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**CYTOGENETICS OF VECTORS
OF DISEASE OF MAN**

**Report
of a WHO Scientific Group**

WORLD HEALTH ORGANIZATION

GENEVA

1968

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OF DISEASE OF MAN

Geneva, 31 October - 6 November 1967

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CYTOGENETICS OF VECTORS OF DISEASE OF MAN

Report of a WHO Scientific Group *

A WHO Scientific Group on the Cytogenetics of Vectors of Disease of Man met in Geneva from 31 October to 6 November 1967. Dr A. M.-M. Payne, Assistant Director-General, opened the meeting on behalf of the Director-General. Professor J. B. Kitzmiller was elected Chairman, Dr J. Itard Vice-Chairman, and Dr L. E. LaChance Rapporteur.

1. INTRODUCTION

The suppression and elimination of the major epidemic and endemic diseases of man, such as malaria, yellow fever, haemorrhagic fever, and filariasis, require effective vector control. The development of resistance to insecticides and the fact that it is not desirable to depend completely on such chemicals for the control of vectors necessitate a search for new approaches to vector control.

During recent years many advances have been made in the control of insects by genetic manipulation. For example, the well-known sterile male technique (using males sterilized by ionizing radiation or by chemical action) has been used in eradicating the screw-worm fly from Curaçao and from the south-eastern and south-western areas of the USA. The successful field experiment in Rangoon, Burma, in which the cytoplasmic incompatibility technique was used, and by which *Culex fatigans* was eliminated from a small village, was made possible by WHO-supported research on the genetics of vector species.¹ Other methods, including the use of hybrid sterility, meiotic drive chromosomes, genes that distort the sex ratio, lethal genes, chromosomal mutations, sterility resulting from chromosome translocations, and genetic manipulation of sex chromosomes, show promise. The tremendous potential of these methods, and the economic gains that can be achieved by their application, have been discussed elsewhere.²

* This meeting was supported by the World Health Organization and by Research Grant No. CC 00261 from the National Communicable Disease Center, United States Public Health Service, to WHO.

¹ Pal, R. (1967) *WHO Chronicle*, **21**, 343.

² WHO Co-ordination Group on Genetic Control of Insects of Public Health Importance (1967) *Report* (mimeographed document WHO/VBC/67.47).

Cytogenetics, the study of chromosomes in relation to morphological and physiological manifestations, is an integral part of research on the genetics of vector species. It provides the most precise method known for differentiating extremely closely related species or populations and those that cannot be differentiated by conventional means and that may differ in their vectorial capacity. The basic process of gamete formation and numerous aberrations of this process (such as distorted sex ratios and meiotic drive phenomena), and the effects of radiation and of chemicals on chromosomal material are all directly related to cytogenetics. The Scientific Group believes cytogenetics to be a fundamental area of study from which future control methods may evolve, and recommends that WHO encourage the continuation and development of research in the cytogenetics of vector species. WHO has profited from cytogenetic research that has been carried out, and at the same time has served a valuable function in supporting and encouraging further research to achieve the ultimate goal of controlling vector species by nonchemical methods.

Cytogenetic studies of insect vectors provide a foundation for all genetic control methods and yield valuable information on the basic biology of such vectors. For this reason, WHO convened the present Scientific Group to review the status of knowledge of the cytogenetics of disease vectors and to suggest areas of study that might lead to the most rapid advances in knowledge. The Group was informed that WHO intends to establish a research unit on *Aedes aegypti* in East Africa. Such a project is ideal for specific genetic and cytogenetic studies (see Annex 1).

2. IMPORTANCE OF VECTOR CYTOGENETICS

Every characteristic of a vector species (except inheritance through cytoplasmic genes) is transmitted to its progeny via the chromosomes. Cytogenetic mechanisms control both the transmission of genetic information and the production of gametes, functions that are basic to the process of reproduction and that must be interrupted if a vector species is to be controlled. Some of the important aspects of cytogenetic studies are listed below; they are discussed in greater detail in subsequent sections.

2.1 Speciation; cytotaxonomic and evolutionary studies

Cytogenetic information provides knowledge of the taxonomic and evolutionary relationship between species. Many disease vectors form species complexes in which it is impossible to distinguish the various species except by chromosome studies. If the species or populations differ in their vector ability, it is apparent that only through cytogenetic studies may it be possible to determine what species should be controlled. The economic

wisdom of using a relatively small amount of resources to select the susceptible target species is obvious. Furthermore, if two morphologically identical populations maintain distinct cytogenetic identities, they must represent sexually isolated populations. Consequently, any genetic control method must be based on the release of insects that are sexually compatible with the vectors in the field. This further emphasizes the need for cytogenetic knowledge (see section 4).

2.2 Basic genetics of disease vectors

Cytogenetic studies provide a basis for other studies of vector genetics. For example, recent studies¹ have dramatically illustrated the use of cytogenetic methods of translocation analysis to assign each linkage group in the house-fly to a specific chromosome. With this information, all new mutations can now be assigned to a particular chromosome by simple linkage studies. Such studies have resolved the serious semantic difficulties that existed in this field as a result of (1) the mandatory random assignment of the various linkage groups to numerically designated chromosomes and (2) the incorrect use of the word "chromosome" to signify a linkage group. Similar studies of other vector species will promote more rapid and thorough understanding of the genetics of disease vectors (see section 3).

2.3 Cytogenetic mechanisms for vector control

2.3.1 *Hybrid sterility*

The inducement of sterility by the hybridization of closely-related species is an ideal technique for use in control programmes. Such species may be hybridized in the laboratory and then released in the field, or wild population may be flooded with a species that will cause hybrid sterility. In order to extend work on hybrid sterility, further knowledge of cytogenetics is necessary.

When combined with genetic analyses, studies of hybrid sterility are of great value in revealing the degree of structural changes in the chromosomes of hybrid species and in determining the type of sterility involved. With this knowledge it is possible to show the degree of isolation that the species have attained and to predict whether such species could be used in genetic control projects (see section 6.2.6).

2.3.2 *Induced sterility*

Cytogenetic information on a vector species is invaluable in planning investigations of the effects of ionizing radiation or chemosterilization. For

¹ Wagoner, D. E. (1967) *Genetics*, 57, 729.

example, knowledge of the chromosome number and of the structure of a species enables the radiation geneticist to estimate the sensitivity or resistance of the species to treatment with radiation or a chemosterilant, and helps him to select dose ranges to be investigated for sterilizing effects. Within a species, knowledge of the cell types being treated and of the condition of the chromosomes in these cells at the time of treatment is of great value in predicting the sensitivity of the cells to mutagenic agents. For example, numerous studies have shown that mature spermatozoa, spermatids, and spermatocytes differ greatly in sensitivity. Similarly, prophase oocytes are much more radiation-resistant than metaphase or anaphase oocytes. Cytological study of the reproductive organs also indicates whether infertility, aspermia, or mature gametes with dominant lethal mutations are likely to result from treatment with sterilizing agents. Cytogenetic information concerning the effects of ionizing radiation or chemicals on reproductive cells also indicates whether sterility will be temporary or permanent (see section 6.1).

2.3.3 *New sterility methods*

Studies should be undertaken to detect irregularities in gametogenesis that might be useful as a basis for genetic control. For example, chromosomes that exhibit meiotic drive are known to occur in *A. aegypti*, and it is reasonable to expect that they will be found in other vector species. Deleterious genes could be linked with the meiotic drive chromosomes to provide a method of genetic control.

Cytogenetic studies are also required to elucidate the different mechanisms of sex determination in insects. The possibility of genetic control of the sex ratio of vector species, the production of sterility through the formation of nonfunctional gametes, and genetic control by other mechanisms are all related to the cytogenetics of sex determination. For example, genetically contrived XO males and X-incomplete Y males in *Drosophila melanogaster* are sterile because they produce nonfunctional or nonmotile spermatozoa. More complete understanding of sex chromosomes (and of sex determination) may make it possible to alter them so as to bring about sterility in other ways.

High levels of sterility are often found in individuals that are heterozygous for chromosome translocations. The use of vectors that are partially or totally sterile because of inherited translocations or other gross chromosomal abnormalities offers great promise. Such strains have been synthesized in the house-fly and in *A. aegypti* (see section 6.2).

2.4 Cytogenetic studies of vectors other than mosquitos

Most of the work that has been done on the cytogenetics of disease vectors has been carried out on mosquito species. However, other vectors

of diseases of man should not be ignored. Cytogenetic investigation of the medically important Hemiptera and of tsetse flies, fleas, and ticks should be encouraged, since these insects certainly transmit diseases of man. Many Diptera (e.g., sand-flies and biting midges) also transmit diseases. Cytogenetic information on these species is extremely meagre, and few genetic control studies have been carried out on them. Since both males and females of many of these vectors transmit disease, any genetic control method should take into account the fact that there are serious objections to the release of additional vectors. Cytogenetic studies in support of autosterilization research should be encouraged.

