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WORLD HEALTH ORGANIZATION

TECHNICAL REPORT SERIES

No. 268

# **GENETICS OF VECTORS AND INSECTICIDE RESISTANCE**

**Report of a WHO Scientific Group**

WORLD HEALTH ORGANIZATION

GENEVA

1964

WHO SCIENTIFIC GROUP ON THE GENETICS OF VECTORS  
AND INSECTICIDE RESISTANCE

Geneva, 5-9 August 1963

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# GENETICS OF VECTORS AND INSECTICIDE RESISTANCE \*

## Report of a WHO Scientific Group

The Scientific Group on the Genetics of Vectors and Insecticide Resistance met in Geneva from 5-9 August 1963. Dr P. M. Kaul, Assistant Director-General, opened the meeting. Dr G. B. Craig was elected Chairman and Dr H. Kikkawa, Vice-Chairman. Dr A. W. A. Brown was appointed Rapporteur.

### 1. INTRODUCTION

The campaigns promoted by WHO against arthropod-borne disease in all parts of the world have during the past 15 years relied heavily upon the use of insecticides, which even at the present time are being employed with remarkable success. However, the development of resistance to some insecticides by populations of certain vector species, as well as the intrinsic undesirability of depending exclusively upon highly persistent chemicals to provide a healthy environment, demand that alternative methods of control be considered. Besides the development of less persistent alternative insecticides, one of the most promising lines for future research and development lies in the field of genetic control. Studies on the nature of insecticide resistance, particularly those carried out since the meeting of the WHO Expert Committee on Insecticides in 1956,<sup>1</sup> have shown that the phenomenon is due to pre-adaptations which have usually proved to involve single gene alleles, and that the emergence of insecticide-resistant strains is thus a consequence of Darwinian selection. Such studies have contributed to the understanding of the basic physiological nature of gene action, and, in addition, have stimulated research on the basic genetics of vector species with particular reference to *Musca domestica*, *Aedes aegypti* and *Culex pipiens*.

This is a fortunate outcome, since the time is now ripe for the development of methods of genetic control of insect vectors, which in turn require a background of knowledge of the genetics of these species. There are tremendous potentialities for utilization of genetic mechanisms for vector control, with a possibility of success comparable to that already achieved

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\* This meeting was supported by the World Health Organization and by a United States Public Health Service Research Grant (EF. 194) to the World Health Organization.

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1957, 125.

in the control of the screw-worm fly by using the so-called sterile male method. In another programme, chemosterilants are being used successfully to control houseflies in certain islands in the western Atlantic. The fact that success was not attained in pilot experiments with irradiated sterile males of *Anopheles quadrimaculatus*, *Aedes aegypti* and *Culex pipiens fatigans* does not preclude further attempts. There is little doubt that success can be achieved if treatment techniques are substituted that are less inhibitory to mating ability and the experiments are pressed home to their conclusion. Failure of any genetic method of control at an early stage of development only serves to underline the very real necessity for a background of adequate genetic studies and knowledge. Events have shown that the rather complicated and large-scale logistics behind such operations, the effectiveness of which can still be vastly improved by better techniques, have not been more costly than insecticide operations. An additional advantage of certain genetic methods is that they may lead to eradication, not only of the disease, but also of the vector population.

The task of this Scientific Group has been to review the present state of knowledge of the genetics of vectors and insecticide resistance, to outline the prospects for future research in the former field and to assess the possibilities of the application of genetic knowledge in meeting the resistance problem and in developing new methods for genetic control of vectors.

## 2. INSECTICIDE RESISTANCE

### 2.1 Current state of research on genetics of resistance

The genetics of resistance was recommended as an important field of study by the WHO Expert Committee on Insecticides in 1956. The impetus given by this recommendation to WHO has resulted in a considerable increase in knowledge of this field of vector control. Study of the inheritance of insecticide resistance has revealed single genes to be responsible for specific resistances, and has resulted in the differentiation, on a genetic basis, between the various resistance entities, of which the principal ones are: resistance to DDT and its analogues; resistance to dieldrin, other cyclo-diene derivatives and BHC; and resistance to organophosphorus compounds. Differentiation between these entities has been achieved notably in 5 species of anophelines, and also in *Musca domestica*, *Culex p. fatigans*, and *Aedes aegypti*. In addition, the inheritance of one type or other of resistance has been investigated in about 20 arthropods of public health importance. In several cases, the position of the gene has been established on a particular chromosome, and thus the gene has aided the genetic mapping of that chromosome (formal genetics). In a few cases the gene allele has been

causally related to the detoxication mechanism responsible for the resistance (physiological genetics), and the allelism can even be shown to change the activity of a specific enzymic protein.

The character of dieldrin-resistance in anopheline and other mosquitos has permitted such a clear separation of phenotypes that it has become possible to discern the monofactorial nature of this resistance. A single gene for dieldrin-resistance has been discovered in 6 species of anophelines, 2 species of culicines, the bedbug, the body louse, the housefly, a blowfly and a cattle tick. In nearly all cases, the dieldrin-resistance was such that the hybrids were intermediate in susceptibility.

A single gene for DDT-resistance, first demonstrated in the housefly in the early 1950's, has been detected in 5 species of anophelines, 3 species of culicines and 2 species of *Drosophila*, as well as in the German cockroach and a cattle tick. The expression of the character DDT-resistance does not allow the clear separation of phenotypes that has been obtained with dieldrin-resistance. In some species, genes for DDT-resistance are recessive and in others they are dominant; even within a species they may be recessive in one strain and dominant in another, or both dominant and recessive factors for resistance may co-exist. Moreover, modifying alleles for DDT-resistance have been detected in several species.

The development of organophosphorus-resistance in recent years is alarming because organophosphorus compounds at present represent the principal alternative insecticides in the two preceding types of resistance. Monofactorial inheritance for this character has been detected in the housefly, in *Culex tarsalis*, and in an African blowfly, *Chrysomyia putoria*. In the case of the housefly, there appear to be two types of resistance, one to methyl-substituted phosphates, such as parathion methyl and malathion, and the other to ethyl-substituted phosphates, such as parathion and diazinon. In the case of *Culex tarsalis*, the resistance is specific to malathion by virtue of its ester side-chain. However, this is probably a special case because the organophosphorus-resistance that has developed in *Aedes nigromaculis* is apparently non-specific and extends to all insecticides of this group.

Resistance to carbamate compounds has not yet been detected in insect vectors in the field but has been induced in laboratory strains of houseflies. Whether this resistance is a separate entity from organophosphorus-resistance is an important problem in vector control; the evidence so far obtained indicates that the two types of resistance have certain factors in common. An increase in tolerance to pyrethrins sufficient to vitiate control measures has been observed in Swedish populations of houseflies; however, the general usefulness of this expensive reserve insecticide has not been destroyed by the development of the types of resistance outlined above. The fact that no resistance to thiocyanate insecticides has yet been recorded is perhaps worthy of mention.

The location of the genes for resistance on the chromosomes was first established in the case of *Drosophila melanogaster* in 1953. The subsequent availability of markers in laboratories concerned with formal genetics has made it possible to locate the genes for DDT-resistance, dieldrin-resistance and organophosphorus-resistance in *Musca domestica*, and for DDT-resistance and dieldrin-resistance in *Aedes aegypti*. It is hoped that similar results can be obtained with *Culex p. fatigans* and with anophelines. The value of such work lies in the establishment beyond doubt that the resistance in question is a specific genetic entity, as well as in the isolation of different resistance entities and the study of their inheritance. Work with marker strains has revealed that in houseflies DDT-resistance in different geographical strains may derive from at least three different genes on chromosome 2 and one gene that has become associated with the Y chromosome, probably by a translocation with chromosome 2. That there may be separate genes for the same character has also been suggested in work with *Anopheles gambiae*.

A combination of biochemical and genetic studies of resistance has contributed in a few instances to a basic understanding of gene action. The parallel inheritance of DDT-resistance and DDT-dehydrochlorinase in the housefly has provided evidence of gene-enzyme relationships. The reduction of alioesterase and a concomitant increase in breakdown enzyme observed in organophosphorus-resistance in the housefly has given evidence that the gene acts by converting one biocatalyst into another. The modification of carboxyesterase by the allele for malathion-resistance in *Culex tarsalis*, which produces more of a "softer" enzyme, is consistent with what has hitherto been known of the action of genes in synthesizing active proteins in micro-organisms. Further study of linkage relationships with marker genes on the one hand and their relationship with detoxication enzymes on the other promises to be the most useful line of research into the genetics of insecticide resistance. At present, studies of polytene chromosomes do not appear to have the same usefulness in this respect. Initial research indicated a correlation between an increase in inversion heterozygotes and increased tolerance to DDT or dieldrin in *Anopheles atroparvus*. However, more recent work has shown that this is not a cause-and-effect relationship, but rather that they are both themselves effects of the same cause, namely their hybrid vigour. This does not mean that polytene chromosome studies will not eventually be extremely useful in ascertaining the precise topographical location of resistance genes.

