Genetic Control of Insect Pests

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PREFACE

The idea of employing genetically manipulated insects to control an insect population evolved from problems created by the use of insecticides. Indeed, resistance to insecticides could be considered as one of the prime reasons for the development of genetic control. An additional impetus was provided by a concern about the growing pollution of the environment caused by the use of persistent toxic chemicals.

The deliberate rearing of sterile insect pests on a large scale and their subsequent release into natural populations, has the added attraction of controlling only those insects which pose a threat. Thus, harmless or beneficial animals of the same ecosystem are not adversely affected, as is often the case with the use of insecticides. Moreover, insect resistance to such control methods seems only a remote possibility.

The initial success of the screw-worm campaign in the United States of America stimulated an interest in the application of such low cost methods to all major agricultural, medical and veterinary pests. This book records the results of laboratory and field trials of different control methods used against some of these pests.

The subject is now passing through a difficult period, in which the general applicability of genetic control is being questioned. Such methods may not succeed when used to control insects of a high biotic potential and which are capable of enormous increases in numbers from very low densities. In addition the logistics of control over large continental areas seem to some to be beyond man's ingenuity. What is needed at this stage in the development of these new methods are carefully planned trials in island situations free from external invasion. Increasing success under these conditions will inevitably lead to more serious consideration for their extension to continental areas.

Having spent a lifetime in the field of tropical medical entomology, the author is still convinced that insecticides have an important role in the control of the insect-borne diseases of man and that, in the way in which they are normally used for this purpose, they contribute little to any pollution of the environment. Of more concern is their cost and the organization required for their efficient application. The ever-increasing threat of resistance and the lack so far of suitable chemical and biological alternatives add urgency to a situation where even now a large section of the world's population is exposed to the ravages of preventable disease. The final solution to pest control must surely come from a combination of methods, strategically applied. One such category is likely to be genetic.

May 1974 G. Davidson

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1. Introduction

Most people agree that the control of the insect pests of man, his domestic animals and his food crops is a necessity. The control of those insects carrying human disease has led to an alleviation of the sufferings of millions. It has also undoubtedly contributed to an increase in human life expectancy and to the growth in population numbers about which there is so much concern nowadays. While there is considerable argument about whether or not man should impose his own curb on population growth by some form of family planning no-one advocates the abandonment of the control of insect-borne diseases. Many people are of the opinion, in fact, that there is room for many more individuals on this planet and that adequate food resources can be created. It is not the purpose of this book to elaborate this discussion. What seems obvious is that control of the insect pests of food crops and domestic animals is as essential as it ever was if everyone on earth is to have sufficient food. This utopian situation has never been achieved in the whole history of the human race, even in times of much smaller populations.

Insecticides, particularly the stable and persistent ones, have provided a rapid and efficient means of combatting the ravages of all three kinds of insect pests but it is precisely because of their stability and persistence, that problems of resistance and environmental pollution have presented themselves. Insecticide resistance is nature's answer to the widespread use of long-lasting chemicals whose presence constitutes a selective influence killing off those individuals not genetically endowed with protective genes and allowing those that have them to survive and pass them to their offspring. Persistence and wide dissemination combine to maximize this selective influence.

According to Brown (1971) and Brown and Pal (1971) some 130 species of arthropods of agricultural and veterinary importance have shown resistance to insecticides and 102 species of medical importance. Most of these are insects. There are in fact very few of the major insect pests that are not resistant to one insecticide or another in some part of their distribution. Once resistance appears and the insecticide can no longer be

used it has been customary to change to another, and with many insects these changes have been so frequent that alternatives are becoming exhausted. The common housefly (*Musca domestica*) is a prime example. Georghiou (1971) cites a single population in California resistant to DDT and to the following organophosphates: malathion, diazinon, ronnel, fenthion, naled, dimethoate, zytron and dichlorvos. In other populations resistance to cyclodiene chlorinated hydrocarbons, e.g., BHC and dieldrin, and also carbamates, e.g., carbaryl, isolan and propoxur is known and there was even a case of resistance to pyrethrins in Sweden (Davies *et al.*, 1958). Among agricultural pests the Egyptian cotton leafworm (*Spodoptera littoralis*) is beginning to compete with the housefly in showing resistance to DDT, cyclodienes, organophosphates and the carbamate, carbaryl.

