

Studies on Genetic Control of Insects and Public Health Importance: A Survey

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Abstract

During recent years many advances have been made in the development of insect control by genetic manipulation. These methods include the sterile male technique, now well known, which depends on ionizing radiation or chemo sterilization. The recent field experiment carried out by WHO in Rangoon, Burma, on *Culex fatigans* has demonstrated that naturally occurring cytogenetic mechanisms such as cytoplasmic incompatibility can be used successfully without the use of radiations or chemosterilants. The paper not only describes the experiment on *Culex fatigans* but also discusses basic concepts and theoretical considerations involved in genetic control of insects of public health importance. There are a number of problems which require study before genetic control can be used on an operational scale.

Keywords: Genetic Manipulation; Auto sterilization; Mass Production.

INTRODUCTION

Effective vector control is fundamental to the suppression of many of the major epidemic and endemic diseases of man such as malaria, yellow fever, plague and filariasis. Before the Second World War, transmission of some of these diseases had been interrupted by the use of non-persistent insecticides or by environmental manipulation, (Kenit 2008)¹² but this was usually on a limited scale and at a high cost. The discovery of long lasting synthetic organic insecticides, such as DDT,

revolutionized the whole concept of vector control and for the first time it was possible to contemplate the control or even eradication of the majority of arthropod borne diseases through out the world. (Rittsch of Rookee et al. 2018)¹⁰ However, the development of insecticide resistance and the discovery of behavioural characters which impede control have seriously modified this optimistic outlook and vector control has again become one of the important problems confronting health authorities. (Wolf et al.. 1995)⁴

Interest in the biological control of insects has been heightened by the development of insecticide resistance and the consequent partial failure of the chemical approach to control, as well as by problems of environmental contamination and associated health hazards.(Saltz 2013)¹³ During the last few years, considerable advances have been made in research on the control of insects by genetic manipulation of populations. The successful application of the sterile male technique for the control of the screwworm and tephritid fruitflies has given impetus to the use of this method for the

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control of many other insect species. Since 96, this technique has been successfully used in the control or eradication of at least 8 species of insects either in laboratory experiments or in field trials. (Schneider, Levine, 2016)²

Genetic control of insects is not limited to the use of insects sterilized by radiation or chemicals.

Other mechanisms known to the geneticist are available and may be adapted for vector control. These include cytoplasmic incompatibility, hybrid sterility, meiotic drive, distorted sex ratios and let al. factors. A major break through in medical entomology has recently been made in the elimination of *Culex fatigans* from an isolated village in Burma by the release of males of a genetically incompatible strain. The importance of these striking results can hardly be over emphasized. (Saltz(2013)¹³

Chemical control and source reduction programmes may reduce a population to a relatively low level, but even low level populations can regenerate often in a short period. Thus, chemical application or source reduction measures must be repeated or maintained, and these may be very expensive. Further more, the low insect population which remains may be resistant, or may simply be so small that it is not detected by control workers. If genetic methods are available, this residuum may be eliminated; released males can seek out niches and habitats that cannot be discovered by conventional methods of detection. Genetic control could be used as a final weapon to knock out those insects which escape conventional control. In completely isolated areas populations may be eradicated; in less isolated areas the continuous release of insects at low population levels may effectively control the vector population indefinitely at a cost substantially below that for current methods of control. In an integrated control programme, initial population reduction by insecticides or sanitary measures or both would be followed by genetic control. In certain situations, natural decline of populations due to seasonal changes should make it possible to use genetic methods by themselves. The most appropriate and most economical integrated procedures to follow will be governed by several factors including the natural density levels, cost of rearing and releasing the number of insects required and the degree of annoyance or damage caused by the released insects. (Avalos et al. 2020)⁷

Certain economic features of genetic control may be pertinent. First, the recurring cost of application of insecticides over a number of years will either not be necessary or will be much reduced. Second,

although the initial cost of genetic control may be high, the maintenance cost could be very much lower. It should be emphasized that genetic control is still largely theoretical from a field operational stand point. The limitations imposed by field conditions must be investigated carefully for each species, before the true utility of this method can be evaluated. In all cases the approach to genetic control must be carefully considered. The method requires a most thorough and detailed knowledge of the biology of insects and it requires well trained investigators who are conversant with the rather sophisticated techniques. Thus, the stimulus to entomological science and education may prove to be a very substantial additional benefit from this approach. (Schneider, 2017)²

The application of this concept provides a valuable additional weapon in the arsenal against vector species. The theoretical considerations and laboratory experiments indicate that the method is technically sound. Successful field trials on a small scale further reinforce confidence in genetic control. (Sokolowski 2010)⁸

Concepts and Theoretical Considerations: A Survey

Basic Principles

The basic principle in genetic control of insects is to utilize factors which will lead to reproductive failure. Induced sterility is one kind of genetic control but other causes of reproductive failure can be utilized. (Vrontou 2006)¹⁸ When a sterile male mates with a normal female there will be no progeny; if adequate numbers of vigorous and competitive sterile males are introduced systematically into a natural population, the population will soon cease to exist. The sterile males should outnumber the fertile males in the natural population so that the chance of a sterile male mating with a native female greatly exceeds that for the fertile native male. For example, a population containing 00 normal females, 00 normal males, and 000 sterile males should result in a 9% reduction of the progeny in the first generation. Continued introduction of 000 sterile males each generation will soon result in the elimination of the original population, because the ratio of sterile males to normal males becomes so great that the normal males have no chance to mate.

It has been shown that this principle will work successfully in cages (and in some instances in field populations) on numerous insects including the codling moth, tobacco hornworm, tropical fruit-flies, *Drosophila*, the screw-worm fly, and several vector species. Over wider areas, the screw-worm,

a serious pest of livestock, has been exterminated from extensive continental areas; tropical fruit flies have been eradicated from certain islands; and experimental work has progressed to the extent that practical field trials will soon be initiated on the codling moth, pink bollworm, tobacco hornworm, *Drosophila* and cotton boll-weevil. (Carter et al., 2019)¹¹

The sterile male method may be used in either of two ways. One method involves the rearing,

sterilization and release of enough insects over a sustained period to overwhelm the natural population, the second involves treatment of the natural population with an agent (chemosterilant) which will induce sterility in the native insects. The theoretical trend of an insect population subjected to these two types of genetic control is shown in Tables 1 and 2.

The two methods of employing the sterility

Table 1: Population trend in a stable insect population subjected to continued release of sterile males each generation.

