MATING COMPETITIVENESS OF CHEMOSTERILIZED HYBRID MALES OF *AEDES AEGYPTI* (L.) IN OUTDOOR CAGE STUDIES

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ABSTRACT. An investigation was conducted to study chemosterilized males of *Aedes aegypti* L. in outdoor cages. The objectives in this work were two-fold: (1) to determine the effects of the sterilants on mating competitiveness and (2) to determine whether heterosis could improve the performance of sterile males. The results clearly indicated that the chemosterilized males were equal to normal males in mating competitiveness, and heterosis improved the mating competitiveness of sterile males.

The success of the sterile male technique for insect control relies heavily on the production of vigorous sterile males that are competitive with native males in their natural environment. However, mass rearing, handling, and sterilizing procedures can be detrimental and cause loss of competitiveness of sterile males, thus requiring the production of larger numbers of sterile males to compensate for the loss. The release of partially competitive males may be acceptable, within certain limits, but obviously the overall effect of releasing debilitated males could easily be crucial to success. Consequently, we believe it is imperative that researchers consider manipulation of the genetic composition of colonized insects to provide more vigorous individuals for release in the field. One method for improving vigor is the use of heterosis which is usually found in hybrid progeny from the cross of inbred strains but can also be observed in hybrid progeny from the cross of strains from different locales. Different strains of an organism differ in the genes at many loci, so hybrid progeny are heterozygous at more loci than either parent, even if the parents are chosen from rather large, genetically heterogeneous populations. Although heterosis is probably manifest to a lesser extent in insects from crosses of

strains that are not highly inbred than in hybrids from inbreds, there should be a measurable effect.

The present paper reports the findings of an investigation of the mating competitiveness of chemosterilized males of *Aedes aegypti* (L.). The objectives in our work were two-fold: (1) to determine the effects of chemosterilization on competitiveness of males in outdoor cages, and (2) to determine whether heterosis could improve the performance of chemosterilized males. The first part of the work was confined to the laboratory to evolve sterilization techniques, and then the outdoor cage portion was completed during the summers of 1972 and 1973.

The present paper has relevance to other types of genetic control techniques because the use of hybrids will probably be of some importance to any type of control program whose success requires the release of insects capable of competing with a field population.

MATERIALS. The strains of *A. aegypti* used were as follows:

RED—A mutant marker strain with the markers *red eye* (*re*) on chromosome 1, *spot* (*s*) on chromosome 2, and *black tarsi* (*blt*) on chromosome 3. This stock is also referred to as GVR by Lorimer et al. (1972).

OCALA—Wild type, field-collected in Ocala, Florida, in 1970.

VOYLE—Wild type, field-collected in Gainesville, Florida, in 1972.

BANGKOK—Wild type, obtained from WHO *Aedes* Research Unit, Bangkok, Thailand.

Larval rearing for laboratory studies was conducted in an incubator at 30° C. A group of 150 larvae was placed in an enamel pan containing 800 ml of distilled water infused with 0.67 mg liver powder and 0.33 mg brewer's yeast per larva; 4 days later, 2 mg per larva of pulverized ground hog supplement was added. The larval rearing for the outdoor cage tests was done outdoors in water from a subterranean well. A group of 1400 larvae was set in a tray containing 6 liters of water and fed the same food at the same rate as in the laboratory rearing.

The sterilization of male mosquitoes was accomplished by treating pupae in an aqueous solution of 0.75% tris(1-aziridinyl)phosphine sulfide (also known as thiotepa) for 3 hours, or 1% *P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide for 30 minutes. The optimum sterilizing treatments were determined by treating pupae at various concentrations and time intervals using techniques previously described (Seawright et al., 1971, 1973). Assays of sterility were based on the percentage egg hatch observed in the progeny of a cross of 50 treated males mated to 50 virgin females.

The mating competitiveness of sterile males in the outdoor cage populations was calculated according to a method developed by Fried (1971), which uses observed egg hatch and the calculation of an expected egg hatch based on the fertilities and frequencies of the various types of insects in a population. Our laboratory data, which excluded sterile males, were analyzed in a similar manner, except that the recovery of mutant markers was scored instead of egg hatch.

Competitiveness was estimated by:

$$c = \frac{fw}{fm} = \frac{a}{1-a}$$

where: fw = frequency of wild males
fm = frequency of mutant males
a = observed frequency of wild progeny from mixed population.

For the tests involving the hybrids, it was necessary to modify the formula slightly, because a hybrid (for 2 mutant genes) produces 0.75 wild gametes as follows:

$$c = \frac{fw}{fm} = \frac{a}{0.75-a}$$

These formulas contain no correction term for fitness differences, since no discernible differences were observed.

