

THE SALIVARY GLAND CHROMOSOMES OF *ANOPHELES ALBIMANUS*

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One of the most common and most widely distributed of the neotropical anophelines is *Anopheles albimanus*, the chief vector of human malaria in the Caribbean and in many areas of Central and South America.

This species is a member of the subgenus *Nyssorhynchus* which contains 24 species (Stone *et al.*, 1959) distributed throughout the Caribbean region, Central America and South America.

There has been very little genetic and cytogenetic work done on the members of the subgenus *Nyssorhynchus* (Kitzmiller, 1963). Frizzi and Riciardi (1955) prepared a preliminary map of the salivary gland chromosomes of *Anopheles aquasalis* and made general observations on the polytene chromosomes of *A. albimanus*. Most of the research concerning this latter species has been concerned with the genetics of insecticide resistance, especially to DDT and dieldrin (Davidson, 1963). Rozeboom (1963), however, has described three morphological mutants in the adults, and Hobbs (1962) has published a general account of the cytogenetics of *A. albimanus*, including a salivary chromosome map.

This paper presents a detailed description of the salivary gland chromosomes of *A. albimanus*. The map presented here basically agrees with that of Hobbs; the techniques herein employed have permitted the delineation of further details of the banding pattern.

MATERIALS AND METHODS

Five different geographic populations of *Anopheles albimanus* were utilized in this study. Four of these were field collected in Central America during the summer of

1964 by Drs. J. B. Kitzmiller and R. H. Baker in Costa Rica, Guatemala, Nicaragua, and Mexico. The El Salvador strain was derived as a subcolony of a dieldrin-resistant colony maintained at Johns Hopkins University and sent to our laboratory through the courtesy of Dr. L. E. Rozeboom.

The cytological techniques used are those basically reported by French, Baker, and Kitzmiller (1962). All dissections, however, were performed in Buck's dipteran saline solution (1942). Forty-five percent glacial acetic acid and Carnoy's have been the standard fixatives for all preparations. For routine staining of the chromosomes, 2 percent lacto-aceto orcein (Gurr's synthetic orcein) has been the standard stain. The Feulgen reagent has been employed for DNA staining utilizing both azure A and basic fuchsin Feulgen. The technique utilized is that given by the Biological Stain Commission with a modification of the acid hydrolysis with 6N HCl at 72° F for 5 minutes.

Rough maps of the salivary gland chromosomes were obtained by taking photographs of satisfactory chromosome figures, printing at a standard magnification, measuring arm lengths and widths, and locating diagnostic chromosome bands and puffs. Detailed banding patterns were drawn from a careful study of 300 slides observed under oil immersion at 1000X.

THE SALIVARY GLANDS. In *Anopheles albimanus* the salivary glands in fourth instar larvae are paired bilobed organs which lie antero-laterally in the prothorax on either side of the esophagus. Each gland consists of two parts of different shapes separated by a constriction. Jensen and Jones (1957) have described the histology of the glands in *Anopheles albimanus*. The most satisfactory chromosome preparations (Figure 1) come only from the an-

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FIG. 1.—Salivary gland chromosome complement of *Anopheles albimanus*. X, 2R, 2L, 3R, 3L are the X chromosome, the right and left arms of chromosome two and the right and left arms of chromosome three, respectively.

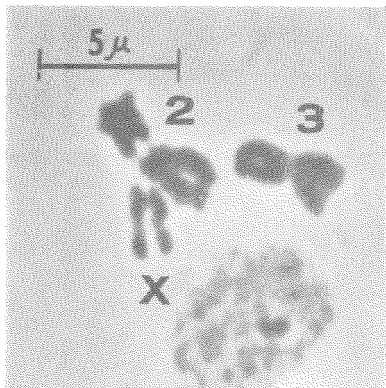


FIG. 3.—Mitotic chromosomes, female. X, second and third chromosomes.

terior spherical lobe of the gland which contains approximately 16 cells. The posterior terminal sac of the gland, with usually 55 cells, yields chromosome preparations which are highly attenuated and generally unsatisfactory for mapping purposes.

SOMATIC CHROMOSOMES. Chromosomes from larval brains, ovaries and testes were examined in the metaphase condition of mitosis and meiosis. In all instances colchicine pretreatment was employed after the methodology of French, Baker, and Kitzmiller, (1962). The karyotype consists of two pairs of metacentric and a pair of submetacentric chromosomes. The autosomes have a v-shaped configuration while those of the sex chromosomes are characteristically rod-shaped. The autosomes are approximately 5 microns while the sex chromosomes are 2 to 2.5 microns in total length (Figures 3 and 4).

X-CHROMOSOME. The telocentric x-chromosome in the salivary glands of this species is about 40 microns in length. For convenience in description, the chromosome has been divided into arbitrary regions 1 through 5 (Hobbs, 1962) starting from the distal end to the centromere using dark distinct bands as the main landmarks (Figure 2).

