THE SALIVARY GLAND CHROMOSOMES OF *ANOPHELES ATROPOS*

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INTRODUCTION. The salivary chromosomes of several North American anophelines have been mapped. (Kitzmiller, Frizzi and Baker, 1967). All thus far belong to the subgenus *Anopheles* and show clear homologies in each of the autosomal arms, particularly at the free ends and at the centromere regions. The internal part of each arm has been variously modified by inversions, but the changes which have occurred may be postulated, at least theoretically, by a close comparison of the banding patterns.

The present paper describes the banding pattern in the salivary gland chromosomes of *Anopheles* (*Anopheles*) *atropos*. This species is distributed along the coastal regions of the United States from New Jersey to Texas, in Cuba, Jamaica and various other islands in the Caribbean. It is a brackish water species with a fairly wide range of salinity tolerance.

All specimens from which the present slides were made were collected in the vicinity of the Entomological Research Laboratory, Florida State board of Health, Vero Beach, Florida. Larvae were collected in the field, kept and fed for a short period, and slides prepared. Techniques used follow those described previously (French, Baker and Kitzmiller, 1962). Measurements of chromosomes and the location of principal bands were taken from standard photographic enlargements, and details of the banding pattern were filled in by direct observation at 1000X with a Zeiss phase contrast system. The salivary chromosomes complement is shown in Figure 1, and the proposed standard chromosome map is shown in Figure 2.

DESCRIPTION OF THE CHROMOSOMES. The telocentric X chromosome measures 52 microns in length. Chromosome two is metacentric, the right arm measures 122 microns and the left arm 118 microns. Submetacentric chromosome three has one longer arm, 3R, and one shorter arm, 3L. The right arm measures 183 microns and the short left arm 108 microns (Fig. 2). A chromocenter is only rarely found in the salivary preparations of this species. Quite frequently, however, both arms of the same chromosome are joined at the centromere. Because the centromere region does not show the usual constriction it is difficult to determine the centromere region of the chromosome on some preparations. The numbering procedure followed for the *atropos* complement is the same as for the other members of the subgenus, and is as follows: X-chromosome, zones 1 to 5, beginning at the free end of the chromosome and with region 5 ending at the centromere; 2R, zones 6 to 14, the latter at the centromere; 2L, zones 15 to 21 with zone 15 beginning at the centromere and zone 21 at the free end of the chromosome; 3R, zones 22 to 32 beginning at the free end and ending at the centromere; 3L, zones 33 to 39 beginning at the centromere and ending at the free end. The free end of the autosomes, as well as the regions near the centromeres, show definite similarities with other members of the subgenus *Anopheles*.

The telocentric X chromosome may be readily recognized by size alone, being about one-fourth the length of either autosome. The free-end typically is puffed with a heavy band (1B) following a series of light bands. This puff is followed by a smaller one containing one (1C) and ending with two (2C) heavily staining bands. The large puff in region 3 has four consistently broken indistinct bands and a series of three clearer bands in 3B and 3C. A series of four bands in 4B is distinctive. Regions 4C to 5B contain a

series of lightly staining bands. The centromere end contains a series of four dark bands in 5B followed by a prominent single band near the centromere.

The right arm of chromosome 2 contains several distinctive, consistent regions which make its identification certain. Perhaps the three most prominent features are the puff in 7C and 8A, bounded by series of heavy bands; three dark areas in 10A and 10B; and the single heavy band in 14B. Identification of the free end, the center of the arm and the centromere end can begin from these points.

