of laboratory and field tests with Dursban insecticide for mosquito control. Mosq. News 26(3):344–351.

Moore, J. B. and Breeland, S. G. 1967. Field evaluation of two mosquito larvicides, Abate and Dursban, against *Anopheles quadrimaculatus* and associated *Culex* species. Mosq. News 27(1):105–111.

Mulla, M. S. 1966. Toxicity of new organic insecticides to mosquito fish and some other aquatic organisms. Mosq. News 26(1):87–91.

Mulla, M. S., Metcalf, R. L. and Geib, A. F. 1966. Laboratory and field evaluation of new mosquito larvicides. Mosq. News 26(2):236–242.

Mulla, M. S., Darwazeh, H. A., Geib, A. F. and Westlake, W. E. 1969. Control of pasture *Aedes* mosquitoes by dripping larvicides into flowing water, with notes on residues in a pasture habitat. J. Econ. Entomol. 62(2):365–370.

Porter, C. H. and Gojmerac, W. L. 1969. Field observations with Abate and Bromophos: their effect on mosquitoes and aquatic arthropods in a Wisconsin park. Mosq. News 29(4):617–620.

Sjogren, R. D. and Mulla, M. S. 1968. Drip application of three organophosphorus insecticides for mosquito control. Mosq. News 28(2):172–177.

Tawfik, M. S. and Gooding, R. H. 1970. Dursban and Abate clay granules for larval mosquito control in Alberta. Mosq. News 30(3):461–464.

Von Windeguth, D. L. and Patterson, R. S. 1966. The effects of two organic phosphate insecticides on segments of the aquatic biota. Mosq. News 26(3):377–380.

Washino, R. K., Whitesell, K. G., Kramer, M. C. and McKenna, R. J. 1972. Rice field mosquito control studies with low volume applications of Dursban in Colusa County, California. III. Effects upon the nontarget organisms. Mosq. News 32(3):375–382.

THE SALIVARY GLAND CHROMOSOMES OF *ANOPHELES WALKERI* THEOBALD

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INTRODUCTION

The salivary gland chromosomes of previously mapped Nearctic anophelines have revealed genetic relationships among members of the subgenus *Anopheles* (Kitzmiller *et al.*, 1967). Similarities are usually confined primarily to the centromere regions of the autosomes and to the free ends, while inversion events are responsible for the variations in banding patterns in the center of the arms. The unique banding patterns of the X chromosomes within the subgenus may be used to distinguish one species from another.

This paper describes the salivary gland chromosomes of *Anopheles walkeri* Theobald and proposes preliminary chromosomal affinities with *A. quadrimaculatus* and *A. atropos*, two closely related species. (Klassen *et al.*, 1965; Kreutzer *et al.*, 1969). *Anopheles walkeri* is distributed in southeastern Canada and eastern United States westward to Louisiana and Minnesota. Extremes in its range to the west include Nebraska and to the south Vera Cruz, Mexico. The slides were prepared from specimens collected in the Everglades National Park, Florida, by the technique described by French, *et al.* (1962). A typical complement is shown in Figure 1. The map (Figure 2) was produced from standard photographic enlargements of the complement with finer details obtained through direct observation at 1000X with a Zeiss photomicroscope. Further details concerning the relationships among *A. walkeri*, *A. quadrimaculatus* and *A. atropos* will be reported elsewhere.

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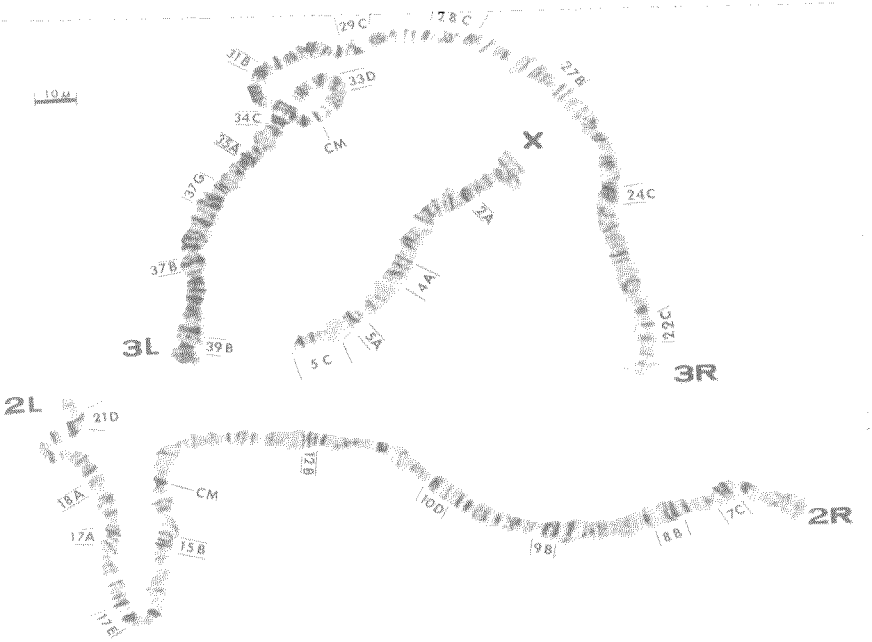