Region 1 represents the flared end por-

tion of this chromosome and starts with a pair of very lightly stained bands which are not found intact in most of the preparations. A pair of medium staining bands constitute a good landmark in this region. A pair of lighter bands follows and then a dark distinct narrow band can be seen at the end of 1A. A doublet of light bands is located in approximately the middle of 1B. A pair of closely situated very dark bands, an excellent landmark, is always present at the end of region 1.

A quartet of light bands is usually present in region 2A which ends at a pair of bands of variable stainability. Parts of 2A and 2B are situated in a constricted area but in some preparations the constriction is lacking. 2B has a dark band followed by a pair of light bands and then a band of medium intensity.

Regions 2B through 3B constitute almost always a wide puff-like area wherein the stainability of the bands is often variable and usually this is the most difficult area of the x-chromosome to map. 2C has a lighter band followed by a pair of very dark bands which stand out as the landmark in this often difficult area. A pair of light bands is present in 3A. In 3B a thin medium staining band precedes

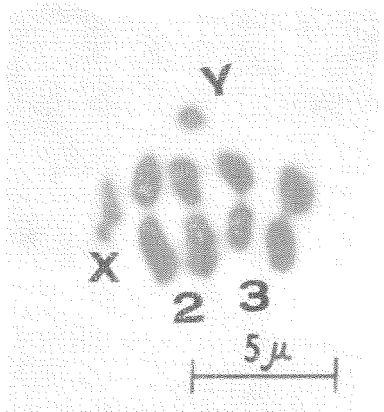


FIG. 4.—Mitotic chromosomes, male. X, Y, second and third chromosomes.

three bands of which the middle one is darkest. Region 3C begins with a dark band usually within a minor constriction. This is followed by a pair of lighter bands, and finally a very distinct dark band, usually a very good landmark and often larger than shown on the map, stands out at the end of 3C.

Region 4A is definitely a constricted area with a pair of light bands. 4B has a pair of bands of medium intensity. There is a pair of light bands followed by a darker and a lighter band before a pair of very distinct and dark bands in region 4C. Each of this pair of dark bands, which is a dependable landmark in this area, may represent a pair of very closely placed dark bands.

5A has a light band followed by a pair of medium staining bands. 5B has a cluster of four darkly staining bands often inseparable and attached to the chromocenter with a dark threadlike connection.

CHROMOSOME TWO, RIGHT ARM. This arm, the longest of the complement, is easily recognized by its length and by several excellent recognition areas. At the free end the tip is usually flared, followed by two dark bands in a narrow constriction. At the centromere end two dark areas in 15A and 15B are at either end of a lightly staining area and clearly mark this section of the arm. At the free end, regions 6 through 9 are often twisted, asynaptic, lightly staining and indistinct, so that the banding pattern as shown on the map can usually be followed only for a short section at a time. It is usually necessary to look at several slides to check this area completely. After the two dark bands in a constriction in 6B are three dark bands at the beginning of 7A, usually broken and involved in a twisted puff. These thin bands are followed by a twisted constriction and then by several dark bands in a puff in 7B, which generally are not as definite as shown on the map. The two dark bands at the end of 8B usually are clear, but the two dark bands at the end of 9A are often weaker than shown and may be much wider. The puff in 9A, be-

tween these two sets of dark bands is never clear and the bands are usually broken. Regions 9B and 10A often appear asynaptic. The closely set bands in the latter part of 9B and the first part of 10A usually appear as a compact dark group, the bands often not capable of individual resolution.

Following a narrow constriction in 10A a group of three dark bands, the central one thickest and darkest, stands out as a consistent landmark for this region of the arm. Regions 10B and 10C often appear as a wide area of the arm, and although several dark bands are shown on the map, this region is often twisted, asynaptic and indistinct, so that the individual bands are not prominent. The four dark bands at the end of 10B often appear as 3, with the middle one heaviest. The dark bands at the end of a 10C are usually heavily stained and constitute the best recognition area for this section. The wide, lightly staining puff in 11A is sometimes twisted and asynaptic, but ends in two heavy bands which are usually distinct. The dark puff in 11B usually appears as a single mass of dark bands and only with difficulty may they be resolved as drawn. Two groups of bands in 11C, at the beginning and at the end of the region, stand out as landmarks and are almost always present as shown on the map. Likewise the five bands in 12A are almost always in the spacing and intensity shown. Following this group is a clear space, then two broad dark bands in 12B. The dark band at the end of 12B is always thin. Regions 12C and 13A are usually weak, indistinct and the bands never sharp. The curved band at the end of 12C, preceded by a lighter band, is usually prominent in a small clear puff and is the best landmark for this section of the arm. In 13A the dark bands are thin, the last one set apart. In 13B the series of 5 dark thin bands usually shows as an indistinct dark area, and the two broad heavy double bands are often broken, not sharp. The heavy bands in 13C are usually wide, but often more broken and less sharply de-