Generation	No. of Insects	No. of Sterile Insects Released	Ratio	No. of Insects Reproducing
1	1000000	9000000	9:01	100000
2	100000	9000000	90:01:00	1099
3	1099	9000000	8000: 1	0

principle have important differences in their effect on population trends, although both methods would theoretically eliminate a population of 1000000 insects in 3 generations. The sterile male release technique (Table 1) becomes more and more effective as the population decreases, and as the ratio of sterile to fertile insects increases. It should be noted, however, that the method of inducing sterility in the natural population by chemosterilants (Table 2) has the same effect in each treatment but the initial impact is much greater. Each treatment is assumed to sterilize 90% of the population and the sterile insects, if fully competitive, can in turn result in infertile matings of a proportionate number of the untreated insects. The combined effect is that reproduction is suppressed by 99%. If an insecticide of similar biological activity were employed, suppression would be only 90%. Thus, the

chemosterilant method at the 90% efficiency level is potentially 10 times as effective as the conventional method using insecticides, in terms of the number of insects reproducing. Like insecticide treatments, but unlike the sterile insect release method, the use of chemosterilants against the natural population has the same suppressive effect at each treatment, regardless of population density. Thus, when the natural population is greatly reduced by initial treatments, it may be advantageous to employ sterile insect releases in place of further use of chemosterilants for the complete suppression of populations and for maintaining suppression if the area is subject to continuous infestation. The advantage of integrating different methods of vector control should be considered in any comprehensive programme. (Robinson, et al. 1971)¹⁴

Table 2: population trend in a stable insect population subjected to a treatment which sterilizes 90% of the population each generation

Generation	No. of insects	No. of sterile insects released	Ratio	No. of insects reproducing
1	1000000	100000	900000	10000
2	10000	1000	9000	100
3	100	10	90	<1

METHDOLOGY

Justification for Exploration of Genetic Control Methods

The new concept of utilizing genetic means for insect population control, particularly when integrated with other methods of control, is

important because numerous species of arthropods have become resistant to insecticides. Furthermore, the wide spread use of insecticides has created problems of possible contamination of the environment as well as potential hazards to fish and wildlife. The use of genetic methods can be species specific and usually innocuous as far as hazards

to the environment or harm to other species are concerned. (Wilson 1971)¹⁵

An advantage of using genetic methods is that although the initial cost of a programme may be high, the control of vector species in an area with a low population density of the species may be comparatively inexpensive. Further more, in situations where a species can be eradicated and the chances of infestation are minor, the saving in costs of year-to-year control can be very great. For example, the cost of eradicating the screw-worm fly from Florida was about \$US 8000000, but the accrued savings to the livestock industry over the last 10 years have been variously estimated to range from \$US 100 000 000 to \$US 700 000 000. (Vrontou 2006)¹⁶

The cost of insecticides for use in developing countries is usually high because of the monetary exchange rate. There now seems to be a good possibility of utilizing genetic control methods at lower over - all costs in many areas; this is particularly true where the insects to be released do not have to be sterilized by specific treatments but are inherently sterile due to genetic manipulations.

Analysis of Genetic Mechanism

Sterility in insects may be induced by exposure to physical and chemical agents or may be based on several types of genetic mechanisms. Generally, the insects must be reared in huge numbers, rendered sterile and introduced into the natural environment to compete for mates with the native insects.

Agents induce sterility

When ionizing radiations or chemical sterilants are used to sterilize males, sterility is usually based on the induction of dominant let al. mutations in the sperm. Sterility can also be achieved by inactivation of the sperm or by aspermia. In females, sterility can result from dominant let al. mutations in the eggs that are deposited or from in fecundity. All of these types of induced sterility are potentially useful for insect control. The use of sterility based on sperm inactivation has not been extensively explored but is nevertheless potentially useful. For example, in the control of monogamous species of insects, aspermic males or those transmitting inactivated sperm could be as effective as sterile males containing dominant let al. mutations, provided that these males produce the monogamous response in the females with which they mate. These females will thereafter refrain from mating and will lay infertile eggs. Because the induction of sterility by ionizing radiation is not suitable for every species, due to the possible

lowering of competitiveness and longevity and the probable changes in behaviour, chemical sterilants may be more useful to sterilize insects in some instances. An amazing number of chemosterilants have been developed and current technology is aimed at assessing the effectiveness of these compounds, their modes of action, the amounts of chemical carried by the sterilized insects and the rapidity of chemical breakdown. Shpigler et al.. (2017)¹⁷ Elzen(2003)⁴³ Winston(1991)³³

However, the vast majority of the currently available chemosterilants are mutagenic and produce their effect by the induction of genetic damage. This is the basis of their effectiveness and also the major principle which limits the manner in which and extent to which these chemicals may be used. There is reason to hope that future research will lead to the development of non-mutagenic chemicals which sterilize insects.

Some possibilities are:

1. The development of chemicals which initiate the monogamous response in females and prevent them from mating
2. The development of chemicals which prevent the normal sequence of spermatogenesis or oogenesis and thereby prohibit the development of gametes by the insect.
3. The production of chemicals which give rise to sperm-inactivation. Some of these chemicals may attack biological systems which are specific for particular insects and therefore stand a better chance of being widely applied. (Seeley 1995)³²

Genetic mechanisms / External Agents

In addition to using external agents to produce sterility, a variety of genetic mechanisms may be used to produce insects incapable of reproduction or insects which may propagate factors through the natural population which will lead to the eventual decline of that population. Examples of these genetic mechanisms are listed below but are discussed more extensively in other sections dealing with the species of insect in which they have been studied. (Abbott 1920)⁴⁰

Cytoplasmic incompatibility

With in some insect species, crosses between various populations are sterile. Sterility is due to a cytoplasmic factor transmitted through the egg, which kills the sperm of the incompatible male after its entry into the egg. Crosses between certain populations give no off-spring at all; in other cases, females of one population may cross with males of

another population and offspring are produced, but the reciprocal cross is completely sterile. Control can be achieved by mass rearing and release of males into an area populated by incompatible crossing types. (Abbott 1920)⁴⁰

Hybrid sterility

In some insect species there are a number of crossing types or races which produce fertile females but sterile males among their progeny. These sterile hybrids are excellent material for use in insect control because, although the males are sterile, they are likely to be fully competitive or even much more vigorous than sterile males produced after radiation or chemosterilant treatment. If research on disease vectors is expanded to include studies on the cross fertility of races and the fertility of hybrids from various populations, it is probable that many instances of hybrid sterility will be found. (Carter 2019)¹¹

Population Replacement

If circumstances arise in which the elimination of a vector population leaves a vacuum which might be filled by a dangerous replacement, it may be possible instead to fill the niche with an innocuous form. In order to assess the feasibility of this approach, further research on the genetics of vector ability is required. Evidence suggests that the ability to transmit disease organisms is genetically controlled and can be studied by standard genetic approaches. Genes controlling the ability of a vector species to transmit disease organisms, especially viruses, should be studied. (Wilson 1971)¹⁵

Other Genetic Approaches

Several types of population control involve the release in the natural environment of insects which are not sterile, but which would introduce factors into the natural population that could eventually lead to the decline of that population. Although there are serious restrictions on the release of non sterile insects into the environment there are many instances where this approach can be envisaged. For many vectors, the release of harmless males into a population at its low seasonal density could conceivably have beneficial results.