FIG. 1.—Salivary gland chromosome complement.

DESCRIPTION OF THE CHROMOSOMES. X-CHROMOSOME. The telocentric X is the shortest of the *walkeri* complement, approximately 75 μ in length. The centromere end is usually attached to a large nucleolus. The free end is consistently poor cytologically but ordinarily occurs in an expanded form as shown. A dark band at the beginning of 1A is separated from the two slightly lighter bands of that region. The puff in region 1B contains bands which are almost always twisted and indistinct. Three consecutive bands of medium intensity in 1C form the first distinctive landmark and are followed by another landmark area in 2A which contains two heavy bands separated by a lighter one. The puff in region 2B is usually indistinct, twisted and asynaptic, but the single dark band is fairly consistent. Other bands in this puff are of variable intensity. Region 2C contains two small puffs, each with one single dark band and several lighter ones. The two dark bands in this region, the second followed by a more lightly staining area, serve as a good landmark. Region 3B is of variable staining intensity but contains three fairly dark bands. The puff in 3C is uniformly poor but in good preparations appears as shown on the map. The dark band at the center of 3C, together with the three or four following bands, may appear as a dark irregular area.

In 4A three heavy bands followed by a pair of broken dark bands are consistently recognizable. Region 4B is also uniform containing a pair of light bands between two bands of medium intensity. Two heavy bands in the center of a dark puff

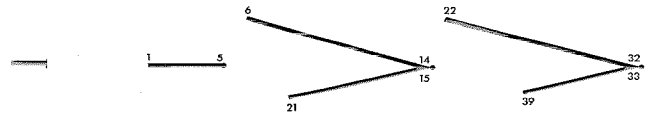
mark region 5A, diagnostic of this end of the chromosome. A consistently expanded puff with two central medium bands is recognizable in 5B followed by a triplet of medium bands. Region 5C is usually stretched and the bands difficult to follow, but in the best preparations is as shown. The last five bands in Region 5C often break apart from the rest of the X during preparation and may appear as a very short arm of the X. This is clearly an artifact due to the preparation process.

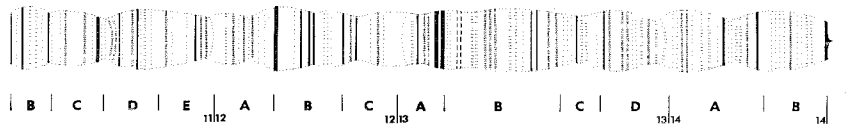
CHROMOSOME 2, RIGHT ARM. The right arm of chromosome 2 contains a good series of landmark areas which may be used for positive identification of the arm. Although the centromere end is variable and has no really good identification areas, the free end and the middle of the arm are easily recognized. The tip of the free end is usually expanded into a lightly staining bulb, which is separated from another lightly staining area in 6C by three dark bands in a constriction in 6B. A heavily staining area of six dark bands in 7B and 7C is followed by a wide, light region, which in turn is followed by a series of seven dark bands in 8A and 8B. Regions 9A and 9B are usually wide, with three evenly spaced dark areas which may be resolved into eight bands as shown on the map. The two heavy bands in a puff in 9C are variable in different specimens. The entire region following in 10A and 10B is also variable both in spacing of the bands and in their staining intensities. These two regions are also often twisted and probably contain an inversion break point. A series of heavy bands in 10C and 10D is always dark and forms a positive recognition area for the center of the arm. A light puff in 11A is bounded by dark bands easily recognized as shown in 11B, 11C, 11D and 11E. The dark bands in 12A are extremely variable, often lighter, often darker. The three dark bands in 12B followed by a light puff, then by three dark bands in 12C, form an always recognizable landmark. A characteristic series of dark bands in 13A is the last consistent region of the arm; from 13B to the centromere the bands are usually as shown, but are subject to extreme stretching, twisting and variation in staining.

