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A Study of the Mitotic Karyotypes of Five Species of Culicine Mosquitoes Found in New Mexico

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A STUDY OF THE MITOTIC KARYOTYPES OF FIVE SPECIES OF CULICINE MOSQUITOES FOUND IN NEW MEXICO

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ABSTRACT

Karyotypic studies were made of five species of mosquitoes: Culex tarsalis Coquillett, Culex pipiens quinquefasciatus Say, Culiseta incidens (Thomson), Psorophora signipennis (Coquillett), and Aedes dorsalis (Meigen). Slides of tissue from the limb buds were prepared by the squash technique. All five species were found to have a diploid number of six. The study shows that Aedes dorsalis, Psorophora signipennis, and Culiseta incidens have virtually identical mitotic karyotypes, with two pairs of large and one pair of somewhat smaller chromosomes. Culex tarsalis and Culex pipiens quinquefasciatus have karyotypes that contain three sizes of chromosomes. In Culex tarsalis there are obvious size differences, but in C. pipiens quinquefasciatus the two large pairs of chromosomes differ only slightly in length. No secondary constrictions were found, and banding appears inconsistent. Species separation on the basis of markings does not appear possible.

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INTRODUCTION

Cytogenetic studies are providing valuable taxonomic information concerning the insects. Species with virtually identical morphology have been separated and ideas on insect evolution have been modified by cytogenetic evidence obtained through studies of mitosis, meiosis, and chromosome morphology. My study was undertaken to determine the feasibility of separating six species of mosquitoes found in New Mexico on the basis of the morphology of their somatic chromosomes. The chromosomes were obtained by squashing limb buds taken from larvae of these species. I hoped to determine the diploid number for each species, the position of the centromere for the chromosomes of each pair, and the lengths of the chromosomes. I intended also to make note of any consistent markings or secondary constrictions present on the chromosomes. I hoped that a comparison of these data would disclose reliable criteria for separation of one or more of these species.

LITERATURE

Out of 2400 species and subspecies of mosquitoes in the world, only about 100 have been examined cytologically (Rai, 1966). Whiting (1917) undertook a study of the chromosomes of Culex pipiens largely because of conflicting reports made by earlier workers on the nature of the chromosomes of this species. He found mitotic figures in limb buds and wing buds and reported a diploid number of six. Hance (1917) also studied the mitotic chromosomes of C. pipiens and reported a diploid number of six. In this species Taylor (1914) had previously reported that some somatic cells contained only three chromosomes, with terminally bifid ends. Hance felt that there were really three pairs of chromosomes in all somatic cells of C. pipiens and that the chromosomes of each pair were sometimes indistinct because of inadequate fixation. Early reports of diploid numbers other than six in mosquitoes are thought by Breland (1961) to be the result of a lack of understanding of polyploid cells and somatic pairing.

The early workers were somewhat handicapped by having only tissue sections to work with. Painter (1934) was the first successfully to adapt the squash technique to a study of the salivary gland chromosomes of Drosophila melanogaster. Prior to this the squash technique apparently had been overlooked by investigators working on dipteran chromosomes. The new technique led to an increasing interest in nuclear phenomena. According to Gillham (1957), Bogojawlensky in 1934 was the first to report the occurrence of polytene chromosomes in mosquitoes. Gillham states that Bogojawlensky found polytene chromosomes in the salivary glands, Malpighian tubules, midgut, and anterior

portion of the hindgut of Anopheles maculipennis larvae. Sutton (1942) suggested that a map of the polytene chromosomes of mosquitoes could be made and that characteristics of polytene chromosomes might be useful for distinguishing species of mosquitoes. Considerable work has been done along these lines. Working with Anopheles maculipennis, Frizzi (1947) developed a technique for preparing salivary gland chromosomes for cytological analysis and found that varieties of A. maculipennis exhibited characteristic banding patterns. Although the work with anophelines has been largely concerned with the polytene chromosomes, several workers have published descriptions and photographs of mitotic and meiotic chromosomes of several species of Anopheles. A synopsis of this work, with literature citations, may be found in Kitzmiller's 1963 paper.