The use of insecticides in the field of public health has been largely confined to the restricted habitat of the human dwelling and has had comparatively little impact on non-target organisms. This is in complete contrast to their use in agriculture where harmful and beneficial creatures alike have been affected. In fact many of the cases of resistance in insects of medical importance have resulted from the contamination of their breeding places with insecticides used to spray crops. The most recent example is the first recorded instance of mutliple resistance involving organochlorine, organophosphate and carbamate insecticides in the malaria vector, *Anopheles albimanus*. Georghiou (1972) describes a population of this mosquito from El Salvador resistant to DDT, dieldrin, the organophosphates: parathion, methyl parathion, malathion and fenitrothion and the carbamates: propoxur and carbaryl, and attributes the organophosphate and carbamate resistance to the intensive use of these insecticides for the spraying of cotton crops and to a lesser extent of rice and corn. Cotton is treated up to 30 times during the 6-month growing season with such insecticides as parathion, methyl parathion, malathion azodrin, trichlorfon, azinphosmethyl, DDT, carbaryl and others against cotton leafworm, fall armyworm, boll weevil, cabbage looper, *Aphis gossypii* and other pests, and most of the insecticide is applied from the air.

Insecticides, then, have contributed to environmental pollution, though more from their use in agriculture than in the control of insect-borne diseases. Rachel Carson (1962) in writing her book "Silent Spring" brought the seriousness of the situation to the general public in the exaggerated and emotional way of presenting the spectre of a world without birds, bees and butterflies. Appearing at a time when resistance was becoming commonplace it served to reinforce the pressing need for alternative methods to those concerned in the control of insects. However, the readiness with which those concerned took to the use of insecticides after the Second World War was in itself an indication of the relative

inefficiency of existing methods. While control operators will undoubtedly take another look at existing and tried methods they will undoubtedly prefer new techniques of comparable efficiency with insecticides.

Special crop culture practices and the search for resistant crop plants will intensify in importance as will general environmental sanitation measures aimed at the reduction of the breeding places of insects of public health importance. These are long-term and often very expensive solutions and far from universally applicable. Biological control methods involving the release of predators, parasites and pathogens have had their successes but there have been many failures also. Knipling (1972) attributes some of these failures to deficiencies in release levels and thinks there is increased likelihood of success now that we know so much more about the mass-rearing of different organisms. Their use is particularly appealing to him as they are methods which are most efficient when pest density is high. However, there is always the uncertainty of whether or not these agents will restrict themselves to the target pests, and in the case of pathogens, and perhaps of parasites too, evidence of a simple genetic basis of susceptibility is accumulating. This implies that refractory individuals already exist and these will of course survive just as insecticide-resistant individuals have done.

Attractant traps have long been recognized as potential safe methods of insect control but really efficient and specific ones are still a rarity. The practical role of natural juvenile hormones and their synthetic analogues is as yet uncertain, and already there are indications of "cross-resistance" to them being shown by insecticide-resistant populations of the housefly (Cerf and Georghiou, 1972) and the flour beetle (*Tribolium castaneum*) (Dyte, 1972).

This book deals with a completely new concept in insect control—the use of insects to control themselves. For the most part it entails the mass-rearing, sterilization and release of populations in the hope that these will mate with wild populations leading to reductions in fertility and perhaps to population elimination. More involved techniques exist which make use of naturally existing population incompatibilities and techniques which can result in population replacement rather than eradication, the intention being to render such replacement populations harmless beforehand by genetic manipulation.

By their very nature these genetic control methods are species specific and non-polluting. Where they lead to population elimination there may be an "upset in the balance of nature" though so far as is known the successful eradication of insect pests in the past by other means has not led to any major catastrophe in this direction. Genetic control methods have the advantage over most other methods of being most efficient when the target insect is in low density as the released insects have the capacity,

if they are competitive, to search out the wild populations. However, they are least efficient against those insects with a high reproductive potential. Against such populations they are best used in seasons of low population numbers or in combination with other methods designed to reduce population numbers.