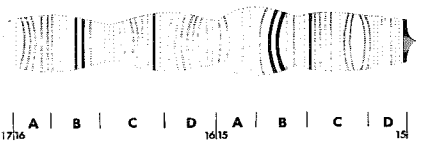
CHROMOSOME 2, LEFT ARM. This distinctive arm contains many readily identifiable areas, as well as many areas which show extreme variability, depending upon the puffing pattern and larval age. Most of the poorly staining areas are in regions in which presumed inversions have taken place (see discussion). At the free end of the arm, a single wide dark band in 21D is followed by four dark bands in 20A. This pattern is usually sufficient to identify the free end of the arm.

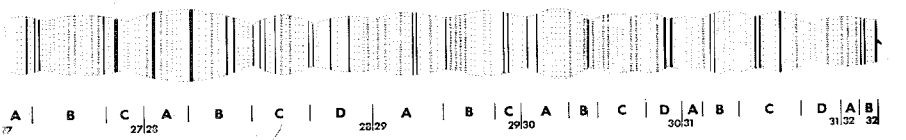
A small puff extends from 20B through 19A and is marked by three heavy bands at its beginning in 20B, a single heavy band at the end of 20C and two dark bands in a constricted area at the end of 19A. The expanded region in 19B is variable, usually wide and lightly staining, but in good preparations shows a series of evenly spaced bands of medium intensity. The darkly stained areas on either side of a constriction in 19C and 18A are always recognizable and are followed by several dark bands at the end of 18B.

A large expanded Balbiani ring is present in 18C and 18D in most specimens, although its appearance is consistently variable, depending upon the age of the larva. The single dark band at the end of 18C is always present, but the rest of the area shows extreme variability. The four bands in a medium-light-light-medium pattern following the expanded area are usually present as shown. Regions 17A and 17B contain a series of dark bands which are always recognizable and perhaps constitute the best recognition region for the central part of the arm. Regions 17C, 17D and 17E comprise an area of extreme variability, and only in a few slides are they as definite as shown on the map. This area is normally twisted, expanded, weakly staining, with broken bands, sometimes asynaptic and can usually be recognized by its variability and lack of definitive staining. However, the four heavy bands in 17E, the two middle ones thinner than the heavy