Some examples of these genetic factors are:

1. Sex-ratio distorters.
2. Detrimental genes incorporated into chromosomes which have meiotic drive.
3. Males heterozygous for chromosome translocations.
4. Insects bearing conditional let al. genes which allow the parents to survive in the

laboratory but which would be let al. to their descendants under field conditions. All of these genetic factors are known to exist in vector species but the search for these factors needs to be intensified. Further more, their role in population suppression should be assessed. (Kent et al.. 2008)¹²

i) Genetic Control/ Auto-Sterilization of Natural Populations Method

One of the most imaginative concepts in the genetic control of insects involves the principle of auto-sterilization. This principle is that instead of massive numbers of insects being reared, sterilized and released into the natural environment, the native insects are sterilized in their natural environment. One method that would minimize the exposure hazard to non-target organisms would be to lure the insects to sites (e.g., bait or trap); at these sites the insects would be sterilized rather than killed. If both sexes of the insect were sterilized this procedure would have a tremendous bonus effect since the sterilized insects could not reproduce but would disperse in their native habitat, seek mates, and negate the reproductive ability of those insects which had escaped treatment. The combination of a species specific attractant with an effective, rapidly degraded chemosterilant seems particularly feasible since the attractants are currently available for some species of insects and are being developed for many others. Further more, chemosterilants are available which could be used in a restricted manner under closely controlled conditions. Saltz 2013)¹³(Giray1994)³⁸

Genetics Mass Production of Insects

The rearing of a few thousand insects daily or weekly usually presents no difficult problems with most species. However, multiplying the numbers to 10000000, 50000000 or 100000000 insects per week presents technical and economic problems that can be solved only with ability and imagination. Obviously, one of the first requirements of any genetic control programme involving insect release is to learn how to rear the species in the required numbers. (Whitfield et al.. 2006)¹⁶

One factor which is not often recognized is that the cost estimates based on rearing small numbers of insects are not always good for estimating the cost of rearing huge numbers. Once many of the procedures are mechanized and a standard procedure is adopted, the cost of 1000000 insects may not be directly proportional to the cost of rearing 10000. In rearing large numbers of insects,

special attention must be given to the stock colonies. Care must be taken to determine that colonization has not resulted in the loss of the biological characteristics that are necessary for the released progeny to survive, disperse and find mates. All aspects of maintaining the stock colony should be linked to the production of insects which resemble the native insects in all biological characteristics except fertility. (Rittsch of, 2018)¹⁰

In large scale rearing operations, many of the procedures for handling the various stages of the insects are mechanized. There is an urgent need to develop additional devices to separate the sexes of mosquitos because it would not be wise to release large numbers of females even though they were sterile. An alternative possibility is the use of genetic mechanisms which would eliminate one of the sexes before the adults emerge. The use of genetic factors such as sex-influenced or sex limited let a.s is a promising feature which could be developed and incorporated into genetic control programmes. This would make possible the rearing and release of unisexual batches of insects. However, until these genes in the proper vector species are available, reliance upon mechanical means of sex separation will be necessary. These techniques should be developed immediately for those vector species that are prime candidates for genetic control.

A further consideration in the mass rearing of insects for release is the production of insects which may be more vigorous than the native insects because of heterosis or of the selection of more vigorous strains. Since the success of any genetic control programme will be dependent on the survival, (Stevenson 1997)⁴¹ dispersal and mating of the released insects, attention should also be focused on the possibility of establishing two inbred strains of the candidate species in the laboratory and of crossing these lines to produce the insects designated for release.

In striving for the production of the greatest number of insects at the least possible cost, it is imperative to guard against the sacrifice of quality for quantity. There is a constant danger in control programmes that when mass rearing is turned over to factory methods of rearing the quality of the insects produced may severely deteriorate. The released insects should be strong, vigorous and able to disperse and compete with the native insects for mates. Only in this manner will the genetic control method be successful in eliminating the last members of the fertile native population. (Huang 1992)³⁹ (Johnson et al. 2009)²³ (Smith 2014)²¹

RESULT

Experimental Area

Experience to date has shown that the selection of a site in the initial field evaluation of the male release technique is of great importance. Ideally, the site should be an island or an area on the main land as isolated as possible so that infestation does not occur or is so slight that it does not distort the evaluation of the experiment. The size of the area will vary according to the species and will depend on rearing capabilities, flight range of the vector and technical and other assistance available. Harpur (2012)³⁷ Harrison1986)³⁴

Natural Population Density Estimates

One of the most important requirements to be considered in developing male-release methods is information on numbers of insects per unit area. The approximate population of an insect species in an area must be known in order to determine the number of males to be reared and released. These population estimates can be determined with varying degrees of success by mark-release-recapture traps and collections of pupae, larvae or eggs. The release of insects bearing genetic markers into the natural environment presents another possibility for estimating population size. With most species, research is needed to develop more accurate methods. Rearing and releasing of tagged or genetically marked insects into the natural population and recapturing them in traps or by hand collection is useful. By studying the ratio of tagged to non-tagged specimens and applying mathematical formulae, a fair appraisal of numbers in the area can be attained.

Studies must also be made of vector incidence on a seasonal basis to determine the low point of the mine the necessity for any further reduction in population that may be required before releasing the males. Releases should start before or at the onset of the lowest population level of the year. (Peter son 2013)²⁸, Henry (2012)²⁶

Method and Efficiency of Reducing the Population Density

Obviously the natural population must be low enough so that the rearing capability can produce the species in excess of the numbers occurring in nature. In most cases it is likely that insecticide treatments or sanitary measures will be necessary to reduce the population to a level where the cost of rearing and releasing will be more economical. The costs of larval and adult control will usually be

lower than the cost of rearing and releasing insects when the natural population is high. The combined use of insecticides, sanitation and genetics makes this approach to vector control or eradication a truly integrated system. (Zhong 2003)³¹

Dispersal of Sterile Males

Insects may be distributed by ground or air or both, depending on the species, size of area, terrain and flight range. Usually, adults are the best stage for release but for certain species the liberation of eggs, larvae or pupae may be satisfactory. If aircraft are used to fly a grid system, local areas of extremely heavy infestations may have to be treated by ground systems or by additional localized treatments from the air. (Steinnauer et al. 2011)³⁰

The timing of release must be studied for each species. Consideration must be given to sexual maturity, and the time of day as well as the time of year during which to release the insects. (Decourtye et al. 2004)²⁷

Problems in the Development and Application of Genetic Techniques

The application of genetic methods of insect control requires highly capable supervisors. The concept is new and highly sophisticated and involves knowledge in many areas including the species' biological behaviour, ecology, genetics, radiation biology, mass rearing techniques and insect reproductive physiology. The preliminary research and development of pilot projects must seek the best trained entomologists and other scientists to initiate and carry out the programme. Special efforts should be directed to the training of competent technical personnel in the country where a project is contemplated. Adequate facilities must be available or constructed even for small scale demonstrations or evaluations. This should not present major problems in small scale evaluations but might be costly in major eradication projects. (Harrison 2002)²⁵

The gaps in our current knowledge on population dynamics, dispersal behaviour and biological assessment techniques are considerable for all the species under consideration. Research should be started as soon as possible on population dynamics and field ecology in the local area on any species for which there is a good possibility of early field evaluations.