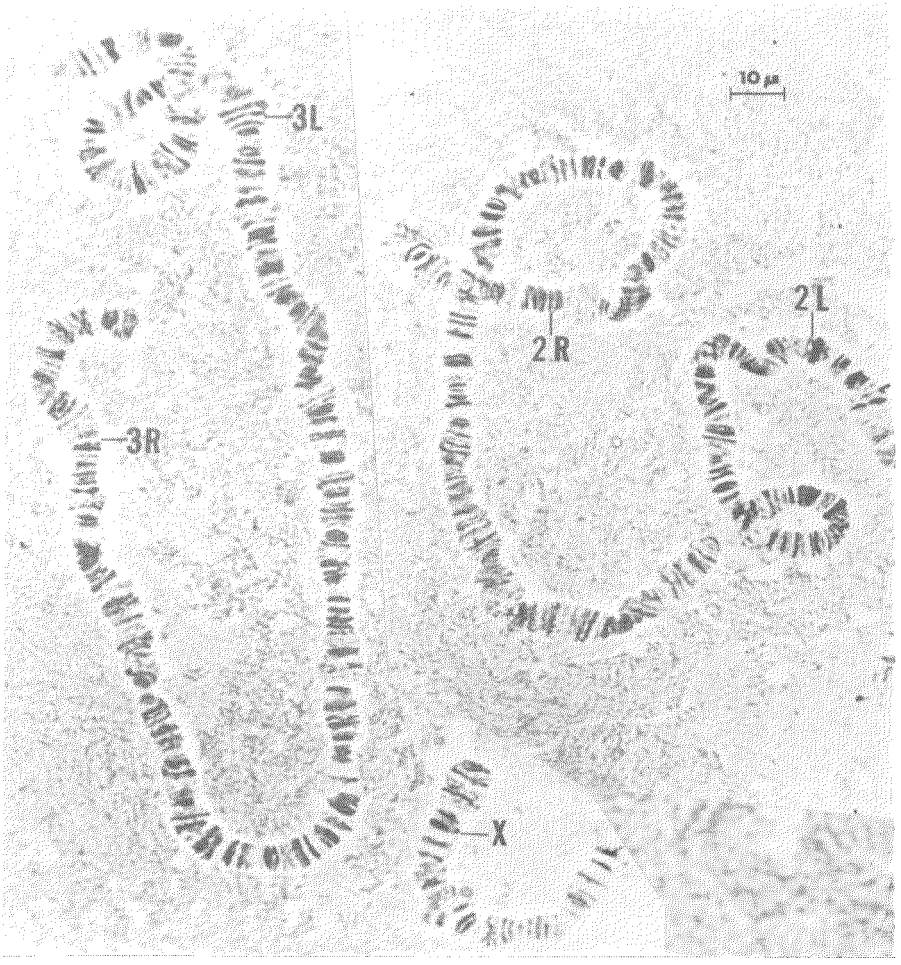

FIG. 1. *Anopheles atropos*. Salivary chromosome complement.

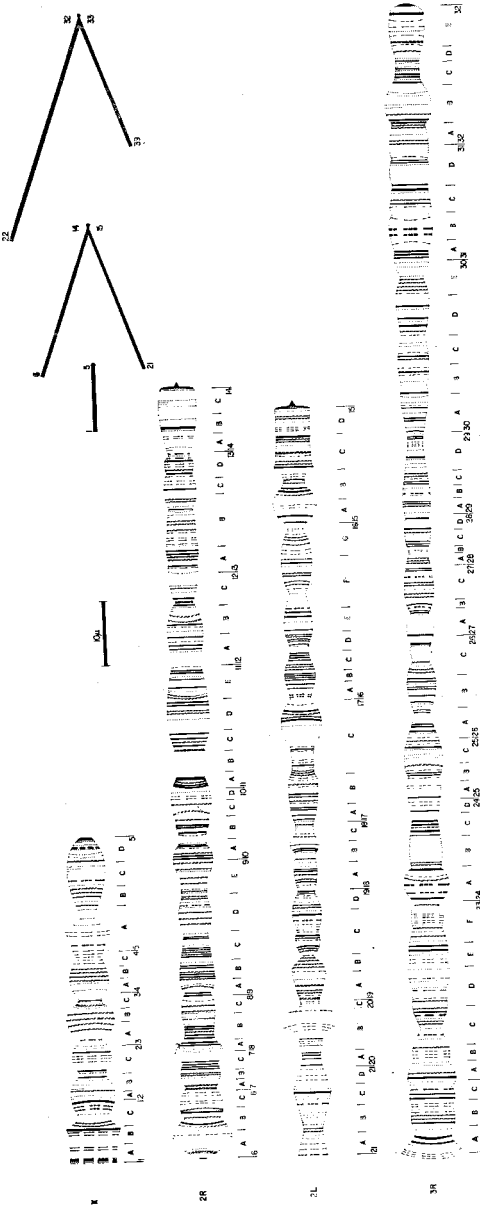

FIG. 2. Salivary chromosome map of *Anopheles atropos*.

