CYTOTAXONOMY AND SALIVARY GLAND CHROMOSOMES OF *ANOPHELES (STETHOMYIA) KOMPI*

J. B. KITZMILLER,[1] R. D. KREUTZER [2] AND M. G. RABBANI [3]

ABSTRACT. The salivary gland chromosomes of *Anopheles* (*Stethomyia*) *kompi*, the first described from this subgenus, differ clearly from chromosomes of the subgenera *Anopheles, Cellia* and *Nyssorhynchus*. A population from Manaus, Brazil contains three paracentric inversions, apparently in Hardy-Weinberg equilibrium. A closely related species, *An. nimbus* occurs in the same larval habitat. The 2 species cannot be reliably distinguished morphologically but are clearly separable cytologically. There is no evidence of hybridization in this population.

INTRODUCTION. The subgenus *Stethomyia* Theobald of the genus *Anopheles* Meigen consists of five closely-related neotropical species distributed from Costa Rica to Peru and Brazil (Stone et al. 1959). Two of these species, *An. kompi* Edwards and *An. nimbus* (Theobald), differ only slightly in morphological characteristics of larvae and adults, are sympatric in many areas of South America and occur together as larvae in breeding sites in the Amazon basin. The salivary gland polytene chromosomes are surprisingly different for 2 species so similar morphologically and ecologically. The chromosomes furthermore differ considerably in banding patterns from those of neotropical species of the subgenera *Anopheles* Meigen and *Nyssorhynchus* Blanchard. The polytene chromosomes of *An. kompi* are described herein; those of *An. nimbus* will be reported separately.

MATERIALS AND METHODS. Salivary gland chromosome preparations (French, et al. 1962) were made from 4th instar larvae collected near Manaus, Amazonas, Brazil during 1971 and 1973. Permanent slides were made using dry ice and mounted in Zeiss Einschlussmittel L-15.

Larvae collected were identified following the keys in Gorham et al. 1967. Larvae with at least one of the prothoracic shoulder hairs extending beyond the tip of the antennae and with palmate hairs with filiform leaflets were classified as *Stethomyia*. Larvae with 6–8 branches of prothoracic hair one were tentatively identified as *An. kompi* and larvae with 11–14 branches tentatively identified as *An. nimbus*. Ten larvae of each type were set aside for emergence and verification of adult characters. All adults had dark tarsi, dark wings, no apical band on the hind tibia; adults from larvae with low (6–8) prothoracic hair counts checked out to *An. kompi*, those with high (11–14) hair counts checked out to *An. nimbus* although even in fresh specimens the coloration difference (Gorham et al. p 19) between the lateral and median mesonotal stripes was not sharp. Chromosome slides were made from individual larvae whose left and right side branch counts had been marked on the slide with a diamond pencil. Thus each chromosome slide was associated with a definite larva. Antennal hair branch counts were not reliable, in this sample, to differentiate the 2 species.

DESCRIPTION OF THE CHROMOSOMES. As in other anophelines the salivary chromosome complement consists of 3 paired elements, a short telocentric X and 2 longer submetacentric autosomes (Figure 1). Average measurements from unstretched preparations are as follows: X, 63 micra; 2R, 175 micra; 2L, 146 micra; 3R, 155 micra; 3L, 89 micra. Numbered and lettered zones have been arbitrarily assigned to each chromosome at follows: X, zones 1–5; 2R, zones 6–15; 2L, zones 16–25; 3R, zones 26–35; 3L, zones 36–45. Lettered areas within zones are com-

[1] Florida Medical Entomology Laboratory, Box 520, Vero Beach, Florida 32960.
[2] Department of Biology, Youngstown State University, Youngstown, Ohio 44555.
[3] I.N.P.A., Caixa Postal 478, Manaus, Amazonas, Brasil.

pletely arbitrary. Numbered and lettered chromosome areas are shown in Figure 2 and the proposed standard salivary chromosome map in Figure 3.

The X is the shortest element in the complement (Figures 1, 2, 3, 5) and may be easily identified by the three dark bands in 1B, the two widely spaced dark areas in region 2 and the two series of dark bands in region 5.