3. STUDY OF CHROMOSOMES IN VECTORS

In 1963 a WHO Scientific Group on Genetics of Vectors and Insecticide Resistance¹ concluded that the status of cytogenetic research on vectors was fragmentary and that relatively few laboratories were working in this field. This statement still holds true, since only a limited amount of work has been carried out during the past few years. The present Group strongly endorses the recommendation of the 1963 Group that descriptive studies of karyotypes, particularly in important genera of insects of public health importance, should be completed as quickly as possible. This relatively simple research would provide a basis for further important studies.

3.1 Karyotypes

In general, the karyotype is a description of the chromosomes of a species in terms of their number, size, and shape. It usually includes a description of the nature and position of the centromere, satellites, and nucleolar organizer region.

The present knowledge of the karyotypes of some insects of public health importance is summarized below.

Culicidae. The diploid number of all species examined is 6. The individual chromosomes are usually numbered according to length and to position of the centromere. The chromosomes are numbered in increasing order of size.

Culicini. Metaphase karyotypes studies of Culicini have been remarkably uniform, showing three pairs of more or less metacentric chromosomes of which two pairs are considerably longer than the third. In general, the two longer pairs may be differentiated by slight differences in arm length.

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1964, 268.

Anophelini. Some species of the genus *Anopheles* differ from *Culex* and *Aedes* in that they have distinguishable heterosomes. In all such species the males are the heterogametic sex. The two pairs of autosomes are metacentric, or nearly so, with slight differences in their over-all length and in the position of the centromere. The variations recorded in the heterosomes appear to be constant for all *Anopheles* mosquitos, differing only in the short arm; the long arms in all species are equal. In the *Anopheles maculipennis* complex there is a long heterochromatic portion and a short euchromatic portion. The X chromosomes of the species *A. quadrimaculatus* and *A. claviger* show very reduced heterochromatic portions. *A. gambiae* species A exhibits an extensive heterochromatic region next to the centromere. Species of the *Nyssorhynchus* group can be classified by the progressive loss of the heterochromatic portion of the X chromosome.

Simuliidae. Karyotypes in the Simuliidae have been established for almost 100 species. The basic chromosome number throughout the family is $2n = 6$. The arm ratios are typically close to 1 : 1 for the largest chromosome, approximately 1 : 1.5 or 1 : 1.6 for the second chromosome, and 1 : 1.2 or 1 : 1.3 for the shortest chromosome. Heteromorphic sex chromosomes are not demonstrable in somatic mitosis. Several species are triploid and parthenogenetic. In approximately 5% of the species small supernumerary (B) chromosomes occur; they are found in variable numbers in different individuals of a population. In the evolution of blackflies, pericentric inversions and whole-arm interchanges have occurred on a number of occasions, leading to distinctive karyotypes for several species. Further karyotype specificity is provided by the nucleolar organizer; its position is constant for each species but shows considerable variation between species. Finally, species differ in the amount of condensed heterochromatin in their interphase nuclei. This feature is sometimes sufficient in itself to differentiate closely related species.

Muscidae. The house-fly, *Musca domestica*, possesses a diploid chromosome number of 12, consisting of 5 pairs of autosomes and a pair of sex chromosomes that are heteromorphic in the males. All the chromosomes of this species are metacentric or submetacentric. It is probable that both the X and Y chromosomes are entirely heterochromatic. However, a considerable amount of polymorphism for heterochromatic sex chromosomes exists in the house-fly. For example, some natural populations contain homomorphic heterochromosomes in both sexes, and in some populations the heterochromosomes appear to have no significance in sex determination. Apparently, a good deal remains to be resolved concerning sex determination in various house-fly populations.

Glossininae. Research on species of Glossininae has shown great differences between *G. tachinoides* and *G. morsitans morsitans*. The species *G. tachinoides* belongs to the *palpalis* group (subgenus *Nemorhina*) and has

a chromosome complement of $2n = 6$. These chromosomes have similar dimensions; two pairs are metacentric and the third pair is telocentric. A species in the *morsitans* group (subgenus *Glossina*), *G. morsitans morsitans*, has a chromosome number $2n = 10$. This number comprises three pairs of large metacentric chromosomes and two pairs of small chromosomes in the somatic tissues.

Blattidae. Chromosome numbers have been determined for only a few species of cockroach. From these determinations it is evident that the chromosome number varies considerably. However, in all species examined the male has been the heterogametic sex and has had the XO condition. In the species *Blattella germanica*, a considerable number of marker mutants have been isolated ($2n = 23$ in the male and 24 in the female). The nucleolus in all species is closely associated with the X chromosome.

Reduviidae. Karyotypes have been described in 25 species of Reduviids (bugs) of the tribe *Triatomini* ($2n = 18$ or $20 - XY$ or XXY male), and four species of the tribe *Rhodnini* ($2n = 20 - XY$). The number and size of the chromosomes, the position of the X and Y chromosomes in the metaphase plates, multiple X systems, and heterochromatic autosomes have also been described. A general homogeneity in the karyotype is found in this group; however, some species with the same number of chromosomes differ greatly in size.

Cimicidae. The karyotype of female bed-bugs comprises 13 autosomes and a number of X chromosomes that varies from 2 to 14. The male has the same complement plus one Y chromosome. The variation in the number of X chromosomes has been shown to be a characteristic of different populations. Two strains of *Cimex pilosellus* from California have been described with different chromosome numbers. One strain ("A") has 12 pairs of autosomes and the other ("B") has 10 pairs. Hybrids of the two strains have shown $2n = 23$ chromosomes and at meiosis two types of spermatozoa have been formed, one type containing 10 autosomes, the other 12.

Ticks. Acarines have a low or moderate number of chromosomes. In most taxa that have been investigated the numbers vary from species to species (from 12 to 33). Monocentric and diffuse centric chromosomes are present.

3.1.1 Changes in chromosome number

Changes may also take place in the total number of chromosomes, so that they are either more or less than a diploid set. There are two principal types of such change: polyploidy, the occurrence of one or more additional complete sets of chromosomes to produce $3n$, $4n$, etc., individuals (see section 6.2.5); and aneuploidy, changes involving the numbers of chro-

mosomes in a set—e.g., $2n + 1$, $2n - 1$, etc. (see section 6.2.4). These changes may be easily observed in polytene chromosomes, but they may also be seen in meiotic and mitotic preparations.

3.1.2 DNA determination

One of the most exciting discoveries of the past 20 years is the finding that deoxyribonucleic acid (DNA) is the genetic information material and that it contains in its nucleotide sequence the code that specifies all the proteins in the cell.

From the point of view of genetic control there are prospects of artificially altering the genetic material of the vector itself. At present this is possible only in microbial systems. However, it is already possible to differentiate closely related or sibling species by determining the DNA content of the nuclei; for example, this technique can readily be used to differentiate two subspecies of chironomid midges, that are otherwise indistinguishable except by the details of their salivary gland chromosome banding patterns.¹ It is recommended that, wherever possible, DNA determinations be performed as an integral part of karyotype investigation.

Techniques for determining the amount of DNA per nucleus range from the comparatively simple to the highly complex. Basically, they involve the squashing of comparable cells (e.g., neuroblasts and spermatocytes) of two species (or suspected species) on the same slide and staining by the Feulgen method, which is specific for chromosomal DNA. By means of photometric extinction determinations at two different wavelengths² it is possible to compute relative amounts of DNA per nucleus. More refined methods are based on a scanning-integrating technique at a single wavelength. Such determinations need not be performed in the field, since material can be readily preserved and studied in a central or base laboratory.

3.2 Salivary gland chromosomes

Long, banded, polytene chromosomes occur in the cells of many tissues in the Diptera. They are particularly evident in the salivary glands of mosquitos, chironomids, blackflies, and many other families of the Nematocera. Such chromosomes often attain a length of 500 μ , present a characteristic pattern of banding within a species, and are extremely useful for many types of cytogenetic research. They are formed by reduplication of haploid chromosomal strands (polyteny), so that they may represent up to 512 or 1024 n strands. They are divided into arms by the position of the centromere and often contain distinctive puffs, Balbiani rings, and nucleolar

¹ Keyl, H. G. (1965) *Chromosoma (Berl.)*, **17**, 139.

² Patau, K. (1964) *Chromosoma (Berl.)*, **15**, 14.

organizer regions. The characteristic bands are DNA-positive and reflect the linear arrangements of genes. The basic importance of these chromosomes derives from the fact that they represent a huge magnification of the diploid chromosomal complement, making it possible to analyse many of the structural and functional properties of the chromosomes.

The consistent banding pattern makes it possible to (1) identify genera, subgenera, species, and populations (which is often of the utmost importance, particularly among groups that cannot be distinguished by conventional means), and (2) recognize structural rearrangements of the linear pattern of the chromosomes, which are usually characterized as inversions, translocations, deletions, and duplications. (Such rearrangements are discussed in section 6.2.)