In common with most other members of the subgenus *Anopheles,* the free end of the arm is expanded into a bulb-like puff, with dark bands as shown in 6B. The lighter bands in region six appear with variable intensities; in some preparations they are darker than indicated on the map. The two dark bands at the beginning of 7A usually are in a constriction. Easily the most prominent feature of the free end of the arm is the sequence, contained in 7C, 8A and 8B; a series of four thin dark bands in 7C, a clear puff in 7C and 8A and seven dark bands in 8A and 8B. The dark areas are often condensed and may appear to consist of fewer, but thicker bands than shown. The puff is also variable and may appear larger than indicated.

The heavy bands in 9A and 9B are also consistent features of this part of the arm. The arm often appears broken in 9A, immediately after the two dark bands. Regions 9C and 9D are usually twisted, weak and indistinct. The three dark areas in 10A and 10B are excellent landmarks; they consistently may be recognized as shown on the map. The puff in 10C and 10D is variable in width and staining intensity, but the two pairs of dark bands, separated by a lighter one, mark the end of the puff, in 11A. The clear puff in 11B is often stretched; it appears to contain no bands. The heavy bands in 11C, in a 2–1–2 pattern, are followed by a clear area, often stretched in 11D, at the end of which occurs a pair of wide, dark bands. Also diagnostic are the four dark bands in 11E.

The clear area in 12A is usually stretched, and followed by four dark bands in a 2–1–1 pattern. The three dark bands in a constriction, the one lighter, is an excellent recognition area in 12C. The clear area in 12C and 13A is usually stretched, but the four dark bands in 13A are consistently good. Region 13B is almost always stretched, weak and indistinct, with the last four bands most consistent. The small puff in 13D is quite variable, often appearing clear and widely puffed, with the bands indistinct. Region 14 is typically as shown, mostly clear, with one prominent heavy band in 14B. The other bands are variable, sometimes darker, especially those in 14C.

In common with other species of the subgenus *Anopheles,* the left arm of chromosome two in *atropos* contains easily recognized areas. Our slides show considerable variation in puffing patterns, although all larvae were of approximately the same age, late fourth instar. The free end of the arm is typical of the subgenus. The pair of dark bands in a constriction at the beginning of 21B sometimes appear as one band. The single dark band, usually in a wider area of the arm in 21C and flanked on either side by a lighter region, is an excellent recognition point. In 20A a constriction contains four dark bands in a 1–3 sequence, followed by four bands, the middle two of which are more lightly staining than the outer two. Region 20B is often expanded into a wide Balbiani-type puff; when it is not puffed it contains the banding pattern shown in Figure 3.

The relatively clear area at the end of 19A is also often expanded into a clear puff. In 19B a series of four dark bands followed by the typical pattern in 19C forms a good landmark. The single dark band at the end of 19C is especially consistent. In 18A the dark bands are usually as shown, followed by an excellent pattern of three dark bands, the last displaced, in 18B. Region 18C is almost always constricted with a series of light bands, flanked by a darker one at each end of the series.

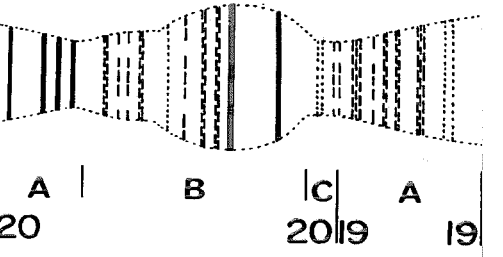

FIG. 3. Banding pattern, region 20, not puffed.

The four dark bands in 17A form a consistent recognition area; they are followed by six bands, in pairs, the first of each pair lighter than the second. The four bands in 17A are sometimes in a puff, but are always dark. Region 17B is often puffed; when it is, the bands are lighter and indistinct. The three bands at the beginning of 18C usually are condensed, so that they appear as one band. All of 18C is variable, sometimes puffed, always indistinct, often folded, twisted or broken. Only rarely do the two dark thin bands appear as shown.