The right arm of chromosome two is slightly longer than the left. At the free end of the arm a series of dark bands in regions 6 and 7 is followed by a lightly staining area which contains a single dark band at about its center, at the beginning

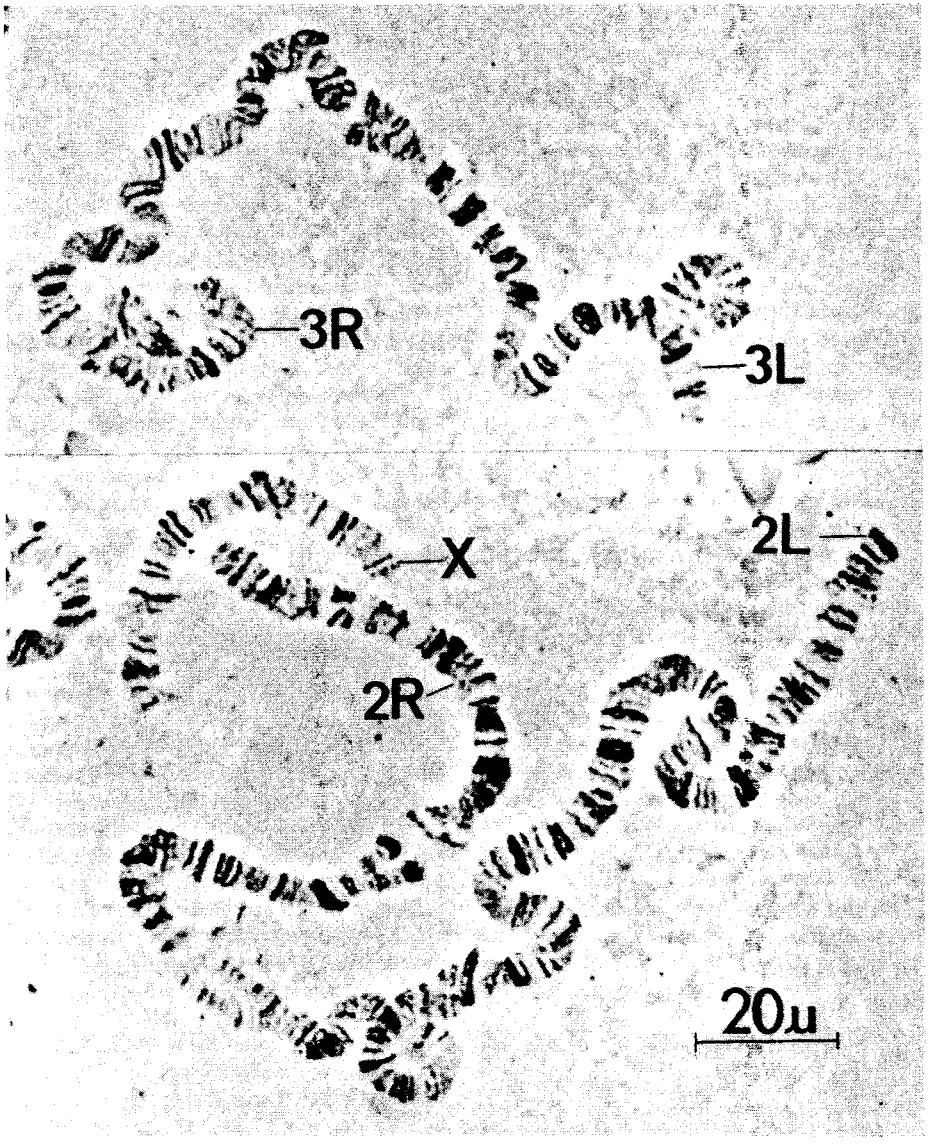

Fig. 1. *Anopheles kompi*, salivary gland chromosome complement.