2. The Principles and Dynamics Involved in the Sterile Insect Technique

The genetic control methods to be considered in this book mainly concern the release of sterilized, sterile or incompatible insects into wild populations with the aim of producing a proportion of sterile matings high enough to result in a significant reduction in wild population size and possibly even in its elimination. Exposure to ionizing radiation or to certain chemicals are ways of deliberately sterilizing insects. Hybridizing closely related species is another way of producing sterile insects, while the incompatibility method involves the release of one sex of certain species which is perfectly fertile with its own opposite sex but incompatible with the opposite sex of another population of the same species. While all three methods owe their sterilizing effects to different genetic mechanisms their dynamic effects are identical. As we shall see this is not the case with the other methods to be considered. Translocations, for example, produce an inherited incomplete sterility. Here the dynamics are more complicated and while in theory they can result in population eradication, they seem more likely to result in population replacement, though this in itself may be a useful attribute. In fact deliberate population replacement without the use of translocations will also be considered. Finally, meiotic drive and sex-distortion mechanisms have a peculiar place of their own with some characters in common with all the other methods. Thus what is to be said in this section on principles and dymanics applies in the first instance to deliberate sterilization, hybrid sterility and cytoplasmic incompatibility but will also have considerable relevance to all the other methods.

The principal requirement for success of all genetic control methods must be the production of sufficient numbers of healthy, competitive (though genetically different) insects and their release in the right place at the right time for them to mate successfully with wild insects. Success will depend on a knowledge of how to rear the species on a large scale, how to sterilize or otherwise genetically manipulate without affecting

mating ability and competitiveness, and a detailed acquaintance with the general ecology and bionomics of the insect to be controlled.

I. Mass-Rearing

The principle of the sterile insect technique involves the inundation of the wild population with sterile insects. Thus a method of rearing the insect in question in large numbers is an essential. Obviously the ideal insect for such large-scale culture is one with a high biotic potential, with a short life cycle and with simple food requirements. In this context high biotic potential is taken to mean that the female is readily fertilized in captivity, lays large numbers of eggs at frequent intervals and lives for a long time and that the immature stages do not suffer high mortalities. Although it is conceivable that mass-rearing could be accomplished with some species from the continuous capture of wild, fertilized females and the rearing of their progeny, advantages accrue from the setting up of self-perpetuating colonies. One of these is the ability to control the incidence of pathogenic organisms and another is to prevent the occurrence of diapause, common in Lepidoptera for example. Diapause is usually initiated by a change in day-length. Keeping a colony under standard conditions of photoperiod may prevent its occurrence. Colonies can be maintained under "aseptic" conditions, though these can seldom be stringent. Containers and food can be autoclaved and various antimicrobial agents such as antibiotics, fungicides, benzoates, sorbic acid, formalin, mercuric chloride, ethanol, sodium hypochlorate, etc., used either as food additives or for the washing of eggs.

Ideally the food for mass cultures should be nutritionally adequate for the species concerned and attractive to it. It should also be cheap and easily prepared. Different diets may be required for adults and immature stages. Attractants may help to encourage feeding on unfamiliar diets and incitants may be needed to release the biting response. These may be particularly necessary in the case of caterpillars accustomed to feeding on the edge of leaves for example. Feeding stimulants are usually provided by some of the diet constituents. Blood-feeding insects present special though not insurmountable problems. Seldom are they restricted to their usual host however, and membrane feeding on citrated, oxylated, heparinized or defibrinated blood can often be substituted for the use of live animals.

A common difficulty in the establishment and maintenance of colonies is to produce confined conditions allowing natural mating behaviour. Encouragement of mating may be achieved by light quantity and quality adjustments, but as we will see later, selective processes may also be involved which eventually produce alterations in mating behaviour. Oviposi-

tion may also present a problem whose solution may lie in experimentation with different oviposition sites or in the use of specific attractants.

