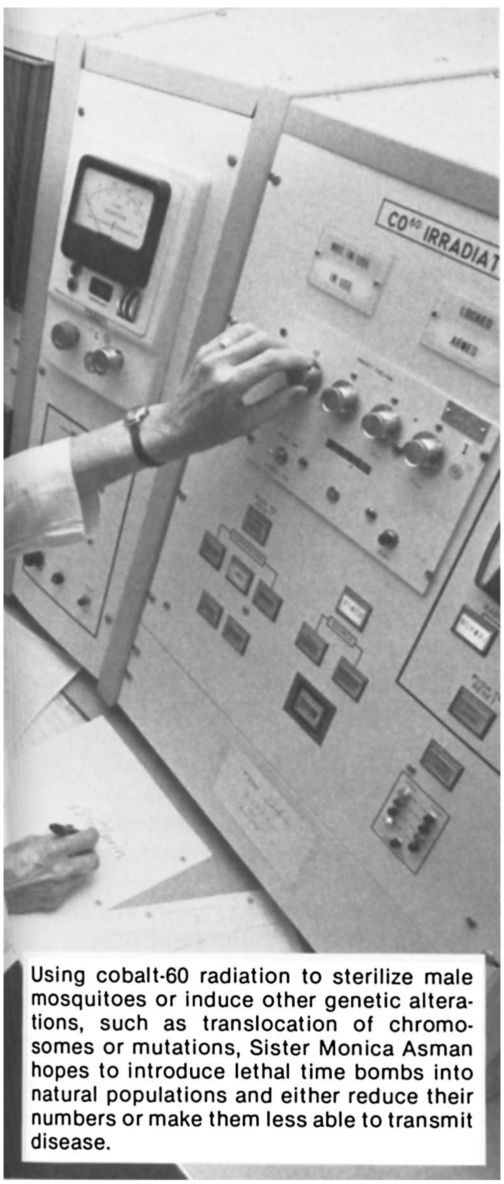
Genetic manipulation of mosquitoes

Sister Monica Asman □ Paul T. McDonald □ Frank G. Zalom

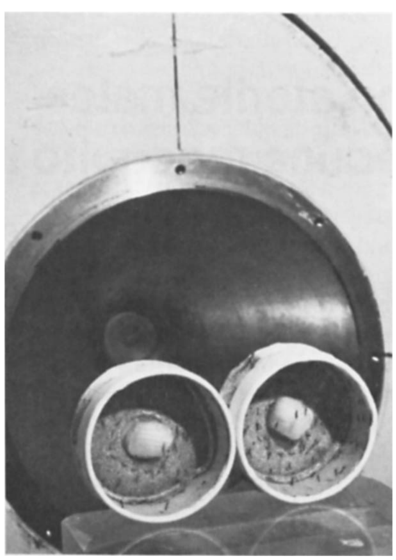
Genetic control involves insects in the reduction or alteration of their field populations. It is a highly selective means of biological control and is directed solely at a target species. The perfection of such systems is technologically complex and requires the development of extensive basic genetic information. The principal thrust of the research is to assess the effects of field introduction of genetically altered and/or sterilized males on the natural population.



Using cobalt-60 radiation to sterilize male mosquitoes or induce other genetic alterations, such as translocation of chromosomes or mutations, Sister Monica Asman hopes to introduce lethal time bombs into natural populations and either reduce their numbers or make them less able to transmit disease.

Translocations are an induced genetic alteration, that when released into a native population, can contribute to its reduction for more than one generation. Translocations can also serve as systems to introduce other genetic factors into a population, which act as lethal "time bombs" later, or which can replace the existing population with one having less competence to transmit disease.

Translocations are induced by ionizing radiation. They occur when pieces of two chromosomes from different pairs are broken off and, in the process of becoming reattached, exchange positions. This creates a false linkage of the genes residing on the two chromosomes affected. Individuals that have the translocation arrangement paired with normal chromosomes (translocation heterozygotes) have unequal distribution of genes during divisions of potential sperm or egg cells, making half of them inviable. This results in a 50 percent reduction in offspring, of which one-half carry the translocation to the next generation. Individuals that have paired translocation rearrangements (translocation homozygotes) can be made by inbreeding. These have the additional potential of being transport systems for other genetic factors.



