an inversion. The inversion covers part of the Y chromosome, which in *An. albimanus* does not lend itself to cytological examinations. Therefore, it is not known whether the inversion is pericentric or paracentric. The MACHO strain has been maintained by inbreeding with SANTA TECLA females since February 1977 and should be quite similar to that strain genetically with the exception of the portion of the right arm of chromosome 2 included in the inversion. MACHO males used in competitive mating tests originated from eggs incubated in a 0.01% aqueous solution of propoxur for 24 hr. This treatment is used to eliminate the *propoxur susceptible* (pr^s/pr^s) females. (3) CAMPO—a new wild-type strain started from females collected on the coastal plain of El Salvador in mid-July 1978. This colony was inbred; however, in an effort to maintain a vigorous, competitive organism for testing, new field-collected stock was added to the colony daily through the conclusion of testing.

COLONY MAINTENANCE. Larvae from the 3 strains were reared in a manner similar to that described by Bailey et al. (1979a). Pupae were separated by the ice water technique as modified by Hazard (1967). One ml samples of pupae from each strain were taken daily to determine the number of pupae per ml and the sex ratio. Pupae were placed in colony cages (61 x 61 x 61 cm); adults were maintained on 10% sugar water and females were blood-fed defibrinated blood through animal membranes. The procedures followed in maintaining adults were previously outlined by Bailey et al. (1979a).

STERILIZATION. Effective chemosterilization of *An. albimanus* pupae by bisazir (*P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide; AI3-61585) has been demonstrated by Lofgren et al. (1973) and Seawright et al. (1973). Pupae were sterilized by placing them in an aqueous solution of 1% bisazir for 1 hr. Then they were transferred to a rinse bath for ½ hr, measured volumetrically, and packaged for transport to the field (Bailey et al. 1979b).

FIELD TESTS. The site of the field releases was the Amayo River, which flows down from the mountains in the north through the coastal plain into the San Diego estuary. During the period between the wet and dry seasons the river provides breeding sites that are typically used by *An. albimanus* (unpublished data) and similar to those described by Breeland (1974). The Amayo River was completely dry during the dry season, but to coincide with the rains which started in June, sterile MACHO males were released along the river for 3 months prior to the competitive mating tests; these releases were stopped in August 2 weeks before the competitive mating releases. The release site was chosen primarily because it was a representative breeding site with a very low indigenous population of *An. albimanus,* and secondly, because there was a general lack of people and domestic animals in the area.

The field releases consisted of 2 replicates, both at a ratio of ca. 5 sterile MACHO ♂:1 CAMPO ♂:1 CAMPO♀. (The 5:1 ratio of sterile to non-sterile males was used in an effort to prevent the establishment of a large population of *An. albimanus* in the area.) Our goal was to release 10,000 females per replicate. To avoid using a mechanical method of sexing the CAMPO strain, a 1 ml volumetric sample of pupae was counted and the sex ratio determined by examination of the pupal terminalia. Then, the desired number of females was calculated and measured for release. The number of

CAMPO males (estimated from the sex ratio) included with the females was multiplied by 5 to give the number of sterile MACHO pupae necessary for an approximate 5:1 ratio. The final ratio was determined by adjusting the release numbers for pupal mortality.

Pupae were packaged for transport to the release site in 500 ml plastic cups (1500/cup) containing 2 ml H_20 each. The cups were placed in styrofoam boxes containing several cups of ice and carried to the release site where ca. 125 ml of H_20 was added.

The test site included: (1) Metal racks to provide shelter for cups containing pupae. These were placed on trees near the edge of the river. (2) Tack-Trap® that was placed on the trees around the racks to exclude predators. (3) A calf trap was placed on each side of the release site approximately 125 m up- and downstream, and a calf was placed in each at 1800 hours and removed the following morning by the collection team.

Daily collections were made in each calf trap and the females returned to the laboratory. Efforts were made to exclude males from capture, and the daily collections were screened for males in a cold room (3.3°C) when they were returned to the laboratory. Females were held in cages in the laboratory for 2 days and then immobilized in a cold room and placed individually in 32 ml plastic vials; several ml of H_20 were added later. The following day the vials were observed for oviposition and females failing to oviposit were transferred to a cage and blood-fed. The subsequent handling of these reblooded females was the same as that previously described for newly captured females. The incubation time for *An. albimanus* eggs is ca. 48 hr, however, the eggs were held for 5 days before they were checked for sterility to ensure an adequate opportunity for all eggs to hatch. An egg batch with 5% hatch or less was counted sterile, and egg batches containing less than 15 eggs were discarded.

