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Mosquito Cytogenetics

A Review of the Literature, 1953-62

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Although an intensified interest in mosquito cytogenetics in the past decade has produced a number of contributions to knowledge on this subject, the available information is still superficial and limited to a few mosquito species only. The author of this review summarizes the research done in this field between 1953 and 1962.

The following are some of the achievements and some of the gaps that remain to be filled. Karyotypes of several species of Anopheles, Aedes *and* Culex *conform to the general pattern 2n=6, with heterosomes distinguishable only in* Anopheles. *At least three different karyotypes are present in* Anopheles. *Salivary gland chromosome maps are now available for several anopheline species, but are still lacking for* Culex *and* Aedes. *No precise correlation may yet be made between the frequency of chromosomal aberrations and the degree of insecticide-resistance. Sexual differences in the salivary X-chromosomes have been reported for several species of* Anopheles. *Chromosomal polymorphism is common in some anophelines, but rare in others. Chromosomal mutation has been induced by means of X-rays.*

In his conclusions, the author stresses that prospects are especially good for evolutionary and genetic studies involving chromosomal polymorphism.

I have arbitrarily limited this review of mosquito cytogenetics to the last ten years, from 1953 to 1962 inclusive, and to the Culicinae, specifically excluding the Chaoborinae and Dixinae. Work prior to 1953 was scattered and scanty, and most of this early work has been adequately summarized elsewhere (Kitzmiller, 1953). The pioneer work of Frizzi in Italy, working with the salivary chromosomes of the European *maculipennis* complex, opened up the field for those of us who have since become interested in the cytogenetics of mosquitos. Frizzi's description of the salivary chromosomes, his discovery that the various sibling species of the *maculipennis* complex could be distinguished by means of inversions, his studies of gene arrangements in natural populations, and his many recent papers continue as outstanding contributions in this area.

In the last ten years there has been a renewed emphasis upon basic research in mosquito biology. The dramatic development of resistance over the world and the unequivocal demonstration of the genetic basis of this resistance have underlined the need for more research, and increasingly intensive research, on the genetics and cytogenetics of mosquitos, yet it is unfortunately a fact that, today, relatively little is known in this field. This is not to say that some progress has not been made. It has. We know considerably more than we did ten years ago, and some important advances in cytological technique offer promise for the future, but at this moment, we have hardly scratched the surface of what can be done.

Perhaps some statistics will make this clearer. I have assembled a bibliography of some 80 papers published in the years 1953-62 which deal with mosquito chromosomes or cytogenetics. This is hardly an overwhelming list, although some papers have undoubtedly been missed.

A tabulation of the species studied is even more revealing. About one-third of the papers deal with the sibling species of the European *maculipennis* complex. Twenty-five more treat other anophelines, about equally distributed among the North American *maculipennis*, the subgenus *Nyssorhynchus*, and a few others. Thus, two-thirds of the papers deal with a relatively small number of anopheline species. Of the remaining contributions only seven deal with the genus *Aedes*. A few technique papers and

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a few but important general ones covering the chromosomes of several species round out the list.

Another feature of the work in the last ten years is that by far the major portion of it is traceable to only a few laboratories. In the United States of America almost all the contributions have come from the laboratories of four investigators: Rozeboom at Johns Hopkins, Craig at Notre Dame, Breland at Texas, and our laboratory at Illinois. In other countries, Frizzi's laboratory has been responsible for the majority of the contributions, with some contributions from the laboratories of d'Alessandro, Schreiber and Mosna. It is also interesting that about two-thirds of these papers have been published between 1958 and 1961.

KARYOTYPE STUDIES

A valuable summary of previous work, a synopsis of workable techniques and the contribution of considerable new material have been made by Breland and his group. His 1961 paper is especially valuable and represents the first cytogenetic knowledge of sixteen additional species. He has described in detail, with excellent photographs, the mitotic and/or meiotic chromosomes of seven species of *Culex*, seven species of *Aedes*, two of *Orthopodomyia*, three of *Psorophora* and one each of *Anopheles*, *Haemagogus*, *Culiseta*, *Toxorhynchites*, and *Uranotaenia*. Included is a contribution to the description of the mitotic process in *Culex salinarius*, and metaphase karyotypes of the other species. All species showed a diploid number of six. All have three pairs of metacentric chromosomes.