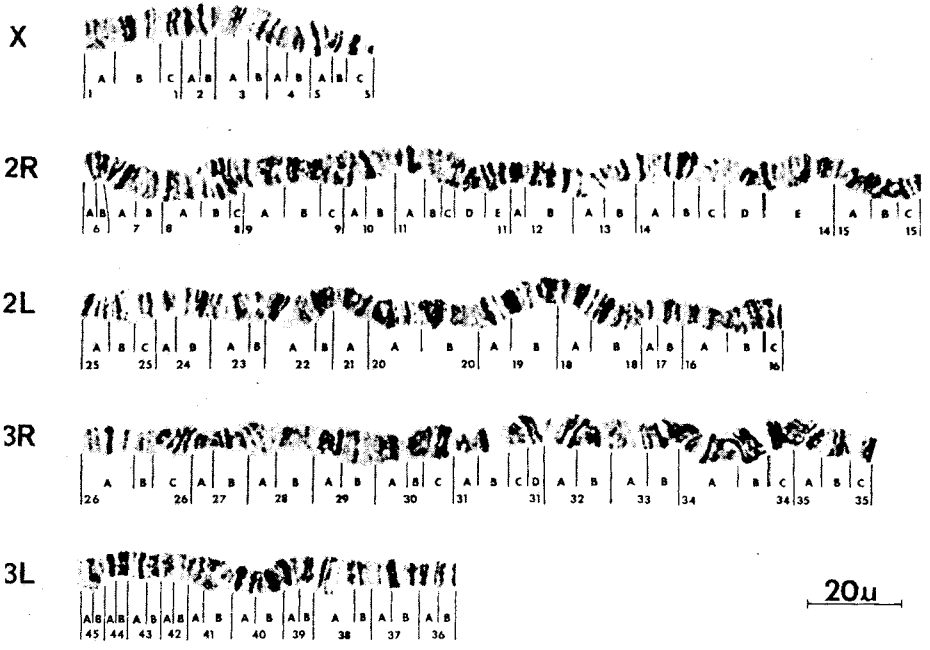

Fig. 2. Paired salivary gland chromosome arms.

of 8A. Two wide, thick, dark bands mark 8C. A series of dark bands starting in 11C and ending in 12B is characteristic of the middle of the arm and another series of dark bands occurs near the centromere in region 15.

The left arm of chromosome two contains many darkly staining bands. At the free end four dark bands are prominent in region 25. Two series of heavy bands, separated by a lighter area, occur in region 22. Another group of characteristic dark bands occurs in 18A and 18B. The remainder of the arm is relatively more lightly staining and variable, especially near the centromere.

The free end of 3R is easily identified by a dark band in 26A, a lighter area following, then a series of five dark bands in 26C. The center of the arm is marked by a series of thin dark bands in 29A, followed by a wide heavy band in 29B. Also easily identified are pairs of heavy dark bands in 30C and 32A. The series of dark bands in region 34 is characteristic of the centromere end of the arm.

The short left arm of chromosome 3 is only slightly longer than the X chromosome. The best recognition areas are the dark heavy bands in regions 45, 44 and 43 at the free end of the arm, the series of dark bands in regions 40 and 39 and the characteristic bands near the centromere in regions 37 and 36.

INVERSION POLYMORPHISM. Three paracentric inversions have been recovered from the Manaus population. We have

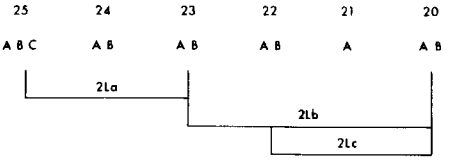

Fig. 4. One possible sequence of three paracentric inversions to produce 2La.

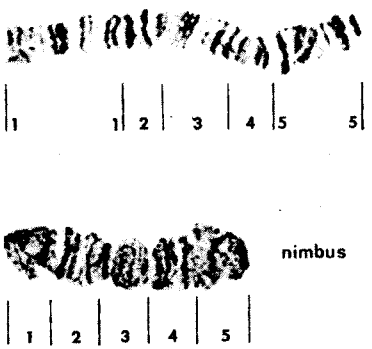

Fig. 5. X-chromosomes, *Anopheles kompi* and *Anopheles nimbus*.

named these inversions following the proposal by Coluzzi et al. (1973) that anopheline inversions be named according to the system widely used by *Drosophila* workers (Wasserman 1963; Carson et al. 1967). This system allows a common nomenclature for interspecific and intraspecific inversions in a group of related species. The inversions and regions involved are as follows:

2R (a/+)	6B-8C
2L (a/+)	25C-20A
3R (a/+)	26G-29A

The designation (a/+) means that the inversion is found in heterozygotes as well as in both homozygotes in the population and thus is indicative of inversion polymorphism.

Inversions 2Ra and 3 Ra are simple paracentric inversions and the inverted sequence can be produced by a simple inversion event, but 2La is complex and requires three additional paracentric inversions to produce the observed sequence. Thus hypothetical inversions 2Lb, 2Lc and 2Ld are necessary and should be looked for in other *An. kompi* populations. One sequence of three inversions (other possibilities exist) which would produce the 2La sequence is as follows:
(1) 25C-23A (2) 23B-20A (3) 22B-20A. This is diagrammatically shown in Figure 4.