These and other studies on the genetics of resistance have added greatly to the understanding of field phenomena that have developed in vector control; whether they can precisely indicate countermeasures is problematical. An example of basic knowledge suggesting possible countermeasures is found in the study of the inability of the gene for DDT-resistance and DDT-dehydrochlorinase in *Aedes aegypti* to detoxify deuterium-substituted

DDT. Similarly, the study of the competitive inhibition of the gene-determined DDT-dehydrochlorinase in houseflies by such compounds as DMC, has led to the development of DDT synergists, to which, however, resistance has subsequently developed. The mutant carboxyesterase in *Culex tarsalis* may be inhibited by EPN, which thus becomes a synergist for malathion against the resistant genotype.

A third type of study that is a great deal more straightforward than the two outlined above is extremely useful, not only in characterizing the resistance entity, but also in uncovering alternative insecticides to counteract it. This is the determination of the cross-resistance spectrum, a technique that has been developed with much success in work on *Anopheles gambiae* and *Musca domestica*. Such cross-resistance studies are essential to an understanding of the operational implications of these resistance patterns.

The discovery of the pleiotropism of the DDT-resistance gene on chromosome 2 of *Drosophila melanogaster*, imparting enhanced susceptibility to PTU, has indicated that DDT and PTU are negatively correlated with respect to resistance and hence that resistance to either can be prevented by the use of the other. The possibility of the detection of other pairs of negatively correlated compounds has been investigated with varying success; the latest indications of negative correlation are between Sevin and DDT in the body louse and *Culex p. fatigans*, and between dieldrin and the carbamate AC-5727 in *Anopheles albimanus*. Whereas in dieldrin-resistance it is clear that dieldrin applications select for one principal resistance allele from the very beginning, with DDT-resistance and organophosphorus-resistance there is good evidence that the emergence of a principal gene takes some time. This delay indicates the necessity for concomitant selection of ancillary alleles to allow the successful survival of the resistant genotype; in addition, there is good evidence that the main gene allele increases in frequency with secondary genes, which act as modifiers and enhancers. Experience has shown that DDT-resistance in houseflies, and more particularly in mosquitos, has taken a much longer time to develop than dieldrin-resistance; often the change is sufficiently gradual to allow the vector control objective (e.g., eradication of malaria) to be attained before full resistance has developed.

These considerations also emphasize the importance of the study of the general genetic background, of the strains and populations in which resistance is developed. Investigations during the past seven years have confirmed that insecticide resistance derives from the selection of pre-adaptive alleles. This has led to the following deductions: (a) that resistance may be expected in populations where such alleles exist or may appear through mutation, and (b) that the speed of development will be directly proportional to the intensity, extent of coverage, and period of time over which selection pressure from the insecticide is maintained. Further

research has emphasized the importance of the mode of expression of the allele, namely, whether it is recessive or dominant, in affecting the speed of resistance development; in cases where the allele is recessive, development may be retarded, owing to elimination of the heterozygote. Precise quantitative assessment of such phenomena in the field appears to be necessary, but opportunities to do this have been overlooked. It seems possible to make an analysis of gene frequencies in field populations in cases of dieldrin-resistance, in which the phenotypes separate clearly and thus the use of discriminating dosages is completely valid. Such studies appear to be warranted in several species of anophelines, and notably in *A. gambiae*.

The cessation of operations with a particular insecticide may be expected to be followed by reversion of the resistant population towards susceptibility, if that population is surrounded by a large reservoir of susceptible insects. This reversion will be facilitated in cases where the resistant genotypes are at a disadvantage as compared with the susceptibles in a normal environment uncontaminated by the insecticide in question. Examples of reversal of dieldrin-resistance have been found in *Anopheles culicifacies* in west India, *A. sundaicus* in Java, and *A. gambiae* in northern Nigeria; reversal of DDT-resistance has been observed in *A. sundaicus* on the north coast of Java, and to a slight extent in *A. sacharovi* in Greece; in addition, reversal of DDT-resistance has been observed in houseflies in Denmark since 1952, but such populations can readily redevelop resistance to this insecticide when it is reapplied, presumably because of their retention of the necessary residual genotypes. Whether reversal could occur in the absence of surrounding untreated populations has not yet been precisely observed in the field.

Laboratory findings have indicated that dieldrin-resistance is liable to be more stable than DDT-resistance. It has been the experience that dieldrin-resistance is of such an intensity that it prevents further effectiveness of this insecticide. On the other hand, DDT-resistance as developed in the field has not been of sufficient intensity to prevent this insecticide from showing a certain degree of effectiveness; in fact, in certain localities where resistance to both dieldrin and DDT was present in the anophelines, the use of substitute organophosphorus compounds has been followed by a return to the application of DDT at increased dosages and shorter intervals. It is tempting to assume that the degree of resistance shown by homozygotes for the DDT-resistant gene in certain anophelines does not reach a level where it cannot be overcome by high doses of DDT.

The fact that the resistances to these two chlorinated hydrocarbons are different in nature has in the past led to the substitution of dieldrin for DDT or *vice versa*. Occasionally, mixtures of the two types of insecticide have been applied, but in the case of houseflies the application of these mixtures has led to resistance to both groups. The effectiveness of

such mixtures against anophelines has not yet been investigated. However, the following facts are known about the control of this genus: dieldrin-resistance does not carry a sufficiently high cross-resistance to BHC to prevent the latter killing the heterozygotes and thus delaying the onset of dieldrin-resistance; on the other hand, BHC and malathion, and dieldrin and malathion, give good, long-lasting kills on non-absorbent surfaces and show no preferential selection of dieldrin-resistant individuals. Whether such results would be obtained on absorbent mud surfaces remains to be seen.

One type of resistance, which has been considered of importance in anti-anopheline operations with DDT, has been loosely termed "behaviouristic resistance". One phase of this concerns a change in the characteristics of the mosquitos, which become irritated by DDT deposits and fly away from them. With the development of resistance to DDT, there may be a decrease in irritability. It appears probable that this is a simple consequence of the increased rate of detoxication that evidently accompanies DDT-resistance. It is thus a response and not a change in intrinsic behaviour and is a correlate of physiological resistance which would make them less irritable.

Another type of "resistance" included under this loose heading is increased exophily, which is an example of behaviour *per se*. The genetic study of the behavioural characteristics of vector species with respect to insecticides is therefore a reasonable possibility. The necessity for such studies appears to have the same urgency as studies on the inheritance of host-selection characteristics. These would also shed light on the selective effect of insecticides in changing populations showing mixed behaviour characteristics.

## 2.2 Research needs

1. Research on the resistances in anophelines linking them with marker genes. At present very few markers in this genus are known, but there is reason to suppose that this situation will improve.
2. Study of the genetics of organophosphorus-resistance in *Culex p. fatigans*. Marker genes are available for this study, which is particularly important in view of the work of the WHO Filariasis Research Unit in Rangoon.
3. Investigation of the potentialities of anophelines to develop resistance to organophosphorus compounds and carbamate insecticides.
4. Cross-resistance in mosquito species and other vectors between organophosphorus compounds and carbamates.
5. Investigation of the relative potentialities for resistance to the various candidate compounds being brought forward for control of *Culex p. fatigans*, *Musca domestica* and anophelines.

6. Assessment of the mutation rates of insecticide-resistance genes as compared with those of other mutant genes.

7. Studies of the genetics of resistance to a given insecticide (e.g., DDT, dieldrin, etc.) in different populations of the same species. Such studies have already commenced for DDT-resistance in *Musca* and could well be pursued for dieldrin-resistance in *Anopheles gambiae* and organophosphorus resistance in *Culex p. fatigans*.

8. Investigations of the specificity of organophosphorus-resistance in *Aedes aegypti* and other culicines with respect to individual compounds. For example, there is reason to believe that organophosphorus-resistance is non-specific in *Aedes nigromaculis* and *Aedes aegypti*. On the other hand, specific malathion-resistance is well known in *Culex tarsalis*.

9. Further investigations on the relationship between resistance genes and physiological mechanisms where the intermediary agent is a detoxifying enzyme. Studies have been commenced on the organophosphorus-resistance of *Musca* and *Culex tarsalis*, but they could well be extended to DDT-resistance in *Musca* and organophosphorus-resistance in other mosquitos, such as *Culex p. fatigans*.

10. Search for the physiological mechanisms of resistance to dieldrin, other cyclodiene derivatives and BHC. Since this resistance can be separated so clearly into three genotypes, and the hybrids are exactly intermediate in susceptibility, the elucidation of its mechanism presents a real challenge. Studies hitherto conducted indicate that detoxication is not involved, but that the mechanism of resistance may lie in the nervous system itself.

11. Work on compounds such as those negatively correlated with DDT in *Drosophila*, and on compound AC-5727 negatively correlated with dieldrin in *A. albimanus*. Work on these questions has already started and should be encouraged.

12. The search for alternative insecticides based upon knowledge of the mechanism of resistance, as for example, deuterio-DDT to remedy the monogenic DDT-resistance in *Aedes aegypti*. In looking for new substitute insecticides preference should be given to those compounds to which resistance does not develop, at least in the strains investigated.

