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SALIVARY GLAND TYPE CHROMOSOMES IN MOSQUITOES

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Mosquitoes have been the subject of several cytological studies, but in these insects very little consideration has been given to the type of chromosome characteristic of the salivary glands and other organs of many Dipteran larvae. In recent work, chromosomes from the large larval mid-gut cells of mosquitoes have been described[1,2] as similar to the salivary gland type, but lacking the regular alternation of bands and achromatic regions and consisting merely of a linear series of chromatic masses.

Giant chromosomes with regular banded structure may be obtained, however, from certain tissues in the later stages of development. These chromosomes will be described here. The most satisfactory preparations were obtained from the Malpighian tubes of the imago, pupa or fourth instar larva, but such chromosomes were also found in the salivary glands, gastric caeca and mid-gut of the prepupal stage.

DESCRIPTION OF PLATE

Figure 1. Nucleus from Malpighian tubes of *Culex pipiens* male, pupal stage. Ca. × 510.

Figure 2. Chromosome from *C. pipiens* male, with nucleolus. Ca. × 1160.

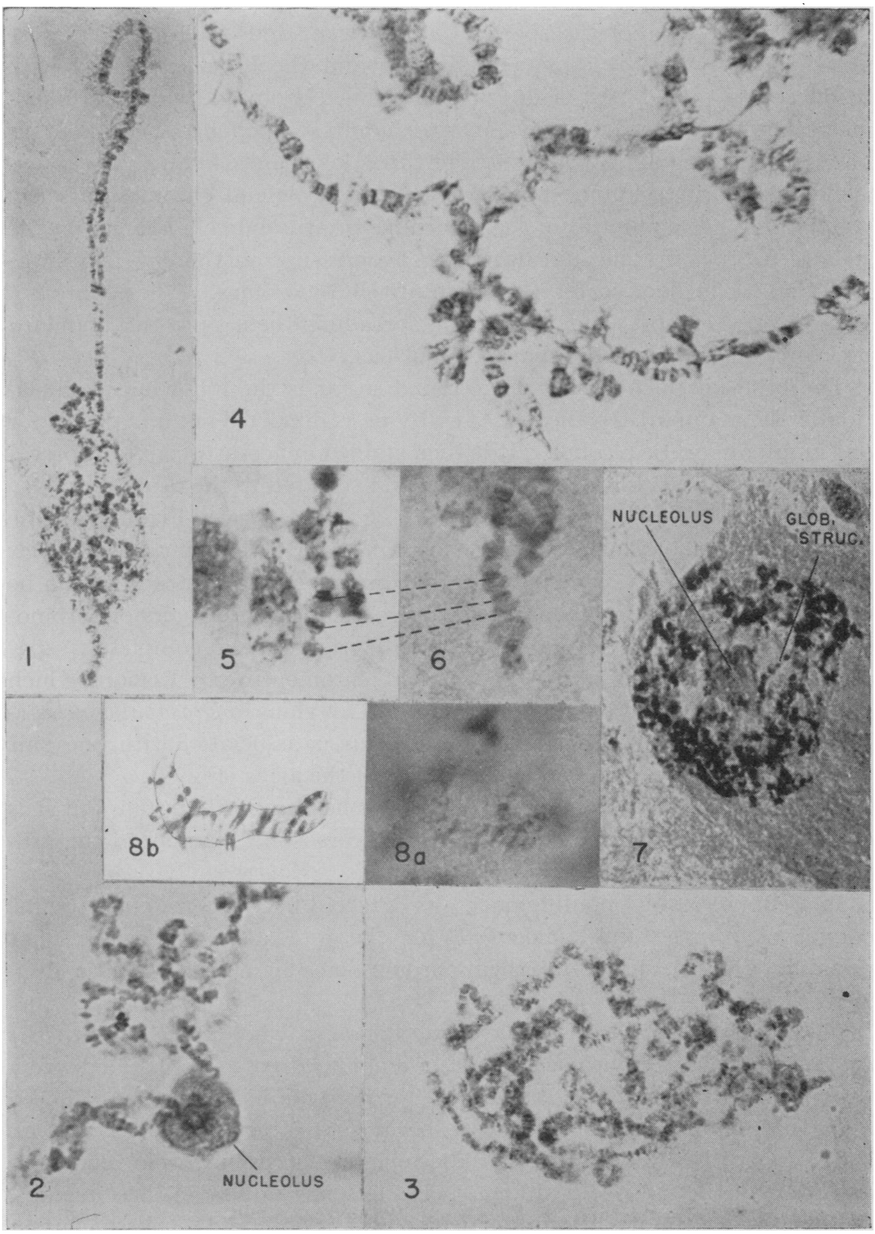
Figure 3. Nucleus from *Aedes aegypti* female. Ca. × 580.