The final sequence of 2La, in terms of the standard map (Figure 3) is:
23A 24B 24A 25C 22B 21A 20A 22A 23B

As had been found to be the case in many anophelines in which inversion polymorphism occurs, common breakpoints are shared, in this case, at 23A-B and 20A-B. The 22B-20A inversion is an included inversion. Chi-square tests indicate that the three inversions are in Hardy-Weinberg equilibrium in the Manaus population (Table 1).

DISCUSSION. The salivary gland chromosomes of *An. kompi* are the first described from the subgenus *Stethomyia*. The banding patterns are unique and are not at all like those of the subgenera *Anopheles, Nyssorhynchus* or *Cellia* Theobald. Although there are many banding pattern homologies among the species within subgenera, chromosomal differentiation between subgenera appears to have been a major evolutionary step.

A relatively high level of inversion polymorphism is evident in this relatively small sample from Manaus. Three different paracentric inversions, in three distinct arms appear to be in equilibrium (Table 1). Inversion polymorphism is also high in populations of *An. darlingi* Root sampled at Manaus (Kreutzer et al. 1972).

The closely related species *An. nimbus* has quite different salivary gland chromosomes. Complete description of these chromosomes will be reported elsewhere but the 2 species may be distinguished easily by the X-chromosome (Figure 5). The X of *An. kompi* is about twice as long as that of *An. nimbus*, with an entirely different banding pattern.

Morphologically, the larvae are separated principally by a minor quantitative trait, the number of branches of prothoracic hair No. 1. A typical couplet in a taxonomic key assigns larvae with 6–8 branches to *An. kompi*, larvae with 11–14 branches to *An. nimbus* (Gorham et al. 1967, p. 33). We scored 151 larvae for prothoracic hair counts and type of X-

