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# Review of previous applications of genetics to vector control

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## Abstract

The idea of genetic control of insect pests and vectors was invented independently three times in the 1930s-40s. Results with releases of radiation-sterilized male tsetse have been encouraging. Much work was done in the 1970s on mosquitoes with sterile males and systems that could potentially be used for gene driving. Chemosterilized *Anopheles* separated from females by a genetic sexing system was successfully released in El Salvador. In India chemosterilization, cytoplasmic incompatibility, translocations and meiotic drive were tested with culicine mosquitoes in field cages, for mating competitiveness in the field and in some cases in village-wide release trials. However, a town-wide eradication attempt with *Aedes aegypti* was stopped due to spurious claims about biological warfare. This experience underlines the need for very careful attention to relations with journalists, politicians and the general public. Transgenic sterile males, which are now available, should have considerable advantages over radiation and chemosterilization and would seem well suited for eradication of urban vector populations.
**Keywords:** sterile male tsetse; chemosterilized mosquitoes; cytoplasmic incompatibility; translocations, meiotic drive; mating competitiveness

## The pioneers

The concept of genetic control was invented independently in three very different environments in the 1930s-40s (Klassen and Curtis in press). In the order in which their ideas were published the inventors were:

**A.S. Serebrovskii**

Serebrovskii (1940) had worked at Moscow State University with H.J. Muller, the discoverer of radiation-induced mutations, including dominant lethals, in *Drosophila*. Serebrovskii proposed the use of chromosome translocations as a source of inherited partial sterility with which to suppress pest populations. He realized that because they caused semi-sterility in heterozygotes, but not in homozygotes, translocations would be selected for in a population if sufficiently released to constitute a majority. However, he did not go on to suggest them as a means of driving genes tightly linked to the translocation and causing inability to transmit disease into populations. Curtis (1968) made this proposal after independently realizing that translocations, a common form of radiation-induced mutation, might have a role in genetic control. Curtis

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(1971) also showed that translocation heterozygotes and homozygotes could be produced in tsetse flies, despite the laboriousness of rearing such viviparous insects.

**F.L.Vanderplank**

Vanderplank (1947; 1948) worked as part of the remarkably active British colonial effort to control tsetse. He and others had found that one could cross closely related species of tsetse and that the crosses were partly sterile and produced partly sterile hybrids. He therefore proposed the collection of thousands of pupae in the breeding range of one species (*Glossina morsitans*) and their release into an isolated sector of the breeding range of another (*G. swynnertoni*) in Tanzania. It was realized that this would only have any useful effect if *G. morsitans* could compete for mating in the field with *G. swynnertoni*. Jackson (1945) released both sexes of both species and found that when couples were collected, during their two-hour copulation, all four combinations of the species could be found at frequencies indicating no behavioural barriers to cross-mating. Therefore Vanderplank initiated a field trial in June 1944, like another more famous enterprise. Over the next two years, releases of about 100,000 *G. morsitans* first led to virtual elimination of the resident *G. swynnertoni*, a phase lasting about a year in which significant numbers of hybrids could be found, and then to replacement by *G. morsitans*. However, in the long term this species could not maintain itself in the arid climate of a *G. swynnertoni* habitat and the introduced *G. morsitans* also declined toward extinction. After two years tsetse densities were so low that local farmers, whose activities completed tsetse eradication, could safely occupy the area. Thus, not only was Vanderplank the first person to release insects causing sterility but he also demonstrated a deliberate population replacement by a conditionally lethal genotype. Unfortunately Vanderplank only published a very brief account of his work. He gave detailed data on this remarkable trial to the present author in 1970 (see Klassen and Curtis in press) by which time release trials of irradiated tsetse were already in progress.