Breland (1961) suggests that *A. pseudopunctipennis* represents a different *Anopheles* karyotype (autosomes with unequal arms). This may well be, and may represent the results of pericentric inversions or translocations. Also of unknown significance, but of great interest, is the presence of satellites in prophase chromosomes in two species of *Orthopodomyia*. Breland (1961) suggests that these might represent chromosomal sex elements, singularly lacking in mosquitos other than *Anopheles*.

Spermatogenesis has also been studied in *Anopheles stephensi* by Rishikesh (1959a). The karyotype is not unusual for *Anopheles*: two pairs of metacentric autosomes and one pair of unequal subtelocentric heterosomes. Most attention is given to the condensation and behaviour of the heterosomes in which a definite heteropycnotic area is incorporated into the X-chromosome. The longer portions of the heterosomes are unequal, rather than the short arms.

Rai & Craig (1961) have reported mitotic metaphases from five species of *Aedes* and one each of *Corethra*, *Anopheles* and *Culex*. The usual diploid picture of 2n=6 (except *Corethra*: 2n=8) was found. They agree with Breland in finding one short pair and two relatively longer ones in *Aedes aegypti*. Essentially the same information on karyotypes is provided by Risler (1959, 1961) in two papers on somatic reduction in the gut and epidermis of *Aedes aegypti*.

In several papers, description of chromosomes and karyotypes is given for several other species, including *Anopheles albimanus*, *A. gambiae*, *A. quadrimaculatus*, *A. freeborni*, *A. punctipennis*, *A. aquasalis*, *A. aztecus*, *A. claviger*, *A. stephensi*, *A. darlingi*, *A. strodei*, *A. noroestensis*, and *A. argyritarsus*.

Karyotypes, heterosomes and chromosomal sex-determining mechanisms in Anopheles

In most non-anophelines, the metaphase karyotypes have thus far been quite uniform and not especially promising for gleaning information about the evolution of these karyotypes. Thus far the non-anopheline genera show three pairs of more or less metacentric chromosomes. However, in *Aedes* (several species) chromosomes of different lengths are apparent, and in some cases chromosomes can be identified by relative arm lengths. In *Anopheles* the prospects are brighter. The two autosomes generally show the same picture; metacentric or nearly so, but with some hints of different arm lengths. The heterosomes are clearly different. The most common picture in the species thus far studied is that shown by the typical *maculipennis* species. In this group the X-chromosome is subtelocentric (point of SFA attachment), consisting of a longer and a shorter portion. In females the two X-chromosomes are usually equal in both the longer and shorter portions (see below for exceptions). The Y-chromosome appears equal to the X as regards the longer portion but the shorter portion appears smaller in the Y than in the X. This difference may be very slight, as excellent preparations are necessary to establish it beyond doubt. This is the condition found in the European *maculipennis*, in *freeborni* and in *occidentalis* (Baker—unpublished).

A second type of karyotype was described by Frizzi (1953b) in *A. claviger* and *A. quadrimaculatus*. In this, only the small dot-like portion of the heterosomes, equal in females but unequal in males,

seemed to be present, and the larger element was lacking. More detailed studies by French (unpublished) in *A. quadrimaculatus* definitely show that this X-chromosome is smaller and subtelocentric, but only slightly so. The Y is much smaller, about half the size of the X, and metacentric or only slightly subtelocentric. Rai & Craig (1961) also report this small pair of heterosomes in *quadrimaculatus* as metacentric, but make no mention of dimorphic X and Y chromosomes. If this preparation were from a female, it is in essential agreement with French's findings in *quadrimaculatus*. The Y-chromosome in *quadrimaculatus* is very interesting. The best preparations show that the Y consists of two small dots, not more than 2 μ each. Our present interpretation of this structure is that one dot represents the sex-differentiating element, homologous to but perhaps smaller than the smaller portion of the X, and the other dot represents a very reduced portion, proximal to the centromere, of the heterochromatic portion, which is much longer in the X.

Breland (1961) has described metaphase chromosomes of *A. pseudopunctipennis*. From his photographs, these clearly are of the *quadrimaculatus* type, rather than the *maculipennis* type. Breland (1961) correctly indicated that this might represent another type of chromosomal arrangement in *Anopheles*. However, the previously described " acrocentric " or dot-like chromosomes in *claviger* and *quadrimaculatus* probably are interpretations of small, " *quadrimaculatus* "-type heterosomes. The description, by Frizzi (1953b) and by Kitzmiller & Frizzi (1954), of these as " acrocentric " is in error. Only in the best preparations can the two arms of these very small chromosomes be distinguished; indeed the sizes approach the resolution limits of the best optical systems. It is therefore probable that *pseudopunctipennis* represents another example of the *quadrimaculatus*-type heterosomes. Breland (1961) was, of course, entirely correct in describing these as different from either of the two previously described forms.