finer than shown. The puff in 14A is almost always twisted and stains with variable intensity; only rarely can the bands be clearly made out. From 14B to the centromere is the most consistently recognizable part of the arm and is probably subject to less change than the middle portion of the arm since it is very similar to the same region in *Anopheles aquasalis*. The sequence of bands in 14B and 14C is a consistent landmark. Following these bands are several dark bands in a wide, somewhat variable, often twisted area in 14D and 14E. The three dark bands at the beginning of 15A, the wide light puff in 15A, and the five dark bands in 15B are always recognizable. The two thickest bands in 15B often appear as a single very dark mass. Region 15C is always light, often asynaptic.

CHROMOSOME TWO, LEFT ARM. Region 25A, at the free end is quite variable, often appears dark and compressed, but the two bands in the center of the region are almost always recognizable. The broken heavy band in a puff at the beginning of 25B may be thinner, or lighter. A series of dark bands occurs in an expanded area in 24A. The dark bands at the end of 24B and at the end of 24C usually stain heavily; between them are two evenly spaced bands of slightly lighter intensity. The twisted dark bands in the center of 23A never appear sharp and distinct and sometimes are seen as a single dark area. In 23B the two wide dark bands stain heavily, their double nature is often obscured, and the puff in which they occur is sometimes expanded and very wide. They are always recognizable, however, as two dark, heavy bands. The thin dark bands in 22A often occur in a wide area; this region as well as regions 22B and 21A are sometimes asynaptic.

The best landmark of the middle of the arm is the series of three thin dark bands in 21B. These bands are usually so close that they appear as a single heavy band, darkly stained. On lightly-stained slides they may be seen as separate bands. Another good recognition area is found

in 20A, consisting of a heavy band, usually broken, and two bands of lighter intensity. The series of heavy bands in 20B marks the beginning of a puff which ends with a single band in 19B. The rest of the wide puff in 19B is often asynaptic and the bands broken. The dark bands in 18A, 18B, 18C, 17A and 17B constitute an excellent recognition area and usually stain clearly. The 3-3 series of dark bands in 16A and 16B can usually be recognized, although they often are twisted and sometimes quite wide.

CHROMOSOME THREE, RIGHT ARM. The free end of this arm, 26A-28B and the centromere end, 34A-35B, are superficially quite similar to comparable regions in *Anopheles aquasalis*. The arm contains several prominent and consistent series of bands which may be used as reliable recognition areas. At the free end, region 26A is usually compressed and dark, but the three thin bands in a 1-2 pattern are usually visible. Following, regions 26B and 26C are usually twisted so that the bands appear weak and broken. The two dark, closely set bands in the middle of 26B often are in a narrow twisted constriction. Region 26C is usually an asynaptic puff. The dark bands in 27A are almost always as figured; the first two are close and may appear to be a single heavy dark band. The constriction in this area is typical. The series of dark bands in 27B through 29B may be easily followed, but this area of the arm is variable in width; 28B often is asynaptic. The three heavy bands in 30A followed by the two thinner but also heavy bands in 30B constitute the most reliable recognition area of the arm. A light area, in 32A, almost always stretched, is bounded by two dark bands in 31B and in 32A and is also a good recognition area, although the group of dark bands at the end of 32A often appears as a single dark area. Likewise 32B and 32C contain a series of dark bands, but this wide area of the arm is usually indistinct and/or asynaptic. A thick, dark band in 33B, followed by a 1-2 pattern of bands of slightly lesser intensity constitute a consistent landmark.

These bands are almost always in a narrower part of the arm. The two thin dark bands at the end of 33C may appear as a single heavy one. In 34A, the four dark bands may appear much closer, and sometimes are seen as two dark bands, or even one. The expanded puff in 34B is always present, although the dark bands are often broken. The widest bands, in the middle of the expanded area, are broken in most preparations. Region 35 is typically asynaptic, as shown, with a series of mostly lightly staining bands.

CHROMOSOME THREE, LEFT ARM. The free end of 3L is variable, and often appears to consist of a large number of bands of rather equal intensities, but in good preparations some reasonably good landmarks are evident. The proximal half of the arm is more distinctive and contains several areas that may be easily recognized.