Grell (1946a, 1946b) published extensive reports on mitosis and meiosis in Culex pipiens and included observations on somatic pairing. In a valuable paper, Breland (1961) presented information on workable techniques, a summary of previous work, and cytogenetic information on 16 previously unstudied species of mosquitoes. This paper seems to have stimulated many investigators to study mitotic and meiotic chromosomes in mosquitoes. Working with Anopheles quadrimaculatus, A. freeborni, and Aedes taeniorhynchus, French, Baker, and Kitzmiller (1962) modified Breland's technique and discussed the preparation of chromosomes from the brains, testes, and ovaries of these species. Breland, Gassner, and Riemann (1964) published a description and photographs of meiosis in Culiseta inornata. Rai (1963, 1966) studied the mitotic chromosomes of 11 species of Culex, and one

species each of Eretmapodites and Wyeomyia.

MATERIALS AND METHODS

The larvae used in this study were collected from ponds and streams within a radius of 60 miles from Albuquerque, New Mexico. Collecting sites and the species of mosquitoes taken from each site are listed in Table 1. Larvae not sufficiently mature for immediate study were held in the laboratory until they developed to the late fourth instar. Larvae in excess of those that could be examined on return from collecting were also held in the laboratory. In the laboratory the larvae were maintained in glass jars containing water from the original habitat and were fed dried Fleischmann's yeast, crushed dog biscuit, and powdered shrimp meat. These foods were given separately and in various combinations. Yeast seemed to be the preferred food, but I found that larvae fed yeast for several days usually had more fat in the thoracic region than did larvae that were dissected shortly after capture. This fat sometimes interfered with dissection.

The final selection of the kind of tissue to be used in this study was made only after studying trial squashes of several tissues. I first attempted to use brain tissue, but I soon learned that removing the brain from the head capsule and making a smear were difficult because of the small size of the brain. Also the removal of the brain resulted in the destruction of the head capsule, making it necessary to identify larvae to species before dissection. An attempt was made to locate the salivary glands, but these could not be found, probably because the salivary glands undergo reorganization in the late prepupal stage (Clements, 1963). Limb buds were finally

used for making squashes as I found that, if the larvae were dissected at the proper stage, the limb buds were easily removed and processed. The head and abdomen were not harmed during dissection and were stored in 70% ethyl alcohol until time was available to make species identification. The larvae were identified with the aid of the keys found in "Mosquitoes of North America" by Carpenter and LaCasse (1955).

After removing the head and abdomen the thorax was placed in modified Carnoy's fluid (Breland, 1961) for approximately 30 seconds; then the Carnoy's fluid was replaced by Belar's saline solution (Breland, 1961). The thorax was submerged in saline solution to prevent drying of the tissues during dissection. Using fine needles I removed the limb buds from the thorax and immediately placed them in either aceto-carmine or aceto-orcein stain. Aceto-orcein gave better results than did aceto-carmine and was used in making most of the smears. A staining period of 20-30 minutes gave satisfactory results.

At the end of the staining period excess stain was removed and a drop of 45% acetic acid placed on the tissue (French, Baker, and Kitzmiller, 1962). Then a cover slip was placed on the limb tissue and the tissue was squashed by a series of sharp taps or by applying as much pressure as possible without breaking the cover slip. Then the slide was placed on dry ice and, after the tissue was frozen, a razor blade was inserted under one corner and the cover slip flipped off. The cover slips used for squashing were treated with a solution of silicone (French et al., 1962), so that they usually flipped off without taking much tissue from the slide. After removing the cover

slip, the slides were passed through an alcohol-xylol series and the squashes were mounted in Canada balsam.

In examining the slides I used a definite pattern of search. Starting at the upper edge of a squash and using a magnification of 1000X, I worked back and forth along the long axis of the slide, moving over a path leading to the lower edge of the squash. I allowed the fields of observation to overlap slightly so that each squash was examined thoroughly. Observations were made on any mitotic figures found and the coordinates of their position were recorded from the scales of the mechanical stage. Drawings were made with the aid of a camera lucida.

Chromosomes were measured with a calibrated ocular micrometer. To make the measurements, nearly straight metaphase chromosomes were positioned so that the micrometer scale lay over the chromosome, parallel to the long axis. It was not possible to make the same number of measurements in each species because relatively few chromosomes were favorably oriented.

DATA

Squashes were made from tissue taken from larvae of the following species: Culex tarsalis Coquillett, C. pipiens quinquefasciatus Say, Culiseta incidens (Thomson), Psorophora signipennis (Coquillett), Aedes dorsalis (Meigen), and Anopheles pseudopunctipennis franciscanus McCracken. The squashes of A. pseudopunctipennis franciscanus tissue were of such poor quality that I could determine only the diploid number, six, and nothing else. Therefore the following data do not pertain to A. pseudopunctipennis franciscanus.