Frizzi & De Carli (1954) substantiate that *freeborni* is very similar to *atroparvus*, and extend observations on *quadrimaculatus*, actually describing the heterosomes as " punctiform " but indicating that the heterochromatic portion is " insignificant." These observations conform to our description of the " *quadrimaculatus* " type, as given above. A survey of the literature shows that the same interpretation can be given other described metaphase sets. In *A. aquasalis* Frizzi & Ricciardi (1955) describe the heterosomes as much shorter than the autosomes, " almost punctiform " but also " metacentric." Schreiber & Guedes (1958, 1959, 1960, 1961) indicate the same situation for *A. noroestensis* and *A. argyritarsus*. These would conform, as far as is possible to determine, to the *quadrimaculatus* type.

Frizzi & Ricciardi describe the heterosomes in *A. albimanus* as rod-shaped and subtelocentric. Hobbs (1962) gives a slightly different description, essentially in agreement with the " *maculipennis* " type, but states that the " knoblike terminal portion is missing from one member of the pair " in males. No photographs are shown and the drawings do not permit definitive interpretation.

Rishikesh (1959a, 1959b) describes *A. stephensi* as very similar to *albimanus*. The heterosomes are subtelocentric, clearly different from the autosomes. As in *albimanus*, they are of different lengths, but the difference is due to a reduction of the larger (heterochromatic) part of the Y-chromosome. The smaller knobs appear to be equal in the X and Y.

Detailed studies by Baker (unpublished) suggest still another karyotype. In *A. punctipennis* from Illinois, he has found females with unequal long arms of the X-chromosomes. These preparations, definitely from females, show two kinds of configurations. In the most common type the long arms of the X are equal in both X-chromosomes; the short arms are also equal. In another type of configuration, the long arms are definitely unequal in the two X-chromosomes, while the short arms appear equal. Any given slide shows only one type of configuration. The longer heterochromatic portion of one X is about twice as long as the heterochromatic portion of the other. If the preparations figured by Rishikesh were from a female rather than a male, the observations would correspond exactly. In *punctipennis*, three types of females and two types of males could be predicted if all were viable. Two of the three types of females have been recovered.

An interesting series of papers (1957 to 1961) from Schreiber's laboratory in Belo Horizonte indicates that in South American *Nyssorhynchus* different species show progressive loss of the X-chromosome material. The authors confirm the small metacentric chromosomes for *aquasalis* and state the same situation for *noroestensis*. Many other species, including *argyritarsus*, *darlingi* and *strodei*, show a reduction in the heterochromatic portion of the X, presumably making it even smaller.

The progressive losses of the X-heterochromatin, according to these authors, carries over to the salivary

gland chromosomes. They find it possible to distinguish species on the basis of the relative amounts of heterochromatin in the X-chromosome of the salivaries.

Certainly this warrants close further investigation. There is no reason to think that the three karyotypes discussed above (*maculipennis*, *quadrimaculatus* and *punctipennis*) represent the only karyotypes to be found in *Anopheles*. It may be possible to sort out at least the large related series of species on this basis. The occurrence of the different types in widely related groups, however, suggests parallel evolution of the karyotype.

SALIVARY CHROMOSOMES, MAPS AND USES

The last ten years have produced at least a beginning on salivary chromosome maps. Frizzi (1947a) has published on *A. atroparvus* and this map is accepted as the standard for the *maculipennis* group. No complete maps have been published for *maculipennis* species other than *atroparvus*; the descriptions of these other species have been limited to diagrammatic representations of inverted regions in which they differ from *atroparvus*. This is a gap in information which can and should be filled.

A few maps for other species are available. Rishikesh (1959b) has produced a map of *A. stephensi*, Hobbs (1962) a map of *A. albimanus*, Frizzi & Holstein (1954) a map of *A. gambiae* and Frizzi & Ricciardi (1955) a map of *A. aquasalis*. This is at least a beginning. Schreiber and his co-workers (1957-1961) have described chromosomes in several South American species and have published photographs of *A. strodei* and *A. argyritarsus*, but no diagnostic maps have been published.