For the production of really large numbers, standardization of rearing methods and some system of automation may be necessary. Counting devices ensuring equal densities of individuals to be reared and precise periodic deliveries of quantities of food related to stage of development are some of the requirements, as well as means of separation of the stage needed for release. Where one sex only is to be released accurate mechanical sex separation aids are an advantage.

There can be no doubt that the continuous maintenance of such colonies under such artificial conditions and the attempted production of as a high a yield as possible must lead to the eventual emergence of insects differing in many respects from those occurring under natural conditions. Numerous geneticists have been highly critical of this emphasis on quantity and uniformity e.g., Coluzzi (1971), Boller (1972), Mackauer (1972) and Boesiger (1972). They all emphasize the loss of heterozygosity through colonization. Mackauer (1972) points out the importance of using a large number of individuals from the centre of distribution of the species for the foundation of the colony. Boller (1972) instances the genetical bottleneck through which most colonies pass during their establishment—a difficult initial period followed by adaptation to the artificial conditions, during the course of which there must be considerable loss of genetic diversity. Boesiger (1972) is of the opinion that while initial marked reductions of population size may result from releases of mass-reared sterile insects, difficulties will arise when the population is reduced and dispersed. The remaining insects may be those which have evaded the attentions of the released individuals and he envisages a kind of resistance to genetic control —a selection in the wild for individuals which will not recognize and mate with the introduced ones. Coluzzi (1971), considering the mass-rearing of mosquitoes, favours a diverse founder colony and insectary conditions simulating field conditions as closely as possible at least with regard to daily changes in light and temperature. All these authors comment on the trend away from diversity resulting from inbreeding in colonies. Craig (1964) considering the mass-rearing of *Aedes aegypti* suggests the keeping of two colonies and the crossing of them to produce material for release. This would produce a high degree of hybrid vigour if the two parent colonies have been inbred for some time. As we shall see when we come to consider cytoplasmic incompatibility and translocations, it is possible by outcrossing to wild populations to transfer the genetic modifications involved to a wild genetic background and thus, theoretically at any rate, eliminate many of the differences accumulated during the isolation of the genetic changes.

What has not been considered seriously is the deliberate selection in

mass-rearing procedures for those characteristics which might contribute most to the efficiency of the sterile insect technique. The most obvious characteristic is mating efficiency. Apparently it is not difficult to change the mating speed in *Drosophila melanogaster*. Manning (1961) selected both slow and fast lines through 25 generations and produced a difference of 80 min. in the slow line and only three minutes in the fast one. Both sexes were affected by this selection. Personal attempts by the writer to improve mating efficiency in *Anopheles gambiae* species A by selecting females seemed to make little difference in four selections even though the final selection was from a female which was mated within 17 h of emergence from the pupa. It remains to attempt male selection.

Gast (1968) considers mass-rearing from an economic point of view. To him the simple objec of the exercise is to produce an acceptable insect at the lowest possible cost. He shows very convincingly that it is cheaper to rear one million boll weevils (*Anthonomus grandis*) using a technique giving only 10% yield than one giving an 80% yield because while egg collection is cheap (10c/1 000) larval diet is expensive—one million from a regime giving a 10% yield cost $1 000 as compared with double that figure for an 80% yield method. Changing larval diets can lead to enormous savings in costs. Substituting cotton seed meal and sugar-cane bagasse for dehydrated carrots and yeast, reduced the cost of rearing the Mediterranean fruit fly (*Ceratitis capitata*) from $80 per million to $4 in one scheme. Colossal savings were made in the same way in the screw-worm campaign as we shall see.

There then are two extreme views—the one concentrating on quantity and costs and the other adhering strictly to the production of insects as similar as possible to the natural ones. In the writer's opinion no rearing method is free from biological criticism and the end product must differ from the wild creature no matter how much care is taken. Lowered competitiveness resulting from colonization and mass-rearing may be compensated for by the weight of numbers of introduced insects. As we shall discuss later the success of such releases may not depend entirely on successful matings between released and natural insect. Overcrowding and aggression effects may contribute. Mass-rearing followed by pilot-release trials designed tc assess competitiveness is really the only practical solution. Trying to simulate all the natural conditions could prove more costly than existing methods of control and could result in the release too few insects to have any effect.