A diploid number of six was found in all species. The chromosomes occurred in three pairs, two pairs of large and one pair of somewhat smaller chromosomes. In Culex tarsalis the chromosomes of one large pair appeared unequal in length to the chromosomes of the other large pair, and although the evidence was not conclusive, this was apparently also true in C. pipiens quinquefasciatus. The presence in a configuration of three sizes of chromosomes was more obvious in C. tarsalis than in C. pipiens quinquefasciatus. Configurations from Culiseta incidens exhibited only two sizes of chromosomes, large and small, and this was apparently also true of Psorophora signipennis. I could not be certain that there were only two sizes of chromosomes in Aedes dorsalis, but if there were three sizes, the two pairs of large chromosomes differed only slightly in length. The average lengths of the longest chromosomes ranged from 4.4 μ in Culex pipiens quinquefasciatus to 6.4 μ in Culiseta incidens. The average lengths of the shortest chromosomes varied from 2.9 μ in Culex pipiens quinquefasciatus to 4.7 μ in Culiseta incidens (Table 2).

All species were found to have metacentric chromosomes. Usually the centromere region appeared as a constriction that stained as darkly as the arms, but occasionally the centromere appeared lighter than the arms.

In all five species terminal granules were frequently observed on prophase and early metaphase chromosomes. The size and shape of these granules were similar in all species. In some chromosomes the granules were round and in others oval. When a granule occurred on only one arm of a chromosome, that arm usually appeared to be shorter than the other arm. Frequently, what appeared to be a granule was found to be simply a folding of the end of the chromosome, but many granules could not be explained in this way. Anaphase and late metaphase chromosomes never exhibited granules.

Occasionally, in all five species, I saw circular-shaped chromosomes, usually formed by one member of the smallest pair of chromosomes (Fig. 1). Rarely, both members of the small pair were circular shaped, and sometimes one of the larger chromosomes was in the shape of a circle. Some of these circular-shaped chromosomes had overlapping ends but in many cases the ends of the chromosomes could not be seen.

There were no consistent markings or secondary constrictions seen on the chromosomes of any of these species. In rare instances dark markings were observed on prophase chromosomes (Fig. 2).

DISCUSSION

Breland (1961) noted three types of chromosome complements among members of the genus Anopheles and suggested that, because of these different karyotypes, a study of metaphase chromosomes in anophelines might provide evidence concerning the relationships of various groups of anopheline mosquitoes. On the other hand, the karyotypes of the culicine mosquitoes appear remarkably uniform, with only slight differences in most cases, and Breland thought that this uniformity rendered a study of their metaphase chromosomes of little value with respect to the relationships of the culicine groups. But further study of the culicines discloses more and more previously unobserved karyotypic differences (Rai, 1963). During this study I examined the karyotypes of five species of culicine mosquitoes, and my present results indicate that only one of these species can be reliably separated from the others on the basis of a consistent karyotypic characteristic.

Culex tarsalis.—Breland (1961) published one photograph of the mitotic chromosomes of C. tarsalis. In the photograph the chromosome pairs seem to be of three different lengths, but Breland makes no reference to C. tarsalis in his text. Other than this, virtually nothing seems to have been published concerning the cytology of the species. I examined 27 squashes made from tissue of larvae of this species, and I found C. tarsalis to be the most distinctive of the five species that I studied because the three pairs of chromosomes were of distinctly different sizes. Usually size differences were plainly visible when late metaphase configurations were examined, and

occasionally late prophase configurations also exhibited three pairs of chromosomes of different lengths. Anaphase configurations did not always support the idea that there were three different chromosome lengths; in many anaphase configurations the large pairs of chromosomes appeared equal in length. However, as chromosomes may stretch somewhat during anaphase, I did not consider results of the study of anaphase chromosomes entirely reliable. Often both prophase and early metaphase configurations showed the two large pairs of chromosomes to be of equal length; this may indicate a difference in the rate of contraction of the different pairs of chromosomes (Breland and Gassner, 1961).