13. Clear differences in behaviour between populations with respect to insecticides should be reported to WHO, and population samples should be referred to a competent laboratory for investigation of the genetic basis of these differences.

14. Studies of the population dynamics of resistance genes in the field to establish the frequency and speed of selection, spread and reversion. The discriminating dosages applicable for dieldrin-resistance provide an ideal tool for such investigations, particularly in anopheline mosquitos.

15. Investigation of the effectiveness against anophelines of mixtures of existing insecticides on both absorbent and non-absorbent surfaces in preventing the development of resistance to one of them, with the aid of discriminating doses to distinguish the genotypes.

16. This list is not meant to be by any means comprehensive. It is considered eminently worth while to investigate the inheritance of any insecticide resistance entity in any vector species. Such inheritance studies will always gain if performed in conjunction with one or more mutant markers, as has recently been done for *Blattella germanica*.

### 3. GENETICS OF VECTORS

The last ten years have produced a sharp increase in the amount of information available on vector genetics. In this period, a considerable body of knowledge has been gathered on the formal genetics and cytogenetics of houseflies, mosquitos and, to a lesser extent, cockroaches. Although the information is still fragmentary and scattered, several important areas have been elaborated, as for example the genetic studies of insecticide resistance in the genus *Anopheles*.

The stimulus for the increased tempo of activity in vector genetics has derived primarily from two sources: (1) the basic need for information on the genetics of resistance, and (2) the fact that many of the vectors have been found to be excellent material for fundamental scientific studies in many areas of genetics.

#### 3.1 Formal genetics

This term is taken to imply the fundamental study of the inheritance of characteristics and mutagenesis, description of mutants, preparation of linkage maps, collection of cross-over data, sex determination, and other matters related to the characterization and transmission of genetic material. Much of the work done in the last ten years on formal genetics has been related to the inheritance of resistance to insecticides. The incompatibility between certain natural populations of *Culex* and *Aedes* is a most interesting genetic phenomenon not only because of its theoretical implications but also because it holds great promise for vector control.

The development of a system of formal genetics provides the tools for the investigation of any genetic phenomenon in vector species. It facilitates full understanding of a situation with respect to populations, subspecies and species. In addition, it is essential for an informed assessment of the

possibilities of genetic control operations and for understanding phenomena such as insecticide resistance and ability to transmit disease. Formal genetics has been developed in four groups of vectors, namely *Aedes*, *Culex*, *Anopheles* and *Musca*; the following is the present status of knowledge in each group.

### 3.1.1 *Aedes*

Most of the recent significant advances in this genus have been made with *Aedes aegypti*. In this species over 50 genes have been isolated, of which some 15 have been mapped on the chromosomes. Marker strains have been developed and used for locating important physiological factors, such as the genes for insecticide resistance and the susceptibility to pathogens. Linkage maps are available. In the course of the *Aedes* work, several other fundamental genetic phenomena have been uncovered, such as meiotic drive, the high frequency of balanced polymorphisms, crossing over in both sexes, and the lack of intra-specific sterility in crosses of strains from many parts of its range. Important theoretical contributions have been made to the knowledge of speciation through the discovery of the genetic basis of certain colour patterns that form the material for taxonomic distinctions. A case of what is evidently introgressive hybridization between *Aedes aegypti* and *A. mascarensis* has been discovered. Hybridization experiments have demonstrated the nature and significance of reproductive isolating mechanisms in *Stegomyia*.

### 3.1.2 *Culex*

About 10 useful markers have been isolated in *Culex pipiens pipiens* and in *Culex p. fatigans*. All three linkage groups have been distinguished. The most striking feature of this work is the evident tendency of the same mutant to occur in independent stocks. Thus scale-row, four-jointed palpi,  $R_2$  (a wing-vein mutant), and *kps* and *kpu* (both palpal mutants) have occurred independently in both "molestus" and in *C. p. fatigans*; a few of these have been recovered several times. In contrast to the work on *A. aegypti*, most of these mutants have been recovered after X-irradiation, but some are also present in natural populations. It is also interesting that similar mutants have been isolated in *A. aegypti*; four-jointed, scale-row, wart, and several types of antennal mutants show close similarities between the two species. As regards cytoplasmic inheritance in the *Culex pipiens* group, a full description is given in paragraph 4.1.1.1 below.

### 3.1.3 *Anopheles*

In spite of much research with various anophelines, there are almost no reports of mutants useful for genetic investigations. Certain mutants, as

for example in *A. gambiae* and *A. albimanus*, await clarification and establishment in stocks. One of the useful morphological markers is the mutant "stripe" recently isolated in *A. quadrimaculatus*. Stripe, a broad dorsal colour pattern, is an autosomal dominant over non-stripe; it is useful in larvae, pupae and adults. It shows independent assortment with the gene for dieldrin-resistance; thus if sex be considered a marker for chromosome 1, all three linkage groups may be distinguished. A similar mutant showing the same mode of inheritance has been isolated in *A. albimanus*.

Another mutant tentatively labelled "extra mouth part" occurs in certain laboratory stocks of *A. quadrimaculatus*. This appears to involve a supernumerary mouth part, often with a terminal bulb or tuft of hairs, suggestive of "proboscipedia" in *Drosophila*. Selection has increased its originally very low penetrance to about 50%.

It seems probable that the present dearth of "good" mutants in *Anopheles* is only a temporary situation. During the course of X-ray experiments, several anomalies in wing-vein pattern, tarsal shape and antennal form were recovered from *A. quadrimaculatus*. None of these could be isolated, although each has occasionally reappeared; intensive work will probably result in their isolation.

#### 3.1.4 *Musca domestica*

To date, over 100 visible mutants have been found; the majority are not adequate in expression and viability and therefore only about 30 have been examined in detail. A number of genes for the two types of resistance to chlorinated hydrocarbons and for organophosphorus-resistance have been isolated. These genes have been named and each has been assigned to one of the 6 linkage groups. The following differences have been observed in *Musca* as distinct from *Drosophila*: firstly, no X-linked mutant has yet been found, possibly because the sex chromosomes are composed of heterochromatin; secondly, maleness is evidently determined by the Y chromosome. In certain strains, mutants belonging to the second chromosome appear only in females, possibly due to translocation between the Y and the second chromosome.

It is of great interest that the second linkage group of *Musca* shows gene analogies with the first of *Drosophila*; in addition, there appears to be a correspondence between the third and fourth linkage groups of *Musca* and the second of *Drosophila* and between the fifth and sixth of *Musca* and the third of *Drosophila*. It is, moreover, possible that the second linkage group of *Musca* corresponds with the X chromosome of *Drosophila*. The fact that cases of holandric inheritance in *Musca* have been observed for mutants normally of the second linkage group seems to support these observations. Further studies may give the clue to the origin and evolution of sex chromosomes in insects.

### 3.2 Cytogenetics

#### 3.2.1 *Culicidae*

A recent review<sup>1</sup> has shown that considerably more information is now available on the cytogenetics of mosquitos than in 1953, but research is still fragmentary and relatively few laboratories are working in this field.

Information on the karyotypes of several species has been added in the last few years. There is general agreement that the diploid chromosome number is 6 for all species. Whereas all *Culex* and *Aedes* so far investigated show 3 pairs of metacentric chromosomes, species of the genus *Anopheles* have distinguishable heterosomes. These consist of a longer heterochromatic portion and a shorter, presumably euchromatic, portion. The shorter elements are subequal in females, unequal in males. This is the classical picture found in the "maculipennis" complex. Other groups of *Anopheles* can be distinguished by means of their heterosomes. In the "quadrimaculatus" group the heterochromatic portion of both the X and Y chromosome is greatly reduced so that the X chromosome is very much shorter than the autosomes. In the "punctipennis" group, which is basically similar to the "maculipennis" type, two different kinds of X chromosome are found. In some species of the subgenus *Nyssorhynchus*, X and Y chromosomes differ in size.

Salivary gland chromosome maps are now available for several species of *Anopheles*, including *atroparvus*, *albimanus*, *gambiae*, *aquasalis*, *quadrimaculatus*, *freeborni*, *punctipennis* and *stephensi*. Maps are also in preparation for all the North American anophelines and should be completed in 1963. All North American species so far studied may be distinguished cytologically on the basis of the salivary X chromosomes. Thus it has become possible in certain cases to use the salivary preparations as taxonomic tools for the identification of populations of doubtful or mixed ancestry.

The most promising aspect of anopheline cytogenetics is the high amount of chromosomal polymorphism present in many species. This has been uncovered in *A. gambiae*, *strodei*, *darlingi* and especially in *punctipennis*. In this last species, at least 9 different inversions and a duplication have been discovered. The occurrence of widespread polymorphisms should facilitate the clarification of the evolutionary picture in *Anopheles punctipennis*.

#### 3.2.2 *Musca domestica*

The karyotype is composed of six pairs of V-shaped chromosomes in both sexes, XX in females and XY in males. Unfortunately, the investiga-

<sup>1</sup> Kitzmiller, J. B. (1963) Mosquito cytogenetics, a review of the literature, 1953-1962. *Bull. Wild Hlth Org.*, 29, 345.

tion of salivary chromosomes in *Musca* is very difficult, and no useful work has been done.