The entire puff as shown in 16A, 16B, and 16C is typical and consistent; it almost always appears exactly as shown on the map. Three heavy bands in 16E are followed by an area which is always stretched, weakly staining, indistinct, comprising regions 16F and 16G. Region 15 is characteristic for the centromere end of the arm. The puff in 15A and B contains two heavy bands at its beginning, then three dark bands immediately before a strong constriction. After the constriction are two dark bands, the second thicker. Several dark bands are present in 16C, although these bands, and those in 16D are often more weakly stained.

The right arm of chromosome 3 is the longest arm in the complement, as it is in all other members of the subgenus *Anopheles* studied thus far. It shows many areas which are consistently found in other species of the subgenus, and like those other species, appears to have been extensively rearranged internally by paracentric inversions. The free end, starting at region 22 has the typical flared tip with two heavy bands, followed by three characteristic puffs in 22B, 22C and 23A. At the end of 23C are two dark bands, the second somewhat thinner and more lightly-staining. These bands, plus another pair of dark bands in 23E and the three initial puffs serve to recognize the free end of the arm. The several bands in 23C are often much condensed, and only in good preparations can the detail shown on the map be made out. Region 23D and the first part of 23E containing a series of light bands is quite variable; it sometimes appears as an expanded puff, is often twisted, weak and indistinct. It is in this area that one end of a postulated inversion occurs.

The puff in 24A is also variable, sometimes quite prominent, sometimes not. In all cases however, the two dark bands at either end of the puff are distinguishable. A clear area in 24B is followed by a prominent series of heavy bands in 24C. This series is one of the best places to begin identification of this area of the arm. The dark bands in 25B usually stand out, and the entire group often appears condensed into one or two dark bands. The following curved band, somewhat set apart, helps identify this group. The heavy series of bands in 25C and 26A are usually as shown, but sometimes appear as a large, expanded puff, in which case the sequences and intensities of the bands are difficult to follow. This area also includes the break point of an assumed inversion. The puff in region 26B and 26C is consistently difficult—it is usually twisted, folded, indistinct, the bands are weak, broken and variable. In this region is also included the break point of a long paracentric inversion.

Region 27 is usually as shown. In regions 28 and 29 are three areas which may be used to identify the center of the arm. Three dark bands in 28C, the third set somewhat apart, a similar series in 29A and a heavy series in 29C are excellent recognition areas. Regions 30B and 30C are usually variable but in 30D a series of widely spaced dark bands stand out, as do the three dark bands in 31A. These three are often condensed so that they appear as a single heavy band, an excellent landmark in this area of the arm. Regions 31B and 31C are consistently weak, indistinct and twisted. Only in good slides do the bands appear as shown. The puff in 32B is consistent as are the two dark bands preceding it. 32C contains a series of dark bands typical of the centromere end of the arm in all species of the sub-

genus and regions 32D and 32E, at the centromere, are typical.

The left arm of chromosome three follows the generally conservative pattern for this arm exhibited by other members of the subgenus *Anopheles*. At the free end, 39A, 39B and 39C contain a typical series of four dark bands, followed by a light puff in 39D which has one dark band. The latter half of 39C, all of 39D and all of 38A are indistinct, with weakly-staining bands. In 38B and 37A two series of dark bands bound a light area, often puffed and asynaptic. The three distal bands are typically thinner than the proximal ones. The large diffuse puff in 37B is characteristic for this section of the arm; it contains bands which vary in staining intensity according to the degree of a synapsis and puffing, but the bands in 37C are usually darker than those in the distal portion of the puff.

In 37D, three dark bands are followed by a single one, spaced somewhat apart. The central part of 37E is usually indistinct and weakly stained. The dark band at the end of 37E and those in 37G and 36A form a diagnostic area for the center of the arm. The large puff in 36B and 36C is typically clear, with only a few weak bands, and is often twisted, collapsed or asynaptic. The "birdseye" in 35A is an excellent landmark, but the rest of region 35 is indistinct, variable and stretched, especially the small puff at the beginning of 35D. The two heavy bands at the end of 35D are followed by a pair of wide, darker bands. Three heavy bands in 34A and 35B followed by four heavy ones in 34C, constitute an excellent recognition area. The heavy doublet in 33B and the 2–1 pattern of dark bands in 33D mark the centromere end of the arm.