Although circular-shaped chromosomes were observed occasionally in all five species, they appeared to be most frequent in squashes made from C. tarsalis. White (1957) states that ring chromosomes do not occur consistently in natural populations of insects because of disruption during mitosis or meiosis. Apparently ring chromosomes previously have not been reported in mosquitoes. Some of the circular-shaped chromosomes were bent so that their ends overlapped but were not fused. However, the ends of many of the circular-shaped chromosomes were not visible. I saw no circular-shaped chromosomes in anaphase or early prophase configurations, and their absence from those stages indicates that they are not true ring chromosomes. In all five species the circular-shaped chromosomes were usually formed from one member of the smallest pair of chromosomes, and only rarely were two such chromosomes found within the same configuration.

The homologue of the circular-shaped chromosome was usually close, but unbent. If these chromosomes are not true aberrations, the force responsible for their shape appears to be highly selective. Their appearance possibly results from the technique used in preparing the chromosomes for study.

Culex pipiens quinquefasciatus.--Breland (1959) reported negative results from squashes made from brains and Malpighian tubules of two fourth-instar larvae of this species. In 1961 Breland published a photograph of metaphase chromosomes of C. pipiens quinquefasciatus and in the photograph the three pairs of chromosomes appear to be of three different sizes, but he said nothing about the species in his text. Breland and Gassner (1961) stated that some workers have considered the chromosomes of the two large pairs to be of equal length in species of the Culex pipiens group because the difference in length is so slight. I had for examination only three squashes of tissue from this species. Although the evidence is not conclusive, I believe that the three pairs of chromosomes are of three different lengths. The difference in length between the two pairs of large chromosomes apparently is not so great as in C. tarsalis, but verification of this depends on further study. Breland (1961) thought that probably the time of maximum contraction of chromosomes varies among the different species of mosquitoes, so it is possible that most of the chromosomes in my three squashes of C. pipiens quinquefasciatus were not at maximum contraction.

Culiseta incidens.--In examining 19 squashes made from larval

tissue of this species I found the two pairs of large chromosomes to be equal in length. This supports the view of Callan and Montalenti (1947) that the two large pairs are of equal length in this species. However, Callan and Montalenti stated that the centromere was very slightly submedian in one pair of large chromosomes and median in the other two pairs of chromosomes. It appeared to me that all three pairs of chromosomes were metacentric.

Psorophora signipennis.--Breland (1961) published a photograph of mitotic chromosomes from P. longipalpus and has done some work on P. confinnis and P. discolor, but he notes only that he has seen no outstanding features in the chromosomes of any of these species. As far as I can determine this is the only published cytological work on the genus Psorophora. I found nothing outstanding in examining nine squash preparations. All the chromosomes seemed to be metacentric, and the two pairs of large chromosomes appeared to be of equal length.

Aedes dorsalis.--I had only two A. dorsalis larvae from which to make squashes, but one of the squashes contained many mitotic figures. Rai (1966), Breland (1961), and Breland and Gassner (1961) examined the karyotypes of several species of Aedes, but apparently did not include A. dorsalis in their studies. Breland and Gassner (1961) reported three sizes of chromosomes in A. aegypti, although they noted that careful examination was often required to see that the two pairs of large chromosomes were unequal in length. Rai (1963) measured the chromosomes of six species of Aedes and found that the two pairs of large chromosomes differed in length by 0.7 μ

to 1.6 μ, depending on the species examined. In A. dorsalis the two pairs of large chromosomes appeared to be equal in length. It is possible that further study will disclose a slight length difference. Both metacentric and submetacentric chromosomes have been reported for Aedes. My observations indicate that the chromosomes of A. dorsalis are metacentric.

General Remarks.—All the species that I studied appear to have metacentric chromosomes. In all species of culicine mosquitoes that have been studied cytologically the centromeres are either median or submedian (Breland and Gassner, 1961). No species of culicine mosquito has been reported as having arms greatly unequal in length. Breland and Gassner (1961) thought that careful study would disclose more species of mosquitoes with submedian centromeres. Future studies may be worthwhile in showing differences in centromere position among karyotypes of various culicines.

Rai (1966) reported a conspicuous chromomere pattern on the prophase chromosomes of Aedes vittatus and also stated that it might be possible to map the chromomere pattern on the prophase chromosomes of A. polynesiensis. Some of the photographs he published in 1963 show chromomere patterns on prophase chromosomes. I observed chromomere patterns in all species at prophase, but the number of prophase chromosomes exhibiting chromomere patterns was small. According to present evidence it seems unlikely that the five species studied can be separated on the basis of markings on the chromosomes. For some species Rai (1963, 1966) reported and published photographs of

chromosomes with achromatic regions, which he stated may be secondary constrictions. In no case did I observe the type of achromatic region described by Rai, and there seemed to be no secondary constrictions on the chromosomes of the five species that I studied.