Preliminary information has been published by Frizzi & De Carli (1954) and by our laboratory (Kitzmiller & French, 1961; Baker & Kitzmiller, 1962) dealing with salivary chromosomes of *quadrimaculatus*, *freeborni*, *aztecus* and *punctipennis*. Maps are in preparation and will appear in the near future.

Relatively less progress has been made in other groups. *Anopheles* chromosomes are shorter, spread more easily and in general are better to work with than either *Culex* or *Aedes*. Our maps of *Culex fatigans* are almost complete.

Very little has been done with respect to *Aedes* chromosomes. Gillham (1957) has studied the distribution of the polytene chromosomes in *Aedes aegypti* and Aldighieri (1961) has attempted preparations of salivary gland chromosomes of the same species, but no other data are available. A lot of work needs to be done on the mosquitos other than *Anopheles*.

In summary, the catalogue of species for which salivary chromosome information is available is disappointingly small. There is evidently a need to recruit more people willing to undertake the laborious and painstaking work that is inherent in the production of salivary maps.

APPLICATIONS OF CYTOGENETICS

The research of Frizzi on the differences in the gene arrangements of the various sibling species of the *maculipennis* complex opened the door to four kinds of use of the cytogenetic method:

1. The identification of *Anopheles* under conditions in which identification was otherwise not possible.

2. An attempt to correlate DDT and other resistance with the frequency of chromosomal inversions.

3. The beginning of the elucidation of evolutionary relationships, using the salivary gland inversion patterns.

4. The discovery of a great deal of chromosomal polymorphism in *Anopheles*, and the evident contribution of such polymorphism to genetic variability, especially in the groups in which there are " difficult species complexes."

Cytotaxonomy

Frizzi's basic contribution, of course, was the preparation of the salivary chromosomes, the mapping of the differences among the sibling species, the construction of a " chromosomal key " to the species, and the demonstration that chromosomal arrangements were validly correlated with taxonomic criteria such as egg morphology and chaetotaxy. Frizzi has also made a valuable contribution in pointing out the further work which should be done, utilizing salivary chromosomes.

Canalis et al. (1956a, 1956b) have used the cytogenetic method to identify larvae from the province of Veneto, in connexion with anopheline surveys following DDT application. This proved fruitful and established the comparative ease and usefulness of the cytogenetic method in actual field situations. Frizzi et al. (1957) have used the same techniques in Sassari. The validity of this method has been proven beyond doubt in the European *maculipennis* complex. It is hopeful that it may also be valid in other anopheline complexes, such as *Nyssorhynchus*,

gambiae, *pseudopunctipennis*, and the North American *maculipennis*.

Inversions and insecticidal selection pressure

The possible correlation of inversions with insecticide-resistance was one of the enthusiastically hoped-for possibilities of the "cytogenetic method." The Italian workers, led by d'Alessandro, Frizzi and Mariani, subjected known strains of *atroparvus* to strong selection pressures, first with DDT, then with dieldrin, and compared the percentage of inversion heterozygotes and homozygotes in the selected populations with the frequencies of inversions in the susceptible reference strains.

At first the results were dramatic. In 1957 and 1958 all preliminary tests showed that under standard laboratory conditions, *atroparvus* showed frequencies (in the susceptible strains) of about 80% of the homozygous standard arrangement, and about 20% of the combined heterozygous and homozygous inverted arrangements. With selection pressure from insecticides,the standard arrangement dropped to about 20% and the combined inversion types rose to about 80%. These results were repeatable and, coupled with Holstein's (1957) field observations on increased chromosome-III inversion frequencies in resistant strains of *A. gambiae*, seemed to indicate that the inversion frequencies were positively correlated with increased insecticidal selection pressure.

Then in 1959 observations on *labranchiae* in Sicily gave the first doubtful results. *A. labranchiae* was shown to be remarkably uniform, both with respect to the lack of inversions and with respect to sensitivity to DDT. In 1960 Bruno-Smiraglia showed that fluctuations in the frequency of inversions was *independent* of selection pressure and suggested that laboratory micro-ecological conditions, primarily temperature elevation, might be responsible. Finally, D'Alessandro et al.[1] seem to have discarded entirely the hypothesis of positive correlation of DDT selection and increased inversion frequencies. They state that once a laboratory population has reached equilibrium between the standard and inverted arrangements, insecticidal pressure is no longer effective in increasing the inversion frequency. Further, the inverted arrangement evidently appears spontaneously in homozygous standard populations. These workers are now turning their attention to a study of day and night temperature fluctuations, salinity and other ecological factors which might be influencing the frequencies of chromosomal arrangements.