3.2.1 Mapping of salivary gland chromosomes

The mapping of salivary gland chromosomes has been successful with some groups (*Simulium* and *Anopheles*), but the nature of the material has prevented progress with other groups. However, the first maps of *Culex pipiens* have been prepared. Some 20 published maps are available for *Anopheles* and about 100 for *Simulium*. All species can be differentiated on the basis of gross features and banding details of the salivary gland chromosomes. It is probable that similar techniques will be helpful in the preparation of analysable chromosome preparations from *Aedes*.

3.3 Linkage maps

The isolation and colonization of visible mutant strains in some species of insect has developed to such an extent that it is now possible to construct linkage maps. For most vector species, further studies are required before it will be possible to assign linkage groups to morphologically identifiable chromosomes. For the house-fly, 5 linkage groups have been assigned to the 5 autosomes.

Culex pipiens. Little information is available on the linkage groups in *Culex pipiens*, despite the fact that there are 10 good marker mutants in the 3 linkage groups. The linkage groups have not yet been assigned to a morphologically identifiable chromosome. Linkage between the mutants is known, but data on the sequence of the genes in the linkage group are limited.

Aedes aegypti. Studies of *Aedes aegypti* show far more promise than those of other Culicinae. Over 50 marker genes have been isolated, and of these 28 have been placed in the 3 linkage groups. As with *Culex*, the linkage groups have not been assigned to specific chromosomes.

Anopheles. Marker mutants have been established on each of the 3 linkage groups in *A. gambiae* species A and B and *A. pharoensis*. A few

mutants are also available in other species of *Anopheles*, but as yet the only identification of a linkage group with a particular chromosome has been with the sex-linked genes.

Musca domestica. Genetic studies of the house-fly have shown the presence of 5 linkage groups, corresponding to the 5 pairs of autosomes. No sex-linked genes have been demonstrated. The 5 linkage groups were originally designated by different arbitrary numbers by two research groups. The house-fly karyotype was worked out by two other groups of investigators and was also numbered differently. As a result, some combinations of linkage groups and chromosomes had as many as 4 different number designations. These different numbers for the linkage groups covered about 60 mutants.

In order to rectify this situation, X-ray-induced reciprocal translocations were analysed cytologically in order to find the particular linkage group located on a specific chromosome. The translocations were induced between selected mutants and subsequent cytological analysis indicated the chromosome pairs involved in interpairing.¹ Where only two mutants were involved in the original isolation, a number of triple and quadruple reciprocal translocations were found during subsequent cytological analysis. Further genetic analysis showed the mutants to be involved in the induced linkage.

By agreement a system has been adopted in which X and Y represent the sex chromosomes and autosomes are numbered from I to V, chromosome I being the longest and V the shortest. Agreement among geneticists on linkage groups in the house-fly resulted in the system described in the WHO *Vector Genetics Information Service*.²

On the basis of genetic tests and analyses of numerous chromosome translocations, it has been determined that the linkage groups containing the following mutants are located on the chromosomes indicated: *Rl* and *ac* mutants: chromosome I; *car*, *cm*, and *ar* mutants: chromosome II; *bwb* mutant: chromosome III; *rb*, *ct*, and *ye* mutants: chromosome IV; *ocra* linkage group: chromosome V. Thus there has been a unification of the nomenclature of the chromosomes and of genetic knowledge. It is now possible to assign all the 60 marker genes to specific chromosomes.

Blattella germanica. The German cockroach, in which 45 mutants have been recorded, provides additional material for the assigning of particular linkage groups to particular chromosomes. Marker genes have been tentatively assigned to 9 linkage groups; in all instances, the linkage distances between visible markers are quite small. However, except for group I, which contains the single-sex-linked mutant, the linkage groups

¹ Wagoner, D. E. (1967) *Genetics*, 57, 729.

² World Health Organization (1968) *Vector genetics information service No. 2*, Geneva (mimeographed document).

have not been correlated with specific chromosomes. Preliminary cytogenetic investigations indicate that the X chromosome is easily distinguished from the autosomes in most meiotic stages. A considerable amount of further work with chromosomal mutations will be necessary before the linkage groups can be assigned to specific chromosomes.

3.4 Conclusion

The mapping of salivary gland chromosomes and the assignment of a particular gene to a particular band or series of bands is the next step that can be taken in the cytogenetic study of species with polytene chromosomes. Radiation-induced translocations can also be used to identify linkage groups and to assign them to a particular chromosome. Genetically marked X-ray-induced reciprocal translocations can be analysed cytogenetically and genetically to determine the exact location of mutant genes.

The Scientific Group recommends that in addition to descriptive studies of karyotypes and salivary gland chromosomes, linkage maps and maps of polytene chromosomes be prepared for as many vector species as possible, particularly those in which taxonomy and disease transmission are problems.

4. SPECIATION, SPECIES COMPLEXES, AND CYTOTAXONOMY

4.1 Chromosomal polymorphism

Gene mutations and changes in gene arrangement (e.g., inversion) are an important evolutionary mechanism. In any given species new mutations and new chromosome sequences are continually injected into the population. In organisms with salivary gland chromosomes, structural changes are directly identifiable under the microscope. Rearranged sequences may differ from the original in the alleles of one or more genes, and certainly will come to do so subsequently as a result of mutation. Such structural changes are important since, in the heterozygote, genes within the limits of the rearrangement cannot readily, if at all, be exchanged by crossing over; they are inherited as a block or "super-gene". Heterozygosity for such "super-genes" may confer a selective advantage on the inversion heterozygote, thus ensuring essentially unaltered proportions of homozygotes and heterozygotes in the population year after year. Apart from its intrinsic interest to evolutionists, this inversion polymorphism is an important diagnostic criterion for the characterization of populations. Furthermore, it may permit inferences to be made regarding the biological and ecological factors responsible for the genetic isolation of neighbouring populations.

4.2 Sibling species

When sufficient genetic diversity accumulates, recombination within each chromosomal type may lead to the emergence of structural homozygotes that are, ultimately, adaptively superior to the heterozygotes. Species formed in this way may initially differ only in homozygosity for one inversion difference and for the assemblage of genic alleles contained in that inversion. Morphological and behavioural differences between such emerging species may initially be so slight as to be undetectable; differences will be found only by examination of their chromosomes. Such species are referred to as cryptic or sibling species.

4.3 Cytotaxonomy

The application of cytological methods to the detection of sibling species has been important in many vector studies—e.g., studies of the *maculipennis* group of the genus *Anopheles*. In this group of morphologically indistinguishable sibling species, differences in the salivary gland chromosomes permitted positive identification of the different species, of which some—but not all—are important vectors of malaria.

Continued research on the salivary gland chromosomes of *Anopheles* has shown that subgenera may be identified on the basis of banding patterns in the autosomes; all species thus far studied can be positively identified by means of the banding pattern of the X chromosomes.

An important practical application of this method has recently been demonstrated. In Africa, the so-called *Anopheles gambiae* is actually a complex of five sibling species, two of which can usually be distinguished morphologically; the other three are identical and can be distinguished only on the basis of breeding tests. It is now possible to identify some species quite simply and rapidly by means of the banding pattern of the X chromosome from the salivary gland cells.

Detailed studies have clarified the phylogeny and the systematics of the blackflies. Numerous taxonomic "species" have been shown to be comprised of biologically distinct and reproductively isolated sibling species. On the basis of inversion differences studied in *Simulium damnosum*, it has been shown that this "species" is made up of eight, and probably more, distinct species. Furthermore, using inversions to trace common descent, it has been possible to draw up a phylogenetic scheme of relationships among such species. Discovery of these sibling species may partly explain the diversity of morphological and behavioural characteristics reported for *S. damnosum*; it may be that such species differ in vectorial capacity and in the bio-ecological properties that determine their susceptibility to control.

It should be emphasized that, at present, examination of the salivary gland chromosomes offers the only practical means of determining the number and distribution of biologically distinct and reproductively isolated entities that exist in a complex of vector species.

5. GAMETOGENESIS

The phenomenal ability of most insects to produce vast quantities of gametes underlies their importance as pests and as disease vectors. Consequently, knowledge of gametogenesis is essential in genetic control. The fertility of every species depends on the precision of this process.

It is important not merely to understand the natural pattern of gametogenesis, which is only of academic interest, but to be able to detect abnormal patterns that could be utilized in genetic control.

5.1 Normal pattern

Gametogenesis is the mechanism by which the diploid cells of insects eventually produce haploid gametes. The process includes the mitotic divisions of gonial stem cells, from which all spermatozoa and ova originate; the duplication of the genetic material; the meiotic divisions during which crossing-over and recombination of genes on homologous chromosomes occur; the random assortment of whole chromosomes; the reduction of the diploid number of chromosomes ($2n$) to a haploid number (n); and the physiological and morphological changes undergone by a haploid cell when it is transformed into a mature gamete. A thorough understanding of all phases of gametogenesis is necessary before the effects of sterilizing agents on the gametes can be assessed. For example, knowledge of the types of cell in the testis or ovary is necessary before insect sterilization studies can be properly conducted. Spermatids and mature spermatozoa differ in their sensitivity to ionizing radiation, as do ova at different stages of development. A certain amount of information is available on several vector species (the house-fly, some mosquitos, some Hemiptera, and some lice), and many more species are being studied. For example, only preliminary data are available on the tsetse fly; there is some information on the stage at which the egg follicles are formed in the adult female, but little is known of the processes of spermatogenesis and oogenesis in the pupae. It appears that the testes and accessory glands are well-formed in 8-10-day-old pupae. In males, spermatogenesis is limited to the pupal stage. The adult male contains the entire supply of mature sperm and apparently all cell division in the testes has ceased. Detailed studies of gametogenesis in all vector species are necessary.