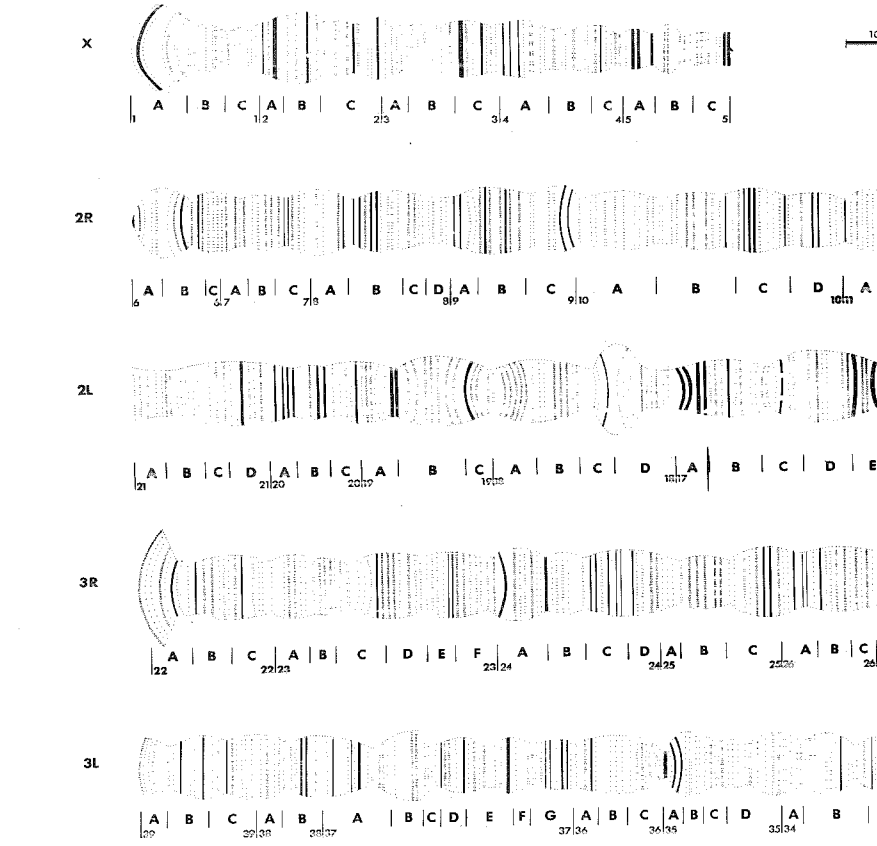






ANOPHELES WALKERI

SALIVARY CHROMOSOME MAP


X
A B C A B C A B C A B C A B C
1 1/2 2/3 3/4 4/5 5
2R
A B C A B C A B C D A B C A B C D A
6 6/7 7/8 8/9 9/10 10/11
2L
A B C D A B C A B C A B C D A B C D E
21 21/20 20/19 19/18 18/17
3R
A B C A B C D E F A B C D A B C A B C
22 22/23 23/24 24/25 25/26
3L
A B C A B A B C D E F G A B C A B C D A B
29 29/28 28/27 27/26 26/25 25/24

flanking bands, can usually be identified, although they are often broken and twisted.

The dark bands in 16A and 16B and the three dark bands at the end of 16B are consistent features as is the single dark band at the end of 16C. Regions 15A and 15B contain a bulbous area marked by two thin dark bands, usually in a twist, at its beginning and two heavy dark landmark bands at its end. Regions 15C and 15D are remarkably uniform for an area so close to the centromere.

CHROMOSOME 3, RIGHT ARM. This arm is the longest of the complement, approximately 215 μ in length, and contains numerous identifying areas. The pattern of landmarks clearly enables division of 3R into three segments, the free end, the middle portion and the centromere end. One paracentric inversion has been found located between 24A and 27C. Along the length of this arm many areas are consistently weak, asynaptic or twisted. This is seen particularly at the breakpoint regions at either end of the inversion. Homology exists with both *atropos* and *quadrimaculatus*, but is more extensive with the former.

The free end is usually of variable quality but in good preparations appears as shown. The three distinctive puffs with their bands immediately following the free end serve as the first identifying landmarks. A heavy band at the beginning of the first puff, region 22B, followed by another heavy band in 22C towards the end of the puff is characteristic. The second puff contains two centrally located dark bands in 23A and is followed by the third puff with its three centrally located bands in 23B. This series of puffs precedes a consistently constricted region containing two closely spaced light bands. This neck, located in region 23C, constitutes a prominent characteristic in this part of the arm. The puff in regions 23C and 23D is frequently twisted but contains a single heavy band followed by three closely-spaced somewhat lighter bands. In 23E, a pair of thin heavy bands in the center of a weak puff are closely spaced as are the six medium bands marking the end of the puff (region 23F). A curved heavy band in 24A occurs at the beginning of the puff in which the inversion begins. Region 24B contains a pair of medium bands bordered on either side by lighter ones. These four bands followed by a clear area at the end of the puff comprise an identifying feature in this portion of the arm. Three heavy bands, a clear space, and a pair of close heavy bands constitute a landmark in region 24C. Region 25A is often asynaptic and generally weak with three bands of light intensity. Five usually dark but variable bands in region 25B precede a pair of heavy bands in 25C. A pair of heavy bands in the neck region of 26A is preceded by a thick heavy band and is followed by a thin heavy one. Regions 26B and 26C are characteristically poor with bands that are twisted, indistinct and of variable intensity. The landmark puff at the end of 27A and the beginning of 27B has a central heavy band with a second heavy band that may be double. The remainder of region 27B along with 27C constitutes an area in which the spacing and intensities of the bands are quite variable. Frequently, these bands are thin and twisted. A single heavy band in 27C which may be double marks the end of the inversion. Other bands in this region are light. In region 28A a single heavy band between two medium bands constitutes the beginning of a landmark puff which is usually twisted and asynaptic. Two additional medium bands followed by a pair of light bands lead to a diagnostic and single heavy band which may be double in region 28B. At the end of the puff in region 28B, a pair of heavy bands appear before a much stretched area that is consistently weak and poor with light and indefinite bands. A lightly staining expanded area in 28C serves as a landmark with its pattern of a single heavy band, a space and a pair of closely spaced heavy bands. Another single heavy band follows. The pair of heavy bands in the constricted region of 28D is usually fol-