The evidently clear picture that increase in inversion heterozygotes was not the direct result of insecticidal pressure led to a most interesting series of observations by Rivosecchi et al.[2] Working on the basic premise that insecticide selection affected some other larval characteristic, and that the increase in heterozygotes was a symptom of these changes, they investigated the sexes of larvae and their chromosomal arrangements. They found that sex of larvae could be determined with 100% accuracy by examination of the gonads of larvae, as observed through a binocular microscope. These larvae were then examined for (*a*) X-chromosomes, to verify sex-determination; and (*b*) relative frequencies of heterozygotes.

The differences in the salivary heterosomes in the two sexes are of special interest. In the female, homozygous for two X-chromosomes, the banding structure is clear and as precise as in the autosomes; in the male, with the paired X and Y, the banding appears lighter and more diffuse. The illustrations also show relatively more of the X-salivary banded in the female than in the male. In the male the "non-banded" portion (presumably heterochromatic) is relatively longer. This correlates well with the larger dot in the female mitotic chromosome. The salivary structure thus described is an important hint as to relative euchromatic and heterochromatic proportions of the X and Y, leads to possible speculations on chromosomal sex-determination, and, of course, provides a way of distinguishing the sex of animals from which salivary preparations have been made.

When the accurately-sexed larvae were examined for their chromosome heterozygotes it was found that in the strains which had been tested for heterozygosity and selection pressure, either all or the majority of the observed heterozygotes were in females (92%-100% in the four strains examined). Evidently females are able to survive as heterozygotes, but only few males are able to do so.

Evolutionary syntheses

In *Drosophila*, Dobzhansky and his co-workers have done brilliant work on the evolution of the

[1] D'Alessandro, G., Mariani, M., Bruno-Smiraglia, C. & Caravaglios, N. (1961) *Investigations of chromosome arrangements, irritability and susceptibility of* Anopheles atroparvus *and* A. labranchiae (unpublished working document WHO/Mal/296).

[2] Rivosecchi, L., Ascher, K. R. S. & Mosna, E. (1960) *Studies on insecticide-resistant anophelines. VI. Sex of larvae and chromosome arrangement in* A. atroparvus (unpublished working document WHO/Mal/263).

Drosophila pseudoobscura group by means of the analysis of overlapping inversions in different populations (for a summary of this work, see Dobzhansky, 1951). This possibility is a bright one in mosquitos, especially in the genus *Anopheles*. A great deal of polymorphism exists in many populations—different inversions in different frequencies—and as more is known about these polymorphic forms, the same kind of evolutionary problem can probably be worked out. Rioux et al. (1959) have made a beginning, with the available information on the European *maculipennis*, using cytological characteristics, but correlating morphological and taxonomic ones at the same time. Frizzi (1954, 1958a, 1958b, 1958c) and Frizzi & De Carli (1954) have outlined the relationships of the North American and European forms of the *maculipennis* complex, indicating the strong similarity in mitotic and salivary chromosomes. The prospects are very good for this kind of analysis of the North American *maculipennis*, and especially for the *Nyssorhynchus* complex.

Chromosomal polymorphism

One of the really encouraging developments in the last ten years is the emerging picture of chromosomal polymorphism, especially in several groups which are taxonomically or physiologically "difficult." In many of the species within these groups widespread chromosomal polymorphism seems to be the usual thing. By polymorphism we mean the presence of several different kinds of inversions, on different chromosomes, covering different areas of the chromosomes. Thus strains of *A. gambiae*, collected in different geographical areas, have been shown by Holstein (1957) to be highly polymorphic—different frequencies of different inversions from different areas. Baker (unpublished) is finding the same to be true of *Anopheles punctipennis* in the USA, in which several different chromosome inversions are found in the X, in chromosome II and chromosome III.

We are now thinking of *punctipennis* as a complex, rather than a uniform species. A great deal of work needs to be done on this species alone in the USA. *A. freeborni* also shows considerable polymorphism.