5.2 Aberrant pattern

The normal process of gametogenesis may be interfered with in both males and females in several ways and at several points in the cycle. Agents such as chemical mutagens, chemosterilants, and different types of radiation produce small or large changes that have important results, chief of which is interference with the normal mechanisms of duplication and division of chromosomes, nuclei, and cells. As a result, sperm or eggs may be produced with deficient or abnormal chromosomes and with other defects that eventually reduce the fertility of the insect.

In addition to induced chromosomal changes, a wide variety of naturally occurring, poorly understood phenomena may offer important opportunities for genetic control. These include meiotic drive or some other form of preferential segregation of chromosomes, including nondisjunction, polyploidy, and distortion of the sex ratio.

Since the process of meiosis, which is most critical in the survival of the species, includes many complex events, it offers many opportunities for the disturbance of such events.

6. INSECT STERILITY

The use of sterile insects in population suppression is a significant entomological achievement. This technique is a new approach to insect control and, since it is the only method that has led to the eradication of a species (e.g., screw-worms) from a large area, its great promise for vector control is obvious. Research in insect sterility is closely related to cytogenetic studies. A great deal is known of the sterilization of many vector species by physical, chemical, or genetic methods. Sterility, the inability to reproduce, may arise from a variety of causes, such as dominant lethal mutations induced in the gametes, interruption of sperm production, infecundity in the female, sperm inactivation in the male, and failure of the mating process. Since all types of sterility are not equally useful in an insect control programme, the type of sterility induced should be known. The basis of sterility can be assessed by cytogenetic studies, which also make it possible to predict the usefulness of a given type of sterility in a given control programme.

6.1 Induced sterility

The exposure of insects during the late pupal or adult stages to either ionizing radiation or chemical sterilants often produces total sterility without otherwise debilitating the insect significantly.

The dosage of radiation or of a chemical required to produce sterility is characteristic for a given species. For example, a radiation dose of

6-7 krads is required to sterilize *Anopheles* species, whereas a dose of 20-25 krads is required for adult male *Glossina*.

Such treatment induces dominant lethal mutations in all the mature sperm contained in the insect. The type of insect sterility that has been studied most extensively is that which arises from the induction of dominant lethal mutations. A dominant lethal mutation is a nuclear change that causes death of the zygote, even though the mutation is carried by only one of the germ cells that unite at fertilization.

Numerous studies have shown that both radiation-induced and chemically-induced dominant lethal mutations arise as the result of chromosomal change in the treated cell (sperm or egg), expressed as chromosome aberrations (chromosome bridges and fragments) and developmental abnormalities (mitotic inhibition and polyploid and aneuploid nuclei) in the developing insect embryo. These cytogenetic abnormalities are sufficiently severe to prevent normal embryonic development and usually result in the death of the embryo before hatching.

Since insects exposed to ionizing radiation or chemicals contain, in addition to sperm and eggs, a variety of immature germ cells, other factors must contribute to sterility. Treatment that induces dominant lethal mutations in the mature spermatozoa of the male very often causes severe damage to the immature spermatocytes and spermatogonial cells. Complete elimination of such cells results in an aspermic male. If the radiation or chemical treatment is not sufficient to eliminate the immature germ cells, the undamaged gonial cells can often divide mitotically and repopulate the testes with undamaged mature spermatozoa, leading to the recovery of fertility. Apart from occasional checks of the permanence of sterility at various intervals after treatment, the recovery of fertility by previously sterile males has received scant attention in past insect sterility studies. Most studies indicate that fertility is not usually recovered after a period of sterility, since doses that kill the mature cells also destroy the immature germ cells. However, there are significant differences in the amounts of radiation required to eliminate gonial cell populations in different species. An investigation of the X-ray dosage required to destroy all spermatogonia in three species of muscoid fly (*Musca domestica*, *Phormia regina*, and *Cochliomyia macellaria*) showed that approximately 3000 R and 5000 R were sufficient for *M. domestica* and *P. regina*, respectively, but spermatogonia occasionally survived in a testis of *C. macellaria* after doses of up to 8000 R.¹ Very little is known about the effects of chemosterilants on the immature germ cells.

Sterility in the female is generally attributed to infecundity or to the induction of dominant lethal mutations in the eggs. The killing of oogonial cells is one factor that leads to infecundity. Damage to the polytene chro-

¹ Reimann, J. G. & Thorson, B. (1967) Unpublished report.

mosomes of the nurse cells that supply the growing oocyte with nutrient material is also observed. During a part of the endomitotic cycle, nurse-cell chromosomes are extremely sensitive to sterilizing treatment, but they are extremely resistant after the endomitotic stage has been reached. Consequently, it is easy to predict whether infecundity will be achieved on the basis of cytogenetic information on the condition of nurse-cell chromosomes and on the stage of development of the egg follicles at a given point in the insect's life cycle.

If the sterilizing treatment reduces the longevity, competitiveness, and general vigour of insects, it is possible that cytogenetic damage to somatic cells has occurred. For example, a serious reduction in the longevity of boll weevils exposed to substerilizing doses of radiation was traced to the destruction of midgut epithelial cells.¹

Sterilizing treatment administered at certain stages of an insect's life cycle often has adverse effects on its vigour, but little is known of the cytogenetic basis of such debilitating effects. However, sterilization without loss of vigour is fundamental to the application of the sterile male technique.

Sterility resulting from sperm inactivation has received scant attention in insect research. Only recently has it been shown that such inactivation is an important factor in the sterilization of male lepidopteran insects. The possibility of inactivating sperm by chemosterilants has scarcely been investigated, yet a chemosterilant that produces sterility by inactivating the sperm might prove far more useful than one that produces sterility by inducing genetic damage,² since genetic damage must be limited to the species under control.

Before the sterile male technique is used in population control experiments, the biological basis of the sterility should be investigated, since every type of sterility cannot be used for every type of insect. Failure to understand the basis of sterility could easily result in the failure of a field test. For example, in a polygamous species where multiple matings occur, an aspermic male or one with inactivated sperm would decrease the population little, if at all. In this situation, only males having active sperm with dominant lethal mutations can be expected to contribute to population decrease. In a monogamous species, however, aspermic males—or those with inactivated sperm—might prove just as useful as males whose sperm have dominant lethal mutations, since the females will not be reinseminated after the first mating. Knowledge of the reproductive habits of the vector and of its ecology, and a thorough understanding of the cytogenetic basis of sterility, are essential for successful insect eradication by means of the sterile male technique.

¹ Riemann, J. G. & Flint, H. M. (1967) *Ann. entomol. Soc. Amer.*, **60**, 298.

² Most of the currently known chemosterilants are mutagenic chemicals and consequently must be used with great care (see Annex 3).

6.2 Genetic sterility

There are many cytogenetic mechanisms that result in varying degrees of sterility in several vector species. Genetically induced sterility is potentially useful in vector control, and in some situations may offer certain advantages over the release of totally sterile males. In some species, males sterilized by radiation or by chemicals may lack vigour and competitiveness. The ideal situation, of course, would be to use completely sterile males that would compete as well as, or better than, males of the local population. Genetic sterility is sometimes only partial; however, insects with this condition give rise to partially sterile offspring and can, therefore, be used to propagate sterility throughout the natural population. Compared with insecticide control or chemosterilization, genetic control has certain advantages: it does not cause chemical contamination of the environment and has no adverse effects on beneficial organisms (see Annex 3). Although most genetic control methods are still in the theoretical stages of development, they are based on well-known cytogenetic mechanisms which, when induced in vectors, will make the practical application of such control a reality.

6.2.1 *Reciprocal translocations*

A reciprocal translocation involves the exchange of chromosome parts, ranging from a small piece to an entire arm, between nonhomologous chromosomes in the same cell. When a sperm bearing the translocated chromosomes fertilizes an egg, the embryo develops into an adult called a "translocation heterozygote", since the chromosomes contributed by the female gamete do not contain the translocation. This "translocation heterozygote" functions normally until it reaches sexual maturity and begins to produce gametes to give rise to the next generation. Since homologous chromosomes pair at meiosis, segregation of translocated chromosomes occurs in such a manner that a certain number of gametes are either deficient in the translocated chromosome portion or have it duplicated. Embryos resulting from such duplication-deficiency gametes die; consequently, the parent is partially sterile.