### 3.2.3 Other vectors

Cytological investigations have been conducted on the karyotypes of 9 species of triatomines. Two species have nine autosomes and the others have 10. The basic sex-determining mechanism is XY heterogamy, i.e., XX in the female, XY in the male. The fragmentation of the X chromosome has led to many types of heterogametic formulae which seem to bear no relation to the systematic picture.

A great deal of significant research has been performed on polytene chromosomes and speciation in *Simulium*. As a result, several groups of sibling species have been distinguished within the Nearctic and Palaearctic forms. Among sandflies only *Phlebotomus longipalpis* has been investigated.

## 3.3 Physiological and developmental genetics

The work done as a consequence of WHO's international programme on resistance has provided excellent examples of physiological genetics, since in some instances success has been attained in relating a gene to a character through the intermediary of an enzyme.

A few other aspects of physiological genetics may be mentioned. The free amino-acids in several species of mosquitos have been analysed by chromatographic and infra-red spectrophotometric methods, and have revealed strain differences undetected by ordinary taxonomic means. Since the proportion of free amino-acids is partially modified by diet, these methods were considered reliable only under carefully controlled laboratory conditions. Recently it has been possible to identify certain fluorescent pigments—the pteridines—which are highly strain specific, so that morphologically identical strains may be readily distinguished from one another.

An important beginning has been made in the study of serological and immunological differentiation of mosquitos. Four species of *Aedes* have been distinguished by serological methods with greater precision than is possible with morphological characters. Sharp serological differences have been found in preparations from North American anophelines. All species and about eight different strains of *A. quadrimaculatus* could be easily distinguished. There are clear-cut differences between the males and females, and distinct characteristics separate the eggs, larvae, pupae and adults within the same strain. All stages of development can be easily distinguished on the basis of uniquely characteristic low-molecular-weight proteins.

Investigations of biochemical mutants are also being performed in *Musca domestica*. For instance, amylase and esterase are controlled by

genes that are located on the third and fifth chromosomes respectively. Similarly, tryptophan metabolism is being investigated by using some of the colour mutants, such as green and ochre, and these studies may be useful in clarifying the relation between the gene and the enzyme.

### 3.4 The genetic basis of vector ability

It is, of course, well known that some strains and species of insects are able to transmit diseases while others are not. It has long been suspected that there is a hereditary basis for the ability of an insect to harbour and/or transmit a pathogen. As early as 1931 it was demonstrated that the ability to transmit bird malaria in *Culex pipiens* was controlled by allelism in a single gene. This important phase, neglected for many years, is now being re-examined. It has recently been demonstrated that the ability of *Aedes aegypti* to transmit *Brugia malayi*, a filarial parasite, is determined by a single, sex-linked, recessive gene. Also in *Aedes aegypti* a single gene has recently been found to control susceptibility to bird malaria. It is important that an immediate search for other such cases be initiated, especially in *Aedes* and *Culex* where markers are available, and that this be given a very high priority in view of the significance of such information to disease control programmes.

### 3.5 Genetics of behaviour

The inheritance of characteristics of behaviour has been clearly demonstrated in instances such as mating habits of *Drosophila* and spinning habits of caterpillars. Emphasis is placed on the proper design of experiments to demonstrate the genetics of behavioural characters. The bases of such a study are: firstly, the existence of two strains that show significant differences in whatever quantitative assessment is made of their behavioural character; secondly, the performance of the usual crosses and back-crosses to ascertain the mode of inheritance and possibly segregation in the resulting  $F_2$  generations; thirdly, that wherever possible this be done in connexion with genetic markers.

### 3.6 Population and evolutionary genetics

This important area of research has been neglected although it has a vital bearing on field control programmes. Reasons for neglect include: (a) lack of the required background knowledge on formal genetics; (b) difficulty of the subject matter, which represents one of the most complex areas in biology; and (c) lack of contact and co-ordination between laboratory and field work. Studies on gene dynamics in populations will contribute to an understanding of the problem of homeostatic regulations of vector populations in nature.

Research on mutation load and gene frequency has demonstrated that certain vector species contain a rich source of genetic variability. High levels of heterozygosity characterize *Aedes aegypti*, *Culex pipiens* and *Musca domestica*. These species may provide continuing difficulties in control programmes. Because genetic plasticity confers resilience and adaptability, some individuals may be able to survive and become resistant to almost any sort of control measure.

Studies on gene frequency in *Aedes aegypti* have demonstrated great variability both between and within laboratory colonies. Different laboratory populations contain markedly different genetic material. Marked changes in levels of equilibrium occur following transfer of populations from one laboratory to another. Therefore, the common assumption that laboratory populations are uniform, stable and unchanging must be incorrect.

The variability in *Anopheles gambiae* in Africa is best explained by considering *gambiae* as a complex of sibling species, at least partially isolated by reproductive barriers. At least five different crossing types have been demonstrated. Reproductive isolation between these types is not complete, at least in the laboratory, since the  $F_1$  females are fertile and may be successfully back-crossed. Very little is known about the degree of isolation in nature. Obviously the recognition of *gambiae* as a group of sibling species is a notable milestone in the eventual control of this vector.

### 3.7 Research needs and priorities

1. Continued search should be made for mutants in any vector species. The need for mutants to serve as markers is especially critical in *Anopheles*, where the lack of adequate markers is beginning to be a definite handicap to further work on genetics.

2. Suitable strains with multiple chromosome markers should be prepared in vector species in which this is possible, and made available for distribution.

3. As mutant strains and laboratory colonies of vector species increase in number, the mere labour of maintenance will become an even more serious problem. In the case of mosquitos immediate attention should be given to research on the storage of *Anopheles* and *Culex* eggs.

4. Population genetics of vector species, which is a virtually untouched field. In those species for which markers are available such studies should be intensified, particularly since the dynamics of genes in populations plays a most important role in the success or failure of control programmes.

5. Physiological, biochemical and behaviouristic genetic studies should receive strong support. Almost no work has been conducted in

these areas apart from that on insecticide resistance, in spite of the fact that vector species provide excellent material for basic scientific research.

6. Descriptive studies of karyotypes, especially in different groups, such as anophelines and triatomines, should be completed as quickly as possible. This relatively simple research provides a basis for further important studies.

7. An intensified study needs to be made of sex-determining mechanisms. This is especially important in *Culex* and *Aedes*, in which distinguishable heterosomes are not present.

8. A search should be made for cytological aberrations (translocations, etc.) that will permit identification of mitotic, meiotic and polytene chromosomes with known linkage groups.

9. Detailed studies on spermatogenesis and oogenesis are needed, especially in vector species that may subsequently be the target of genetic control by radiomimetic chemicals.

10. Study of chromosomal polymorphism in *Anopheles* should be intensified. This unexpected "bonus" in the North American anophelines has already resulted in a better understanding of populations and taxa, and should be exploited fully.

11. The mapping of polytene chromosomes in *Anopheles*, especially in those complexes in which systematics and disease transmission are problems. The proven worth of chromosomal studies as a tool in distinguishing sibling species should stimulate this field.

12. Mosquito chromosomes are exceptionally well suited for cytochemical studies. DNA and RNA should be thoroughly studied, especially in *Anopheles*.

#### 4. GENETIC CONTROL

Problems with chemical toxicants have stimulated interest in the genetic control of insects. In its broadest sense genetic control implies the use of any condition or treatment that can reduce the reproductive potential of noxious forms by altering or replacing the hereditary material. The desired end-result is a reduction or a change in the composition of the insect population. Genetic control has so far been limited to the release of insects sterilized with ionizing radiation or chemosterilants. However, a great many other possibilities exist for the manipulation of genetic mechanisms already present in natural populations. Among these, the following appear most promising :

## 4.1 Incompatibility

### 4.1.1 *Cytoplasmic incompatibility*

Within some species complexes of mosquitos, cytoplasmic agents cause incompatibility between populations. Crosses between certain populations give no offspring at all. In other cases, females of one population may cross with males of another and fertile offspring are produced, but in the reciprocal cross all embryos die. The sterility is due to a cytoplasmic factor transmitted through the egg, which kills the incompatible sperm after entry into the egg and before karyogamy. Control could be effected by mass rearing of males of one crossing type, separation of the sexes in the pupal stage, and release of these males into an area populated by an incompatible crossing type. This principle of control is comparable to that of the so-called sterile-male method used in programmes with radiation treatment, with the difference, however, that other incapacitating effects of radiation are avoided.