Only a few examples of mass-rearing methods will be given here. For further details of insects of medical, veterinary and agricultural importance the reader is referred to a book by Smith (1966) and to various publications by the International Atomic Energy Agency, Vienna (see, References).

The mass-production of the screw-worm (*Cochliomyia hominivorax*) is described in detail by Smith (1967). Adult flies are kept in complete darkness in colony cages of some 50 000–60 000 individuals and the food provided for them is a mixture of ground lean horse meat and honey. To produce 150 million flies per week it is necessary to set up 18 such cages every day. When 8 days old the flies are offered a special oviposition medium and light is admitted. The oviposition medium consists of ground horse meat to which has been added an oviposition stimulant (either extract of heart or of blood albumen). This is presented in a tray containing a heating coil maintaining the temperature around body heat (37–39°C) and a battery of low-powered electric light bulbs (7·5 W) is placed on top of the medium. This acts as a further attractant and oviposition is completed in about 4 hours. Larval rearing is in trays of lean ground meat, blood, water and formaldehyde. In the main rearing plant now at Mission, Texas, the potential exists for the production of 150 million flies a week and for this some 20 000 lb. of dried blood and 200 000 lb. of meat are required. At 4–6 days the larvae are mature and migrate to the sides of the trays and eventually fall into channels of slow moving water which carry them to central collecting points where they are drained off and placed in sawdust to pupate. The pupae are then collected by a sifting process and held for 5½ days at 27°C before irradiation. Gast (1968) refers to a cost of production using this diet of $1 345 per million, and a change to a mixture of blood, fish meal and milk solids leading to a reduction to $800 per million, a saving over a year's production of 150 million flies per week of $330 000.

The fruit flies have proved particularly easy to mass-rear. Nadel and Peleg (1968) describe in some detail the methods used in Israel to rear up to two million Mediterranean fruit flies (*C. capitata*) per day. They used a cage measuring 210 × 30 × 50 cm containing 25 000–50 000 flies as a production unit. Adult food was provided in the form of a mixture of yeast and sugar and oviposition was achieved through the provision of loosely woven cloth through which the female obligingly inserts her ovipositor to let her eggs fall into a collection tray underneath. From three such cages 350 ml of eggs were collected over a period of about two weeks. At 20 000 per ml this represents some 7 million eggs. The larvae were reared in trays measuring 25 × 35 × 1·5 cm containing 1·75 kg of food, at 30 000 per tray. The yield was about 67%. The larval diet was a moist mixture of wheat bran, brewers' yeast and sucrose with two antimicrobial benzoates (Nipagin and Nipasol) added. The pH of the water used was adjusted to 4·3–4·5 by the addition of hydrochloric acid. 10% of the yield was returned to stock and one man could manage the production of nearly two million flies per day at a cost of $5 to $10 per million.

Mosquitoes are also easy to rear on a large scale. This is particularly

the case with *Aedes aegypti*, and Smith (1967) describes the methods used to produce more than 10 million males of this species over a period of 10 months at Savannah, Georgia. However, as numerous references will be made in this book to attempts to control *Culex pipiens fatigans* by the Research Unit on the Genetic Control of Mosquitoes at Delhi it is intended to expand on the mass-rearing of this species. This research unit was established by the World Health Organization, in collaboration with the Indian Council of Medical Research, in 1969 to determine the operational feasibility of genetic control techniques for the control or eradication of *C. p. fatigans* and to obtain data on the reproductive biology and population dynamics of *Ae. aegypti* required for the genetic control of this species. The studies are to last over a period of 6–7 years and may culminate in a large-scale attempt to control *C. p. fatigans* in a suburb of the city of Delhi. The anticipated cost of the project is 2·6 million dollars and financial assistance is being given to it by the United States Public Health Service (World Health Organization, 1971).