Also, 100 of the females that had oviposited in vials were placed together in a cage and blood-fed again. Their eggs were collected in mass, held for 5 days, and assayed for sterility. The observed sterility was then compared with that observed for females in vials.

CONTROLS (field). The following quality control measures were established: (1) Daily collections from the 2 calf-traps were begun the week prior to the initial release and continued for 1 week following the final collection of the second release. The spermathecae of the collected females were observed for sperm. The average number of mated females collected per day for the week preceding the first release was used as a correction factor in determining sterility for that release. Similarly, the data gathered during the final week were used for the last release. (2) The cups that were used in the releases were returned to the laboratory after the eclosion of the adults and mortality was counted. This observed mortality was used to determine the actual numbers released. (3) One hundred CAMPO females and 100 sterile MACHO males were placed in a colony cage for mating. Since the same sample of sterilant was used for all MACHO males, the 100 control males were taken from the final treatment day. The females were assayed for sterility as previously described. (4) Spermathecae were removed from 20% of the ovipositing females that were captured in the calf-traps. They were assayed for sperm since uninseminated females often deposit normal complements of eggs that do not hatch but cannot be distinguished from those resulting from sterile matings. This precluded the necessity of maintaining a CAMPO ♀ X CAMPO ♂ control cage. (5) The CAMPO colony cage was assayed for natural sterility and this value was used to adjust the observed sterility of the females that were mass egged.

CAGE TESTS. The following cage competitive mating tests and respective controls were set up in the laboratory: (1) 200 sterile MACHO ♂:200 CAMPO ♂:200 CAMPO ♀; controls were 100 sterile MACHO ♂:100 CAMPO ♀ and 100

CAMPO ♂:100 CAMPO ♀. (2) 200 MACHO ♂:200 sterile CAMPO ♂:200 CAMPO ♀; controls were 100 MACHO ♂:100 CAMPO ♀ and 100 sterile CAMPO ♂:100 CAMPO ♀. (3) 200 sterile MACHO ♂:200 SANTA TECLA ♂:200 CAMPO ♀; controls were 100 sterile MACHO ♂:100 CAMPO ♀ and 100 SANTA TECLA ♂:100 CAMPO ♀. There were 2 replicates of each competitive mating test.

Pupae were placed in cages and the resulting adults were sexed in the cold room when <12 hr old and the males were counted and placed in holding cages. Female pupae were placed individually in plastic vials for emergence to ensure virginity; after emergence they were placed in holding cages. Adults were maintained on sugar water for 48 hr. Subsequently, the males were immobilized in the cold room, counted, and transferred to an adult colony cage (61 × 61 × 61 cm). Females were treated similarly and added to the cage 2 hr later. Females were blood-fed on the 2 days following initiation of the test, and on the third day placed individually in 32 ml vials. On day 4 the vials were checked for oviposition, and non-ovipositing females were removed, reblooded, and revialed. Eggs were allowed 5 days to hatch.

RESULTS AND DISCUSSION

Table 1 shows the number of each type of adult released per day for the 2 replicates. The first replicate consisted of 4 release days and the second consisted of 2 release days. Six days of calf trap collections were analyzed for the first replicate and 4 days were analyzed for the second replicate. The actual numbers captured are listed in Table 1. As expected, females released day 1 were not captured until day 3 since the majority of pupae emerged within 24 hr and were too young to migrate in search of a blood meal that first night. Migration started the second night which resulted in our collections for

Table 1. Numbers of MACHO (S) and CAMPO males and CAMPO females released during competitive mating tests. The recaptured females were assayed for fertility.