4.1.1.1 *Culex pipiens*. In this species complex, at least 15 different crossing types from many parts of the world have been isolated and are immediately available. These could be utilized for a programme of genetic control in the near future. Population-cage experiments have already demonstrated that repeated release of incompatible males into a population will suppress that population to extinction. Properly conducted, this procedure should reduce field populations at least to a very low level. By maintaining constant pressure through repeated releases, a population could be reduced to the point where transmission of filariasis would be interrupted. Burma is suggested as a logical site for a field trial. An installation for the mass production of males could be established in conjunction with the Filariasis Research Unit in Rangoon. Initial trials could be conducted through the release of incompatible males into small isolated populations of mosquitos. Villages in this area are particularly suitable for a pilot experiment because they are relatively isolated biologically and little mixing of mosquito populations occurs during the dry season. Thus, in effect, one has an island situation for experimentation. Immediate attention should be directed towards development of mass rearing methods for *Culex p. fatigans*, using genetic breeding technology to ensure an end-product of high vigour, mating competitiveness, and insecticide susceptibility. Alternatively, the release of incompatible males could be combined with an insecticide application, provided that resistant males were used. The effect of the release could be evaluated by regular collection of egg rafts and determination of the proportion of rafts that failed to hatch.

4.1.1.2 *Aedes scutellaris*. The incompatibility phenomenon is also known in this species complex. Members of this group are important

vectors of filariasis through most of Pacific Oceania. They are especially difficult to control with insecticides, but their island distribution makes them good subjects for genetic control. Moreover, mass production facilities for *Stegomyia* mosquitos are currently available; for example, the United States Public Health Service has developed a production plant for rearing very large numbers of *Aedes aegypti*. Facilities of this nature could be readily converted for use in rearing *Aedes scutellaris*.

Although little genetic research has been done in the *A. scutellaris* complex, an example of non-reciprocal fertility has been found in this group. It seems probable that further crossing types exist among the various forms. Laboratory colonies from different island groups should be assembled and hybridization experiments conducted to isolate additional crossing types. Population-cage experiments should be made in order to determine the proportion of incompatible males required to reduce or stop production of fertile eggs. Annex 1 gives a more detailed explanation of this scheme.

4.1.1.3 *Other vectors.* It seems highly probable that cytoplasmic incompatibility exists in other species. In addition to *Culex* and *Aedes*, such agents have been isolated from *Drosophila*, *Mormoniella*, and a species of Chironomidae (*Chunio*). Search for such agents in *Anopheles* would seem important, particularly in groups such as *Kerteszia* whose larvae develop in concealed locations difficult to reach with insecticides.

#### 4.1.2 *Hybrid sterility*

Appropriate genetic crosses can be used to produce sterile males for subsequent release. In *Anopheles gambiae*, a number of crossing types have been isolated. Crosses between types in either direction result in an  $F_1$  with fertile females but sterile males. Although the latter have atrophied testes, usually devoid of spermatozoa, their sexual activity is normal and may even be enhanced by heterosis. Sterile males have been introduced into laboratory cages to compete with normal males with respect to copulation with normal females; the result was a reduction in the number of fertile eggs proportional to the number of sterile males introduced. If this method is to be used for field trials with *Anopheles gambiae*, particular attention must be paid to methods of mass production. Annex 2 contains a scheme for the control of *A. gambiae* by this method.

It has been suggested that control of the tsetse fly, *Glossina swynnertoni*, might be accomplished by the massive introduction of *G. morsitans* into its territory. These largely allopatric species cross readily in the laboratory and in nature. Mating seems to be at random yet the crosses yield only a few offspring, almost all of them sterile. In general, wherever isolation is reproductive and not sexual, hybrid sterility would result from the introduction of one form into the territory of another.

#### 4.2 Deleterious genes

Natural populations of insects carry a large load of deleterious genes, including lethal factors, conditional lethals, and genes reducing viability; these can be isolated by inbreeding in the laboratory. The mutation load of field populations may be increased by continued introduction of these genes, and this naturally results in elimination of a proportion of the population in every generation. Sex-linked or sex-influenced lethals should be especially useful. Seasonal lethals and density-dependent factors are also promising. Hereditary factors and infective agents distorting sex ratio should be useful, especially in species where only one sex is noxious, as in mosquitos. Certain candidate genes for such programmes are currently available in some of the well-studied species, such as *Culex pipiens* and *Aedes aegypti*. However, a great deal more work needs to be done in this area. The possibilities with deleterious genes are extensive, but the immediate prospects for genetic control may be better in certain other fields.

#### 4.3 Replacement

Through population replacement innocuous forms could be substituted for pests that fill similar ecological niches. Genes affecting the ability to transmit disease deserve special investigation. Because there are particular dangers involved in population substitution, a careful study of those cases where natural substitution has occurred is required and it is suggested that this be undertaken by WHO.

#### 4.4 Artificial induction of dominant lethality

In the so-called "sterile male method" of control, irradiation and chemosterilant compounds have been used to produce sterility through the induction of dominant lethal mutations in gametes. When sperm from treated individuals fertilize eggs from field females, the embryos die as a result of the lethal genes. The net effect is that the male acts by sterilizing the ova of wild females. This method has been successful for control of the screw-worm fly and certain species of fruit flies. Prospects for its use with *Glossina* seem especially good. Several trials with this method on mosquitos have failed, probably because radiation treatment reduces mating competitiveness.

#### 4.5 Methods for propagating genes in populations

By mass production and release, populations can be overwhelmed by sheer quantity of unfavourable genes. However, certain genetic mechanisms, among them heterosis and meiotic drive, can be used to propagate genes in

populations. Certain chromosome combinations can carry deleterious genes through populations, even though these combinations are disadvantageous in the homozygous condition. Perhaps the best hope for field propagation of deleterious genes is through the distortion of segregation ratios brought about by meiotic drive. During gametogenesis, more meiotic drive chromosomes reach the gametes than do their normal homologues. This mechanism can cause the chromosomes of a few individuals to sweep through a population even though the fitness of the population is thereby reduced. Thus a meiotic drive chromosome containing a gene for female sterility could eradicate a population. This phenomenon is of great current interest among biologists and comprises an area of active research. A meiotic drive chromosome is available in *Aedes aegypti* and could be used if linked lethal factors can be made available.

#### **4.6 Areas for further research and development**

1. Fundamental research on the genetics of vector species. This is expected to produce many more approaches to genetic control of insects and should be encouraged.
2. Extensive studies on ecology and population dynamics to discover how, where, when, and in what numbers insects should be released for the genetic control of populations. In particular, studies of the dispersal patterns best adapted to obtain maximum effectiveness of released material should be conducted. Techniques for estimation of natural population density are necessary to assess the successive levels of reduction of the target population. The use of mutant markers for this purpose offers promise.
3. Population-cage experiments on sexual behaviour and mating competitiveness are required for sound prediction of the results of field release.
4. Considerable attention should be directed towards breeding methods and development of facilities for mass production. The possibility of developing insect material for release, with superior genetic characteristics for survival and mating ability, should not be neglected.

### **5. INTERNATIONAL CO-ORDINATION AND SERVICING OF RESEARCH**

#### **5.1 Stimulation and co-ordination of research**

There is an urgent need to expand research on vector genetics. Although this subject is still in its infancy, it already occupies a central position in vector studies, and opportunities for the future are extensive. Expansion

of work at institutes now engaged in research on vector genetics, the initiation of research at new laboratories and recruitment of research workers are essential. The first objective can be accomplished largely through increased support, possibly in part through contractual agreements with WHO. It will be somewhat more difficult to stimulate research on new aspects of vector genetics, since this may require that research workers transfer their interest from more theoretical areas of genetics to this relatively specialized subject. Because of the importance of this problem, the Group considered ways of increasing the supply of new workers. One way would be to support the training in genetics of promising graduate students and to encourage them to make vector genetics the subject of their postgraduate research. Relatively small research grants could give similar encouragement to persons who have completed their graduate training but have not yet had time to become established in the field. It is emphasized that personnel trained in vector genetics could subsequently be utilized in this expanded programme of genetic control of vectors.

Research on vector genetics is limited by availability of funds and space for research as well as by the shortage of qualified personnel. Funds for buildings and laboratories are particularly difficult to obtain. It is suggested that WHO might consider supporting applications for grants for research on vector genetics made to other agencies. Requests for adequate laboratory space and environmental control apparatus deserve special attention.

It is hoped that WHO will encourage field studies in vector genetics, especially by supporting travel of research workers to problem areas. In most cases, genetic research laboratories are far removed from field populations of the important vector species, and funds for travel to these areas are not usually included in research budgets. Samples of field populations for genetic research are usually obtained through personal requests to field workers in the area of interest, but because these workers are not directly concerned with such research, the results are usually unsatisfactory. The paucity of our knowledge of population genetics in vector species reflects this situation. Many important lines of research would be initiated if laboratory workers had greater access to field material and field situations. Moreover, reasonable availability of travel funds would make it easier to interest geneticists in problems encountered in practical control operations.

In addition to the role currently played by WHO in the co-ordination of research on the genetics of insecticide resistance, the broader field of vector genetics as a whole deserves greater attention, not only for its intrinsic importance, but because rapid developments in widely dispersed laboratories make co-ordination essential if full advantage is to be taken of genetic factors as a means of vector control. Efforts should be directed towards (1) improvement of communication between scientists working in similar or related fields, (2) development of comparable techniques for the collection

and presentation of genetic data and (3) integration between programmes carried out in laboratories in different parts of the world.