There is, of course, little available knowledge on mass rearing techniques for most vector species. Research on this could be encouraged and started in selected areas. Perhaps a goal of learning to rear 50000 specimens a day might go a long way to

developing methods to bridge the gap to millions per day. (Caside et al. 1962)⁶⁷ Johnson et al. , 2006)⁵⁷ Rooke et al. 2020)⁹

Culex Papiens Fatigans

This mosquito species with a world-wide distribution is the major vector of filariasis in most parts of South-East Asia. It is very closely connected with human habitations and is most abundant in man-made breeding places with a high degree of pollution. Owing to urbanization in many parts of the world, the numbers of this mosquito have increased in an alarming way. Sanitation measures have not kept pace with building developments in many cities and towns. Therefore, the danger of filariasis infection is ever increasing. The natural vigour tolerance and the rapid appearance of resistance to insecticides in this mosquito make the development of other control methods imperative. (Liu 1992)⁶

Cytoplasmic incompatibility

Source and synthesis of incompatible strains. The crossing of members of allopatric populations of the *Culex pipiens* complex may result in the production of offspring when the crosses are made in either direction or in one direction only, or the crosses in both directions may be infertile. This phenomenon, involving the lack of development of offspring in one or both directions, has been termed cytoplasmic area of distribution of the *Culex pipiens* complex incompatibility. It has been found throughout the world. There is only one difference between a normal and an incompatible cross. In a compatible cross there is a normal development of the embryo after mating (insemination of the eggs, meiotic division of the egg nucleus and fusion of the haploid egg and sperm nuclei follow the normal course). In an incompatible cross the sperm nucleus is blocked before it can fuse with the egg nucleus. If any development of the embryo takes place, it is in the haploid condition, resulting entirely from the egg nucleus. Blockage of the sperm is a trait which is inherited through an agent (plasma gene) in the cytoplasm of the egg and cannot be changed by chromosome replacement. It remains constant for indefinite numbers of generations in the female line. (Scott et al. 1999)⁴⁹

Owing to the fact that there are at present more than 20 different crossing types known in the *Culex pipiens* complex, it is possible to synthesize at least one or sometimes several strains of the complex which are incompatible with a certain population of *Culex pipiens* anywhere in the world. Introduction of desirable genic traits is possible without a change

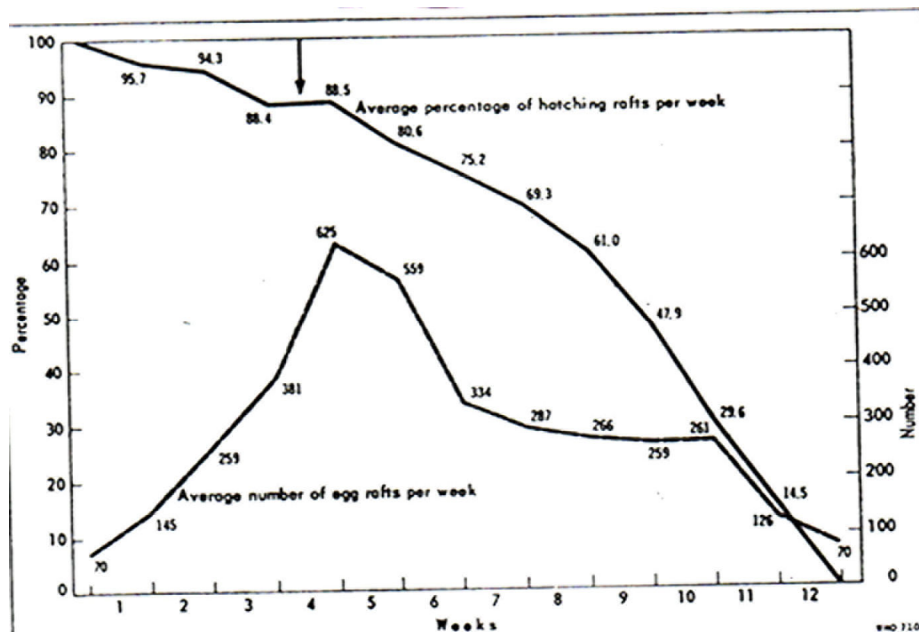
in the cytoplasmic incompatibility. (Rinkerich, 2012)⁵⁵

Cytoplasmic incompatibility appears to provide a suitable mechanism for the control of *Culex pipiens fatigans* by the release of appropriate numbers of incompatible males. Cage experiments have shown that females cannot discriminate between normal and incompatible males. Laboratory populations have been eradicated by the release of appropriate numbers of incompatible males.

Okpo experiment. To explore the possibility of utilizing cytoplasmic incompatibility for the control of *Culex fatigans*, WHO sponsored a pilot experiment at its Filariasis Research Unit at Rangoon, Burma. This work was done with a strain (D1) developed at the Institute for Genetics, University of Mainz, Germany. After a careful survey of breeding places, egg laying sites and estimation of the population size, the village of Okpo about 10 miles (16 km) north of Rangoon was selected. Surrounded by completely dry rice fields during the winter season, this village provided an ecological island. The mosquito population size was estimated to vary, according to the fluctuations

in breeding places, from a minimum of 4000 to a maximum of 20000 on a given day. (Soott, 1999)⁴⁹

The actual pilot experiment with the release of incompatible males was undertaken between February and May 1967; the accompanying figure shows the course of the experiment. During the first 4 weeks, a low, inadequate number of incompatible males was available for release. As a consequence, the percentage of non-hatching rafts from incompatible crosses remained low about 11.5% or less. Soon after the release of the optimum number of 5000 incompatible males per day began, the percentage of non-hatching rafts increased to an average of 19.4% during the fifth week. During the sixth week a slight increase to 24.8% occurred. In the seventh week 30.7% was observed. From then on the percentage of non-hatching rafts increased rapidly. This went up to 39.0% during the eighth week, to 52.1% during the ninth week and 70.4% during the tenth week. During the eleventh week it reached an average of 85.5% and finally in the twelfth week 100% of rafts were non-hatching. No more adults were expected to emerge after about 10 days. (Gilbert et al., 1973)⁶⁴



The Course of Eradication of *Culex fatigans* in the Village of Okpo, Burma

The results of this pilot experiment show clearly that the incompatibility principle can operate in nature and that with the release of an adequate number of incompatible males, a *Culex fatigans* population was eradicated in about 3 months or 5-6 generations.⁹ Robertson, 1992)⁴⁶

Proposed experiment over extended area. Having shown that eradication of a relative small population of *Culex fatigans* is possible, the next

step would be an extension to a larger experimental area. One possibility comes immediately to mind; application of the method in the Kemmendine Experimental Area in Rangoon. This part of the township of Rangoon of about 2.5 km² has been treated with a larvicide (fenthion) and the mosquito population has been reduced to about 3%. Even further reduction of the natural population may be necessary before the eradication of the remaining

mosquitos by the release of incompatible males could be attempted. But it was felt that this area might not be sufficiently isolated and immigration of mosquitos from the outside would interfere in such a way that final extermination could not be accomplished.