Reciprocal translocations may arise spontaneously or be induced by irradiation and chemical treatment. Insects in which they occur differ from completely sterile insects in that, instead of producing inviable embryos, they produce a reduced number of partially sterile progeny. Reciprocal translocations can be maintained in the laboratory by proper crossing; over 100 different translocation stocks of the house-fly have been produced by the use of ionizing radiation and are being analysed for their potential usefulness in vector control. In *Aedes aegypti*, one induced reciprocal translocation results in males of which 80% are sterile; since this translocation involves the sex-determining chromosome, it is transmitted to all

the male offspring of males that carry it. This aberration may be of use in *Aedes aegypti* control; through the release of partially sterile males, which can be bred in the laboratory and which transmit it to their offspring (from the 20% of eggs that hatch), it may be possible to introduce self-propagation of sterility into a population. Laboratory trials and small-scale field experiments utilizing this translocation might be considered by the WHO *Aedes* research units (see Annex 1).

Other disease vectors in which reciprocal translocations are known to occur are *Anopheles atroparvus*, *Periplaneta americana*, *Blattella germanica*, and the *Simulium* species.

Research on similar aberrations is in progress in a number of other vector species, such as tsetse flies. As knowledge of such aberrations becomes available, it is suggested that WHO study their possible application in vector control.

6.2.2 Inversions

An inversion is a chromosomal rearrangement in which a segment or block of genes is rotated through 180° with respect to the normal arrangement. In individuals that are heterozygous for an inversion, crossing-over or genetic recombination in that segment is restricted or inhibited, leading to the formation of gene blocks within which little, if any, recombination occurs. If crossing-over takes place within an inversion loop in an inversion heterozygote, various types of abnormal gametes, which ultimately result in inviable offspring, are produced. Perhaps the most important criterion for the application of inversions for genetic control is the length of the inverted segment. If the inverted region is long enough, the probability of an exchange in this region is extremely high, and this condition, like the translocation heterozygote, will result in semisterility. Since the use of chromosome inversions to produce partially sterile insects would depend upon the frequency of recombination in the inverted chromosome segment, and since the inversions tend to decrease recombination in the inverted segments, the Group felt that it was difficult to foresee any practical application of this type of chromosome change. However, knowledge of such chromosome inversions, and ability to manipulate them, are increasing and it is possible that a system might be developed that would combine multiple inversions in the same genome. This would lead to a greater degree of sterility and might possibly be of use in vector control. In addition, other factors must be considered in the evaluation of chromosome inversions for vector control. For example, in a study of *Chironomus tentans*, matings involving normal females and males that were heterozygous for a long inversion resulted not in the expected 50% sterility, but in the production of nonfunctional double spermatozoa. Whether this type of sterility is usable or not will depend largely on the sperm utilization pattern of a given

vector. The Group believes that, owing to the lack of knowledge, the use of inversions to control vectors is remote.

6.2.3 *Deletions (deficiencies)*

The loss of a chromosome segment constitutes a deletion, which may occur within or at the end of a chromosome arm. In either case, a deletion may produce phenotypes similar to those produced by a recessive or dominant gene. In *Drosophila* the loss of a certain portion of the chromosome acts as a dominant lethal mutation. Natural and artificially induced deletions are known to occur in several vectors, such as *Aedes*, *Anopheles*, and *Musca*. The release of vectors having suitable deletions might be an approach to control.

6.2.4 *Aneuploidy*

Aneuploidy is a condition in which one or more chromosomes (but not a complete set) are gained or lost. Thus, for a given "pair" of chromosomes an individual may be trisomic ($2n + 1$), monosomic ($2n - 1$), or nullosomic ($2n - 2$). Various other combinations are possible. Nondisjunction is usually involved in cell division in aneuploids and may lead to a certain proportion of inviable gametes or offspring. Aneuploids often have associated phenotypic effects — e.g., Down's syndrome (mongolism) in man — and are often sterile, as in many plants and in Klinefelter's and Turner's syndromes in man. The phenomenon is found in blackflies and in *Aedes aegypti*. However, it will not be possible to evaluate the usefulness of aneuploidy for vector control until much further research has been carried out.

6.2.5 *Polyploidy*

Normal sexually reproducing organisms are diploid ($2n$), with 2 sets of haploid (n) chromosomes. Variations in cell division sometimes give rise to individuals that are triploid ($3n$) or tetraploid ($4n$), or have some other multiple of the haploid number of chromosomes. Individuals with $3n$ or more chromosomes are called polyploids. Many polyploids are equal, or even superior, to diploids in viability and fertility, but many are inferior. Among vector species, triploidy is known in blackflies. Many vector species that are diploid in most of their tissues may be polyploid in other tissues. Nothing is known of the possible applicability of polyploidy to control programmes.

6.2.6 *Hybrid sterility*

Species are usually reproductively isolated—i.e., they do not exchange genes. Such isolation may take several forms.

In some insect species, there are a number of crossing types or races whose progeny are fertile females and sterile males. Such sterile male hybrids are excellent for use in insect control, since they are likely to be fully competitive with (and even more vigorous than) the sterile males produced by radiation or chemosterilants. When research on disease vectors is expanded to include studies on the cross-fertility of races and the fertility of hybrids from various populations, it is probable that many sterile hybrids will be found.

This principle may be exploited in the control of *Anopheles gambiae*, which is a complex of five species. Prospects for the control of these species are highly promising, and the Group recommends that WHO consider the possibility of sponsoring field trials in a suitable isolated area. (Details of the genetic system of *Anopheles gambiae* are given in Annex 2.)

6.2.7 Cytoplasmic incompatibility

Crosses between different populations of some insect species are sterile, owing to a cytoplasmic factor that is transmitted through the egg and that kills the sperm of an incompatible male after its entry into the egg. Crosses between certain populations produce no offspring at all. Females of one population may also cross with males of another population, producing completely sterile offspring.

Recent investigations have shown that the basis of such incompatibility is the fact that sperm enter the egg but do not actually join with its nucleus; subsequent development of the larva is entirely haploid and reaches only a certain stage before death results.

Control can be achieved by the mass rearing and release of males into an area populated by incompatible crossing types. This method has been used successfully in a field experiment in Okpo, a small isolated village in Burma. Several thousand male *Culex fatigans* were released daily over a period of 3 months. These males competed successfully for local females, which laid eggs that failed to hatch, resulting in the ultimate suppression of the population. The results of this pilot experiment represent a significant advance.¹ This new approach was made possible by academic research that was carried out for many years, long before its practical application was visualized. The Group recommends that it be used wherever possible, and that studies of its applicability to other vector species be carried out.

¹ WHO Co-ordination Group on Genetic Control of Insects of Public Health Importance (1967) Mimeographed document WHO/VBC/67.47.

7. PROBLEMS IN THE USE OF GENETIC CONTROL

In any use of genetic control methods, it is most important to have exact knowledge of the number and kind of genetic factors to be introduced, and of the time when they should be introduced.

When a new genetic control technique has been tested and evaluated in the laboratory, there is always uncertainty as to its efficacy in field applications. Field trials often have disappointing results, sometimes leading to the abandonment of an apparently sound and logical line of study. Since large-scale trials are so expensive, it would be a great advantage if it were possible to predict, with reasonable accuracy, the outcome of a projected vector control programme. It is common to attempt such prediction by developing a mathematical expression of the functional relationships assumed to exist between the density of the vector population and factors (e.g., fertility and mortality levels and various environmental factors) that can modify the density. (The modifying factors are generally assigned values that would be necessary to maintain the vector population in a stable condition.) By means of this expression, the consequences of artificial modification of one or more factors can be estimated, and operational decisions can be made on the basis of the results.

However, it must be recognized that such models usually over-simplify the situation that exists in nature, since only relatively simple models can be solved mathematically. If many variables are involved, and if their interrelationships are complex, numerical solutions can, in principle, be obtained by trial and error. However, the applicability of this technique is limited by human errors if automatic computers are not available.

The use of the electronic computer has completely changed the situation, since there is practically no limit to the degree of complexity that can be handled by the computer. As a result, more elaborate models, incorporating density-dependent factors that play an important role in regulating population size, can be used. Computer programmes should allow a great degree of variation of the factors thought to have any bearing on the reproductive ability and survival pattern of a given species; trends in population changes can then be simulated and easily repeated for any set of values. The possibility of controlling interaction between such factors is considered a significant step towards a more realistic simulation of population dynamics, and offers great promise.

Although the computer can solve problems involving many complex biological variables, its accuracy depends on the reliability of the information presented to it. Unfortunately, most of the important factors necessary for such calculations are poorly understood. It is difficult to make accurate estimates of factors such as (1) the absolute density of individuals per unit area, (2) the life span of female vectors in nature, (3) the number of eggs

produced by each female in her lifetime, and (4) the relative competitiveness and longevity of normal males and of introduced males. Nevertheless, such estimates are essential if the model produced by the computer is to be of any value. For this reason, much further research will be necessary before the potential value of computer systems can be fully realised.

8. RESEARCH NEEDS AND RECOMMENDATIONS

The areas of cytogenetic research that could be most profitable for vector control are listed in the following sections. However, basic research on all facets of vector cytogenetics must be continued, since it is difficult to predict the usefulness of a given approach (as was the case with cytoplasmic incompatibility).