Rai (1963) measured the chromosomes of 12 species of mosquitoes. Although I did not study any of the species that he measured, it is interesting to note that his measurements of the chromosomes of several species of Culex and Aedes fall in the same general range as mine. Table 2 gives the measurements obtained for each of the five species that I studied and includes the average for each series of measurements. Although the averages differ in all cases, many of the measurements are identical in several of the species. Within a species the measurements differed because it was difficult to find several cells with chromosomes at exactly the same stage of contraction. It seems unlikely that the species can be separated on the basis of chromosome length alone, unless one measures the chromosomes of many configurations and takes the average of the measurements. Even then the reliability of such a method would be questionable. Rai (1963) points out that it is difficult to compare meaningfully the sizes of chromosomes of different species of mosquitoes because it would be almost impossible to measure their chromosomes at exactly the same stage of contraction.

Frequently, in all five species, I observed granules on the ends of the chromosomes. In some cases they could be explained as foldings of the ends of the chromosomes, but in many cases no such foldings

were visible. Whiting (1917) reported terminal gramules on prophase chromosomes of Culex pipiens, and according to his figures they appeared much like the ones I saw. Whiting noted that the terminal granules varied in size and staining reaction but he could not explain their nature or origin. Breland (1961) found some similar structures in Orthopodomyia alba and in O. signifera, and he felt that these may represent sex chromosomes. Rai (1963) observed two Feulgen-positive particles in the resting nuclei of Aedes atropalpus, and he felt that these may be compared with the terminal granules reported by Whiting. But Breland never reported more than two elements in a configuration, and I have seen as many as six granules in one configuration. Furthermore, Breland's photographs show the elements found in Orthopodomyia to be attached to the chromosome by a very thin thread of material, while the granules that I observed were attached directly to the ends of the chromosomes. It is unlikely that the granules I observed represent sex chromosomes because there were frequently more than two present in one configuration. The particles reported by Rai may have no relation to the granules reported by Whiting or to the granules found in my preparations. Rai's particles were seen only in resting nuclei; Whiting's granules and the granules that I observed were visible in mitotic nuclei and apparently were always attached to chromosomes.

I had only one species of Anopheles available for study and the squashes of larval tissue of this species, Anopheles pseudopunctipennis franciscamus, were of such poor quality that

they were useless. The reason for the inadequate preparations is not known, but the tissue seemed to be poorly fixed. Often I noticed that, even though apparently well formed, the limb buds were very soft when removed from the larvae, and this was probably an indication of insufficient fixation. At the time I made the slides I did not know of the experience of Rai (1963) who noted that Anopheles tissue required four to six times longer fixation than required for tissues from mosquitoes of other genera.

SUMMARY AND CONCLUSIONS

1. The chromosomes of five species of culicine mosquitoes were studied in an attempt to find distinctive differences that would make it possible to distinguish one or more of these species on the basis of their karyotypes.
2. The chromosomes were prepared with a squash technique based on the techniques of Breland (1961) and French, Baker, and Kitzmiller (1962). The squashes were made from limb buds taken from late fourth-instar larvae.
3. The karyotypes of Psorophora signipennis, Aedes dorsalis, and Culiseta incidens were found to be virtually indistinguishable, all having three pairs of apparently metacentric chromosomes. In each of these species, the two large pairs of chromosomes appear equal in length.
4. The karyotypes of Culex tarsalis and C. pipiens quinquefasciatus are similar as there appear to be three sizes of metacentric chromosomes in both species. Although the present evidence is not conclusive, the difference in length between the two large pairs of chromosomes appears to be greater in C. tarsalis than in C. pipiens quinquefasciatus.
5. There were no secondary constrictions or consistent markings observed on the chromosomes of any of these species.
6. The results of this study indicate that Culex tarsalis is, at this time, the only species of those studied that can be distinguished from the others on the basis of a karyotypic characteristic. The separation can be made because of the

presence in C. tarsalis of three distinct sizes of chromosomes.