DISCUSSION. The salivary chromosomes of *Anopheles atropos* show many similarities to the salivary chromosomes of the other members of the subgenus *Anopheles*. The ends of the autosomes are very similar to *freeborni* and to *quadrimaculatus*.

The X chromosome is very distinctive and quite unlike that of any other anopheline thus far studied.

The right arm of chromosome two appears to show most similarities with *freeborni*, and only few good homologies with *quadrimaculatus*. At the free end of the arm, regions 6, 7 and 8 are quite similar in both *freeborni* and *atropos*. Region 6 is especially good, region 7 somewhat less comparable, but region 8 is quite similar. Region 9 contains some similarities but also many differences, and cannot be securely homologized. In *atropos*, 10A through 11A appears to be an inversion of 10F through 11D in *freeborni;* the three dark areas in 10A and 10B of *atropos* are almost certainly those found in 11D and 11C in *freeborni*. Next, 11C through 12B in *atropos* appears to correspond closely, but not exactly, with 10A through 10E in *freeborni*, in a normal, noninverted order. This sequence could have been derived by a series of two paracentric inversions, involving 10A through 11D in *freeborni*, then a reinversion of 11C–12B. Regions 12C, 13A, 13B, 13C and 13D do not homologize well with the corresponding areas in *freeborni*. Region 14 is very similar in both species.

The left arm of chromosome 2 resembles that of *freeborni* more closely than that of *quadrimaculatus*. With minor differences in banding and intensities, the free ends are quite similar from 21A through 20A. The *freeborni* map was made from condensed chromosomes, the *atropos* map from excellent, well-stretched preparations, therefore the detail is richer in the *atropos* map.

The puff in 20B is difficult to homologize, but the non-puffed banding pattern is similar to that of *freeborni*, although not exactly so. Region 19 in both species contains a large number of dark bands, but the pattern is not the same, although similar in some respects. However, regions 18 and 17A are almost exactly the same in both species. The first three doublets in 17B of *freeborni* are very similar to those in 17B of *atropos*.

The next good similarity is in region

16A through 16E, which is identical in both species. Region 15 is also probably identical, keeping in mind the variation in staining intensity which is characteristic of the centromere region. Such a comparison leaves 17C and 16F and 16G of the *atropos* arm unaccounted for, likewise part of 17B, 17C and 17D of the *freeborni* arm. The puff in 17D of *freeborni* is possibly that in 17C of *atropos;* it is in the proper position. If this be true, then 16F and 16G in *atropos* contain a pattern not too different from that in the latter half of 17B and 17C in *freeborni*. Such a rearrangement could have been effected by an inversion of a segment starting at the middle of 17B and continuing through 16D (*freeborni*). A reinversion of 17D–16E would then produce the pattern found in *atropos*. The presumed end point of these inversions is found in the weakly-staining, "difficult" areas of the arm.

The right arm of chromosome three clearly shows structural affinities with the other members of the subgenus *Anopheles*. Similarities are especially striking when this arm is compared with the same arm of *quadrimaculatus* (Klassen et al., 1965). Basically, the banding pattern of this arm is "standard" at the free end and close to the centromere, that is, it closely resembles most of the other species of the subgenus in these areas. Internally, the arm appears to have been rearranged by a series of paracentric inversions. Similarities are closest with *quadrimaculatus;* resemblances and differences will be detailed with respect to this species.

The banding patterns are essentially similar in both species from the free end through 23B. Also a long area, 23E through 26B in *atropos,* is essentially similar and in the same order (noninverted) as 23C through 26B in *quadrimaculatus*. However, an intercalated region 23C and 23D in *atropos* is similar to regions 25C and 25D in *quadrimaculatus*. Such an arrangement in *atropos* may theoretically be accounted for by two inversions in the *quadrimaculatus* arrangement. First, the entire area 23C through the first part of 25D (*quadrimaculatus*) is inverted. Next, the segment 23C through 25B is reinverted, so that this segment is in the same (noninverted) order as in *quadrimaculatus*. This would account for the insertion (shift translocation) of 23C and 23D (*atropos*), corresponding to 25C and 25D in *quadrimaculatus*.