Schreiber and Memoria (1957) have reported a high degree of polymorphism in *A. strodei* and *A. darlingi*. Some polymorphism is present in *A. noroestensis* and, so far, none in *A. agyritarsus*. It is of especial significance that the two complexes *albimanus* and *argyritarsus*, which are relatively difficult taxonomically, are the richest so far in polymorphism. The polymorphism in the *maculipennis* complex has already been discussed. It must be pointed out that most populations in nature (Frizzi, 1956) are homozygous, although laboratory strains exhibit polymorphism.

At first this may seem more of a curse than a blessing. The taxonomic picture is difficult enough; why compound it with a complicated chromosomal picture? The answer, of course, lies in the mechanism of evolution itself: in these groups we are able to study primary evolutionary mechanisms, and once the cytogenetic picture is straightened out, we shall have a much clearer idea of what has gone on, and what is going on, in these complexes.

It is also encouraging to note that some correlation can be made between certain types of inversions and certain ecological conditions, although these studies are only beginning.

CYTOGENETICS AND CONTRIBUTIONS TO GENETIC THEORY

As an example of an interesting kind of cytogenetic problem available in mosquitos, I should like to cite one of the facets of the work of Dr W. L. French in our laboratory. In the course of radiation experiments on mutagenesis, he attempted to obtain a strain of *quadrimaculatus* homozygous for all three chromosomes—that is, without heterozygous inversions. In the laboratory we had six standard strains and two wild-caught strains from Illinois. All of these proved to have about 85% of the individuals heterozygous for an extensive rearrangement in III-S. It involves an inversion, an extensive asynaptic area, and probably at least two lethals (perhaps deletions), one on each chromosome.

This poses an interesting problem. Do homozygous populations of *A. quadrimaculatus* exist? So far we have not discovered any. It is certainly possible that laboratory conditions might favour the preservation of the heterozygotes, but all populations thus far examined in nature show the same rearrangement.

The presence of this inversion in practically all individuals permitted another basic observation. Crossing-over does not occur in the males of *Drosophila* but presumably does in most other Diptera. Gilchrist & Haldane (1947) have cited genetic evidence for crossing-over in males of *Culex* and Craig & VandeHey (1962) have shown it in *Aedes*. No such information is available for *Anopheles*, but

French has clear evidence of segmental interchange during spermatogenesis in *A. quadrimaculatus*. Segmental interchange, which is related to genetic crossing-over, occurs during prophase I. If such segmental interchange takes place within an inversion, or involves the inversion and a non-inverted region, typical anaphase I plates show, in addition to normal figures, dicentric bridges and acentric fragments. The presence of such figures, presumptive evidence of " crossing-over " within an inversion, are found routinely in *quadrimaculatus*. Segmental interchange therefore takes place in males, and genetic cross-overs can be predicted when markers are available on chromosome III.

Chromosomal mutation

Frizzi (1961) has irradiated *A. atroparvus* with dosages of 2000r to 6000r. This latter dose gave complete sterility, but 3500r produced the highest rate of chromosomal mutations. He found that both pericentric and paracentric inversions were most frequent, but that deficiencies and translocations were rare. Most of the inversions were in chromosomes II and III, very few in the X. This work opens the way towards the creation of polymorphic laboratory strains, which could prove useful in detailed studies of the salivary chromosomes.

Prospects for future work

The fragmentary data available on mosquito cytogenetics clearly indicate the kind and amount of future work to be done. Of the 2400 kinds of mosquitos thus far described, we have information on fewer than 100, and much of this is very superficial.

The most obvious need is for more labourers in the vineyard. Even three or four additional laboratories would provide a great deal more information than we now have.

Certainly much of this information is of a descriptive sort, and easy to obtain by present techniques. Karyotype studies in additional species can be simply done, and should be carried out in a standardized way so that data on heterosomes and relative arm lengths of mitotic chromosomes, for example, may be obtained.

We need intensive work, in depth, in restricted groups. *Anopheles* provides a good example, but the same sort of work should be undertaken in other groups. Comparisons of karyotypes, preparation of salivary maps, the correlation of these two sets, the studies of chromosomes from species hybrids, would contribute to an understanding of the evolution of these groups. What has been done for the European *maculipennis* can be done for the North American *maculipennis*, for *Nyssorhynchus* and for many other complexes.

Immediate attention should be given to chromosomal polymorphism. This picture is now incomplete and therefore confusing, but it offers the possibility of understanding the evolution, taxonomy and speciation of difficult complexes. It also offers the hope of clarifying the position with regard to known " ecological races " and may contribute to the understanding of such phenomena as behaviouristic resistance and transmission or non-transmission. If mosquitos follow the *Drosophila* pattern, we may expect to find correlations of chromosomal patterns with altitude, temperature, salinity and all sorts of ecological conditions.