7. Further cytological study of these species may disclose other presently unobserved karyotypic differences. The present study did not include all aspects of mitosis in the five species.

TABLE 1. Collecting sites and species of mosquito larvae taken from each site

Collecting sites	Species taken
Isleta Indian Reservation, 15 miles south of Albuquerque near U. S. Highway 85, Bernalillo County	Culex tarsalis
4.1 miles south of U. S. Highway 66 along N. M. Highway 10, Bernalillo County	Culex tarsalis Culiseta incidens
West bank of Rio Grande, directly east of 1412 Riverview Drive N. W., Albuquerque, Bernalillo County	Culex pipiens quinquefasciatus
Cinco-C Ranch, 5 miles south of Chilili along N. M. Highway 10, Torrance County	Culex tarsalis
Tajique, Torrance County	Anopheles pseudopunctipennis franciscanus
Manzano Lake, at town of Manzano, Torrance County	Anopheles pseudopunctipennis franciscanus Culex tarsalis Culiseta incidens
San Ysidro, Sandoval County	Aedes dorsalis Anopheles pseudopunctipennis franciscanus Culex tarsalis Psorophora signipennis
3.6 miles south of Jemez Springs along N. M. Highway 4, Sandoval County	Anopheles pseudopunctipennis franciscanus
7.5 miles south of U. S. Highway 66 along N. M. Highway 10, Bernalillo County	Anopheles pseudopunctipennis franciscanus
Abo State Monument, Torrance County	Anopheles pseudopunctipennis franciscanus

TABLE 2. Chromosome lengths in the five species of mosquitoes studied

Species	Length of longest chromosome (μ)	Average length (μ)	Length of shortest chromosome (μ)	Average length (μ)
Aedes dorsalis	5.7 5.7 5.7 5.7 5.7 5.0	5.6	4.3 4.3 4.3 4.3 5.0 5.0	4.5
Culex pipiens quinquefasciatus	4.3 4.3 4.3 4.3 4.3 4.3 5.0	4.4	2.9 2.9 2.9	2.9
Culex tarsalis	5.7 5.7 5.7 5.7 5.7 5.7 5.0 4.3 4.3	5.3	3.6 4.3 4.3 4.3	4.1
Culiseta incidens	5.7 5.7 5.7 6.4 6.4 7.2 7.2 7.2	6.4	4.3 4.3 4.3 5.0 5.0 5.0	4.7
Psorophora signipennis	5.7 5.7 5.7 5.7 5.7 6.4 6.4	5.9	4.3 4.3 4.3 4.3 4.3	4.3

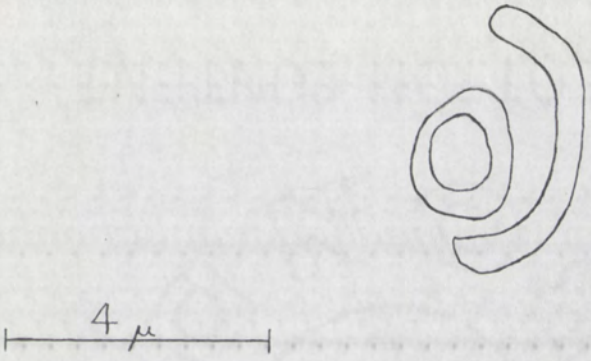

FIGURE 1. Culex tarsalis, a typical circular-shaped chromosome and its homologue.

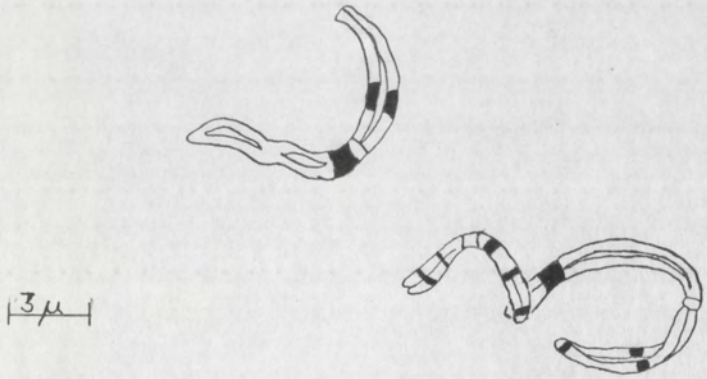

FIGURE 2. Culex tarsalis, prophase chromosomes showing markings on each pair of homologues.

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