Figure 4. Part of nucleus from *A. aegypti* male showing banded regions and "weak spots." Ca. × 1160.

Figures 5 and 6. Comparison of the same region in two different cells of *A. aegypti*, fourth instar larva. Ca. × 1160.

Figure 7. Nucleus from *A. aegypti*, fourth instar larva, showing globular structure and nucleolus. Ca. × 580.

Figure 8 (*a*). Section of a chromosome from *A. aegypti* pupa, with peculiar banded structure. Ca. × 1160. (*b*) Camera lucida drawing of same.



DESCRIPTION ON OPPOSITE PAGE

In the salivary glands of the adult mosquito the chromosomes are apparently of the normal somatic type.

Technique.—Eggs of the species *Culex pipiens* and *Aedes aegypti*[3] were raised in tap-water at room temperature and the larvae were fed with breadcrumbs. The best slides were obtained from individuals (fourth instar larvae, pupae or newly emerged adults) which had been kept at low temperature (10–18°C.) for two or three days before killing.

The standard technique for smearing salivary gland chromosomes (by staining for a few minutes in aceto-carmine or acetic orcein placing on the slide in a drop of stain and flattening by pressure on the coverslip) was found to be ineffective for the mosquito chromosomes. These chromosomes are very soft, and instead of spreading them out, the standard procedure merely squashes the whole nucleus.

The best treatment that I have found so far is the following modification of the standard technique: The tissue is dissected out and placed for one minute in acetic alcohol. It is then stained in acetic orcein (1% orcein in 45% acetic acid) for about one hour, and smeared in the usual way.

The Salivary Type Chromosomes.—(1) *Culex pipiens.* Figure 1 in the plate shows a whole nucleus from a smear of the Malpighian tubes of *Culex pipiens.* One chromosome pair lies free, and the banded structure can be clearly seen. In some preparations I have seen the three pairs of chromosomes ($2n = 6$), synapsed along most of their length, and completely separated from each other, with no apparent chromocenter. Regions which appear heterochromatic like the chromocenter regions in *Drosophila melanogaster* have not been observed. A nucleolus is associated with one pair of chromosomes near the distal end of one of the arms (Fig. 2).

No detailed study of the banding pattern has been attempted, but it is clear that a map could be made for each chromosome, similar to the salivary gland chromosome maps of Drosophila.

In a cursory study, no difference was detected between the chromosome complexes of males and females. It may be noted that the sexes are not certainly distinguishable by their chromosomes in normal mitotic divisions.[4]

(2) *Aedes aegypti.* Figures 3 and 4 show a whole nucleus of *Aedes aegypti* and part of a nucleus at a higher magnification. The banded structure is clear in some places, but the pattern of banding cannot be followed along individual chromosome arms, because it is interrupted by "weak spots." At these places the chromosomes often fragment in smearing, and parts of the same or of different chromosomes adhere in these regions, so that the continuity of any particular arm is obscured. These contact points can be seen in figure 4. Parts of chromosomes are very often connected by attenuated threads.

As in *Culex pipiens* there is no single chromocenter such as is found in salivary gland nuclei of Drosophila. It is possible that the contacts are due to non-specific pairing between heterochromatic segments which are interspersed between the banded regions all along the chromosome arms.