Therefore an experiment in a large area or areas with suitable isolation should be undertaken. Because an incompatible strain for Indian *C. fatigans* is already available, India may be the country where the experiment for control of this mosquito in an extended area could be performed. Preparatory work on the ecology, life cycle and density of mosquitos in the village or town selected would have to be done before incompatible males were released.

If complete suppression of a population in an extended area is accomplished the problem of filling the biological vacuum thus created might become serious. It can be envisaged that with the removal of *C. fatigans* with its high density of population in most breeding places a wide open niche will be available for insects with similar ecological requirements. The possibility cannot be denied that another mosquito species could occupy the niche. It could be a harmless one, but it could also be a vector of the same importance. Therefore, the filling of a gap created by the elimination of a species should be considered. There are possibilities of breeding a non-vector strain of *C. fatigans*, less likely or with no drive to bite man. Once such a strain has been established it could be liberated in the area. But this strain should be at the same time incompatible with the surrounding original population. (Mao 2011)⁵⁸ (Liu et al.. 2005)⁵⁵ (Kammer et al.. 1978)⁶³

A model can be designed for the simultaneous eradication and replacement of a given population (strain A) by introducing a new population (strain B) which is incompatible with the original population A in both directions (i.e., $A\♂ \times B_0 = -$; $B\♂ \times A = -$). Furthermore, it is desirable that strain B should be resistant to insecticides and a non-vector. A visible genetic marker, e.g., ruby eye, included in this strain could also be very helpful to distinguish strains A and B and to estimate their numbers. (Laurino et al., 2013)⁴⁴

Operational procedure in an integrated programme. *C. fatigans* frequently breeds in tremendous numbers. Control by the incompatibility principle alone would therefore require immense numbers of males for release. It appears that it would not be feasible economically to breed in the laboratory the number of insects required for a ratio of 1:10 between local and

released males. Therefore, the initial step would be the depression of a given population by larval and perhaps by adult control. In this way a population could be reduced to the level necessary for control by male release. A theoretical model demonstrating the economic feasibility of this method is given in the Annex. (Wujy et al.. 2011)⁴⁸

Sterile-male technique: radiation

Principle. The basic concepts and principles of the sterile male technique have been discussed above. Theoretical considerations of the biology and behaviour of *Culex fatigans* place it among the species of mosquitos likely to be amenable to control by this technique. Some information on the effect of irradiation on this species is already available.

It has been demonstrated that radiation of male pupae of *Culex fatigans* with 7700 R of gamma rays rendered adults sterile with a minimum of mortality. Results in competitive mating experiments have shown that the proportion of rafts of non-viable embrocated eggs increased with rise in the proportion of sterile to normal males. (i was a et al.. 2004)⁵¹

Village experiment. A field experiment in India with released sterile males produced no reduction in larval or adult densities; with an increase in sterile males in *C. fatigans* populations, the percentage of embrocated but unhatched egg rafts increased, but not adequately. Perhaps this experiment would have been successful if sufficient numbers of sterile males had been released. This experiment indicated the possibility of using males sterilized by radiation to reduce a population of *C. fatigans* in the field.

Proposed Ceylon experiment. The desirability of additional field tests with irradiated males has been considered by the Government of Ceylon. This Government maintains a filariasis control programme, and has a valuable store of information on the distribution and abundance of the mosquito species in various localities. In 1966, a consultant of the Joint FAO/IAEA Division of Atomic Energy in Agriculture visited Ceylon at the request of the Government, to assess the feasibility of making control experiments in the area. He concluded that the ecological aspects of certain isolated areas of infestation were favourable, and that field experiments could be recommended if and when specific prerequisites were met. The prerequisites included assurance that necessary facilities and personnel would be provided for the mass rearing and distribution of insects, for the evaluation of the programme, and for the acquisition of additional

information on certain aspects of the ecology and biology of *C. fatigans*. (Kammer, 1978)⁶³

Sterile male technique: chemosterilization

Chemosterilants provide an alternative to radiation for sterilizing the insects to be released. Sterility in *Culex fatigans* has been induced by exposure of the larvae in water treated with various compounds, including apholate, tepa and metepa, but the concentrations required to cause complete sterility usually cause some mortality in the larval or pupal stages. Sterilization by treatment in the pupal stage has not been demonstrated with this species; however, *Aedes aegypti* has been sterilized by treatment of the pupae with thiotepa. The induction of complete sterility with the least undesirable side-effects has been obtained by treatment in the adult stage. Both males and females have been sterilized by feeding them treated food, by exposing them to treated surfaces, and by dusting or spraying them with various chemosterilants. (Johnson et al., 2006)⁵⁷

Auto-Chemosterilization

If a sufficient number of mosquitos could be attracted to light or bait traps which contained a chemosterilant, the insects might be sterilized before escaping from the trap. Traps have been designed for this purpose. Using a CDC trap fitted with an ultraviolet light source, mosquitos were drawn into a chemosterilizing chamber treated with tepa. *Culex fatigans* adults escaping from the chamber showed about 87%-93% sterility. A pilot field experiment is currently in progress on a *C. fatigans* population in a forest near Gainesville, Florida. (Liu 1992) casida 2011)⁵²

ANOPHELES

General

At present, 4 species of anopheline mosquitos, *A. gambiae*, *A. albimanus*, *A. stephensi* and *A. bala bacensis*, appear to warrant more detailed investigations on the feasibility of genetic control since they have not always proved amenable to control in problem areas by means of the conventional method of residual insecticides. Sufficient data are at present available to warrant genetic control investigations only in *A. gambiae*. Some genetic studies have been carried out on *A. albimanus* and *A. stephensi*, but practically nothing is known about the genetics of *A. balabacensis*. Because of their obvious importance, genetic and cytogenetic research on these species should be encouraged and supported. (Jones et al., 2006)⁵⁴

Anopheles gambiae

On the continent of Africa, *A. gambiae* is the principal vector of malaria. House construction materials, daily living patterns and periodic movement of the human populations, ecological factors, insect behaviour and resistance to insecticides have all contributed to the overall lack of effectiveness of conventional control methods.