Close-up of the business end of the cobalt-60 irradiator showing mosquitoes about to be radiated.

Fourteen mutant laboratory colonies of *Culex tarsalis*, as well as 14 lines with markers on all three chromosomes, have been established to genetically identify translocations and the position of the chromosomal breaks and reattachments. These lines are useful for mapping chromosomal positions for this species and for use as markers when inserted into stocks for laboratory and field experiments.

Chromosomal abnormalities such as translocations also can be identified by studying dividing cells. In addition, the involved chromosomes can be determined by observing their length. Through such observations and the use of multiple-marker stocks, it was shown that the longest chromosome carries the sex-determining gene in *Cx. tarsalis*. Conversely, in other culicine mosquitoes examined to date, the shortest chromosome carries the sex-determining gene. Now the large salivary-gland chromosomes of this species also can be prepared and studied. These are about 100 times larger than other chromosomes, and because they have distinctive patterns of transverse bandings, aberrations can be clearly identified.

Over the past three years a double sex-linked heterozygous translocation that includes all three chromosomes in the two interchanges was studied in detail. Only males inherit this linked combination, which is passed to each succeeding generation from males to male progeny. Males of this line had only 28 percent fertility and also competed well against the males of field populations in preliminary mating tests.

Pilot releases made in 1977 field studies indicated that this interchange could be mass-produced, that immature and adult males could survive in the field, and that the development time of their offspring in the field was similar to that of the native populations. The first release underscored the need for improved release strategies and field monitoring techniques. Unfortunately, the native population was larger in 1977 than in the two previous years when we had monitored field population size. Consequently, the release of 76,000 males was too small to compete effectively with the native males and to decrease the field population. The release was 24,000 fewer males than planned, and about 11,000 females were inadvertently included. A genetic sexing mechanism is currently being developed to completely eliminate females in future mass-production of males for releases.

The 1978 release focused on two objectives carried forward from the previous year: to reduce the population significantly with an insert of males carrying the heterozygote translocation, and to recover the interchange in progeny collected in the field. Quality control tests of release mosquitoes indicated that 99.4 percent of the males released were carrying the translocation. The number needed for a successful release was again ascertained by computer simulations based on previous population estimates. Male adults instead of pupae were released in the field to ensure against unwanted introductions of females.

In 1978, 180,000 translocated males were released. The progeny of females collected in the field were genetically tested to determine male parentage. In 22 cases tests confirmed that released translocated males had mated with native females. Unfortunately, parallel tests in outdoor cages revealed that the release stock had become less competitive with time; thus again, the release did not have sufficient impact to cause a continuing population reduction in the field. The data, however, did show a four-week delay before the population reached a peak, when compared with that of previous years, and the insert may have contributed toward keeping the population down during the release period. The population increased considerably once the release ended, and the peak population was twice that of the previous year.

A new method for rapidly identifying homozygote translocations was perfected. Currently, simulated field releases of various combinations of homozygous lines are being conducted in large outdoor cages. The advantages of homozygotes over heterozygotes would allow use of a field-replacement mechanism in addition to a self-destruction scheme.

One desirable genotype to be carried into field populations is decreased competence of *Culex tarsalis* as a vector of encephalitis viruses. A strain that rejects infection with western equine encephalitis (WEE) has been isolated by selection techniques. Although we lack conclusive genetic data on this resistance to WEE, the strain is a candidate for induction of translocations that might carry resistance to viral infection into field populations. Attempts are in progress to isolate a gene that acts as a lethal at low temperatures only. Such a conditional lethal would be a desirable "time bomb" for insertion in the summer to kill overwintering populations.

