3

Lessons from the past: an overview of studies by the University of Maryland and the University of California, Berkeley

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Abstract

Males of *Anopheles culicifacies*, *Culex tritaeniorhynchus* and *Culex tarsalis* were sterilized using chromosomal rearrangements, chemosterilants or irradiation, marked with fluorescent dusts and released to determine their ability for disperse and compete with field males for field females and laboratory strain females. Released males were uncompetitive for field females but supercompetitive for lab-reared females with a similar genetic background; i.e. mating was assortative between field and laboratory populations. Apparently field populations were not freely interbreeding and colonization selected for only that part of the field gene pool that was able to survive and mate under cage conditions. Mating barriers could have a significant impact on the dispersal of genetically engineered characters destined to impede pathogen transmission.

Keywords: genetics, Sterile Insect Control, mosquito, review, *Anopheles culicifacies*, *Culex tritaeniorhynchus*, *Culex tarsalis*

Preface

My first two positions in medical entomology after receiving my PhD were to serve as the mosquito ecologist with teams of geneticists and arbovirologists attempting to release laboratory-crafted genetic strains of mosquitoes in the field for population suppression or replacement. My job was to investigate when, where and how many males were to be released and to devise assessment protocols. All of the research and releases essentially could be termed failures, because we did not reduce target mosquito population size and the males we released did not compete well for mates against field males. However, we learned a lot about male-mosquito biology, mosquito mating and population ecology. We now are poised to initiate the same types of research, only to find that we are faced with the same problems and that little research has been done to advance these aspects of field mosquito ecology in the past 15 years.

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Introduction

The present paper summarizes field-release experiments of mosquitoes performed during the 1970s and 1980s to understand the mating competitiveness of male mosquitoes that were altered genetically, sterilized by irradiation or chemically, or both. My purpose is to extend an earlier review of this work (Reisen 1985) and assess what was accomplished, what was learned, and what research is needed to prevent similar failures in future releases. Although there have been remarkable advances in understanding and manipulating the genetics of mosquitoes, far less effort has gone into understanding mosquito-population genetic structure and the process of mate selection.

Research summary

The Pakistan Medical Research Centre in Lahore, Pakistan, focused detailed genetic studies initially on *Culex tritaeniorhynchus* Giles, an important arbovirus vector in Asia. Elegant laboratory studies detailed the formal genetics of this species using procedures that were state of the art for the 1970s (Baker and Sakai 1974) and selection experiments focused on producing a strain refractory to West-Nile virus infection (Hayes et al. 1980; 1984). The field aspects of these studies largely failed, because little was known about the mating behavior of this mosquito, population sizes were huge, movement including immigration excessive, and colonized males were uncompetitive for field females (see Table 1). Releases focused on the mating success of males carrying several different chromosomal translocations in an attempt to use sterility as a marker to estimate mating competitiveness of released males against field males for field females (Baker et al. 1979; Reisen et al. 1980). *Cx. tritaeniorhynchus* populations were very large, vagile, dynamic and rested outdoors, thereby providing no visible target at which to focus our releases. Despite the production of large numbers of males with a target-population genetic background, the numbers released were comparatively few compared to the field population size and uncompetitive. None and one mating with a target female was detected during 1977 and 1978 experiments, respectively (see Table 1). In 1977 matings were detected using the unselected female progeny of wild-caught females that were reared in the laboratory, marked with fluorescent dust and released concurrently with the sterile males. Collectively these data indicated that most target-population matings occurred away from the sites we chose for our male releases. In contrast, released males mated competitively for their sisters ($e>2.0$), indicating that assortative mating had occurred.