**E.F.Knipling**

Knipling (1955; 1959) observed in the US Dept. of Agriculture's Screwworm Fly laboratories that Screwworm (*Cochliomya hominivorax*) females are monogamous. It was apparently this observation that set him thinking about the possibility of the Sterile Insect Technique (SIT) if only some means of sterilizing male insects could be found. In the 1940s Knipling and Bushland made contact with H.J.Muller who pointed out that, based on 20 years of experience in *Drosophila* (Muller 1927), irradiation with gamma rays or short-wavelength X-rays readily induced dominant lethals (broken chromosomes) in sperms killing the embryos when mitosis begins after fertilization. A dominant-lethal rate of virtually 100% could readily be obtained by irradiation of Diptera at a dose far less than that needed to kill a pupa or adult insect in which development, including the radiation-sensitive process of mitosis, had already been completed. Knipling soon realized that, because radiation induced sterility in Diptera via dominant lethals, which did not inactivate sperm, his original observation of female monogamy in Screwworm was by no means a requirement for the SIT to work – an interesting example of being right for the wrong reasons! The belief that monogamy is a requirement for the SIT is still entrenched among journalists and, judging by recent comments of a referee on a grant application, among some biologists who ought to know better! Knipling, Bushland and the USDA team quickly showed that Screwworm pupae could be given a radiation dose that would ensure lifetime male sterility, that mass-rearing was possible and that, after release, sterile males could compete for mates. They demonstrated eradication from the island of Curaçao and then from Florida. The programme expanded to achieve complete eradication of Screwworm from the southwestern states of the USA, Mexico and Central America as far south as Panama and of an accidentally introduced population of *C. hominivorax* from Libya (Wyss 2000; Lindquist 1993). A programme of aerial releases which moved steadily forward was able to deal with the problem of immigration by ensuring that females were engaged in sterile matings before beginning their migratory flights, achieving eradication of this major veterinary pest, which can also cause myiasis in humans, at a cost that was rapidly repaid by elimination of the cattle losses caused by the Screwworm. At one point it was claimed that behavioural barriers existed between different sub-populations of Screwworms (Richardson, Ellison and Averhoff 1982), but this was never supported by many data (Krafsur 1998) and the successful eradication all the way to Panama with flies reared from a single captive population indicates that this kind of biological complexity was not a real problem.

## Induced sterility in vectors

### Tsetse

Combined trapping and SIT programmes were carried out against the riverine sub-group of tsetse in Burkina Faso (Politzar and Cuisance 1984) and Nigeria (Takken et al. 1986). More recently *G. austeni*, the only tsetse species in Zanzibar, has been eradicated by SIT from that island (Msangi et al. 2000). In a few of the trials, late pupae have been irradiated but in most cases adults have been irradiated. Because of the extraordinarily low natural fertility of tsetse, the numbers that can feasibly be reared are far less than with Screwworm but, conversely, far lower sterile:fertile male ratios are presumably needed to initiate a downward population trend. In tsetse one can collect predominantly males for release from the pupae deposited on a given day, as the males emerge first and the females can be retained to provide breeding stock for the colony. Male tsetse bite but various methods have been attempted to minimize the risk of disease transmission by the released sterile males. The only recorded attempts to use the auto-sterilization principle has been carried out in tsetse (Hargrove and Langley 1990; Oloo et al. 2000), i.e. attraction of wild flies to a bait which will sterilize them and then allowing them to return to the wild population and mate.

There are strong disagreements about whether the eradication of tsetse in Zanzibar should be taken as a precedent for major efforts to eradicate tsetse from large parts of continental Africa or whether such an attempt would be a hopeless task and a serious diversion of resources from less ambitious but more hopeful anti-trypanosomiasis measures.

### Mosquitoes

In mosquitoes, irradiation of late pupae with a male-sterilizing dose causes severe reduction in competitiveness of the emerging adults. This was shown for *Culex* mosquitoes 35 years ago (Smittle et al. 1968) and confirmed for *Anopheles* recently (Andreasen and Curtis in press). Irradiation of adults is apparently not harmful but irradiation of millions of adult mosquitoes would be very inconvenient. It was found in the 1970s that immersion of pupae in an alkylating agent such as thiotepa or bisazir could cause nearly 100% dominant lethality in sperms with little apparent damage to the emerging adults. The treated pupae were carefully rinsed before emergence and the residues of the alkylating agents, which are mutagens, were very low (LaBrecque et al. 1972). However, from a so far unreplicated study it was reported that spiders fed a diet of nothing but chemosterilized mosquitoes were themselves sterilized (Bracken and Dondale 1972).