	Replicate 1		Replicate 2	
Day	No. released[a]	No. ♀ collected	No. released	No. ♀ collected
Prerelease		7±1.22		
1	16,946 S ♂		29,318 S ♂	
	3,274 ♂		5,681 ♂	
	3,546 ♀		4,312 ♀	
2	7,736 S ♂		22,322 S ♂	
	1,242 ♂		3,631 ♂	
	1,946 ♀		3,454 ♀	
3	7,389 S ♂	167		163
	1,406 ♂			
	1,353 ♀			
4	4,304 S ♂	357		456
	772 ♂			
	875 ♀			
5		144		204
6		120		26
7		93		
8		63		
Total	36,375 S ♂		51,630 S ♂	
	7,720 ♂		9,312 ♂	
	6,694 ♀	944	7,766 ♀	849
Postrelease				1.5±0.37

[a] Estimated numbers after mortality.

day 3. It is interesting to note that for both releases there were essentially no release females in our capture zone 5 days following the final release.

The results of the field competitive mating tests using sterile MACHO males, CAMPO males and CAMPO females are given in Table 2. (The numbers for the test population shown in the table have been corrected for mortality.) CAMPO emergence for the first and second replicates was 66.9% and 77.7%, respectively, which is unusually low. However, the CAMPO strain was not well adapted to routine colony maintenance so there are numerous factors such as nutrition and shock from cold water treatment that may have adversely affected emergence and subsequent competitiveness.

The number of sterile or fertile females also required adjustments. Since ovipositing females without sperm would be classified as sterile without some sort of correction, the figures were adjusted by using the insemination rates observed in the samples of field-collected females. The percentages of unmated, ovipositing females for the first and second replicates were 2 and 13.6, respectively. Also, the number of fertile females was adjusted downward in accordance with field collections from the 2 calf traps for the week preceding the first release and the week following the second release. The mean number of mated females per trap-night prior to the first replicate was 7 ± 1.22 and the mean for the week following the second replicate was 1.5 ± 0.37.

The competitiveness factors (c) calculated by using the formula of Fried (1971) were 0.76 and 0.82, respectively, for the 2 replicates and 0.785 for the combined data. These findings support the rejection of the null hypothesis that the competitiveness of the 2 male types is equal. (The calculated total $\chi^2 = 8.81$; $P<0.05$.)

The calf traps proved to be a useful tool for sampling the artificial population. The numbers listed in Table 2 represent only ovipositing females, and the actual numbers captured for the 2 replicates (Table 1) were 944 and 849, respectively,

Table 2. Results of field competitive mating tests using sterile MACHO males, CAMPO males and CAMPO females.

Replicate	Test population[a]				Mating of[b] field-collected ♀		% Sterility of field-collected ♀		
	Sterile MACHO ♂	CAMPO ♂	CAMPO ♀	Male ratio	Sterile	Fertile	Observed	Expected	Competitiveness (c)
1	36,375	7,720	6,694	5.43:1	447	108	80.54	84.45	0.76
2	51,630	9,312	7,766	5.54:1	295.5	65	81.97	84.7	0.82

[a] Corrected for mortality.
[b] Corrected for ovipositing females without sperm and fertile indigenous population.

which is 12% of the females released in the first replicate and 11% in the second replicate.

Sterility of ovipositing females that were reblooded and egged in mass was 87.1% (observed) vs. 83.5 (expected) for the first replicate and 87.4 (observed) vs. 84.7 (expected) for the second replicate. Natural sterility in the CAMPO cage was used to compute expected hatch. The difference in sterility between egging in mass and collecting eggs from individual females in vials was significant; collecting eggs in mass, though much simpler, does not appear to be an accurate method of determining male competitiveness in *An. albimanus*.

Several previous reports are available showing the degree of mating competitiveness of genetically-altered, male mosquitoes under natural conditions. Grover et al. (1976a) noted that chemosterilized males of *Culex pipiens fatigans* (=*quinquefasciatus* Say) were 20–26% competitive compared with native males in experiments conducted in India. In other release experiments, Grover et al. (1976b) reported that chemosterilized male *Aedes aegypti* (L.) were 68–139% competitive and that double translocation heterozygote males of that species were quite variable in their competitiveness (range of 28–155%) against native males. In tests conducted in Florida, chemosterilized males (Seawright et al. 1977) and translocation males (Seawright et al. 1975, Seawright et al. 1976) of *Ae. aegypti* were about equally competitive under field conditions. However, the experiments in Florida were conducted using known ratios of laboratory-reared mosquitoes in an isolated location as compared to the totally natural situation in India, where the genetically-altered males were competing against native males.

The only data available concerning the mating competitiveness of genetically-altered male *An. albimanus* are from cage tests; Rabbani and Kitzmiller (1975) reported that translocation males were fully competitive in the laboratory.