The resurgence of malaria in Pakistan switched the research priorities of the Pakistan Medical Research Centre from field releases of *Cx. tritaeniorhynchus* to *Anopheles culicifacies* Giles, the primary malaria vector in Punjab. Because this species obligatorily rests indoors and feeds mostly on cattle, rural villages formed "island" populations that were semi-isolated (Reisen, Mahmood and Parveen 1980; Reisen, Mahmood and Azra 1981) and, therefore, more conducive for release experiments than were large, undelineated *Cx. tritaeniorhynchus* populations. Initial releases used males carrying a complex chromosomal rearrangement (see Table 2, Exp. 1). These males were equally competitive for field females when male ratios were based on collections from resting sites, but were not competitive if male ratios

Table 1. Summary of mating competitiveness of male *Culex tritaeniorhynchus* released near Lahore, Pakistan, carrying translocations to induce sterility for their sisters (Lab), progeny of wild caught females (WC F1) or unmarked host-seeking females (Target) (Data summarized from Baker et al. 1979; Reisen et al. 1980)

	1977 Experiment			1978 Experiment	
Males	Trans.	Trans.	Trans.	Trans.	Trans.
Released	167,291			107,759	
All males	5,584	4,427	12,999	5,446	3,079
Released	578	859	1,615	353	298
w	0.90	0.81	0.88	0.94	0.90
Females	Lab	WC F1	Target	Lab	Target
Total rafts	94	266	2,541	155	800
Sterile	18	9	0	23	1
f	0.81	0.97	1.00	0.85	1.00
Comp. (E)	2.05	0.15	0.00	2.51	0.01

Trans., males carrying translocations.
w = proportion of fertile or target males among all males
f = proportion of females mating with target males among all males
E, competitiveness = w/(1-w)x(1-f)/f (Grover et al. 1976)

were estimated from samples from swarms, the actual site of mating (Baker et al. 1980). Because releasing fertile males or females potentially could increase focal malaria transmission, emphasis was switched from partially fertile chromosomal rearrangements to chemosterilized males that had been produced using a genetic sexing system (Reisen et al. 1981b). In a second experiment (see Table 2, Exp. 2), chemosterilized males were not competitive in nature, although in quality-control laboratory trials they were equally competitive against wild type colonized males (e = 0.82).

Table 2. Summary of mating competitiveness of male *Anopheles culicifacies* released at villages near Lahore, Pakistan for females from the unmarked target population (UM) or released as pupae (P) or adults (A) with Sattoki (SAT) or Kot Baghicha Sing Walla (KB) genetic backgrounds (Data summarized from Baker et al. 1980; Reisen et al. 1981b)

	Exp. 1	Exp. 2				
Males	Trans.	GS+Chem	GS+Chem	GS+Chem	GS+Chem	GS+Chem
Sterile male	1,604	1,182	946	1,066	809	571
All males	1,172	715	551	948	799	2,972
Released males	113	97	121	159	181	313
w	0.90	0.86	0.78	0.83	0.77	0.90
Females	UM	SAT-P	SAT-A	KB-P	KB-A	UM
All matings	538	45	204	105	218	1,417
Sterile matings	68	4	14	4	33	49
f	0.87	0.91	0.93	0.96	0.85	0.97
Comp. (E)	1.34	0.62	0.26	0.20	0.61	0.31

Trans. = translocation, GS = genetic sexing system, Chem. = chemosterilized
w, f and E abbreviations follow Table 1.

A parallel genetic-control programme was initiated on *Culex tarsalis* Coquillett in California during the 1970s. This mosquito is the most important arbovirus vector in western North America, and like *Cx. tritaeniorhynchus*, is an outdoor-resting mosquito that forms large, vagile populations that exploit the extensive agro-ecosystems of the Central Valley. Initial trials were conducted in outdoor cages and focused on males carrying several translocations (McDonald et al. 1978; McDonald 1980; Terwedow et al. 1977). These males were not highly competitive and mass production facilities were limited at this time, precluding large field-release experiments. Therefore, initial control attempts used males that were collected in the field as pupae, sexed using a mechanical device, irradiated, and released into a semi-isolated foothill population (see Table 3, Exp. 1). Encouragingly, irradiated males were equally competitive with field males for field females; however, the growth rate of this semi-isolated population exceeded our ability to collect field mosquitoes, and control was not achieved (Reisen et al. 1981a). The following winter a mass-production insectary was constructed and a new colony from the target population was amplified for 3 generations in a circular mating scheme to conserve variability. The following spring inundative releases with irradiated males failed to suppress the vernal rise in the field population or produce >10% sterility (see Figure 1). Failure here again was attributed to assortative mating (Reisen et al. 1982); that is, released laboratory males were not competitive for field females, but were supercompetitive for sibling females (see Table 3, Exp. 2).