These aims might be achieved through personal contact, private exchange of information, visits between laboratories (including technicians) and meetings of specialists. The latter might include the formal type of committee at which such matters as standards, definitions and nomenclature would be established, and seminars at which scientists and public health specialists would be brought together for the communication of research findings and operational developments in this field. It seems appropriate to note that most of the progress in vector genetics to date has been made by geneticists whose interest is academic rather than applied, and to whom vector control is of only secondary importance. WHO can play a major role in co-ordinating basic research and the application of research findings to field situations.

## 5.2 Servicing of research

Research on vector genetics would be greatly facilitated if WHO could provide regularly certain services and information as elucidated below. These are as follows :

### 5.2.1 Terminology

A review of the literature on vector genetics indicates that the problem of communication on the subject is growing and that standardization of nomenclature is necessary. While it is not suggested that a new and specialized system of terminology is required it does appear advisable that an investigation be undertaken to determine to what extent existing systems might be adopted or adapted to meet this situation. It is recommended, therefore, that WHO undertake this study in collaboration with a group of geneticists and that on the basis of the results the advice of an expert committee on genetics be sought.

### 5.2.2 Dissemination of information

In the fast-growing field of vector genetics, the prompt exchange of information is of paramount importance. The well-established and widely-distributed *Information Circular on Insecticide Resistance* currently issued by WHO is an ideal means of achieving this. It is therefore recommended that this publication be expanded to include a section dealing with vector genetics, and that its circulation be increased to include individuals and laboratories engaged in all aspects of research on this subject.

The documentation prepared for this meeting would constitute an excellent starting point for a monograph on vector genetics, which would summarize existing data on the subject and include information on termi-

nology and methods. Such a publication would both serve as a reference and provide a great stimulus to further progress in this field. It is considered that WHO is the only organization that could logically undertake the preparation of such a monograph and it is hoped that this recommendation will be given a high priority.

### 5.2.3 *Standard procedures and techniques*

Although standard genetic techniques are familiar to geneticists, entomologists engaged in vector research and control are not generally conversant with these methods. Therefore a service could be performed by assembling information on techniques of crossing and genetic analysis, cytogenetic methods, and breeding procedures for use by interested workers. The provision of such a service would necessitate discussion among workers in the field, possibly at a meeting of an expert committee.

In studying the genetics of insecticide resistance a number of toxicological procedures have been used. The standard methods recommended by the WHO Expert Committee on Insecticides have frequently proved extremely useful, based as they are on exposure to multiple dosages for a constant time. However, in genetic analysis of resistance other procedures have proved more useful for detecting segregation. Some of these are based on a single dosage and the determination of knock-down times. Advantage should be taken of such tests, and WHO could perform a service by assembling these methods and making them available for further studies.

A wide variety of techniques will be involved in the development of genetic control operations, e.g., assessment of gene frequency, analysis of gametogenesis and chromosome fracture, and determination of embryonic development. As new workers are employed in increasing numbers for genetic control, it will become essential to make these methods more readily available.

### 5.2.4 *Standard strains*

That the work of WHO in developing a standard strain of houseflies has been appreciated is shown by the large number of requests for samples that have already been received. It is a tremendous advantage for any worker, in any part of the world, to know that on application to a central agency, at any time, he can obtain material of known constitution. The group wished to support the present activities of WHO in developing standard strains for additional vector species, such as *Aedes aegypti*, *Culex pipiens fatigans*, *Anopheles gambiae*, *Pediculus humanus humanus* and *Blattella germanica*.

The standard strains already developed and to be developed fall into the following three categories :

*Reference strains.* These should be of reduced genetic variability. Since their principal use is in toxicological and biochemical work, it is important

that they be homozygous for susceptibility to the known insecticides. It is not necessary for them to be completely isogenic because a certain amount of genetic heterogeneity will probably be necessary in some species to ensure a healthy strain in a condition of physiological homeostasis. In certain cases, the reference strain may be constituted in each generation by hybridization of two inbred lines. This method has the advantage that it ensures both maximum genetic uniformity and maximum quality due to heterosis.

*Inbred strains.* Many genetic and physiological problems require the use of inbred strains, such as those already developed for some species of mosquitos. These strains should be isogenic as far as possible. The number of generations required for the process of inbreeding depends on the genetic system of the species in question. Maintenance of the inbred line should continue even after mass-culturing has been started, as in certain species the mutation rate, at least at some foci, is so high that it can restore genetic variability in inbred strains.

*Marker strains.* Certain strains carrying specific mutant markers are intended mainly for gene analysis. Their value generally increases with the number of marked loci evenly spaced on each linkage group. The basic requirement of these strains is absolute purity for the markers involved, or balanced heterozygosity might be equally useful in some instances. Genetically marked strains should be made available as soon as it is possible.

The numerous colonies of different species of varying origin present in laboratories throughout the world have been called "strains" for convenience, and the Group would not presume to condemn this practice. Nevertheless, it wished to stress the desirability that, whenever such colonies are forwarded to other laboratories or research work on them is described in publications, their origin and laboratory history be specified. Lists of these strains have appeared from time to time as supplements to the WHO *Information Circular on Insecticide Resistance*. These strains are of such value that WHO might consider ensuring their preservation by designating certain laboratories as stock culture centres. The ultimate objective is the establishment of an international laboratory or institute to maintain useful colonies and standard strains of vectors.

#### 5.2.5 *Transportation of insects*

With regard to the transportation of insects from the field to the laboratory and between laboratories, the mailing of *Aedes aegypti* eggs presents few difficulties and the eggs of anophelines, the pupae of *M. domestica*, and adults of *C. p. fatigans* have also been sent successfully by air over long distances. However, the subject still requires a considerable amount of investigation, especially with respect to the development of suitable con-

tainers. It is recommended that WHO support and co-ordinate such studies with the aim of developing shipping procedures applicable to insects of public health importance in general.

It is stressed that all shipments of insects should be made in accordance with national and international quarantine requirements.

#### 5.2.6 *Culture procedures for insects of public health importance*

Emphasis is placed on the importance of developing effective procedures for the culture of insects for laboratory research and for control programmes. It is emphasized that this problem involves not only suitable rearing methods (food, cages, etc.) for small laboratory cultures, but also the production on a mass or assembly-line basis of the large numbers of insects likely to be needed for certain types of genetic control operations. The Symposium on Culture Procedures for Arthropod Vectors and their Biological Control Agents being sponsored by WHO in September 1963 is expected to provide a considerable amount of information on this subject, and it is recommended that this be amplified and issued by WHO as a comprehensive guide.

### 5.3 Training

In view of the urgent need for training new workers in all phases of vector genetics, it is recommended that WHO give consideration to the following :

1. The training of technicians and laboratory assistants in the collection and maintenance of colonies of insects and their vertebrate hosts and in cytological and toxicological techniques. This might be achieved by the organization by WHO of courses of about three months' duration.

2. The training of young scientists for periods of at least two years and their subsequent placement, if feasible, in suitable laboratories for periods of up to five years.

3. The exchange of senior scientific workers between laboratories for periods of up to three months.

4. The provision of opportunities for laboratory research workers in vector genetics to obtain field experience with vector species, possibly through travel fellowships.

In addition to the *ad hoc* training of entomologists taking up the study of vector genetics, an increase in the genetic content of courses given to students of entomology in general, and medical entomology in particular, is eminently desirable. Furthermore, it is hoped that the valuable contributions to the science of genetics made by research on the genetics of vectors and insecticide resistance will be given more extensive treatment than at present in textbooks on genetics.

## 6. ASSESSMENT AND RECOMMENDATIONS

It is evident from the foregoing that the science of vector genetics comprises a large and growing body of knowledge, and that it makes an important contribution to almost every aspect of vector control. The genetic thread is intimately woven into the fabric of life and must always be taken into account in any study of living organisms. Specific recommendations for further research on genetics of resistance, basic vector genetics and the application of genetics in the control of vectors have been outlined at the end of each section of this report. For ease of reference, however, they are summarized in the following over-all assessment.

### 6.1 Insecticide resistance

Studies of insecticide resistance have stimulated research on insect genetics and have provided an excellent example of biochemical genetics on the one hand and of evolutionary change on the other. It is therefore considered that, apart from its obvious economic value, there is every justification for continuing genetic research on resistance and extending it to species not yet studied and to field investigations of population dynamics. Specific recommendations for further research on the resistance problem are listed in section 2.2.

### 6.2 Vector genetics

The following recommendations are made :

1. Development of fundamental genetic information for vector species.

(a) For species such as the housefly, the *Culex pipiens* complex, *Aedes aegypti* and the *Anopheles gambiae* complex, a considerable amount of work has been done. The construction of linkage maps and the search for new mutants should be continued.

(b) A limited amount of work has been done on the genetics of *Blattella*, *Triatoma* and *Simulium*. This research should be intensified.

(c) Some species readily adaptable to rearing for genetic purposes have been neglected. These include certain *Culex* and *Anopheles*, aedine mosquitos of the subgenera *Finlaya* and *Stegomyia*, body lice, mites and bed-bugs. Work on mutant isolation, pair mating and inbreeding should be commenced.