EXPERIMENTATION AND RESULTS. To ex-

amine the idea of using hybrid vigor, tests were conducted in the laboratory during the winter months of 1972. In these tests, comparisons were made of the mating competitiveness of hybrid males (from RED x OCALA or RED x BANGKOK) or wild males (OCALA or BANGKOK) with RED males for RED females. After confirmation of the appropriate phenotype, 3-day-old adults were placed in 0.5 m^3 cages. Each population was comprised of 100 RED females, 100 RED males, and 100 of either wild or hybrid males. Competitiveness was based on recovery of the mutant markers in the progeny. Analysis (χ^2) of the data in Table 1 nation and confirmation of phenotypes, all the cage populations were initiated when the adults were 3 days old. Each population consisted of 200 individuals of each type and the following tests were executed:

1. Sterile F_1 ♂ (RED x OCALA) : RED ♂ : RED ♀
2. Sterile F_1 ♂ (RED x OCALA) : OCALA ♂ : OCALA ♀
3. Sterile OCALA ♂ : OCALA ♂ : RED ♀
4. F_1 ♂ (RED x OCALA) : RED ♂ : RED ♀

For the last population, competitive mating was assayed on the basis of progeny scoring for the mutant markers. Com-

Table 1. Results of competitive mating tests in the laboratory with OCALA (wild), BANGKOK (wild), F_1 (RED x BANGKOK), and F_1 (RED x OCALA) males against RED males for RED females.

Population type	Phenotype of progeny[a]				Competitiveness index (c)
	++	*s*+	+*blt*	*sblt*	
RED ♂ OCALA ♂ RED ♀	299 (315.5)	.....	.....	332 (315.5)	0.9
RED ♂ BANGKOK ♂ RED ♀	426 (414)	.....	.....	402 (414)	1.0
RED ♂ F_1 ♂ (RED x OCALA) RED ♀	145 (81)	148 (81)	123 (81)	230 (403)	6.1[b]
RED ♂ F_1 ♂ (RED x BANGKOK) RED ♀	127 (80)	110 (80)	111 (80)	290 (398)	2.6[b]

[a] Expected number in parentheses.
[b] Significantly different from expected (χ^2 0.05).

showed no difference between the RED and wildtype males, but both hybrid types outcompeted RED. Even though the hybrid males were more competitive in the laboratory cages, a more definitive test was needed to determine their vigor under more natural conditions and when sterile.

Competitive mating tests in outdoor cages (1.2 x 0.75 x 1.12 m) with sterile F_1 ♂ (RED x OCALA) were conducted during the summer of 1972. Thiotepa was used as the chemosterilant. After examipetitiveness of the populations that included sterile males was assessed on the basis of percentage egg hatch. Controls consisted of RED x RED, OCALA x OCALA, and similar crosses with sterile males.

The results (Table 2) of these tests clearly demonstrated that the hybrids, either sterile or fertile, were more competitive than the RED ♂. However, there was no difference between the sterile hybrid and OCALA ♂, and sterile OCALA

Table 2. Egg hatch from competitive crosses in outdoor cages using sterile or fertile hybrid (RED x OCALA) males or OCALA and RED males. (Chemosterilant treatment with thiotepa designated by T.)

Population type	Number eggs	Number eggs hatched		Competitiveness index (c)
		Observed	Expected	
T F_1 ♂ (RED x OCALA) RED ♂ RED ♀	4318	1115	1878	2.4[a]
F_1 ♂ (RED x OCALA) RED ♂ RED ♀	4703	4086	4162	4.2[a]
T F_1 ♂ (RED x OCALA) OCALA ♂ OCALA ♀	7090	3320	3403	1.0
T OCALA ♂ OCALA ♂ OCALA ♀	3025	1331	1361	1.0

[a] Significantly different from expected ($\chi^2_{0.05}$). Competitiveness of fertile F_1 ♂ (RED x OCALA) calculated from mutant marker analysis.

♂ were equally competitive with untreated OCALA ♂. On the basis of the laboratory tests (Table 1), the hybrid ♂ were expected to outcompete both RED and OCALA ♂, but apparently the behavior of the OCALA ♂ was different in the larger, outdoor cage. These results were encouraging, since the hybrid exceeded both parental types in the laboratory and was equal to OCALA and more competitive than RED in the outdoor cages. The hybrid males were derived from the cross between wild type and RED, a mutant marker stock; however, in spite of the fact that half of the genotype of the hybrid was derived from the inbred RED, it was equal to the wild type in the outdoor cage. The chemosterilant, thiotepa, had caused no obvious debilitation, so we felt the sterilant procedure was sound.

All of the observations up to this point led us to consider the possibility that hybrid males resulting from crossing two different wild-type strains might exhibit the type of vigor that would result in mating performances superior to that of either parent type. Therefore, we proceeded to test sterilized reciprocal hybrid males from crosses between VOYLE and OCALA in competition with the parental type males in large outdoor cages (14 x 14 x 10 ft) (4.3 x 3.0 m) during the summer of 1973. Outdoor cage tests were conducted with the following populations:

1. Sterile F_1 ♂ (OCALA x VOYLE) : VOYLE ♂ : VOYLE ♀
2. Sterile F_1 ♂ (VOYLE x OCALA) : OCALA ♂ : OCALA ♀
3. Sterile VOYLE ♂ : VOYLE ♂ : VOYLE ♀
4. Sterile OCALA ♂ : OCALA ♂ : OCALA ♀