Table 3. Summary of mating competitiveness of male *Culex tarsalis* released into semi-isolated foothill populations near Bakersfield, California for females from the unmarked target population (UM) or released females with a field or laboratory genetic background (Data summarized from Reisen et al. 1981a; 1982)

	Experiment 1		Experiment 2				
Environment	Cage	Field	Cage	Cage	Field	Field	Field
Males	Irrad.	Field Irrad.	Field Irrad. Lab	Irrad. Lab	Irrad. Lab	Irrad. Lab	Irrad. Lab
Sterile male		71,000			85,000		
All males	2,000	1,132	1,600	1,600	862	256	256
Irrad. males	1,000	93	800	800	112	47	47
w	0.50	0.92	0.50	0.50	0.87	0.82	0.82
Females	Field	UM	Field-F1	Lab	Target-P	Field	Lab
Total rafts	16	1,309	31	34	349	101	196
Sterile	7	131	18	23	28	12	88
f	0.56	0.90	0.42	0.32	0.92	0.88	0.55
Comp. (E)	0.78	1.24	1.38	2.09	0.58	0.60	3.62

Irrad., sterilized by irradiation; P, females emerging from field-caught immatures
w, f and E abbreviations follow Table 1.

Concurrent with sterile-male research, a recessive genotype of *Cx. tarsalis* refractory to western equine encephalomyelitis virus infection was produced after repeated selection (Hardy et al. 1978). To simulate a release to establish this or other engineered genotypes into a field population, we mass-produced and then released males homozygous for the recessive eye-colour mutant, carmine eye (Reisen et al. 1985). Carmine eye was an excellent candidate for this experiment, because although immatures were easy to detect morphologically in all instars, adults appeared wild type (Asman 1975). Despite inundative releases of both males and females during