(d) Many species are currently considered to be unsuitable for genetic research. Development of improved culture methods might allow genetic investigations of ticks, fleas, *Phlebotomus*, tsetse flies and other higher Diptera, certain anopheline and aedine mosquitos and others.

2. Development of strains with multiple chromosome markers for the identification and locating of genes affecting physiological characters. This work should be continued in the *Culex pipiens* complex, *Aedes aegypti* and *Musca domestica*, and extended to other forms such as the *Anopheles gambiae* complex. These strains should be made available for resistance research.

3. Investigation of specific genes for ability to transmit pathogens. The main emphasis at present should be on genes for susceptibility to filarial worms in *Aedes aegypti*, the *Culex pipiens* complex and the *Aedes scutellaris* complex, and susceptibility to malaria in both anopheline and culicine mosquitos. Work on the genetics of the ability to transmit virus diseases offers great promise.

4. Studies on polytene, mitotic and meiotic chromosomes of various vector species. The excellent work on polytene chromosomes in certain *Anopheles* and *Simulium* and on gametogenesis in a few culicine species should be extended and expanded. The preparation of polytene chromosome maps should be continued in anophelines and further research encouraged in *Aedes* and *Culex*.

5. Biochemical investigation of the relationship between genes, enzymes and characters. Biochemical characterization of strains and species should be continued, especially along the lines already commenced in research on resistance.

6. Comparative studies of genetic plasticity in vector species, possibly along the lines of those made in *Aedes aegypti*. In addition, population-cage experiments should be conducted in order to understand population dynamics and genetic equilibrium, especially in species such as *Aedes aegypti*, the *Culex pipiens* complex, *Musca domestica* and the *Anopheles gambiae* complex.

7. Genetic study of certain complexes in vector species that require taxonomic clarification. Research on polymorphism in polytene chromosomes has been of great value in establishing routes of speciation and defining the existence of sibling species in some *Anopheles*; such work deserves support, expansion and extension.

8. Laboratory investigation of hybridization as an aid to studies in speciation. Crossing experiments within and between species contribute important information on reproductive barriers, genetic isolating mechanisms, and sibling species.

9. The question of the inheritance of behavioural characteristics in vector species remains one of the untouched fields. There is an urgent need, firstly for quantification and precise measurement of behavioural responses, and secondly for crossing experiments between two strains that differ in a behavioural characteristic.

### 6.3 Genetic control

The following lines of research are recommended :

1. Exploitation of currently available genetically incompatible strains to control field populations of vectors such as the *Aedes scutellaris* complex, the *Culex pipiens* complex and the *Anopheles gambiae* complex. Population-cage experiments and limited field trials could be conducted in the near future. Additional incompatibility factors should be sought in these and other vector species.

2. A systematic effort to isolate and study lethal factors and other deleterious genes in those species that have been well studied genetically.

3. Investigation of the phenomenon of meiotic drive in *Aedes aegypti* and other organisms in order to understand its influence on populations. The incorporation of lethal factors in chromosomes exhibiting meiotic drive should provide possibilities for genetic control of vectors.

4. Investigation of the mutagenic action of radiomimetic chemicals, compared with radiation, on the chromosomal material and the gametocytes. In addition, research should be undertaken on the population dynamics of dominant lethal alleles thus introduced into populations for the control of vectors.

5. Development of breeding methods to produce standard insects for research and for field use. Both isogenic strains and strains with a high but constant level of genetic plasticity would be of value. Work on inbreeding and heterosis should be emphasized.

6. Development for other vector species of mass production methods similar to those for *Aedes aegypti* and the screw-worm. The most important species for this work include the *Culex pipiens* complex, the *Anopheles gambiae* complex, *Triatoma* and *Glossina*.

### 6.4 Role of WHO

The role of WHO in implementing an extended programme of research in vector genetics has been discussed in detail under section 5. However, the Group wished to reiterate the following particularly important recommendations :

#### 6.4.1 *International co-ordination and stimulation of research*

A programme of international co-ordination and stimulation of research in vector genetics could be developed along the lines that proved so successful for insecticide resistance. It is suggested that work on this subject now in progress in some institutes should be expanded and that, in addition, other laboratories and individuals should be encouraged to enter this field. Furthermore, WHO could facilitate communication between these

institutes and laboratories by sponsoring meetings and seminars and visits between laboratories. The Group considers that the field would be stimulated, and continuity ensured if a formal committee on this subject could be established.

#### 6.4.2 *Support of research*

It is hoped that research on vector genetics will receive more adequate support in terms of funds, laboratory space and technical assistance. A programme of contractual grants might be established ; in addition, WHO might wish to support applications for grants made to member states and other fund-granting agencies.

#### 6.4.3 *Servicing of research*

An international system of nomenclature, procedures and techniques for use in work on the genetics of vectors is urgently required. WHO might consider convening a formal committee to establish such a system. The Group recommends that the *Information Circular on Insecticide Resistance* be expanded to include a section on vector genetics and that WHO should consider the publication of a monograph summarizing the current status of vector genetics. The development of standard and marker strains of different species is a basic requirement for progress in all fields of vector research, and should be carried out along the lines already established by WHO for *Musca domestica*. It would be advisable to establish stock culture centres for each species by designating certain laboratories as WHO reference centres. Ultimately an international institute might be established to maintain valuable genetic material of the various vector species.

#### 6.4.4 *Training*

WHO could assist the training of graduate students and technicians by the award of fellowships. Both geneticists and entomologists should be encouraged to undertake work in vector genetics, possibly by fostering the exchange of senior scientific workers, or by providing refresher courses.

### ACKNOWLEDGEMENTS

The Scientific Group acknowledges the contributions made by the following staff members of WHO :

Mr N. G. Gratz, Vector Control, Division of Environmental Health

Dr M. Laird, Chief, Environmental Biology, Division of Environmental Health

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Division of Malaria Eradication.

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### Annex 1

#### A SCHEME FOR THE GENETIC CONTROL OF *Aedes scutellaris*

Members of the *Aedes scutellaris* species complex serve as the primary vector of filariasis in many islands in Pacific Oceania. These mosquitos are particularly difficult to control with insecticides. Larvae breed in a variety of small, inaccessible habitats, including artificial containers, tree holes and coconut husks. On most islands, removal or destruction of larval habitats is impractical. Attempts at biological control through introduction of *Coelomomyces* (i.e., the Tokelau Project) are currently under way but have not yet met with success.

*Aedes scutellaris* (Walker) belongs to a complex that includes a number of species, subspecies and races of doubtful rank (see table, p. 37). Some of the names in this table undoubtedly represent distinct species. However, taxonomic "splitters" have assigned species names to many other forms based on geographic distribution and minute morphological differences. The group presents an interesting example of incipient speciation. Distinct populations are evolving on different island groups but this evolution has not proceeded very far. Most forms have very similar habits. Hybridization studies have demonstrated that reproductive barriers between some of the so-called species are incomplete (Woodhill, 1950; Perry, 1950; Rozeboom & Gilford, 1954; Woodhill, 1954). Most significantly, behavioural barriers to mating are not apparent in many cases, and in the laboratory mating between forms occurs readily.

A peculiar sort of non-reciprocal fertility between members of the *scutellaris* complex was described by Woodhill (1949, 1950). He crossed *Aedes scutellaris scutellaris* (S) from New Guinea with *Aedes scutellaris katherinensis* (K) from Northern Territory, Australia. In the cross of S female to K male, normal fertility was obtained and no reduction of viability was observed in subsequent generations. However, no offspring were produced from the reciprocal cross of K female to S male. Inheritance of this sterility was entirely maternal, passing from K females to their female offspring. Smith-White & Woodhill (1954) suggest that the sterility may be due to a factor in the cytoplasm of the egg of K which is incompatible with the sperm of S, killing the latter before karyogamy.

In an important series of papers summarized in 1959, Laven conclusively demonstrated a cytoplasmic sterility factor in *Culex pipiens*. In a study of *C. pipiens* populations from various areas of the world, Laven found many different crossing types. There are no barriers to mating or insemination between the types but unidirectional sterility occurs in various crossing combinations. There is little doubt that the cytoplasmic sterility in *C. pipiens* is similar to that in *A. scutellaris*. Unfortunately, the latter case has not been so well studied, especially with regard to distribution of the sterility factor(s) among populations. By analogy from *Culex*, one would expect numerous crossing types among the various so-called species and varieties in the *scutellaris* complex.

It seems possible that this sterility phenomenon might be used for genetic control. The success of the sterile-male method in eradicating the screw-worm fly from the south-eastern area of the USA is well known. Flies were mass-reared, irradiated to produce dominant lethals in sperm, and released in the field, with the expectation that dominant lethality would operate in the zygotes from field females. Unfortunately, radiation reduces mating competitiveness in mosquitos. However, it is well known that sterilization and/or genetic death can be brought about by appropriate genetic techniques and breeding procedures, without recourse to radiation or chemicals.

Mass production and release of infertile or incompatible males offers marked promise for mosquito control. Laven (personal communication) has suggested mass release of males of *Culex pipiens* into an area with females of an incompatible crossing type. Unfortunately methods of mass production of *C. pipiens* are not currently available. For this species, genetic control must await bio-engineering developments.