lowed by a stretched area. In region 29A another stretched section follows a pair of closely spaced heavy bands. An always complex area in 29B contains bands which are twisted and of variable intensity. In good preparations, however, the bands consist of a single heavy band followed by four medium bands as shown. Diagnostic of this portion of the arm is the extended clear area at the end of 29B, interrupted by two centrally placed light bands. Region 29C has another pair of closely spaced heavy bands, typical of this portion of the arm. Two separated heavy bands followed by a series of light bands occur in a difficult and twisted area in 30A and 30B. At the end of region 30B a heavy band indicates an always dark and variable area. A series of four characteristic banding patterns occur at the centromere end of the arm. In region 30D a pair of heavy bands is followed by another pair of bands of similar intensity but thinner in region 31B. The landmark puff in 31C contains a pair of heavy bands, followed by a clear area and then two bands of consistent intensity, the first heavy, the second lighter. Immediately before the centromere is a pair of heavy bands in 32B.

CHROMOSOME 3, LEFT ARM. The left arm of chromosome three is the shortest autosomal arm, approximately 125 μ in length. It is consistently a well staining portion of the complement containing many reliable landmark areas and showing remarkable similarity to 3L of other members of the subgenus *Anopheles*.

In the puff occupying regions 39B and 39C, two dark bands in region 39B followed by two more dark bands in 39C comprise the first landmark at the free end of the arm. A puff in region 39C and 38A is characterized by four light bands. Region 38B is a consistently poor area but in good preparations contains two closely-spaced dark bands. A single dark band at the beginning of 37A is followed by two dark bands.

The puff which is centered in 37B is similar to that in many species of the subgenus. This puff is variable, usually expanded, and contains a pair of dark bands which are often broken or indistinct.

The four dark bands in region 37D constitute another identifiable area of this arm. The heavy band in a small puff at the end of 37E is a recognizable feature as are the four dark bands in 37G. A series of 6 puffs extend from 36B to 34C. These puffs are consistently present in all members of the subgenus studied thus far, and contain mostly light bands. Region 35A contains the typical "birds-eye" configuration characteristic in most members of the subgenus *Anopheles*. The "birdseye" characteristic is undoubtedly the most recognizable feature of the central part of the arm. Region 35D is usually indistinct and always of poor quality. The single dark band of variable intensity in 34B is followed by three thin, dark, distinct bands in 34C. At the centromere end of the arm two distinct landmarks exist; the first of these, in region 33B, contains two heavy bands which are of different thicknesses. The second is composed of a triplet of dark bands in 33D followed by a single band of dark intensity occupying the center of the puff of that region. The beginning of region 33E is consistently poor and often composed of an area that is frequently twisted and contains broken bands.

DISCUSSION

Following the previously observed phenomenon in the anophelines, the X-chromosome appears to have a unique banding pattern, not readily homologizable with the X of any other species. The autosomes however show clear and evident banding pattern relationships with the autosomes of other species, especially with the closely related species *atropos* and *quadrimaculatus*.

In 2R, the free ends of the arms are identical in *walkeri* and *atropos* from 6A through 9C. Likewise the centromere ends of the arms are identical from 12A through 14C. Thus fully two thirds of the arm appears to be homologous and in

the same order. The middle third of the arm has many similar areas and the pattern is probably the result of paracentric inversions, but detailed analysis of hybrids will be necessary to identify the inversions with certainty. When compared with *quadrimaculatus* the homologies are not as clearly evident, but 6A through 9C in *walkeri* is closely similar to 6A through 8B in *quadrimaculatus*. With less certainty the similarities can be traced from 8C through 9C of *quadrimaculatus* corresponding to 10A through 11B of *walkeri*. At the centromere end of the arm only 14B in *walkeri* corresponds with 14C in *quadrimaculatus*, but 12A through 14B in *quadrimaculatus* corresponds very well with 13A–14B in *walkeri*, inverted. Again, the central third of the arm shows some similarities, but details must be studied in hybrids.

The left arm of chromosome two shows clear, unmistakable and surprisingly close homologies with 2L of *atropos*. The *walkeri* arrangement differs by 3 paracentric inversions from that of *atropos*. At the free end, 21A and 21B are identical in both species. Regions 21C and 21D in *walkeri* are 21C and 21D, inverted, of *atropos*. Except for minor differences in band intensities, regions 20B through 19B of *walkeri* are identical with 19C (except for the first three bands) through 18A of *atropos*. Region 19C through 18D of *walkeri* is an inversion of 20B through 19C (first three bands) of *atropos*. The small puff in 18B and 18C of *atropos* is apparently not present in *walkeri*, but 17A to the centromere is apparently identical in both species except for a few band intensity differences and the apparent lack, in *walkeri*, of regions 16A, 16B and 16C of the *atropos* arrangement.