A common break point at the beginning of 23C is shared by both inversions. Similar phenomena have been postulated often in the derivations of the banding patterns of other species (Kitzmiller, Frizzi and Baker 1967). Regions 23C and 23D (*atropos*) appear to be in the noninverted order as well, corresponding to 25C and part of 25D (*quadrimaculatus*), although this is not certain. If so, a third inversion has occurred, to place 25C and 25D again in the noninverted arrangement. Regions 25C, 26A and 26B (*atropos*) thus follow sequentially, and represent an area in *quadrimaculatus* (the last part of 25D, 26A and 26B) which has not been involved in an inversion.

The half of the arm closest to the centromere has undergone more extensive rearrangement. Only regions 32C, 32D and 32E, at the centromere, are similar. The *atropos* arrangement may theoretically be derived from the one in *quadrimaculatus* by four inversions, three of them included inversions. First, the entire area 26C through 32B is inverted. Then, three included inversions, 27B through 28A, 28B and 28C, and 28D through the first part of 31A, would produce the *atropos* arrangement. During these rearrangements regions 31A (part) through 32A of the *quadrimaculatus* chromosome has evidently been lost; it is not found in *atropos*. The three included inversions could of course have occurred in any sequence.

The right arm of chromosome three, the longest arm in all species studied, appears quite susceptible to inversion rearrangements. Several inversions are known to exist in various species in this arm, and hypothetical rearrangements,

postulated to account for comparisons among species, add many more. In the *atropos-quadrimaculatus* comparison, at least 6 and perhaps 7 inversions have modified the banding pattern in this arm.

The left arm of chromosome three also is very similar to 3L in *quadrimaculatus*. The central portion of the arm is almost exactly similar in the two species, and it is at the free end and at the centromere end that small inversions produce differences from the "normal" pattern. Regions 39A and 39B are exactly the same. 39C in *atropos* consists of an inversion of the first three bands in 39E of *quadrimaculatus*, and 39D is the same in both species. Regions 38A, 38B and part of 38C in *quadrimaculatus* are entirely lacking in *atropos*. The latter half of 38C and region 38D in *quadrimaculatus* are the same as 38A and 38B in *atropos*.

With very minor differences the banding patterns in the two species are identical from 37A through 36A. Especially striking are the similarities in the heavy bands in 37G and 36A. Regions 36B and 36C in *atropos* represent an inversion of these two regions in *quadrimaculatus*. Regions 36D and 36E in *quadrimaculatus* are lacking in *atropos*. The "birds-eye" at the beginning of region 35 is typical of this region of 3L in all members of the subgenus thus far studied. With only minor differences, region 35 is the same in both *atropos* and *quadrimaculatus*, as are regions 34A and 34B. Region 34C in *atropos* is an inversion of 34C in *quadrimaculatus*.

Regions 33A and 33B are essentially the same in both species, and 33C and 33D in *atropos* represent the same regions, inverted, in *quadrimaculatus*. Region 33E is somewhat more condensed in *atropos* than in *quadrimaculatus*, and 33F in *quadrimaculatus* is not present in *atropos*. In *quadrimaculatus*, 33C through 33E is extra material, not found in, for example, *freeborni*, but present in *atropos, occidentalis* and *aztecus*. Some of the material, but not all, is present in *atropos*. A comparison between *atropos* and *freeborni* is also good, especially in the middle of the arm.

SUMMARY. The salivary chromosome complement of *Anopheles atropos* consists of five paired elements, a telocentric X chromosome, a metacentric chromosome two with arms of approximately equal lengths and a submetacentric third chromosome in which the right arm is considerably longer than the left. The banding patterns of these chromosomes are described and a map presented for the species. The banding pattern of the X chromosome is unique, but the autosomes show banding patterns quite similar to those of *Anopheles freeborni* and *Anopheles quadrimaculatus* and similar in general to other members of the subgenus *Anopheles*.

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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., and BAKER, R. H. 1963. The salivary chromosomes of *Anopheles freeborni*. Mosq. News 23:254–261.

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.