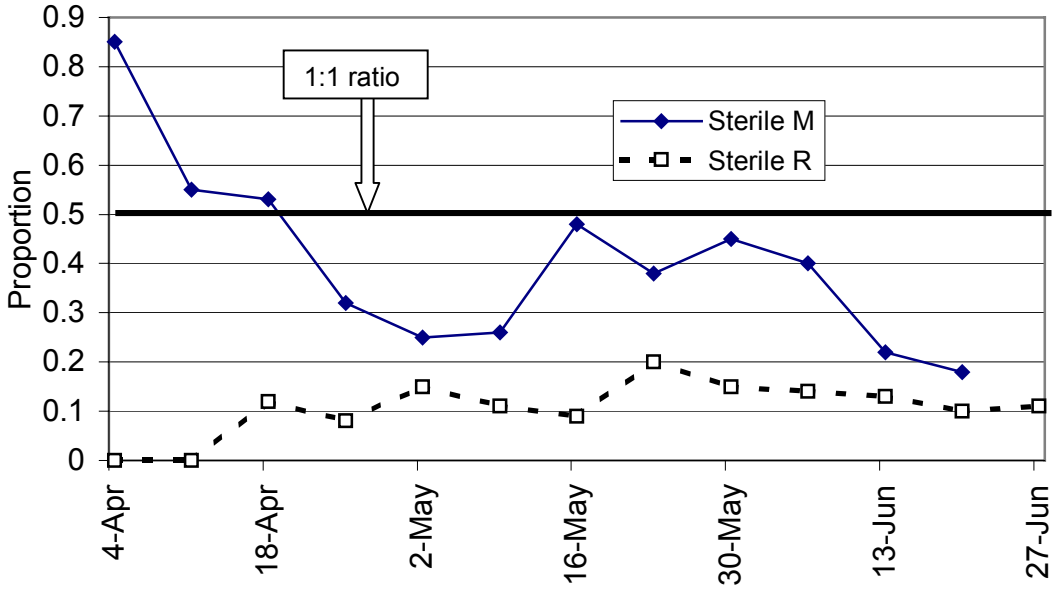

Figure 1. Proportion of sterile males among all males (Sterile M) and sterile egg rafts among all rafts (Sterile R) following the release of irradiated Br80 males at a semi-isolated site in the foothills near Bakersfield, California, during 1981 (Figure redrawn from Reisen et al. 1982).

spring, the mutant genotype failed to become established at high levels in a semi-isolated foothill area (see Table 4). Released males were found to be competitive for their siblings, but not for field females. Interestingly, the heterozygous +/*car* genotype was detected 27 months later at a low rate (1 pos/107 families tested) by backcrossing phenotypically wild-type males from the field to *car/car* females. Poor mating competitiveness of laboratory-reared *Cx. tarsalis* males may have been related to assortative swarming behaviour, because released males swarmed mostly in space

Table 4. Percentage of *Cx. tarsalis* that were carmine eye (*car*) based on the numbers marked with fluorescent dust or whose progeny were genetically *car/car* (data from Reisen et al. 1985)

Male sampling	Number collected	% *car* dusted	Families	% *car/car* mated
Red boxes	795	47	102	69
Top swarms	623	3	67	5
Space swarms	503	61	69	65
Females				
Red boxes	301	45	340	59
Traps	5,121	55		
Competitiveness for *car/car* females				1.62

swarms near the ground within breaks in vegetation, whereas males from the target field population swarmed predominantly above the vegetation. Colonization apparently selected for a deme that swarmed and mated most frequently within space-delineated swarms, but this deme comprised only a small portion of the target population.

Lessons

Important lessons learned from our field-release experiments (Reisen 1985) may be summarized as follows:

1. **Sex.** Males were chosen for release, because release of blood-feeding females may increase disease risk by enhancing the rate of transmission. Because much less is known about male than about female biology, design of male-sampling protocols, understanding target-male population age and reproductive structure, and selecting the time and place for releases was less well informed than if females had been released. Clearly future emphasis should include detailed studies on male biology and mating behaviour to develop the most effective release strategy.
2. **Release.** Males must be released at the correct time and place to swarm and mate competitively immediately after release. Correct photoperiod entrainment in the laboratory is critical. In one release of *An. culicifacies* males failed to egress from shelters and swarm at the correct time of the day, because they had been entrained on an aberrant midsummer insectary photoperiod (Baker et al. 1980).
3. **Colonization.** Only demes able to reproduce in laboratory cages become colonies; field genotypes may be lost in as few as three generations in the laboratory (Reisen et al. 1982). Research is needed to improve colonization techniques so that the field genotype is retained within laboratory strains.
4. **Competitiveness.** Laboratory, outdoor cage, and/or field evaluations must use female and male genotypes representative of the target population. Even vigorous laboratory strains may mate assortatively when released back into the parent population and, therefore, may not be competitive with wild-type mosquitoes.
5. **Addition rate.** Improved methods are needed to estimate the addition rate (emergence + immigration) into the target population to estimate the number of inseminations required per day. Inundative releases of *Cx. tarsalis* during the spring with either irradiated males or an eye-colour mutant failed to prevent the normal increase of the vernal population or to introduce the eye-colour mutant successfully into the field population at high levels.
6. **Transgenic mosquitoes.** Releases of transgenic mosquitoes probably will face similar challenges as did sterile-male releases, even if both males and females are released. Development of mechanisms to drive engineered genotypes through the target population would seem essential for success and to prevent repeating the mistakes made in the past.

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