Each test was replicated twice, and each population consisted of 1000 sterile males, 1000 normal males, and 1000 normal females. Control groups included the normal egg viability for each type of mosquito in the tests and a monitor of the efficacy of the chemosterilant treatment. In these tests, chemosterilization was achieved by treating male pupae for 30 minutes in an aqueous solution of 1% *P,P*-bis(1-aziridinyl)-*N*-methylphosphinothioic amide. This chemical was used because the much shorter treatment time allowed more flexibility in working schedules. In these tests, as before, the competitiveness of the sterile males was based on percentage egg hatch. In the results shown in Table 3,

Table 3. Egg hatch from competitive tests in large outdoor cages using sterile hybrid males and sterile or fertile VOYLE and OCALA males. Chemosterilant treatment with *P,P*-bis(1-aziridinyl)-N-methylphosphinothioic amide designated by T.)

Population type	Number eggs	Number eggs hatched		Competitiveness index (c)
		Observed	Expected	
T F_1 ♂ (OCALA x VOYLE) VOYLE ♂ VOYLE ♀	2287	913	1057	1.36[a]
T F_1 ♂ (VOYLE x OCALA) OCALA ♂ OCALA ♀	2112	809	997	1.47[a]
T VOYLE ♂ VOYLE ♂ VOYLE ♀	1635	765	756	1.03
T OCALA ♂ OCALA ♂ OCALA ♀	1247	581	590	0.98

[a] Significantly different from expected ($\chi^2_{0.05}$).

all the data were corrected for natural sterility. The hybrids outcompeted the parental types, thereby demonstrating the desirability of using sterile males that are genetically heterogeneous.

DISCUSSION. Heterogeneity appears to be the normal state in many insect populations that have been studied for lethal or visible mutation load. McDonald and Overland (1974) found that the incidence of recessive lethals on chromosome 3 in a field population of the house fly averaged 23.2%. Similar lethal frequencies have been studied in *Drosophila* populations (Dobzhansky et al., 1963; Dobzhansky and Spassky, 1953; Goldschmidt et al., 1955; Pavan et al., 1951). VandeHey (1964) studied genetic variability in *A. aegypti,* based on visible mutants, and found an average mutation load of 1.3% for four populations. Craig et al., (1961) observed a range of mutation loads in field-collected *A. aegypti* of 0.52 to 2.96% in African populations.

From these field studies there appears to be a necessity for heterogeneity. This is in contrast to events in most laboratory colonies used for mass rearing where a conscious effort is made to produce sterile males in an orderly, efficient system. Efforts to maximize efficiency can inadvertently change the original genetic pool through inbreeding and by upsetting the process of natural selection because the species is in an alien environment. The degree of inbreeding will depend mostly on the size of the initial population and the ease of colonization as related to factors such as mating behavior. Also, inbreeding is likely to be a problem if an effort is made to expedite time schedules by making the population conform to rigid development rates. Natural selection will occur in a laboratory colony, but the direction will favor those genotypes best suited for the laboratory. Some of the problems created by typical laboratory rearing could be overcome by the production of hybrid release insects.

Hybrid varieties have revolutionized yields in plant and animal breeding, and the results of our experiments demonstrate the feasibility of this approach in sterile male releases. The hybrids are genetically heterogeneous but are also fairly uniform in developmental rates, thus allowing the release of viable insects using a standard set of rearing conditions. In our tests, the hybrids clearly outcompeted the parent strains, and this could be significant in the success of the sterile male technique.

Past experimental work with different insects has demonstrated the need for releasing sterile males with behavior patterns similar to the wild counterpart. Fye and LaBrecque (1966) found that a strain of house flies from Grand Turk Island, Bahamas, had mating behavior characteristics that were different from those of a Florida strain which was used in a sterile-male release on Grand Turk. Dame et al. (1964) found that males from a laboratory colony of *Anopheles quadrimaculatus* Say would not mate readily with wild females. Mosquitoes are often very difficult to colonize in the laboratory because of mating behavior patterns, but creating genetic variability in laboratory colonies is possible. For example, Haeger and O'Meara (1970) observed that wild males of *Culex nigripalpus* (Theob.) would mate readily with females from a laboratory colony in cages in the laboratory, but wild females would not mate under these conditions. We have observed the same thing for *Aedes taeniorhynchus* Wiedemann, collected in Duval County, Florida.

On St. Croix, Virgin Islands, it was necessary to cross a laboratory colony of *Heliothis zea* (Boddie) from Tifton, Georgia, to the St. Croix males to produce laboratory reared, sterile males that were competitive (Young et al., 1975). Currently, the *H. zea* colony at Tifton is maintained by a series of crosses designed to promote a genetically heterogeneous population from which males are drawn for sterile male projects.

While starting colonies from wild material is not always simple, there are usually manipulations which can be used to retain characteristics that facilitate laboratory colonization on a massive scale and to perpetuate traits that make a more vigorous insect.

The data presented in the present paper are not conclusive of what would occur in a field situation. On the other hand, this work does prove that a fairly simple manipulation can be fruitful. We believe that any type of control program that depends on insect release should include an effort to release insects that are as heterogeneous as possible.

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