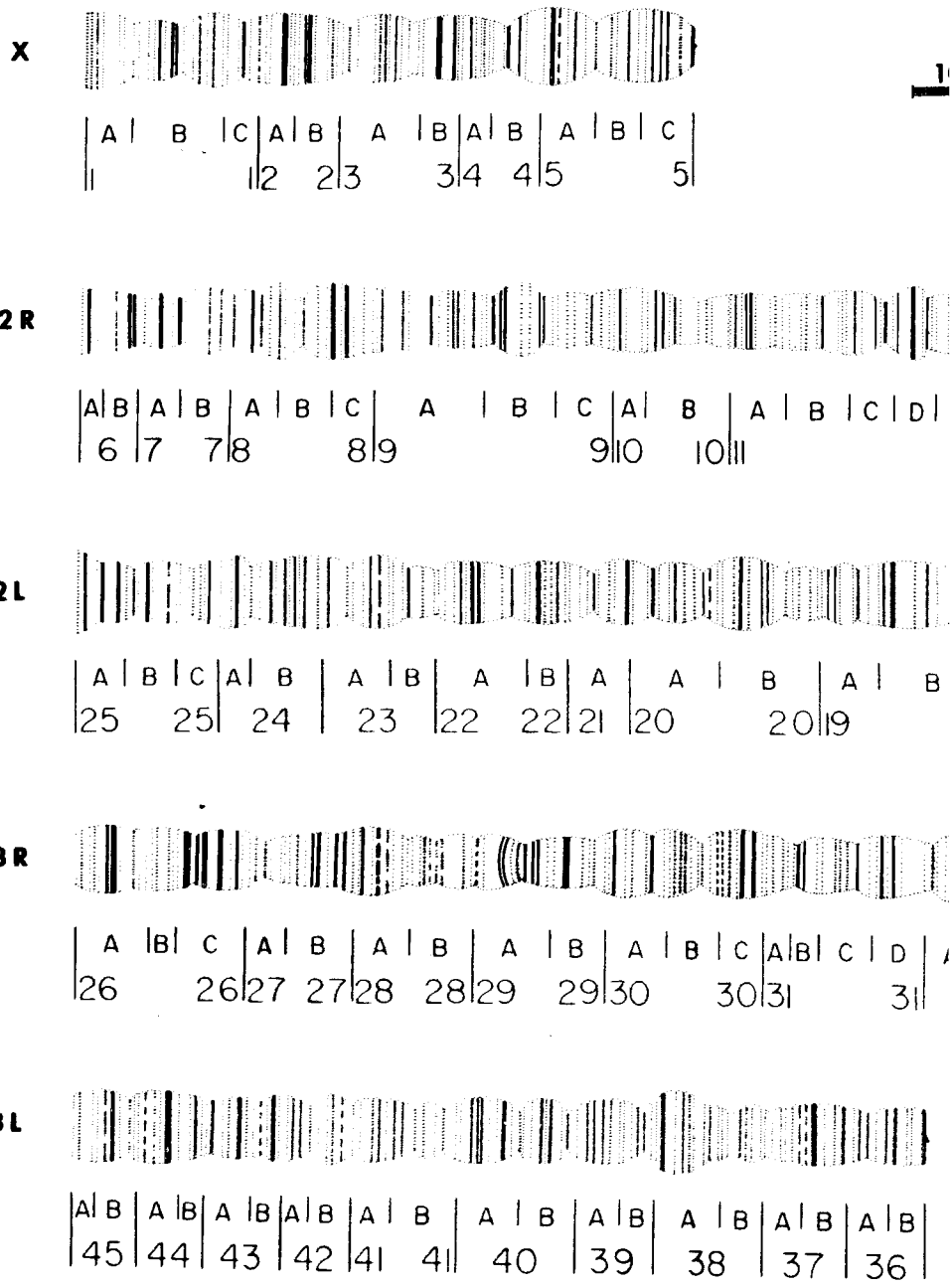

Fig. 3. Salivary chromosome map.