The *A. gambiae* complex consists of 5 sibling species. All the 20 possible crosses between these 5 species result in hybrid male sterility to varying degrees. F1 males of these crosses have atrophied testes, yet show normal mating behaviour. When these males mate with normal females of any member of the species complex, no offspring are produced. The sterile males are vigorous and competitive in mating ability at least under cage conditions. Among the 20 possible crosses between the 5 species, 6 produce F1 progeny that are predominantly male. A cross producing all male, or nearly all male, sterile progeny eliminates any need for the separation of sexes in the mass production of insects for genetic control; moreover, introduction of the experimental population into the wild population can be made in any stage, including the egg. (Mao, 2011)⁵⁸

In laboratory cage experiments, sterile hybrid males were added to normal males and females in varying proportions. These introduced males successfully reduced the number of viable eggs laid by the females. More recent cage experiments have been carried out using sterile and dieldrin resistant. These were equally successful in reducing the females from a cross producing virtually only males. tility of females. An extension of these experiments in which bowls of normal larvae were seeded with eggs from this cross (known to produce virtually only males) was also successful in reducing the number of females laying viable eggs when the adults emerging from these bowls were allowed to mate in cages. (Sehali 2000)⁴⁵, Rinkevich 2013)⁵⁰ Johnson et al. 2006)⁵⁷

In the light of these experiments, it now seems appropriate for a preliminary field experiment to be made in a restricted locality in Africa. Initially this would involve selection of a suitable test site and the testing of hybrid males against females of the local species. Further tests would have to be made to compare hybrid males with the local males with respect to mating competitiveness and other behaviour patterns. Detailed studies of population structure, population density and related ecological factors would be needed for the local mosquito population. (Iwasa et al., 2004)⁵¹

Once these parameters had been determined, field trials with F1 sterile males could be carried out on a limited basis. This could be done in two ways. Initial releases of adult males should be made, so that the immediate effect upon the population could be determined. And, after an appropriate time, eggs which would develop into sterile F1 males should be distributed in as large numbers as possible, in local breeding sites. In both cases, the effectiveness of competition by the sterile males should be tested by assessing the percentage of eggs from females inseminated by native males which have the potentiality to hatch. (Johnson et al., 2006)⁵⁷

Preliminary experiments to develop sterile hybrid males have been started at the London School of Hygiene and Tropical Medicine.

DISCUSSION

This species is a major vector of the arboviruses that produce yellow fever, dengue and haemorrhagic fevers. In the past decade, epidemics of haemorrhagic fever in cities in South-East Asia have presented a major public health problem. The resistance to the chlorinated hydrocarbon insecticides in the *A. aegypti* population in these areas as well as in South and Central America and in the Caribbean islands has created a serious obstacle to control or to eradication or both. Extensive investigations are now being made which should lead to the development of an improved control methodology including both chemical and biological measures, either alone or on an integrated basis.

A. aegypti is a promising subject for genetic control since it is easily maintained and manipulated, and genetic knowledge of this species is relatively well advanced. Several mechanisms potentially useful for genetic control have been discovered. In addition, the limited dispersal of adults from a breeding source is advantageous for field studies. (Haarmann et al., 2002)⁶² (Kent 2008)¹²

Methods for mass production

Techniques for mass production of *A. aegypti* are simple, efficient and economical and have been used to produce about 1000000 mosquitos per week on a routine basis. In mass production techniques, the application of genetic methods should be used to facilitate production and to improve the uniformity and quality of the product. Heterosis has been shown to reduce development time, to improve synchrony of pupation and emergence

and to increase vigour, fecundity, longevity and competitiveness. Where male preponderance is desirable, the sex ratio can be distorted by genetic means to produce 90% males instead of the expected 50%, thereby increasing the production capacity for males by 80% Genetical methods also make it possible to incorporate into the mass produced populations many of the characteristics of the field population that are essential to successful competition and integration. (Liu 1992)⁶¹

Candidate genetic mechanisms

(1) In the production of sterilized males of *A. aegypti* the sterilizing technique (use of chemosterilants or radiation) preferably should be applied to the pupal stage. At this point, the sexes can be accurately and rapidly separated so that only the males are treated and the females remain for colony production. Further, for pupae, the handling time and space requirements are reduced and in this stage the insects are readily transported to release sites at some distance from the production source.

Pupae of *A. aegypti* are easily sterilized by certain chemosterilants or by radiation. Recent work with exposure of pupae to thiotepa solution for a few hours appears to offer a successful method involving little mortality or adverse side-effects. This technique also has a minimum effect on the survival and competitiveness of the treated insects in the field. (Mao, et al., 2011)⁵⁸

The irradiation of *A. aegypti* pupae by cobalt 60 has produced sterile males. In laboratory tests it was shown that when the ratio of sterile to normal males was 20:2 the viability of the eggs dropped to 1.5%. Release of such irradiated males in a field study did not prove successful but the absence of effectiveness may have been due to the reduced competitiveness of the released males or to the pattern of release or both. (Liu, 1992)⁶¹

(2) A meiotic drive factor distorts sex ratio in mosquitos. The level of distortion varies in different strains and may result in the production of 90%-100% males. When released into laboratory populations, distortion effects may persist at a high level, establish an intermediate plateau or disappear after a few generations depending upon the sensitivity of the test population. To discover appropriate field populations for genetic control by distortion of the sex-ratio, samples should be collected from various areas of the world and tested for susceptibility to distortion of the sex-ratio. A radiation induced chromosomal translocation transmitted by males will prevent 80% of eggs from

hatching. The effectiveness of this sterility factor in depressing populations is being laboratory tested. Several genes are known to confer sex limited sterility in *A. aegypti*. Combining such genes with a meiotic drive chromosome might cause the deleterious factor gradually to become predominant in the field population.