Figures 5 and 6 show comparable banded fragments from different nuclei. By a series of such comparisons it would be possible to map the banding pattern for short regions, but it is doubtful whether the sequence for a whole chromosome could be worked out.

Although a nucleolus is present earlier in the development of the nuclei (Fig. 7), it is not visible at later stages after treatment with acetic orcein, in marked contrast to the nucleolus of *Culex pipiens*.

Development of the Salivary Gland Type Chromosomes.—The development of the fully banded chromosomes in mosquitoes has not been studied to any considerable extent, but a few observations, made in the course of dissecting tissues from individuals at various stages of development, may be of some interest. In this respect the two species studied are similar.

The regular banding is not present in chromosomes at the earlier stages (up to third instar larvae), nor do all chromosomes in the Malpighian tubes of later stages necessarily attain this type. In young larvae all, and in later stages some of the cells in question have chromosomes in which the nucleic acid is accumulated in more or less spherical masses of different sizes, separated by achromatic regions (Fig. 7).

Sometimes the globules appear to be connected in a linear series only by fine chromatic threads. In a few figures, short sections of chromosome showed a peculiar banded structure, in which the bands appeared to be connected by paired threads (Fig. 8).

It seems that the dense accumulations of nucleic acid in the globule stage are redistributed (perhaps spread out laterally, or perhaps partially lost) in the formation of the banded type of chromosome. Some intermediate cells are found in the later stages, where well-defined bands are seen in some sections of the chromosomes, while other sections approximate to the globule type.

Tentatively, a comparison may be drawn between this shift in nucleic acid attachment and the experimental results obtained by Kodani.[5] The globule stage described here bears some resemblance to the γ 1 stage in his treated chromosomes. It must be remembered, however, that while the transition in mosquito is a normal developmental process, the apparent reversal of this process obtained by Kodani is induced by somewhat drastic treatment, and it is not yet known whether this induced effect is reversible.

Summary.—A brief description is presented of the development and characteristics of salivary gland type chromosomes in two mosquito species (*Culex pipiens* and *Aedes aegypti*) in both of which the diploid chromosome number is six. The differences observed between the two species indicate

that these chromosomes might be useful as a diagnostic character in distinguishing different species of mosquito.

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PURINES AS GROWTH REQUIREMENTS OF SPIRILLUM SERPENS

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The recognition of the purine and pyrimidine bases as factors affecting the growth of microörganisms has been reported with increasing frequency during the past few years. Richardson[1] in 1936 found that under certain conditions uracil was essential for the growth of *Staphylococcus aureus.* Möller[2] showed that adenine was required for the growth of *Streptobacterium plantarum,* while Pappenheimer and Hottle[3] found adenine to be necessary for the growth of a strain of group A hemolytic streptococci. In the latter case adenine could be replaced by hypoxanthine, guanine, xanthine, guanylic acid or adenylic acid. Furthermore, it was observed that purines were not required by this organism if the carbon dioxide tension above the medium was maintained sufficiently high. Snell and Mitchell[4] have reported the requirements of several lactic acid bacteria for purine and pyrimidine bases. *Streptococcus lactis* was found to require adenine and thymine for growth. Guanine was found to be essential for the growth of *Leuconostoc mesenteroides,* while uracil stimulated the growth of the latter organism and of *Lactobacillus arabinosus.* Robbins and Kavanagh[5,6] have recently described the effect of guanine and hypoxanthine on the growth of *Phycomyces.* In the present communication we wish to report the essential nature of several purine bases for the growth of a strain of *Spirillum serpens.*

Investigation of the cultural requirements of this organism (carried by American Type Culture Collection as No. 8084) showed that although it could not be grown in simple synthetic media the addition to such media of small amounts of natural extracts caused growth to occur. The active principle of such extracts was isolated and was found to consist of purine bases. Hypoxanthine, adenine and guanine were found to affect the growth of the organism.