In studies on the feasibility of using the sterile male technique to control *Cx. tarsalis*, radiation sterility curves were established, mating competition tests were performed in the laboratory, and extended tests were conducted in walk-in cages outdoors. Egg hatch was reduced to 43 percent when irradiated males and nonirradiated males competed in a 1:1 ratio as compared with a 92 percent hatch in the nonirradiated control cage, and a 3 percent hatch in the irradiated control cage. In August 1979, a small-scale field release of sterile males reared from field-collected pupae was made in an isolated area outside Bakersfield. Only 1 of 50 egg rafts from females collected before the release had hatch rates lower than 70 percent, while 22 of 112 females collected during the first four days after the release laid rafts with low or medium hatch rates. Such results warrant further research with this method.

The behavior and ecology of *Cx. tarsalis* in the field, primarily in relation to reproduction, have been investigated continuously. Studies on reproductive vigor of laboratory-adapted and field-collected populations indicated that laboratory populations were more successful in insemination, oviposition, and the production of hatching egg rafts in small cage tests. The results also documented a substantial reproductive disadvantage of newly colonized populations during the initial colonizing process.

Current priorities are to keep field populations that are captured and colonized for genetic studies biologically and genetically close to the original stock and to improve the fitness of genetically altered strains to withstand field releases. A strict quality-control program has been established to ensure that specific attributes essential for normal behavior in the field are not lost during or after colonization. Representatives of translocated stocks and wild-type populations are being reared as continuously as possible in large outdoor cages under overwintering conditions.

Studies have been initiated to identify genetic selection factors that reduce competitive fitness. Study of genetic markers for several enzymes in *Cx. tarsalis* strains are under way. Analysis of the wild-type populations as they become colonized or become less competitive because of other selection factors will be made. The enzyme markers can also contribute to our formal genetic information, to release experiments where we can follow these "fingerprinted" strains, and to the study of reproductive behavior.

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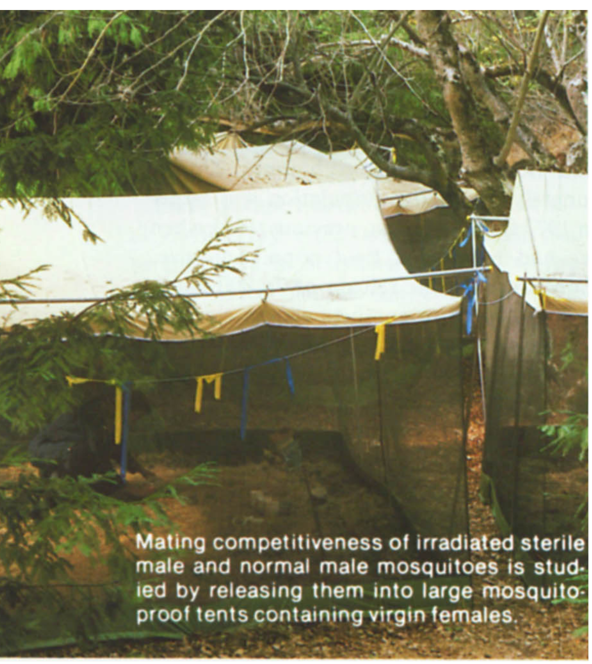

Mating competitiveness of irradiated sterile male and normal male mosquitoes is studied by releasing them into large mosquito-proof tents containing virgin females.

Using sterile males to reduce mosquito numbers

John R. Anderson □ Sister Monica Asman

Control of the treehole mosquito, *Aedes sierrensis*, is difficult, because it develops in hard-to-find treeholes. For this reason, the release of sterilized males is being researched as one possible component of an integrated management program. Initial laboratory experiments determined the most effective sterilization doses, and established that Gamma-irradiated, sterilized males competed equally with normal males in all mating experiments. Since females mate only once, regardless of whether mating with a fertile or sterilized male, sterilized males could be released and used to help reduce populations of this common pest.

In experiments at the University of California Russell Tree Farm Field Station (near Briones Regional Park, Contra Costa County), laboratory-reared mosquitoes were released into large tents having screened sides and natural turf floors. For these experiments, males mass-produced in the laboratory were sterilized by exposure to 7 kR of Gamma irradiation when less than a day old. The tents were set up in a