Cytogenetic studies

Of the research needs listed below, nos. 5-8 are considered to be most likely to lead to application in the field and are, therefore, most urgent; nos. 3, 4, 9, and 10 are next in order of priority.

(1) *Karyotypes*. Research should continue on karyotype description of as many vector species as possible. For example, karyotype information is available for only about 5% of all mosquitos, and studies of *Glossina* karyotypes have been limited to only two species. Such information will naturally be obtained in the course of other studies, but karyotype studies should be constantly encouraged.

(2) *Idiograms*. WHO should collect and publish in the *Vector Genetics Information Service* a series of idiograms of karyotypes for vector species, information on which is at present scattered. The information should be recorded as follows: genus, species, diploid complement in germ cells and somatic cells, heteromorphic sex chromosomes, and supernumary chromosomes.

(3) *Salivary gland chromosomes*. The salivary chromosomes of vector species should be studied wherever possible. Technical problems are involved in the preparation of salivary gland chromosomes of *Culex*, *Aedes*, and *Anopheles (Kerteszia)*, and further work on these species should be encouraged.

(4) *Salivary gland chromosome maps*. Considerable progress has been made on the mapping of salivary gland chromosomes in *Anopheles* and *Simulium*. The global mapping programme of WHO should continue, with particular attention to Africa, South America, and Oceania. The maps should include localization of diagnostic puffing patterns and nucleolar

organizer regions. It is suggested that WHO maintain a file of maps and photographs, to be available on request.

(5) *Linkage maps.* Of all vector species, linkage maps are available only for *Musca domestica*, for which the genes are assigned to a particular chromosome. Work in progress on linkage maps of other vector species, particularly in the genera *Aedes*, *Culex*, and *Anopheles*, should be developed along similar lines. The present Group endorses the recommendations to this effect made by the WHO Scientific Group on Standardized Strains.¹ Encouragement should be given to a further search for mutants and to the establishment of mutant strains.

(6) *House-fly stock centre.* More mutations have been studied in the house-fly than in any other vector species. Most house-fly strains are maintained in scattered laboratories in Italy, Japan, and the USA; furthermore, maintenance is difficult and expensive, and constant technical supervision is required. The Group recommends that consideration be given to the possibility of using the WHO International Reference Centre on House-Flies as a stock and supply centre for all mutant strains. All pure-breeding strains showing visible mutations, and strains showing chromosome translocations and sex-distorting factors, should be preserved for further study.

(7) *Gametogenesis.* Although some information is available on gametogenesis in vectors, basic research on this fundamental process should continue. Many of the mechanisms that are potentially important for control were first discovered during studies of gametogenesis. Although knowledge of spermatogenesis is relatively extensive, much less is known about oogenesis. Research on methods of suppressing egg formation in insects should be encouraged. The Group recommends that research on gametogenesis be encouraged and that consideration be given to the convening, at a later date, of a meeting of experts on insect gametogenesis.

(8) *Sex determination.* The complex mechanism of sex determination in vectors is poorly understood. Particular attention should be given to this problem, clarification of which might lead to the discovery of cytogenetic mechanisms that would be useful for control. Sex-determination and sex-distortion studies should be carried out in vectors other than mosquitos.

(9) *Chromosomal aberrations.* Translocations and other aberrations should be studied in order to identify known linkage groups with optically identifiable chromosomes and to evaluate their effect on the fertility of vectors. Such aberrations should be studied in as many vector species as possible.

¹ *Bull. Wld Hlth Org.* (1966) **34**, 437. Attention is also drawn to the usefulness of the WHO *Vector Genetics Information Service*.

(10) *Speciation*. Cytogenetic studies of vectors have revealed species complexes that were previously indistinguishable. Different populations of important vector species should be systematically studied for chromosomal polymorphism and isolation mechanisms. Studies of *Anopheles gambiae* have identified five species in the complex; further studies should include a search for other X chromosome types that would be indicative of more species within the complex. Special attention should be given to the cytogenetics of both interspecific and intraspecific hybrids.

(11) *DNA, RNA, and molecular biology*. Several studies of molecular biology, using vector species, are in progress. Material from vector species (e.g., the salivary gland chromosomes of Diptera) frequently offer considerable advantages for this type of work. Where possible, molecular biology studies should be instituted in laboratories that carry out research in vector genetics, and molecular biologists should be encouraged to utilize vector species.

Sterility mechanisms

Research in the following areas is considered most likely to lead to control methods for use in the field, and is therefore of the same degree of priority as research on cytogenetic studies 5-8 above.

Since sterility mechanisms are basic to control programmes, the search for new mechanisms and for further examples of known mechanisms should be continued both in the laboratory and in the field. Further search should be made for barriers to reproduction such as cytoplasmic incompatibility and hybrid sterility.

The competitiveness of males used in control programmes must be at least as good as that of "natural" males. Research on methods of enhancing male competitiveness by genetic or other methods should be encouraged.

Further research is necessary on the possibility of recovery from induced sterility and on the cytogenetic basis of somatic damage resulting from exposure to chemosterilants.

The search for attractants in vector species should be continued, to facilitate research in autochemosterilization and vector ecology.

Studies of chemosterilants, and particularly the search for nonmutagenic agents and sperm inactivators, should be continued. Suitable ways should be found to interfere with the production or metabolism of nurse cells or endocrine systems and thus prevent oogenesis.

Pilot field studies

Studies in the areas noted below should be given the same priority as research on sterility mechanisms (see above).

The highly successful experiment in Burma, resulting in the eradication of *Culex fatigans* from the village of Okpo, has demonstrated the feasibility

of the sterility method. The Group recommends that a larger integrated experiment be undertaken as soon as possible, but cautions that it is essential to select personnel and plan the procedures to be used with great care.

The highly effective hybrid sterility in crosses between species of the *Anopheles gambiae* complex should be exploited as soon as possible (see Annex 2).

The Group recommends that studies of the genetics and cytogenetics of *Aedes aegypti* be undertaken by WHO field units. Particular attention should be given to the study of field populations from East Africa, the ancestral home of this species, since most of the existing knowledge is based on populations that have been colonized in the laboratory over several years. During the first few years, emphasis should be placed on basic studies; field experiments on the genetic control of the species may then be carried out. The present status of knowledge of this species, and recommendations for further studies, are summarized in Annex 1.

Control programmes are affected by several highly variable factors. Guidance on such matters can be obtained from mathematical models and computer-simulated control programmes, and ecological studies will provide the necessary data on the absolute numbers of vector species in a given area.

Role of WHO

In the past few years, WHO has been largely responsible for stimulating research on the cytogenetics of vector species,¹ and the Group recommends that WHO continue to encourage and co-ordinate such research, including studies of vectors other than arthropods (see Annex 4).

Encouragement should be given to the arranging of visits to laboratories and to the holding of seminars. Such seminars could train a nucleus of field workers from different countries, who would be of assistance in the over-all WHO vector genetics programme. The exchange of personnel between different institutions would also be valuable.

The Group suggested that consideration be given to the possibility of making WHO field units available for laboratory and field experiments on cytogenetic mechanisms in vectors. Specialists in the latter subject could use the facilities of such units and could obtain field populations for further work in their own institutions.

¹ Specifically, WHO has established an International Reference Centre for the Maintenance and Distribution of Standardized Strains of *Musca domestica* in Pavia, Italy; has undertaken publication of the *Vector Genetics Information Service*; and was responsible for the editing of the volume *Genetics of insect vectors of disease* (Wright, J. W. & Pal, R., ed.), Amsterdam, Elsevier, 1967.

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Annex 1

AEDES AEGYPTI

During the past few years a great deal of information has accumulated on the cytology, cytogenetics, and genetics of the yellow fever mosquito, *Aedes aegypti*. This is summarized below.

Karyotype

The somatic karyotype consists of three chromosome pairs, of which two are relatively large and one small. The three pairs can be distinguished on the basis of their length and on the position of their centromeres, and have been designated I, II, and III. In brain tissue their lengths are about 5.4 μ , 6.9 μ , and 7.6 μ , respectively, at metaphase. Chromosome II is slightly submetacentric, the lengths of the two arms being in the ratio 1 : 1.2; chromosomes I and III are metacentric. The shortest chromosome is probably involved in sex determination and one of the two larger pairs contains the nucleolar organizer. The known linkage groups should be assigned to these chromosomes.

Chromosome ultrastructure

The fine structure of *A. aegypti* chromosomes has been analysed by means of electron microscopy. Based on this work, the presence of a highly complex structural entity, designated the synaptonemal complex, has been demonstrated during meiotic prophase. It appears that the synaptonemal complex is responsible for homologous chromosome pairing, and thus has a function in genetic exchange of chromosome segments during meiosis. A chromosome model has accordingly been proposed for *A. aegypti*; this model probably applies to meiotic chromosomes of all organisms.

DNA determination

It is well known that deoxyribonucleic acid (DNA) is normally present in the nucleus, associated with the chromosomes. However, in recent work the interesting observation was made that, in *A. aegypti*, approximately 40% of the total soluble DNA is present in the cytoplasm. The implications of this finding are not yet clear and further study is required. Biochemical studies on DNA and several distinctive enzymes in several mutants, such as bronze (*br*) and yellow (*y*) larvae, are also in progress.