In the 1970s it was considered acceptable to go ahead with releases of chemosterilized *Cx. quinquefasciatus* at the WHO/ICMR Unit on Genetic Control of Mosquitoes in Delhi (Yasuno et al. 1978) and *An. albimanus* in El Salvador (Lofgren et al. 1974; Dame, Lowe and Williamson 1981). There would have been almost no contact of these mosquitoes with humans after release because highly effective sex separation was developed so that virtually all were non-biting males. In culicines the male pupae are markedly smaller than females and a carefully adjusted sieving system (Sharma, Patterson and Ford 1972) routinely achieved 99.8% males among releases of 300,000 per day (Singh et al. 1975; Ansari et al. 1977). Demonstration to villagers near Delhi that the males that were being released did not bite satisfied them that the mosquito release activities were at least doing the villagers no harm. In *Anopheles* the pupal size difference between the sexes is not reliable and instead dominant genes causing insecticide resistance were translocated on to the Y chromosome so that females could be selectively killed early in the egg or larval stage, leaving the resistant males unharmed in the *An. gambiae* complex (Curtis, Akiyama and Davidson 1976; Curtis 1978) and *An. albimanus* (Seawright et al. 1978). In the latter work an inversion was used covering the chromosomal segment between the resistance gene and the translocation breakpoint to minimize recombination in this segment that would otherwise progressively reduce the accuracy of sex separation over successive generations. For unknown reasons great difficulty has been experienced in producing comparable sex separation systems in *An. stephensi* (Robinson 1986; Andreasen 2003). This is unfortunate as the latter species, with its urban distribution and role as a serious malaria vector in India, renders it a suitable target for attempts at eradication of urban ‘island’ populations by SIT as discussed below.

Studies on mating competitiveness were carried out in India with released chemosterilized *Cx. quinquefasciatus* and *Ae. aegypti* (Grover et al. 1976a; 1976b). In the tests with the culicine mosquitoes, males marked with fluorescent powder were released so that the ratio of released sterile to wild fertile males could be determined. Then similarly marked virgin females of wild origin were released, left long enough to mate and then recaptured and made to lay eggs in the laboratory. The proportion of sterile and fertile egg layings was found to be not much less than the sterile:fertile ratio among the males, indicating quite good competitiveness for these females whose marking indicated that they had mated within the village where the males had been released. Similar good competitiveness was found with chemosterilized *An. albimanus* (Dame, Lowe and Williamson 1981).

When releases were made with chemosterilized *Cx. quinquefasciatus* daily over many weeks the percentage sterility among the egg rafts laid was initially disappointingly low and only slowly rose to 80%, which was still far less than the proportion of sterile males within the release village (Yasuno et al. 1978). Because the hypothesis of poor competitiveness of the males had been ruled out by the above described competitiveness test, it was concluded that many of the fertile rafts had been laid by females which had mated outside the release area and then migrated into it, despite attempts to maintain a 3km-wide barrier zone free of breeding by use of larvicide. Because of the generally monogamous behaviour of mosquito females they had refused to re-mate on arrival in the release village. Thus, far from female monogamy being a requirement for the SIT to succeed, it is actually an unfortunate fact of life, which in this and several other trials has made non-isolation of the target area a major barrier to success.

In the El Salvador project a target population around one lake was apparently adequately isolated; 100% sterility of the eggs laid was achieved and the normal surge in the wild vector population was prevented when the seasonal rains arrived (Lofgren et al. 1974). However, extension to a larger target area again encountered problems of immigration despite maintenance of a barrier zone (Dame, Lowe and Williamson 1981). Nevertheless, a histogram of the data (Benedict and Robinson 2003) on *An. albimanus* densities in the release and a comparison area emphasize how successful the chemosterilized males were in preventing a normal seasonal rise in vector density. Latterly these males came from the strain described above with genetic sex separation and this allowed production and release of a million males per day.