An important but difficult job that must be done soon is identification of genetic markers with known chromosomes and specific regions on chromosomes. This will require more sophisticated genetic analysis and will necesstiate visible translocations or other known chromosomal markers.

Although the situation in the European *maculipennis* is negative, work should be continued in other forms to establish whether any chromosomal indicators of resistance may be found. Studies should be concentrated on field strains if possible.

An inevitable result of more information will be the possibility of cytotaxonomic keys, which, as in *maculipennis*, will perhaps aid in the identification of closely related species, otherwise difficult to separate.

As more detailed knowledge of salivary maps becomes available, and as genetic markers become available, we shall be able to locate genes on chromosomes and identify the particular bands with which they are associated.

A virgin field, extremely important, and untouched in mosquitos, is the cytochemistry of the chromosomes. The identification of DNA and RNA with puffs, centromeres and other regions of the chromosomes urgently needs to be done. Mosquito salivary glands are excellent for such studies—they simply have not been worked on. This needs immediate attention.

As a sobering reminder of the inadequacy of our information, it may be recalled that even such a fundamental concept as the mechanism of sex-

determination in mosquitos is still unproven for most species.

Another important need is for field studies—chromosomal studies of natural populations in the field. This is more a problem of time, money and logistics than anything else.

In summary, a beginning has been made. A great deal needs to be done. The present state of our knowledge of mosquito cytogenetics is somewhat like certain highway construction programmes. The over-all plan is apparent, but only small sections have been started, and even smaller ones completed. Many of the sections end abruptly in cornfields, and many more hardly have the bulldozers working on them. We may hope that the next ten years will see several completed sections and enough men, machines and money, to complete the job.

RÉSUMÉ

L'auteur passe en revue les travaux consacrés depuis 1953 à la cytogénétique des moustiques. En dépit de l'intérêt considérable suscité par ces problèmes, nos connaissances sont encore superficielles et bien réduites.

L'établissement de caryotypes des différentes espèces ont fait considérer comme normal un nombre de chromosomes égal à 6 (3 paires). *Culex* et *Aedes* possèdent 3 paires de chromosomes essentiellement métacentriques (à centromère submédian); l'on n'a pu identifier avec certitude des hétérosomes que chez *Anopheles*. Dans le genre *Anopheles*, l'on distingue au moins 3 caryotypes d'après la structure des chromosomes X. Il semble que dans plusieurs espèces, en particulier dans le sous-genre *Nyssorhynchus*, existe une réduction de la partie hétérochromatique du chromosome X. Cette réduction est également visible au niveau des cellules des glandes salivaires.

Des atlas cytogénétiques des glandes salivaires sont — ou vont être bientôt — à la disposition des chercheurs en ce qui concerne *A. atroparvus*, *A. gambiae*, *A. albimanus*, *A. stephensi*, *A. aquasalis*, *A. freeborni*, *A. quadrimaculatus* et *A. punctipennis*. Il est difficile d'obtenir de bonnes préparations salivaires chez *Culex* et *Aedes*, et l'on sait peu de choses à leur sujet.

L'utilisation des méthodes génétiques comme moyen d'identification d'espèces impossibles à différencier autrement s'est avérée fructueuse pour l'étude du groupe *maculipennis* d'Europe. Cette méthode sera probablement intéressante pour situer certains complexes de classement difficile.

L'on ne peut pour le moment établir aucune correspondance entre la fréquence des anomalies chromosomiques et le degré de résistance aux insecticides. De toute évidence, des anomalies peuvent être produites sous l'action de conditions particulières d'habitat, au laboratoire ou sur le terrain, mais une fois qu'une population donnée a atteint un certain équilibre, l'on ne peut obtenir de nouveau changement par sélection avec les insecticides.

Plusieurs espèces d'anophèles présentent un polymorphisme chromosomique. C'est ainsi que *A. gambiae*, *A. punctipennis*, *A. strodei* et *A. darlingi* sont hautement polymorphes. De nombreuses autres espèces semblent n'être que peu polymorphes.

Le genre *Anopheles* offre de riches perspectives aux recherches cytogénétiques. Les autres genres sont d'étude plus difficile; les recherches devraient se porter avec ardeur dans ce sens.

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