Salivary gland chromosomes

Owing to a number of technical difficulties, it has not yet been possible to map the salivary gland chromosomes of *A. aegypti*. Even considerable refinement in techniques (e.g., the rearing of the larvae at low densities, the maintenance of a temperature of 18–28°C, and the addition of small amounts of brewer's yeast and ribonucleic acid) has not improved the quality of "squashes" sufficiently to make mapping possible, although some laboratory strains have given consistently better results than others in the spreading of the polytene chromosomes. Attempts should be made to overcome these difficulties.

Mutants and linkage maps

More than 80 mutants have been isolated, about half of them being useful as genetic markers. Most of the mutations are expressed as structural aberrations, primarily affecting appendages; approximately 30 mutations are known to affect colour pattern. Several genes with identical functions are located on the same chromosomes; for example, linkage group I contains three genes that affect eye colour and two that influence pigmentation of the entire body. In addition to the gene for sex determination, the genes for sex-ratio distortion and susceptibility to filaria are located on this linkage group.

Linkage group II has the genes for resistance to DDT and dieldrin, in addition to intersex (*ix*), yellow larva (*y*), spot abdomen (*s*), and several other genes. On linkage group III are located the genes for miniature appendages (*min*), black tarsi, compressed antenna, and many other appendage mutants.

Considerable progress has been made in studies of the extent of mutation in both laboratory and field populations, and on heterosis, polymorphism, meiotic drive, intersexuality, and other areas of fundamental genetic interest.

Populations of *A. aegypti* are characterized by tremendous genetic variability, and heterozygosity is probably an important factor in the fitness of this species. Furthermore, a significant level of heterosis has been shown in crosses between strains.

A chemical substance recently isolated from the male accessory glands, if passed to the female during the first insemination (or by injection), makes subsequent inseminations impossible. A similar substance has been discovered in the house-fly. Extensive studies of the chemistry of this substance are in progress; if it can be synthesized, it may provide a new approach to the control of *A. aegypti*.

Normal gametogenesis

Spermatogenesis and oogenesis have so far been studied in only a few laboratory strains. Details of meiosis during spermatogenesis have been analysed, and the normal meiotic pattern has been established. It seems likely that during the early prophase visible leptotene and zygotene stages do not occur in this species. If this is so, it must result from the fact that during the earliest stages of meiosis the chromosomes retain the intimate pairing of the previous mitotic anaphase and telophase.

The frequency of chiasmata per pair of homologous chromosomes in this species is 1.5. More than 2 chiasmata per pair have not been observed.

Aberrant gametogenesis

Gametogenesis associated with certain types of structural rearrangement of chromosomes has recently been studied. A radiation-induced chromosomal interchange in males causes about 80% of the sperm produced by such males to be genetically imbalanced (defective). A case of spontaneous polyploidy and chromosome breakage (occurring in certain lines of the "notch" wing mutant) has resulted in aberrant spermatogenesis.

Several strains of this species are characterized by the distortion of sex-ratios in favour of males (up to 90% males). Spermatogenesis studies of high-male-producing lines have indicated that such males produce fewer functional sperm than do normal males. The meiotic details underlying this phenomenon are not yet understood.

The greatest need in gametogenetic research is to study the process in natural populations; very little is known of the way it occurs in a natural population of *A. aegypti*. The survival and evolutionary advance of a population depend on the successful culmination of meiosis during gametogenesis. Study of the process in natural populations might reveal the existence of mechanisms that could be manipulated for control purposes.

Cytogenetics of sterility

Induced sterility

Radiation and chemosterilants have been used to induce sexual sterility in both sexes of *A. aegypti*, as discussed elsewhere in this report.

Treatment by various mutagens may inhibit or completely prevent the production of gametes, particularly in females. If gametes are produced, sterility results from the induction of gross chromosomal aberrations (dominant lethality) in spermatozoa and ova. The cytogenetic basis of such induced sterility described in section 6.1 also applies to *A. aegypti*.

Genetic sterility

Chromosomal sterility. As noted previously, certain types of chromosomal rearrangement result in varying degrees of sterility. During the past few years, chromosomal aberrations have been induced in *A. aegypti* by gamma irradiation. One of these, a reciprocal translocation between chromosomes I and II, has been studied in detail and is of considerable interest. Lines heterozygous for this translocation have been established. Since the radiation-induced breakage of chromosome I occurs close to the male-determining allele (*M*) of the sex locus, this translocation breeds true in male progeny. Furthermore, 80% of the eggs from normal females inseminated by translocated males fail to hatch. The remaining (20%) zygotes produce normal females and translocated males in equal numbers.

Genic sterility. The sex-linked gene bronze (*bz*) results in complete female sterility, since the eggs laid by bronze females are defective. Females heterozygous for this gene are fertile.

Little is known about the existence of such factors in natural populations, and it is recommended that extensive hybridization studies of such populations be undertaken. It is conceivable that such studies might reveal the existence of incompatibilities of the type that exists in *Culex* and *Anopheles*. Although crosses among strains of *A. aegypti* from diverse geographic origins have not thus far revealed the existence of any reproductive isolation, it is possible that such isolation exists. For example, recent work has shown that although reciprocal crosses between *A. aegypti* and *A. mascarensis* result in fertile progeny, crosses between *A. aegypti* females and F_1 males from *aegypti* female \times *mascarensis* male crosses result in the production of males with genital abnormalities. The proportion of such abnormal males among the back-cross progeny depends on the extent of the *aegypti* genome in the F_1 male; more than 90% of the males from certain back-crosses have been abnormal. Thus, these abnormalities are associated with some sort of incompatibility between the male-determining chromosome (or parts of it) and the *aegypti* genome.

Genetic mechanisms for population control

Induced sterility

A field trial using radiation-sterilized males for the control of *A. aegypti* was carried out in Pensacola, Fla., USA, but was unsuccessful. It is not known whether failure resulted from behavioural differences in males released from a laboratory colony or from a loss of competitiveness following radiation sterilization. The use of chemosterilants rather than radiation might overcome the latter difficulty.

Distortion of sex-ratios

An inherited factor in *A. aegypti*, designated distorter (D), results in excess production (up to 90%) of males. These high male ratios are not the result of postfertilization mortality. The distorter functions only in males of the genotype M^Dm^d , distorting segregation of chromosome I in favour of the homologue bearing M^D ; no distortion occurs in females and males of the reciprocal genotype M^dm^D . Thus, if given female and male populations are of the right genotype, their progeny will include a high proportion of males. The potential applicability of this phenomenon to the control of *A. aegypti* populations has been demonstrated by extensive experiments using population cages containing varying proportions of male-producing and normal males with so-called sensitive females (i.e., females with the genotype m^dm^d).

Translocations

The possibility of using translocations for the control of *A. aegypti* was mentioned in section 6.2.1. Under laboratory conditions translocated males are competitive with normal males. Theoretically it should be possible to control a population by means of successive introductions of translocated males.

Discussion

It is recommended that the applicability of the above methods be tested in the laboratory, using large population cages. Particular attention should be given to their applicability to female *A. aegypti* from South-East Asia or East Africa, areas where eventual field trials are contemplated.

It should be emphasized that the success of any of the above methods depends on the ability of males reared in the laboratory to inseminate wild females. This has not been demonstrated for *A. aegypti*; however, a field experiment is being undertaken in Meridian, Miss., USA. The dominant gene *silver mesonotum* (*Si*) has been incorporated in the Meridian strain, and males of this "synthetic" strain will be released at several locations. Eggs will be collected from time to time and hatched; if adults reared from such eggs are found to contain the dominant markers, proof will have been obtained of the ability of the males to inseminate wild females.

Additional details concerning the genetics and cytogenetics of *A. aegypti* have appeared elsewhere.¹

¹ Coker, W. (1967) *Bull. Wld Hlth Org.*, **36**, 555; Craig, C. B., jr & Hickey, W. A. (1967) *ibid.*, 559; Rai, K. S. (1967) *ibid.*, 563.

Annex 2

ANOPHELES GAMBIAE

The *Anopheles gambiae* complex consists of the following five species: *merus*, *melas*, A, B, and C.

Karyotypes

In *Anopheles* the two autosomes are metacentric or nearly so, but there is a visible difference in the smaller pair of chromosomes, the male in all cases being the heterogametic sex. The mitotic figures of *A. gambiae* fit this general description. The two pairs of autosomes are metacentric and submetacentric, respectively. The sex chromosomes are readily distinguishable since they are equal in size in females and heteromorphic in males. The X-chromosome is J-shaped in this species and the Y-chromosome is acrocentric. Figures from testicular and brain cells confirm that the long arm of the X-chromosome is homologous with the long arm of the Y-chromosome; the two chromosomes differ in the length of the short arm. Curiously enough, the homology of the long arms of the X and Y chromosomes is not reflected in the polytene configurations. Salivary gland spreads from male *A. gambiae* show a normal double thickness for the short arm of the X-chromosome and single thickness for the long arm—the reverse of what one would expect from the mitotic configurations. It is interesting that the X-chromosome obtained from testis preparations differs from the X-chromosome in female ovary spreads. In all male metaphase I karyotype figures the X-chromosome has an elongated heterochromatic region adjacent to the centromere, between the latter and the long arm of the X-chromosome. The female metaphase karyotype X-chromosome from ovary nurse cells does not show this heterochromatic region.