The sterilized MACHO male is capable of inducing high levels of sterility in a wild-type population under natural conditions when the emerging MACHO males are in close proximity to the wild population. However, biological data such as breeding site location and mating behavior, which presumably are necessary for the successful application of the sterile male technique, have been lacking for the coastal populations of *An. albimanus*. Breeland (1974) pointed out that breeding habits change drastically throughout the year, so it is likely that mating also occurs in different locations corresponding to the changes in breeding sites. Therefore, a successful sterile male release would necessitate widespread distributions of the release males, and subsequent dispersal to the mating locations. Obviously, our releases did not measure the dispersal abilities of the MACHO male. However, the level of competitiveness, which agrees favorably with that of males used in other successful sterile male release projects of other researchers, notably, Weidhaas et al. (1974), Lowe et al. (1974) and Grover et al. (1976a, 1976b), clearly demonstrates the ability of the sterile MACHO male to inseminate wild females in the field.

The results of the indoor cage competitive mating tests are shown in Table 3. It is not surprising to note that the CAMPO male performed poorly indoors in a cage. Our goal was to maintain a vigorous wild-type strain for field testing, so we did not expect the CAMPO strain to compete well in the laboratory. Sterile males showed no adverse effect of sterilization and though the comparison is indirect, slightly outperformed their fertile counterparts: S MACHO (c = 1.36), MACHO (c = 1.16); S CAMPO (c = .84), CAMPO (c = .64).

The sterile MACHO and SANTA TECLA males appear equally competitive. This is significant because MACHO males have been inbred with SANTA TECLA females for over a year so their genomes are quite similar. The exception of course is that the MACHO male is heterozygous for a translocation and an

Table 3. Results of laboratory cage competitive mating tests using MACHO, SANTA TECLA, and CAMPO males and CAMPO females.

Populations	Test population ♂	♀	Female matings Sterile	Fertile	% Female sterility Observed	Expected	Competitiveness (c)
1a	S MACHO	CAMPO	50	35	58.8	50	1.43
1b	S MACHO	CAMPO	61	47	56.5	50	1.30
1c	S MACHO	CAMPO	46	1	98.0	100	
1d	CAMPO	CAMPO	1	56	2.0	0	
2a	S CAMPO	MACHO	60	71	45.8	50	0.85
2b	S CAMPO	MACHO	62	74	45.6	50	0.84
2c	S CAMPO	CAMPO	53	2	96.0	100	
2d	MACHO	CAMPO	2	55	4.0	0	
3a	S MACHO	SANTA TECLA	62	55	53.0	50	1.13
3b	S MACHO	SANTA TECLA	57	55	50.8	50	1.04
3c	S MACHO	CAMPO	46	1	98.0	100	
3d	SANTA TECLA	CAMPO	1	65	2.0	0	

inversion. The results of the test indicate that the chromosomal aberrations of the MACHO male do not inhibit its mating competitiveness under laboratory conditions.

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BOOK REVIEW

The Sand Flies (*Culicoides*) of Florida (Diptera: Ceratopogonidae), 1979. F. S. Blanton and W. W. Wirth. (Vol. 10 in the series Arthropods of Florida and Neighboring Areas). Florida Department of Consumer Services. 204 pp.

This substantial monograph is the latest in an extraordinary flow of important taxonomic contributions that collaboration between these authors has produced over the years. The publication has been very well prepared by the publisher.

There is an extensive introductory section preceding the formal descriptions of species. In addition to sections on historical, economic importance, disease transmission and control aspects, the biology section includes information under 8 subheadings. Colonization is discussed as well as methods of collection and morphology. Particularly interesting is a brief but illustrated account of the geography and life zones of Florida. Each section is in the form of a review, with important references contributing to a total of about 350 in the entire monograph.

There are 4 keys: to females, male genitalia, known pupae and known (mature) larvae. Also, there are 4 very useful diagnostic tables for quick identification, for the same stages. A total of 45 species is described with illustrated distributional records both in detail within Florida, and also for the U.S. and adjacent territories through the central Americas and Caribbean.

This is an important publication which will be a valuable acquisition for concerned workers in the eastern U.S. and Caribbean regions. The standard of publiction has, fortunately, done justice to the content.

—J. R. Linley