Genes affecting vectorial capacity, such as those already discovered for *Brugia malayi* and *Plasmodium gallinaceum*, are also of interest. Theoretically, a gene conferring an inability to transmit a virus could be incorporated into a field population by mass release of laboratory reared carriers, especially when such populations are at a low level. The use of these genetic mechanisms might be preferable to the conventional sterile-male technique. These mechanisms avoid the reduction in mating competitiveness from radiation and the potential health hazard from chemosterilants. Moreover, self propagating factors such as the sex-ratio distorter and the chromosome translocation may continue to act in populations, even beyond the generation in which they were released. (Gilbert et al. 1973)⁶⁴

Reproductive biology

To date there has been only one field experiment on genetic control of *A. aegypti* and it was unsuccessful. In the Pensacola project of the Public Health Service in the USA, 4 770 000 radiation sterilized males were released but no significant reduction in the field population occurred, most probably because the radiation dose used was high and the treated males were not competitive. It therefore remains to be demonstrated whether released males can successfully compete with wild males. Female mosquitos are inseminated only once, even though multiple copulation occurs. It has been suggested that females mate initially with males from their own breeding site; such females would then be unaffected by released males. However, insemination does not take place until 2 days after the female emerges. This fact should improve the chances of successful competition by released males. (Whitefield et al. (2006)¹⁶ Cartner et al. (2019)¹¹

Experiments to evaluate the competitiveness of released males should be made in the near future. A dominant genetic marker gene can be used to identify the effect of released males upon the wild population. The recovery rate of the marker gene in field collected eggs will measure the competitiveness of the released males and this in turn would contribute to the assessment of the feasibility of any male release technique in

the control or eradication of this species. Similar experiments could be used to evaluate different patterns and methods of release. Marker genes for such experiments are available from the University of Notre Dame, Indiana, USA. (Decourtage et al. 2005)⁴⁷

Aedes Scutellaris

Members of the *A. scutellaris* species complex are the primary vectors of filariasis in many islands in Oceania. They are especially difficult to control with insecticides because of the relatively inaccessible larval habitats. The complex includes a number of species, subspecies and races of doubtful status. Non-reciprocal fertility exists between some of the members of this complex. This barrier to crossing is due to cytoplasmic incompatibility comparable to that found in the *Culex pipiens* complex. This incompatibility phenomenon might be used for genetic control. (Liu 1992)⁶¹ Kent 2008)¹²

Mass production techniques are available for other closely related *Stegomyia* mosquitos with a similar laboratory biology. Mass produced incompatible males should be released into an experimental area containing a population of the opposite crossing type. Suitable island areas are available for this experiment which should involve the establishment of laboratory colonies from different islands. Incompatible crossing types should be sought by hybridization experiments. Mass production methods should then be adapted for this species complex and a pilot field trial could be considered. (Smirle et al. 1988)⁶⁵

Tsetse Flies

Tsetse flies are serious pests of man and animals over extensive areas of Africa. Species of these biting flies are vectors of trypanosome infections of both man and animals and, consequently, prevent the economic development of large areas of Africa. Current methods of control are costly and time consuming, particularly where eradication is the aim. The sterility method of control, especially when integrated with existing methods, offers promise as a means of more effective and more economical tsetse fly control. The theoretical potential of the method has been given considerable attention.

Current research has shown that two species of tsetse fly, *Glossina morsitans* and *G. pallidipes*, can be sterilized in the laboratory by the chemosterilants tepa and metepa or by gamma radiation. Laboratory tests and limited tests in field cages show that sterilized males are competitive with untreated males in mating. Chemosterilants can be administered via tarsal contact with residual

deposits and by contact sprays. (Rittschof et al. 2018)

Gamma radiation (Co) experiments with *G. morsitans* pupae produced adults which were sterile but which had reduced longevity. However, irradiation of older pupae or young adults did not adversely affect longevity. Females were more radiation sensitive than males. Eggs failed to develop in irradiated females, whereas mature and motile sperm was transferred by irradiated males but this sperm was non-functional. Preliminary experiments with *G. pallidipes* gave similar results.

Under the auspices of the Agency for International Development, the International Atomic Energy Agency, the US Department of Agriculture and the African Research Council, a small-scale pilot test is in progress to evaluate the feasibility of releasing sterile males in combination with the use of insecticides for the control of tsetse flies. Research was first conducted to determine the population dynamics of *Glossina morsitans* on an island in Lake Kariba, Southern Rhodesia. A protocol was developed for the application of two rounds of insecticide treatment followed by the release of chemosterilized males. These males will be obtained from pupae collected in the field. (seeley 1995)³²

Mass rearing of tsetse flies continues to present a serious obstacle to the development of the sterile-male release technique. Although laboratory colonies of a few species exist, they are not of sufficient magnitude to provide the needed flies for release. It is suggested that research be encouraged to develop methods of rearing and mass producing tsetse flies and to develop more effective means of sterilizing large numbers. More field experiments and research on attractants to collect tsetse flies for auto chemosterilization are also needed.

Genetic control of *G. swynnertoni* might be accomplished by the massive introduction of males of *G. morsitans* into its territory since these largely allopatric species cross readily in the laboratory and in nature. Mating seems to be random yet the crosses yield only a few offspring, almost all of them sterile. Research on the genetics of tsetse flies should be encouraged in order to find additional reproductive isolating mechanisms. (Kent et al. 2008)¹² Moore et al. 1997)³

Ticks

Many species of ticks throughout the world are serious pests of man and animals. These arthropods inflict annoying and debilitating bites and are capable of spreading many diseases. Species of ticks occur in widely varying ecological

habitats, parasitize a variety of host animals, and develop through diverse types of lifecycles. Current methods of protection from attack by ticks depend upon personal protection through the use of repellents and application of insecticides to the environment or to animals. (Danka et al. 1986)⁴²

The concept of genetic control appears to hold less promise for ticks than for other arthropods. Ticks do not disperse readily, their development cycle is long and many species attach to host animals before they mate. Finally, the presence of large numbers of released males would be undesirable. However, in special situations, the sterile-male technique may be of value, especially to eliminate extremely low residual populations. A proposal for the evaluation of the release of sterile males has been made to study the feasibility of this technique for control of *Ornithodoros tholozani*, the vector of relapsing fever in Iraq, Syria, Cyprus and Israel. In view of the unique occurrence of this species in isolated situations in caves, the potential of the sterile male might usefully be evaluated by developing sterilization and rearing procedures capable of permitting a small scale field evaluation of the technique.⁹ Rotherbuhler 1958)³⁶

Houseflies

The housefly has been an extremely useful species in the screening of chemosterilants. More than 5000 compounds have been screened, and more than 400 effective chemosterilants have been found, including many that are not alkylating agents. The housefly has also served as a model to demonstrate that males may be sterilized while attempting to copulate with sterilitant carrying females. Colonies selected with low dosages of chemosterilants have also shown that treatment in successive generations may sometimes result in the accumulation of reproductive deficiency and at other times in the development of low levels of resistance. Baits containing chemosterilants have given exceptional control of houseflies in some situations, and little or no control in others, depending largely on the degree of isolation. The habits of houseflies often bring them into association with human food; hence any general use of chemosterilant baits would require the use of non-hazardous compounds. If such materials can be found, baits might at some future time have a place in an organized, large scale integrated fly control programme. The most important areas for research at present are the search for safer materials and the discovery of more powerful attractants.

Flies can be sterilized by radiation or by chemicals with little loss in longevity or mating competitive-

ness, and their field behaviour suggests that the species would be susceptible to control by the release of sterile males. However, the possible annoyance from the released insects would have to be considered.