It is recommended that the karyotype for all five species of the *A. gambiae* complex be studied, with particular attention to the size and position of the nucleolar organizer.

Salivary gland chromosomes

The chromosome map of the banding pattern in *A. gambiae* species A is at present being revised and made more detailed. This map has served as a standard of comparison for the members of the complex. At present only species A and B have been studied extensively, although studies of the other species are in progress.

Cytogenetic studies of species A and B hybrids reveal distinct chromosomal heterozygosity. All show an asynaptic region in chromosome arm 2R. The X-chromosome in female hybrids also appears to be almost completely asynaptic, except for a few bands near the tip and the centromere. More surprising is the fact that length and banding on the tips of the X-chromosomes differ. Other aberrations that need further clarification have been found in these hybrids.

It is essential that the polytene chromosome banding pattern be recorded for all the members of the complex.

In the *A. gambiae* species complex it appears that the chromosomes have become differentiated both in gene arrangement and in genotype, resulting in hybrid male sterility (and, in some crosses, in female lethality) before morphological divergence has occurred. Present evidence indicates that there is complete reproductive isolation between the different species of the *A. gambiae* complex. The results of crossing the various forms are given in Table 1.

TABLE 1
RESULTS OF CROSSING *A. GAMBIAE* SPECIES

Cross	Results
species A × species B (reciprocal)	normal sex ratio ; males sterile, females fertile
species A or B male × <i>melas</i> , <i>merus</i> , or species C female	F ₁ ; all males
<i>melas</i> , <i>merus</i> , or species C male × species A or B female	normal sex ratio ; males sterile, females sterile

It can be seen that the hybrid females from reciprocal crosses between species A and species B, and females resulting from crosses between *melas*, *merus*, or species C males and species A or B females, can be successfully back-crossed to either group in the laboratory. Gene flow thus can take place between the species. Although some of the species are sympatric, no hybrid has been detected in nature. It appears that the reproductive isolation responsible for the maintenance of these species is highly efficient.

When different members of the *A. gambiae* complex are crossed, the degree of atrophy of the reproductive system of the hybrid male varies, depending on the mating types involved. It does not necessarily follow that the sterility, as manifested in the male testis, is an index of the efficiency of the isolating mechanisms. Thus, although spermatogenesis is interrupted at various stages, in the hybrid males this only indicates that different

factors are involved in each species cross. The general features of the testes from the different crosses are listed in Table 2.

TABLE 2
TESTICULAR EFFECTS OF CROSSING *A. GAMBIAE* SPECIES

Cross	F ₁ sex ratio	Testicular effects
species C male × species A or B female	normal	partial spermatogenesis
species A male × species B female	normal	spermatids and a few spermatozoa
species B male × species A female	normal	spermatocytes only
species A or B male × <i>melas</i> female	mostly males	mixture of spermatocytes, spermatids, and spermatozoa
<i>melas</i> or <i>merus</i> male × species A, B, or C female	normal	reduced accessory glands, testes, and vas deferens; primary spermatocytes only

The absence of female progeny of some of the crosses seems to indicate that the combination of the X-chromosome from species A or B with the X-chromosome and egg cytoplasm from species *melas*, *merus*, or C is lethal. In studies of such crosses, only half of the eggs hatched, the unhatched eggs showing no signs of embryonic development. F₁ females produced by certain crosses between species A females and species B males were backcrossed to species A and testis morphology was studied in the offspring. The results indicate that a combination of the X-chromosome of species B with the Y-chromosome of species A causes sterility regardless of the autosomes. Some of the smallest testes measured were those of individuals with an X-chromosome from species B; all the other individuals with small testes had an X-chromosome from species A. A combination of chromosomes homozygous for species A (except for heterozygosity in one of the two pairs of autosomes) also caused sterility. However, some individuals with these chromosome combinations, particularly those that were only heterozygous, were considered normal under visual examination of the testes. In these two combinations heterozygous for one pair of autosomes a reduced number of mature motile sperm was observed, and to all intents and purposes they could be considered sterile. Some individuals that were heterozygous for both autosomes showed similar sterility or reduced fertility, but only when a species B female was used in the parental cross. All the hybrid testes showed varying degrees of development; however, the size

of the testes and the stage of spermatogenesis reached were indicative of the chromosome complement. Cytogenetic studies of this species show the X-chromosome to have undergone extensive rearrangement. A number of asynaptic regions are also present, indicating overlapping inversions. However, these rearrangements are not sufficient to cause the degree of sterility attained by hybrids, and it is concluded that the sterility in A-B hybrids is for the most part genic.

It is recommended that *A. gambiae* hybrids be studied cytogenetically and by means of genetic analysis in order to determine the exact cause of sterility and its potential for genetic control.

The fact that *A. gambiae* forms a complex of species suggests a possible means of genetic control. In the *A. gambiae* complex, male sterility is genetic in origin and not artificially induced (as is the case with the sterile male technique); it is invariably linked with hybrid vigour and is perhaps associated with increased mating ability. Consequently, it may have a considerable advantage over sterility produced by irradiation or chemosterilants. Preliminary cage experiments with this type of sterility have been highly successful in significantly reducing the fecundity of females. WHO has made plans to conduct a small-scale field experiment on the control of *A. gambiae* by means of this type of hybrid sterility in a suitable area in Africa.

Annex 3

CHEMOSTERILANTS

When fed to insects in low concentrations, a number of chemicals induce male sterility. Most of the chemicals that have been tested in the field belong to the group of substances loosely termed alkylating agents. Toxicity studies have shown that chemosterilants such as TEPA (OMS-CS-3) and METEPA (OMS-CS-2) have a highly specific and localized action on the developing sperm of mammals and of insects, and that this action takes place at dose levels that do not produce other signs of toxicity (e.g., depression of bone marrow activity).

Hopes of developing a safe chemosterilant were recently raised by the demonstration that hexamethylphosphoramide (OMS-CS-42) and hexamethylmelamine (OMS-CS-41), which are not alkylating agents, are effective chemosterilants for insects and that they have low toxicity in mammals. However, experiments on rats have shown that OMS-CS-42 produces damage to the testis, and clinical trials and animal toxicity tests with OMS-CS-41 have indicated that it can behave as an alkylating agent. It seems probable that, in insects and mammals, the molecules of OMS-CS-

41 and OMS-CS-42 are transformed in such a way as to lead to the production of an alkylating radical.

Since these compounds have a striking cumulative effect on the sperm cells of rats, particular care is needed to protect operators in prolonged field work.¹

The problem of the safe use of chemosterilants was considered in the sixteenth report of the WHO Expert Committee on Insecticides,² where it was recommended that there should be no exposure of the public to the compounds thus far examined. At present it seems that chemosterilants of low selectivity can be used only in combination with attractants for certain special methods.

Annex 4

CYTOGENETICS OF VECTORS OTHER THAN ARTHROPODS.

Snails

It has been demonstrated that the susceptibility of snail vectors to infection with trematodes depends on the biochemical structure—and, consequently, the genetic constitution—of the host. However, there is still a great deal of work to be done on the cytogenetics of snail vectors of disease.

It has been found that the determination of chromosome numbers alone is of value in only a few cases. It should, therefore, be accompanied by detailed morphological studies of the mitotic chromosomes (e.g., their length, the position of the centromere, and the presence or absence of secondary structures). Several populations of the same species of snail from different habitats should be studied to determine the effect of the environment as indicated by chromosomal polymorphism and special gene arrangements.

For cross-breeding experiments, the only genetic marker that has been available in species of the hermaphrodite *Biomphalaria* is albinism. If it were possible to tag sperm with radioactive isotopes, it might be possible to differentiate the sperm of one snail from another.

The Group is of the opinion that collaboration between malacologists and cytogeneticists should be improved, so as to promote research on the cytogenetics of snail vectors.

¹ Barnes, J. M. (1963) *Trans. roy. Soc. trop. Med. Hyg.*, **58**, 327.

² *Wld Hlth Org. techn. Rep. Ser.*, 1967, 356.

Rats

Although a great deal of information is available on the genetics of laboratory rats and mice, there have been few cytogenetic studies of wild rodent vectors and reservoirs of human diseases. The recent appearance of resistance to anticoagulant rodenticides in certain strains of *Rattus norvegicus* has emphasized the need for such cytogenetic studies. In addition, more specific information on the genetics of rats must be obtained before it will be possible to use sterilization or infertility techniques to control these vectors.

The Group recognizes the importance of the genetics of vectors other than arthropods and suggests that WHO consider the possibility of convening a meeting of experts to study these problems.

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