At the free end region 45A is typically spatulate, containing three evenly spaced bands of medium intensity followed by three bands which are usually darker. A light band followed by two dark ones in a constriction are at the end of 45A. In heavily stained slides, or in condensed ones, the three heavy bands in the middle of the region and the two heavy ones at the end may appear as single dark irregular areas, preceded and followed by lighter regions. Regions 45B and 44B are uniformly present as shown on the map, but most of the area is usually puffed and expanded, so that the bands are broken, often twisted and may be more lightly stained. There is probably a small (2-3 band) inversion in the distal portion of 44B. A wide puff in 45B contains three dark bands, usually broken. Following a lightly stained section are two dark regions with a pair of broken, dotted bands in each at the end of 44A. The wide puff in the distal portion of 44B contains light bands and is usually asynaptic. The most consistent landmark of this portion of the arm is a series of four thin dark bands, evenly spaced in 44B, followed by a dark double at the end of 44B; the double appears as a single thick dark band in some

preparations. The expanded puff in 43A has an asynaptic region which is probably due to a 3 or 4 band inversion. The bands in the area are variable and often are darker than shown. At the end of the puff are two dark bands, followed by another which is also dark, but variable in shape. The heavy dark band at the beginning of 43B is the most prominent band in the area and is followed by two bands of medium intensity, then a pair of thin, wide but dark bands. These five bands in 43B are usually clearly recognizable.

The light broken bands in 42A are followed at the end of the region by two bands of medium intensity, which, along with the three dark bands in 42B are usually involved in a twisted area. The heavy band in 42B is usually the widest of the group, best defined and most intensely stained. All other bands in region 42 are usually as shown on the map, although this area is not always clear.

A heavy thick band, followed by two thinner ones stands out in 41A. A small puff in 41B contains a 2-1-1 pattern, the last band darkest. The three dark bands in 40A, usually in a constriction, often mark the best area to begin the examination of this arm. The two dark bands in 40B are usually in a wide area, as are the three dark bands in 39A. The following region, 39B, is usually twisted or asynaptic and probably contains a small inversion. The four dark bands in 38A sometimes are closer than shown on the map, and then appear as an irregular dark area. Region 38B is dark, often appears as shown, but also may appear to be an irregular series of dark bands. The first band in 38B is always thickest and darkest, and a lighter space usually separates the two pairs of dark bands. The two series of bands in 38C are usually as shown, except for variable spacing; they often are seen as two dark areas. The four heavy wide bands in 37B, preceded by a single dark band in 37A, constitute an excellent landmark which may be used to recognize the centromere end of the arm.

Region 36 is usually stretched, the bands lightly staining, but two narrow bands in 36A are usually darker than the others.

DISCUSSION

The salivary chromosomes of *Anopheles albimanus* present a complement typical of the genus *Anopheles*, a short X-chromosome and two longer pairs of autosomes (Figure 1). Labelling of 2R, 2L, 3R and 3L has been completely arbitrary except that 2R definitely is attached to 2L and 3R to 3L. We have followed Hobbs' system of designating the arms, and the numbered zones in each arm (Figure 2).

The salivary chromosomes are remarkably uniform in samples of all five strains studied. In our laboratory populations, at least, apparently no banding pattern differences exist. This is unusual. Furthermore all five strains show complete reciprocal fertility (Keppler and Kitzmiller, 1969). Among more than 1000 slides examined, none was heterozygous for an inversion. This uniformity among populations is unusual, and not consistent with findings in other species of *Anopheles* (Kitzmiller *et al.*, 1967). It is possibly due to strong laboratory selection of these strains, but many of the slides were made in the field in Mexico and in Central America. Certainly an intensive sampling of natural populations is necessary in order to estimate the amount of chromosomal variability in this important species. Almost all anopheline salivary gland chromosome preparations to date have been made from the subgenera *Anopheles* and *Cellia*. The banding patterns of these two subgenera are distinct from one another, but clear and definite homologies may be seen within subgenera, especially within closely related species groups. The detailed maps of *albimanus* indicate a banding pattern quite distinct from either *Anopheles* or *Cellia*. Other studies, (*aquasalis*, Kitzmiller and Chow in press; *oswaldoi*, Kitzmiller, Kreutzer and Narang in preparation; *nuneztovari*, Kitzmiller, Kreutzer and Tallaferro, in press) indicate that

close banding pattern homologies exist within the subgenus *Nyssorhynchus* as well.

The salivary X chromosome in *albimanus* has a single arm as do *darlingi*, *oswaldoi* and *triannulatus* (unpublished preliminary observations). In *aquasalis* and *nuneztovari* the X is clearly metacentric with two arms which differ only slightly in length. Similar to the situation found in the other subgenera, the banding pattern of the X is distinctive in each species and may readily be used as a taxonomic characteristic.

SUMMARY

The salivary gland chromosomes of *Anopheles albimanus* consist of a short telocentric X chromosome and two longer pairs of metacentric autosomes. The banding patterns of X and autosomes are distinct from those in species of the subgenera *Cellia* and *Anopheles*, but show clear autosomal homologies with other species within the subgenus *Nyssorhynchus*. Supported in part by Grant AT-03486, USPHS.

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