Extensive research has been accomplished on the genetics of houseflies. Future work may lead to the discovery of genetic mechanisms which might be useful in control programmes. (Kent et al. 2008)¹²

Blackflies

Black flies are important vectors of onchocerciasis and other filarial infections of man and animals in Latin America and in Africa. Cytogenetic research on black flies has shown that species complexes exist, similar to those among mosquitoes, and the individual species can be identified by chromosome examination. Most of the infested areas are well circumscribed, and the breeding is often localized in a river bed.

Blackflies might be suitable candidates for genetic control because of their restricted habitat. However, our present inability to rear these insects in the laboratory sets any such programme well into the future. (Rittsch of et al. 2018)¹⁰

Triatomids

These insects transmit Chagas' disease which causes damage to the heart and other organs in several million people in Latin America. Triatomids are difficult to control with insecticides because of their inaccessible habitats cracks and crevices in human dwellings. Perhaps populations might be controlled by the release of sterilized males which would seek out females in their natural habitat. However, both males and females bite man. Even if the basic ecological and other biological data were known and if mass production and sterilization were feasible, the acceptance of the method by the public is doubtful. Already, preliminary research on genetic control of triatomids in Venezuela has been started; research on attractants should now be stimulated since triatomids may be suitable candidates for auto-chemosterilization techniques. (Wilson 1971)¹⁵ Rooke et al. 2020)⁹

CONCLUSION

Suggestions For Future Research

Basic Research on Genetic Mechanisms

Experience has shown that when extensive crosses between strains of the same species are made, genetic isolating mechanisms are sometimes discovered. Every effort should be made to

encourage collection, maintenance and crossing of numerous strains from diverse geographical areas in order to discover useful genetic mechanisms which might be utilized in the control of insect vectors of disease.

Genetic stock centres should be established and the investigation of new methods of maintaining stocks should be encouraged. The possible existence of genes for inability to transmit pathogens should be sought and work on insect viruses should be emphasized. Further studies should be carried out on the synthesis of strains with a combination of characters suitably linked to a specific situation. For example, released males might contain an incompatibility factor, insecticide resistance, a gene for inability to transmit disease and a visible marker for ready detection. (Rooke et al. 2020)⁹

Basic Research on Ecology

Information on population dynamics of the target species is essential. Techniques for estimation of absolute density must be developed and life budget tables be constructed. In view of the paucity of information currently available in this field, ecological investigations are urgently needed. The mathematical model approach could well be emphasized during the early stages of studies on population dynamics and the resulting models could be improved as reliable data were accumulated. A good model could then be used to study the effects of varying any number of factors, so that the most important factors could be identified and manipulated.

Studies on the range and distribution of candidate populations are particularly desirable; flight ranges of the species must be determined in the area under consideration. Pilot experiments on genetic control will have to be done in geographic or ecological island situations to minimize infiltration from adjoining areas, and information on mating behaviour and site of insemination in the field must be obtained. (Live et al. 2005)⁵⁵

Mass Production Technique

Mass production techniques for species such as *Culex fatigans* and *Anopheles gambiae* need investigation in order to determine the feasibility of producing these insects by the million and to allow estimations of the cost to be made. Research on development of bio-engineering techniques and standardized methods is also needed. It is suggested that the changes which occur in colonized insects in the laboratory be studied. As far as possible, released insects should have the same genetic constitution as the field population to be controlled.

(Wolif 1998)

It would also be valuable for research to be made into ways of applying genetic technology to the production process; for example, selection procedures, heterosis, sex linked or sex limited characters can be used to produce a better product. (Sphigler et al. 2017)¹⁷

Sterilization by Radiation or Chemicals

Intensive search is needed to discover chemosterilant compounds which will not damage the genetic material but will, rather, induce aspermia, sperm immobility, or inability to mate or inseminate. Methods of chemosterilization of insects by dipping, dusting, baiting or by the use of females treated with chemosterilants, require further development, and the rate of degradation of the chemical should be determined so that insects that are sterilized by dangerous mutagens are not dispersed in the environment. The investigation of methods of auto-chemosterilization might also yield valuable results; attractants, traps and baits should be developed so that insects will be drawn to a source of chemosterilant where they may come into contact with the material and sterilize themselves. (Scott 2011)⁴⁴

Toxicological investigations also need to be encouraged, taking into consideration the cytogenetic and developmental effects of chemosterilants on vertebrates. For example, studies of cell cultures for chromosomal damage would demonstrate whether a given dose via a specific route of entry is hazardous. In addition, damage to the nervous system could be assessed by studies of behavioural responses. Suitable animals for such studies might include mice and chickens. (Danka et al.)⁴²

In radiation experiments, the total dose, dose rate and age of the organism being irradiated are important factors in determining the desired effect. Greater attention should be given to these parameters in the production of sterilized insects. The objective of the sterile male technique is to flood a field population with the defective gametes. However, complete sterility of released insects may not be advantageous because the dose required for 100% sterilization may adversely affect competitiveness. Therefore, insects that are 95%-99% sterile may be more effective in achieving control. (Elzen. et al. 2013)

Release of Sterile Insects

The pattern of release is of extreme importance and investigation is required into the manner, timing and site of release as well as the optimum

numbers, stage, age and physiological condition of released insects. Releases of males must be made in a pattern which will ensure that released insects will reach the site in the field where mating and insemination occur. The possibility of dispersal in stages other than adult needs investigation for certain species. (Kent 2008)¹²

Behaviour of strains considered for genetic control must be assessed carefully in the field for such factors as vigour, dispersal, mating competitiveness and longevity. (Abbot 1925, Johnson et al. 2009)²⁶

Priority For Investigation

Many vector species are potentially suitable candidates for genetic control. However, certain species deserve high priority because of their importance in the transmission of disease and because research on these species has revealed genetic mechanisms which can be exploited for control. There is an urgent need for further laboratory and field research leading to pilot and operational experiments on the following species: *Culex fatigans*, *Anopheles gambiae*, *Aedes aegypti*, *Aedes scutellaris*, and *Glossina* spp.

Malaria eradication programmes are seriously hampered by our inability to control by conventional methods such species as *Anopheles stephensi*, *A. albimanus* and *A. balabacensis*. While some genetic work has been carried out in these species, much remains to be done. Extensive genetic and ecological studies on these species should be undertaken as soon as possible; *A. stephensi* and *A. albimanus* are potentially suitable insects for genetic control. (Scott, 1999)⁴⁹ Liu 1992)⁶¹

A considerable amount of work is currently being carried out on houseflies. The results of these investigations may well result on the development of suitable methods of genetic control for this important vector. Certain other species might be satisfactory subjects for genetic control; these include *Culex tritaeniorhynchus*, *Culex tarsalis* and *Aedes albopictus*. Other insects such as *Simulium* and *Mansonia*, might be considered if suitable methods for mass rearing could be developed. (Haarmann et al. 2002)⁶², Kammer et al. 1978)⁶³, Caside,1962)⁶⁷, Smith, Smirle et al. 1988)⁶⁵

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