The prospects for genetic control of *A. scutellaris* are more promising. Mass production techniques for certain *Stegomyia* mosquitos have been developed by the Technical Developments Laboratory of the United States Public Health Service, Savannah, Georgia. Morlan, McCray & Kilpatrick (1962) report production of 10 620 000 males of *A. aegypti* in 43 weeks. This operation was conducted in a small area and could have been expanded considerably without great expense. The laboratory biology of many forms in the *A. scutellaris* complex is little different from that of *A. aegypti*. Colonies of *A. pseudoscutellaris* and *A. polynesiensis* are maintained by the same methods as those used for *A. aegypti*. It seems probable that the Savannah production facility could be converted for rearing *A. scutellaris* without serious problems. At present, this facility is not being used and has been dismantled : however, it could be reconstructed in a very short time.

Assuming that mass-produced males are available, the next step is obvious—repeated release of males into an experimental area containing a population of incompatible crossing type. Such an experiment could be

done at present in the Northern Territory of Australia. Thus, males of *A. scutellaris scutellaris* would be mass-produced and released into habitats of *A. s. katherinensis*. Unfortunately, continental land masses and large, mixed mosquito populations do not provide the best conditions for an experiment of this sort. A small island in Oceania with a single mosquito species of the *scutellaris* complex would provide the best testing ground. Such islands are not at all unusual.

The following lines of research must be pursued if this experiment is to be attempted :

1. A large number of laboratory colonies of members of the *scutellaris* complex from different islands must be assembled.
2. Hybridization experiments between the different laboratory colonies must be conducted in order to find additional crossing types. Search must be made for crosses where isolation is reproductive but not sexual.
3. Cage experiments must be made in order to determine the proportions of compatible and incompatible males in a population necessary to reduce or stop production of fertile eggs. Naturally, mating competitiveness of the two types must be ascertained. A start on this work could be made at once, using populations of *A. s. scutellaris* and *A. s. katherinensis*.
4. Mass production methods must be investigated for the *scutellaris* material.
5. Field trials must be designed and carried out. Particular attention must be given to methods of assessment of population size and to safeguards in case unexpected population phenomena occur. Both problems would be best surmounted on an island.

Ancillary research should be conducted on vector capacity for filariasis in the *A. scutellaris* complex. Backhouse & Woodhill (1954) found remarkable differences among certain forms in the complex with respect to susceptibility to *Wuchereria bancrofti*. For example, 58 % of 103 individuals of *A. pseudoscutellaris* from Fiji showed complete development of larval worms ; on the other hand, no development occurred in any of 91 individuals of *A. scutellaris scutellaris* from New Guinea. This must be considered in relation to recent work by Macdonald (1962) who has isolated a single gene controlling susceptibility to filaria in *A. aegypti*. He found that a sex-linked recessive factor confers susceptibility and that this allele is present with different frequency in various laboratory strains. It seems probable that similar gene alleles exist in other *Stegomyia* mosquitos and a search for such factors should be initiated in the *A. scutellaris* complex. Obviously it would be advantageous to incorporate filaria-refractory factors in mosquitos that are mass-produced for genetic control through incompatibility.

To summarize, the *A. scutellaris* complex provides an ideal opportunity for demonstrating genetic control through incompatibility, with the following advantages :

1. An important vector, difficult to control in other ways, is involved.
2. The vector occurs on islands, simplifying experimental evaluation and control.
3. Mass production facilities for the vector are available.
4. Genetic incompatibility mechanisms are available for some strains.

**DISTRIBUTION OF MEMBERS OF THE *AEDES SCUTELLARIS* SUBGROUP,  
GROUP C, SUBGENUS *STEGOMYIA* \***

<i>Taxon</i>	<i>Author</i>	<i>Distribution</i>
<i>alorensis</i>	Bonne-Wepster & Brug, 1932	Alor Island
<i>andrewsi</i>	Edwards, 1926	Christmas Island
<i>aobae</i>	Belkin, 1962	Banks Island, New Hebrides
<i>cooki</i>	Belkin, 1962	Niue Island
<i>futunae</i>	Belkin, 1962	Horne Island
<i>guamensis</i>	Farner & Bohart, 1944	Mariana
<i>gurneyi</i>	Stone & Bohart, 1944	Solomon Island
<i>hebrideus</i>	Edwards, 1926	Solomons, Santa Cruz, Torres, Banks, Wurulu, New Hebrides, Nuguria
<i>hensilli</i>	Farner, 1945	Carolines, Palau
<i>hoguei</i>	Belkin, 1962	Solomons
<i>horrescens</i>	Edwards, 1935	Fiji
<i>katherinensis</i> <sup>a</sup>	Woodhill, 1949	North-East Australia
<i>marshalensis</i>	Stone & Bohart, 1944	Marshalls, Gilberts, Carolines
<i>paullusi</i>	Stone & Farner, 1945	Philippines, Tarona, Sangin, ?Celebes, ?Moluccas, ?Sumatra
<i>pernotatus</i>	Farner & Bohart, 1944	New Hebrides
<i>polynesiensis</i>	Marks, 1951	Fiji, Ellice, Samoa, Cook, Tokelau, Marquesas, Horne, Society, Tuamotu, Austral
<i>pseudoscutellaris</i>	(Theobald, 1910)	Fiji
<i>quasi scutellaris</i>	Farner & Bohart, 1944	Solomons, Bismarck Archi- pelago
<i>rotumae</i>	Belkin, 1962	Rotuma Island
<i>scutellaris</i>	(Walker, 1859)	New Guinea, Moluccas, New Hebrides, Philippines, Hainan, Bismarck Archipelago, Solo- mons, Palau, Caroline
<i>tongae</i>	Edwards, 1926	Tonga, Solomon, ?Santa Cruz
<i>upolensis</i>	Marks, 1957	Samoa
<i>varuae</i>	Belkin, 1962	Solomons, Santa Cruz

\* After Woodhill (1949), Stone, Knight & Starcke (1959) and Belkin (1962).

<sup>a</sup> Retained as a subspecies of *scutellaris* by Stone, Knight & Starcke (1959).

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## Annex 2

A POSSIBLE METHOD OF CONTROL  
OF *ANOPHELES GAMBIAE* GILES

The successful eradication of the screw-worm (*Cochliomyia hominivorax* (Coquerel)) from the island of Curaçao has recently been achieved by the release of flies of this species previously sterilized by gamma radiation from cobalt-60 sources (Baumhover et al., 1955). This species mates only once in its lifetime. Irradiation of the male does not prevent the production of spermatozoa and in the act of mating these are apparently actually transferred to the female. However, eggs laid by such mated females are invariably sterile.

The effect of such radiation on the tsetse-fly, *Glossina morsitans* Westw. has also been shown to lead to a considerable measure of sterility, though no release of such sterilized insects has yet been attempted in the field (Potts, W. H., 1958). The effects of radiation on *Anopheles quadrimaculatus* Say are also promising, for it has been shown that the release of sterile males into cages of normal males and females in the ratio of 6 : 1 : 1 and 10 : 1 : 1 has resulted in a reduction of about 80 % in viable egg production (Davis, A. N. et al., 1959).

In the course of investigations on the mode of inheritance of dieldrin-resistance in *Anopheles gambiae* Giles it was found that the crossing of certain strains of this species resulted in the production of hybrid males with atrophied testes, the hybrid females being reproductively normal (Davidson, G., 1958). When the two sexes are allowed to emerge together in a cage and the females are fed on blood, eggs are readily laid but are almost invariably sterile; approximately, only one egg in ten thousand hatches. This contrasts with some reproductively normal strains of the same species which for some reason do not readily mate under laboratory conditions. Here, although the unmated females may feed and develop their eggs, these are not readily laid. For this reason it is believed that the sterile hybrid males with atrophied testes are still capable of carrying out the act of copulation, but without (or very rarely with) any transfer of spermatozoa.

In a preliminary laboratory experiment, such sterile males have been produced by crossing the males of a strain of *A. gambiae* from Lagos with the females of a strain of the same species from Italian Somaliland. Two hundred and sixty-three of these sterile males were introduced into a cage containing 26 males and 26 females of the normal Lagos strain. A control cage containing 26 male and 26 female Lagos *A. gambiae* was also set up. The females in both cages were fed and eggs obtained. A total of three successive egg-layings from each cage over a period of two weeks has shown a normal hatching from the control cage and no hatching at all from the cage containing the sterile males.

This, then, offers a potential means of control of *A. gambiae*, a species which is proving difficult to control by the use of insecticides.

The sterilization of the males does not in this case involve the handling and transport of radioactive materials but merely the crossing of two strains of the same species. The production of large numbers of hybrids should not present great difficulties, although some efficient and rapid method of separation of the sexes would be required. Methods of transport and release of the sterile males and ways of estimating population densities of the species will certainly present some difficulties, but a preliminary field trial at least would seem worth while and in the event of failure it must be remembered that the introduced mosquitos are male and, therefore, incapable of transmitting malaria.

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