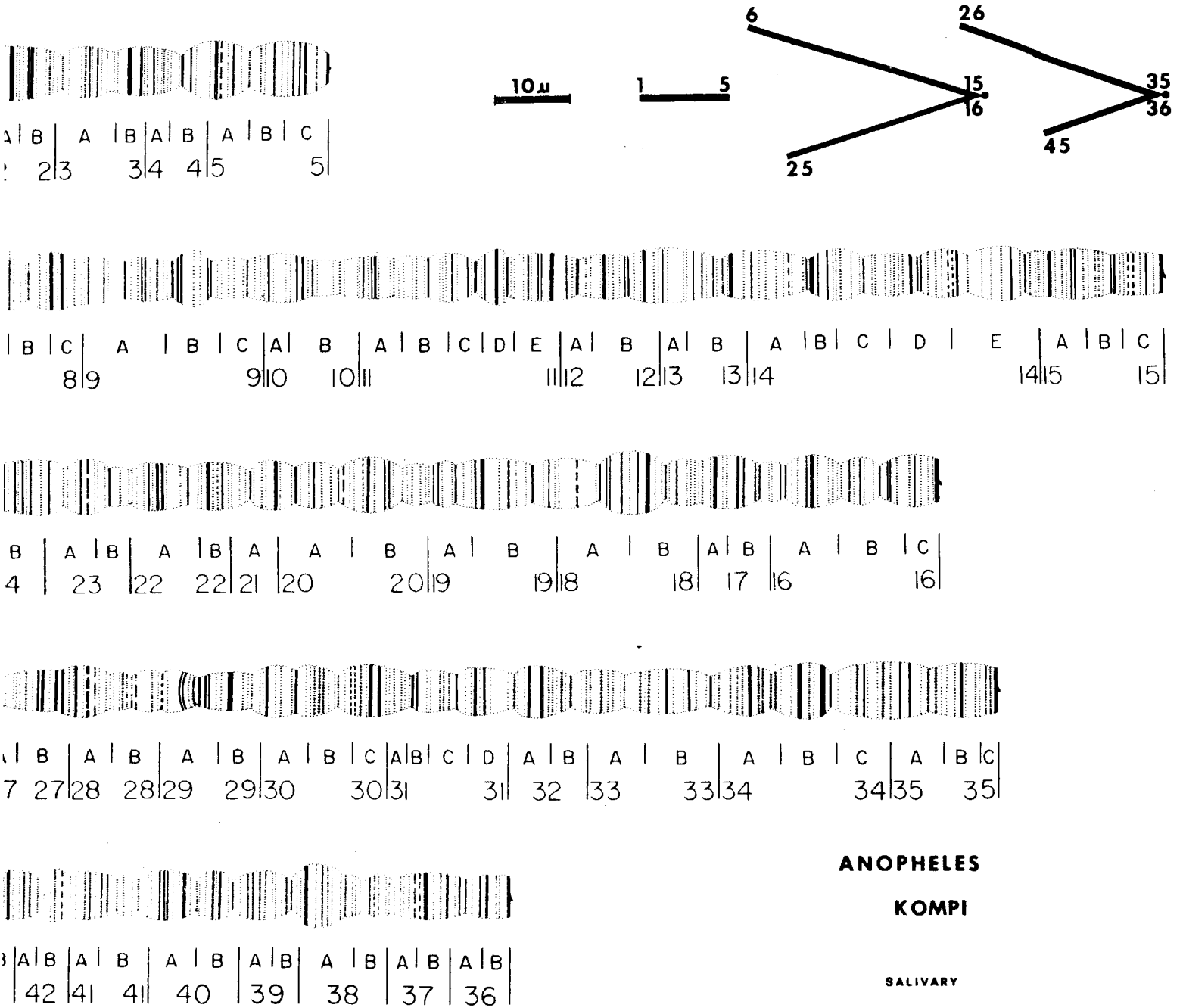

Fig. 3. Salivary chromosome map.

Table 1. Hardy-Weinberg frequencies of homozygotes and heterozygotes for three inversions in *An. kompi*. Variations in N result from some slides not being readable for a given inversion.

	+/+	a/+	a/a	N	X^2	P
2Ra	23	37	7	67	1.95	.39
2La	21	41	9	71	2.51	.28
3Ra	16	28	19	63	.74	.69

chromosome. Seventy-six larvae had *An. kompi* chromosomes and 75 had *An. nimbus* chromosomes. Of the larvae with *An. kompi* chromosomes, 28 had hair counts above 8, and of the larvae with *An. nimbus* chromosomes, 22 had hair counts fewer than 11.

The presence of such a large number of morphologically overlapping types (50 out of 151) and the fact that both types occur in the same larval habitat might suggest hybridization in nature. No chromosomal hybrids were found. Hair counts are highly variable between both sides of a single larva. Summing the branches on both sides of an individual larva gives a much better separation, at least in this sample. Larvae with a total of 19 or fewer branches (75) had *An. kompi* chromosomes and all but one of those with a total of 20 or more (75) had *An. nimbus* chromosomes. Only one larva of 151 in this sample had 10 branches on each side (20 total) but had *An. kompi* chromosomes. Chromosomal identification appears to be the only sure way of separating larvae of these species.

ACKNOWLEDGMENTS. It is a pleasure to thank Dr. Agostinho Cruz Marques, Director of SUCAM, Manaus, Amazonas, Brazil for space, facilities and logistic support during several visits to Manaus. Dr. Glenn Fleming of PAHO assisted in many ways. The chromosome map was drawn by Miss Jody Grenga. Supported by grants E-3486, USPHS and by Youngstown State University research grants 184 and 191.

References Cited

Carson, H. L., F. E. Clayton and H. D. Stalker. 1967. Karyotypic stability and speciation in Hawaiian *Drosophila*. Proc. Nation. Acad. Sci., U.S., 57:1280–1285.

Coluzzi, M., M. di Deco and G. Cancrini. 1973. Chromosomal inversions in *Anopheles stephensi*. Parassitologia 15:129–136.

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

Gorham, J. R., C. J. Stojanovich and H. G. Scott. 1967. Clave Ilustrada para los mosquitos anofelinos de sudamerica oriental. U.S. Public Health Service, Center for Disease Control, Atlanta. 62 pp.

Kreutzer, R. D., J. B. Kitzmiller and E. Ferreira. 1972. Inversion polymorphism in the salivary gland chromosomes of *Anopheles darlingi* Root. Mosquito News 32:555–565.

Stone, A., K. L. Knight and H. Starcke. 1959. A synoptic catalog of the mosquitoes of the world. Entomol. Soc. America. The Thomas Say Foundation. Vol. 6:1–358.

Wasserman, M. 1963. Cytology and phylogeny of *Drosophila*. Amer. Nat. 97:333–352.

WHERE? New Orleans, La. WHEN? March 27–30, 1977. WHAT? The joint AMCA and LMCA meetings.