## Tests of systems with the potential to drive genetic factors into populations

### Cytoplasmic incompatibility

Sterility was found to exist in crosses between different geographical populations of the *Culex pipiens* complex. In some cases both reciprocal crosses were sterile and in other cases only one of the reciprocal crosses was sterile. By repeated backcrosses in one of the latter cases Laven (1967b) showed that control of crossing type was wholly maternally inherited and the phenomenon was given the name cytoplasmic incompatibility. It was possible to choose strains which yielded 'ready made' sterile males with respect to a given target population. By an appropriate backcrossing programme, sometimes involving an intermediate strain, it was possible to equip a strain for release with the chromosomes of a target population and therefore presumably make it well adapted to its environment, without affecting the sterility of crosses between the strains (Krishnamurthy and Laven 1976).

Laven (1967a) demonstrated eradication of a small village population in Myanmar by release of males only of an incompatible strain. It was realized that 100% sex separation was not possible on a large scale and released females could engage in fertile matings with released males leading to population replacement and not eradication. The idea therefore arose of equipping the released strain with another genetic factor with desirable properties and using bi-directional cytoplasmic incompatibility as a means of driving this factor into the population. This should be achievable on the principle of selection for the majority type, where crosses are less fit than either of the pure matings, as described above with regard to autosomal chromosome translocations. It was hoped eventually to drive in genes for non-susceptibility (refractoriness) to filariae but it was more feasible to produce male-linked translocation complexes causing about 65% sterility (Krishnamurthy and Laven 1976). Because they were male-linked, homozygotes could not be produced so they would behave quite differently to autosomal translocations and could not themselves act as gene-driving systems. The idea was that the bi-directional cytoplasmic incompatibility would be the gene-driving system and would also prevent recombination of the cytoplasmic type with the translocation. In a field cage it was shown that this did indeed happen. As releases of the translocation-incompatible strain proceeded there was a phase when many fully sterile (incompatible) egg rafts were produced but, after several weeks, the frequency of these declined to zero, leaving only egg rafts showing partial sterility due to the translocation in matings between males and females with the same cytoplasm (Curtis 1976).

The mating competitiveness in the field of the males of this translocation-incompatible strain was tested by the same method as described above for chemosterilized males and very much the same results were obtained (Grover et al. 1976a). Several weeks of large-scale field releases into a village followed (Curtis et al. 1982) and, as with the chemosterilized releases, the continued appearance of some egg rafts with full fertility indicated that considerable immigration was occurring despite efforts to prevent it.

While these field studies were going on it was shown that complete bi-directional incompatibility of Indian wild strains and laboratory strains of European origin could not be relied on because there was an effect of male aging on incompatibility (Singh, Curtis and Krishnamurthy 1976) and because there was polymorphism for cytoplasmic types in wild populations (Subbarao et al. 1977). At about this time Yen and Barr (1973) produced strong evidence that the maternally inherited factors that caused cytoplasmic incompatibility were symbiotic *Wolbachia*. These occur naturally in *Culex* and some *Aedes* populations but can be eliminated with antibiotic treatment, resulting in strains with males that are universally compatible and females that are only compatible with males from which *Wolbachia* has been removed. It is deduced that *Wolbachia* in males inactivates sperms but these can be ‘rescued’ if the female carries *Wolbachia* that are compatible with the strain in her mate. This hypothesis explains the crossing properties of strains from which *Wolbachia* have been removed and of naturally existing incompatibility between different geographical populations. With *Aedes* and *Drosophila* it has been possible to inject back *Wolbachia* into strains without them and the expected incompatibility is then expressed (Sinkins, Curtis and O'Neil 1997). It is therefore hoped that *Wolbachia* could be introduced into *Anopheles* where it does not naturally occur.