The derivation of 2L from one species to the other can be most simply explained by invoking three paracentric inversions. The small inversion in 21C and 21D of both species is clearly obvious. The second and third inversions evidently involved the entire region 20B through 18A of the *atropos* map and then the subsequent reinversion of the included region 19C–18A (except for the first three bands of 19C). Both latter inversions share a common break point at the end of 18A.

There are also homologies between 2L of *walkeri* and 2L of *quadrimaculatus* but these homologies are not as clear and obvious and remain to be worked out in detail.

In 3R the free ends of *walkeri* and *atropos* are quite similar, with allowances for band intensity differences, from 22A through 26A. At the centromere end, only the bands in 32A and 32B appear to be the same as 32E in *atropos*. About half the *walkeri* arm and about two thirds of the *atropos* arm are not readily subject to comparison. 3R of *atropos* is definitely longer than 3R of *walkeri*. One section in *atropos*, 30D through 32D appears to be very similar to 26B–29B in *walkeri*. If so, at least two inversions would be necessary to account for this shift translocation. This leaves 26B–30C of the *atropos* map unaccounted for in *walkeri* and 28D–31D of *walkeri* unaccounted for in *atropos*. Again, the internal portion of the arm appears to have been rearranged by paracentric inversions. The distinctive 3R of *quadrimaculatus* does not appear to have much in common with *walkeri* except in 22A and in 32A and B *(walkeri)* which are similar to 22A and the last part of 32E in *quadrimaculatus*.

3L is a conservative, well-marked arm in most species of the subgenus *Anopheles*. *Anopheles walkeri* exhibits this same conservatism, and except that the arm is noticeably lighter in staining intensity than in most other species, the pattern is essentially the same. "Puff 37," the "birds-eye" in 35A and the successive series of wide puffs are characteristic of most North American species. When compared with *atropos*, *walkeri* shows the same pattern in the same sequence, without inversions. The chief differences are a generally lighter staining intensity in *walkeri* and the absence of several groups of heavy bands present in *atropos* absent in *walkeri*. The principal groups of bands in *atropos*,

not present in *walkeri*, are in 39C, 38B and 36A. The same basic similarity exists between *walkeri* and *quadrimaculatus*, although there appears to be more material in the *quadrimaculatus* chromosome, and it stains more heavily. The basic sequence of puffs is however the same.

SUMMARY

The salivary gland chromosomes of *Anopheles walkeri* are described and figured. In common with all other nearctic anophelines the chromosome complement consists of a short X-chromosome and two paired autosomes, one with equal, the other with unequal arms. Chromosomal banding patterns of *walkeri* are closer to those of *atropos*, less close to those of *quadrimaculatus*.

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References

French, W. L., Baker, R. H. and Kitzmiller, J. B. 1962. Preparation of mosquito chromosomes. Mosq. News 22:377–383.

Kitzmiller, J. B., Frizzi, G. and Baker, R. H. 1967. Evolution and speciation within the *maculipennis* complex of the genus *Anopheles*. In: Genetics of Insect Vectors of Disease. Wright and Pal, Editors. Elsevier Publishing Co., Amsterdam. Chapter 5, pp. 151–208.

Klassen, W., French, W. L., Laven, H. and Kitzmiller, J. B. 1965. The salivary chromosomes of *Anopheles quadrimaculatus* Say. Mosq. News 25:328–334.

Kreutzer, R. D., Narang, S. L. and Kitzmiller, J. B. 1969. The salivary gland chromosomes of *Anopheles atropos*. Mosq. News 29:223–230.

NOTICE

The "1971 Global Mosquito and Mosquito-Borne Disease Situation" by Helen Sollers-Riedel is off the press. This Supplement to the New Jersey Mosquito Extermination Proceedings contains 51 pages and is a review of the literature on mosquitoes for 1971. The booklet is divided into sections on taxonomy and distribution; techniques; genetics; behavior, biology and ecology; anatomy, morphology and ecology; arboviruses and other vertebrate viruses; filariasis; malaria; yellow fever; adulticides and larvicides; sterilization methods; biological control; resistance and susceptibility; attractants and repellents. The cost is just $2.50 U.S. It may be obtained from the N. J. Mosquito Extermin. Assoc. Fund No. 25, P.O. Box 19009, Washington, D.C. 20036, USA.