The unit has a capability of producing some 5 million mosquitoes a week, or some 360 000 male pupae per day.[1] The basic units are cages 72 × 60 × 60 cm stocked initially with 15 000 females and 5 000 males with additions of 3 000 females and 1 000 males on alternate days. 8 such cages are needed. They are kept in a room maintained at 30°C and 80–85% relative humidity with a 13 h light and 11 h dark cycle. 3–4-month-old chickens held still in a restraining device are left on top of the cages overnight on alternate nights to provide blood for the females. On the nights without the chicken ovipositing trays of water which has been previously used for rearing larvae are put into the cage. A total of some 6 000 egg-rafts are obtained each day. The larvae are reared at 31°C in plastic trays 68 × 63 × 9 cm containing 24 l of water at a density of 30 000 per tray. Measurement of numbers involves the use of tubes calibrated from the number of rafts completely filling the surface area of the contained water and assuming 200 eggs per raft. The food consists of equal parts of ground dog biscuit and brewers' yeast and is distributed at 5, 6, 13, 20, 25 and 20 gm/per tray on days 0–5. 204 such trays are available. Pupation occurs on the sixth day and separation from larvae is achieved by straining and immersing in iced water. In such water the larvae sink and the pupae float (Ramakrishna *et al.*, 1963; Weathersby, 1963). As in this species males pupate first and the primary interest is in males for release, pupae are only normally collected on the sixth and seventh days after seeding the larval rearing trays. Collected pupae are then released beneath a grid, the holes in which are of such a size that most of the male pupae pass through but very few of the female ones, and a combination of

[1] Singh, K. R. P., Patterson, R. S., LaBrecque, G. C. and Razdan, R. K. (1972). World Health Organization mimeographed document WHO/VBC/72.386.

this technique and the cropping of pupae on the first two days only produces pupae which are 95–98% male (Sharma *et al.*, 1972). Costs are reckoned at $10 per million exclusive of manpower ($40 inclusive). The unit is now organizing for the production of 70 million mosquitoes per week.

Anopheline mosquitoes are somewhat more difficult to mass-rear than *Ae. aegypti* or *C. p. fatigans* but not markedly so. The first species to be released on a large scale, *An. quadrimaculatus*, is now considered to be one of the more difficult. Yet more than 10 years ago nearly half a million males of this species were irradiated and released over a 14 month period (Weidhaas *et al.*, 1962). Since that time *An. stephensi* has been reared on a large scale for chemotherapeutic work (Gerberg *et al.*, 1968) and a very recent field release of chemosterilized *An. albimanus* has taken place in El Salvador involving the production of 100 000–120 000 pupae per day.[1] Though the difference in size of male and female pupae is not as marked in anophelines as it is in culicines it proved possible in this trial to separate fractions containing 86% males using a pupal separator (Fay and Morlan, 1959). Altogether 4 360 000 sterile males were released.

A much more modest production was achieved in the hybrid sterility field trial of Davidson *et al.* (1970). Here two species of the *An. gambiae* complex had to be reared and crossed, in one direction, to produce the sterile males. Cages of 30 × 30 × 30 cm were stocked with 600 pupae and emergent females fed on immobilized rabbits. Oviposition was on to free water containing 7 g/l of sea salt (equivalent to 20% sea water) in the case of *An. melas* and on to filter paper moistened with plain water in the case of *An. gambiae* species B. Larval rearing in flat enamel trays at a density of about one larva per square inch (6·25 cm^2) of water surface area, anophelines being mainly surface feeders. *An. melas* had to be reared in 20% sea water; species B in local river water. Food was provided in the form of a finely ground proprietary cereal baby food containing added vitamins and minerals (Farex). Pupation usually started on the eighth day after hatching of the eggs. The pupae were handpicked for the most part. The capacity of the insectary was such that approximately 3 000 *An. melas* and 2 600 species A pupae could be produced each day. Of the 3 000 *An. melas* pupae 600 were used for colony maintenance while the other 2 400 were set aside for emergence and for the isolation of approximately 1 200 virgin females (separated within about 12 h of emergence) for the cross. Of the 2 600 species B pupae, 200 were used for colony maintenance while the other 2 400 were used as the source of approximately 1 200 males for the cross. The cross between species B males and *An. melas* females was then made in cages of 300 males and 300 females. Thus

[1] Vector Genetics Information Service, 1972. World Health Organization mimeographed document VBC/G/73.1