**Meiotic drive**

Hickey and Craig (1966) showed in *Aedes aegypti* that combination of a ‘driving’ *M*, male-determining, gene with susceptibility of its *m* allele led to distortion of the sex ratio in favour of males. This was not due to selective mortality of female embryos but to excess production of male-determining sperm (i.e. meiotic drive – “breaking of Mendel’s first law”). At the unit in Delhi this phenomenon was combined with two translocations. These fortunately enhanced the strength of sex-ratio distortion to 13:1 and the double-translocation heterozygote showed about 65% sterility (Suguna et al. 1977a). This combined system, when tested in the field by the above described method, showed good competitiveness for mates (Grover et al. 1976b). In a field cage trial extending over several generations and with simulated density-dependent regulation of the population, the sex-ratio – translocation system showed as good, but no better, population-suppressing ability as chemosterilized males (Curtis et al. 1976).

Apart from a use for attempted population suppression, meiotic drive has been suggested as a possible means of driving refractoriness genes into populations. However, it was found that in Indian wild *Ae. aegypti* populations there are some resistant *m* alleles (Suguna et al. 1977b) which would be selected for and prevent indefinite spread of the meiotic-drive factor. Furthermore, meiotic drive has not yet been reported in *Anopheles*.

**Claims about biological warfare**

As indicated above there was evidence for a serious level of immigration of *Culex* between Indian villages. It was noted in the 1970s in India that *Ae. aegypti* was limited to urban areas where there were water tanks. Because of the importance of this species as a dengue vector it was proposed to attempt eradication of an *Ae. aegypti* population from a whole town by sterile-male release (Reuben et al. 1975). Unfortunately an Indian journalist noted that *Ae. aegypti* is commonly called the 'yellow-fever mosquito', that there is no yellow fever in Asia and that yellow fever had been considered in the 1960s as a biological warfare agent. He jumped to the conclusion that the purpose of the research at the unit in Delhi must have been to obtain data of use for biological warfare (Oh New Delhi, Oh Geneva (editorial) 1975; WHO 1976). Powell and Jayaraman (2002) attempted to revive these claims, but it was pointed out to them that the mass releases were to be with males which, because they do not bite, could not be relevant to biological warfare. This press campaign was adopted by opposition politicians and led to cancellation of the town-wide eradication attempt just two days before it was due to begin. From this experience must be drawn the conclusion that any future mosquito release project must pay close attention to relations with, and information to, journalists, politicians and the general public. The WHO restrained its staff from replying to the slanderous accusations on the grounds that the accusations should be viewed as a political matter internal to the host country, but with hindsight this was a very unwise decision.

**Transgenic sterile males**

Apart from the biological warfare and other accusations the journalist raised the question of the use of chemical mutagens to induce sterility. Though these were handled with great care and the residues in released males were minute as mentioned above, it seems unlikely that their use would be authorized nowadays. There are also problems with radiation-sterilization as mentioned above. It is therefore notable that a transgenic form of sterile male has now been produced in *Ae. aegypti* (L. Alphey, H. White-Cooper, M. Andreasen and P. Coleman, pers. comm.). This uses the system known as RIDL (Release of Insects carrying a Dominant Lethal, Thomas et al. 2000). By adding tetracycline to the rearing medium, the construct is 'switched off' to allow mass rearing. The effect is genetically dominant so that progeny of released RIDL males mated to wild females will die in the absence of tetracycline in normal breeding sites. A multi-centre trial of mating competitiveness of RIDL males is about to begin in cages. At present the additional refinement of making the lethal action sex-limited so that only females die has not yet been achieved. Yet, when it is, it will provide a replacement or a back-up for conventional sex separation systems somewhat extending the impact in the field, as male progeny will be heterozygous for RIDL and will be 50% sterile.

The most hopeful type of sites for eradication using RIDL releases is urban areas having one species of vector, but where the surrounding rural area has another. Based on earlier work it would seem that examples of such cases are *An. stephensi stephensi* in Indian cities (Ramachandra Rao 1984) and *An. arabiensis* in southern-Nigerian cities surrounded by *An. gambiae* in rural areas (Coluzzi et al. 1979; Kristan et al. 2003). *Ae. aegypti* still transmits dengue in Singapore in a human population which now has little immunity (Ooi et al. 2001) because of the impact of legally enforced larval control. However, this form of control has not been able to eradicate *Ae. aegypti* and an attempt to do so with RIDL males would seem to be appropriate.

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