Chromosome Studies of Oriental Anophelines. I. The Salivary Gland Chromosomes of *Anopheles barbirostris*

Increasing emphasis is being placed at present on the cytogenetic studies of mosquitoes, the vector species in particular. Karyotypes and maps of salivary gland chromosomes for several of the anopheline species are now available. However, paucity in the cytogenetic knowledge of the tropical anopheline fauna, especially of the oriental region is enormous. Mention may be made here of two preliminary papers describing the salivary gland chromosomes on some Indian anophelines[1,2]. For a review of the pertinent literature on the genetics and cytogenetics of mosquitoes, see KITZMILLER[3-5] and KITZMILLER, FRIZZI and BAKER[6]. The present paper, which proposes a 'standard' chromosome map of *Anopheles barbirostris*, is the first of a projected series for the oriental anophelines.

Anopheles barbirostris Van der Wulp, used in the present study, is the type form of the 'barbirostris group' which belongs to the series Myzorhynchus in the subgenus Anopheles. It is confined to India, Ceylon, Burma, Thailand and Cambodia[7]. Larvae prefer both sunlight and shaded clear water of deep ponds, pools and other large bodies of water covered by growth of plants such as *Pistia* and *Jussiaea*. A neglected irrigation well with a marshy bottom about 7 miles south-east of Bangalore, was a source of larvae throughout the year. Chromosome slides were prepared by the standard method for anophelines[8]. The diploid chromosome number is 6 (Figure 1). In salivary gland preparations the chromosomes appear as 5 paired polytene elements representing 3 pairs of chromosomes – a short telocentric *X* chromosome and 2 pairs of longer metacentric autosomes with arms of distinguishable lengths (Figure 2). The autosomal arms are designated as 2R, 2L, 3R and 3L. The *X* chromosome and the autosomal arms are further divided into zones and subdivisions by the same general numbering system as the one used for other species of the subgenus Anopheles thus far studies (Figure 3).

X Chromosome. This short chromosome can be readily recognized by its length. The free end in 1A is normally diffuse. The expanded puff that follows is characteristic for this region. A single heavy band in a constriction at the beginning of 1B is distinctive as are 2 heavy bands at the beginning of 2A. Several diagnostic areas which serve as landmarks for the *X* chromosome include a series of heavy bands in 2B, 3C, 3D and 5B. 3 dark bands found in 5C mark the spindle-shaped centromere end of the chromosome.

Chromosome 2, right arm. The free end of this arm terminates in a diffuse expansion in 6A containing a series of lightly staining bands. This is followed by a series of dark bands in 6B of which 2 heavy bands in a constriction at the beginning stand out. Perhaps the best landmark for this end of the arm are a heavy triplet in

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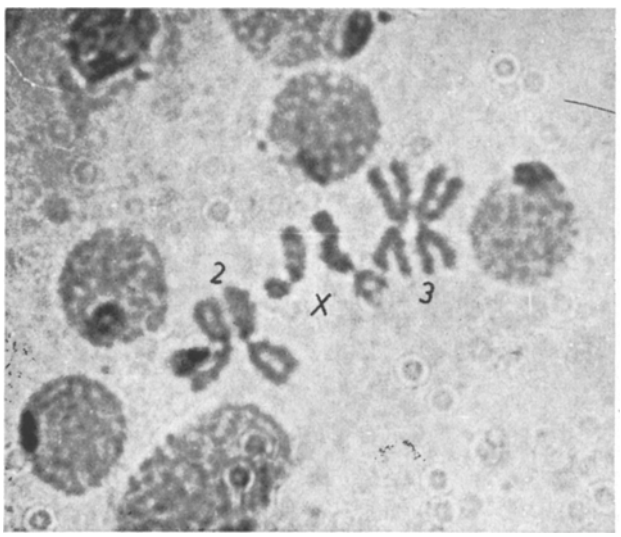

Fig. 1. Mitotic chromosomes (female fourth instar larval brain) of *Anopheles barbirostris*.

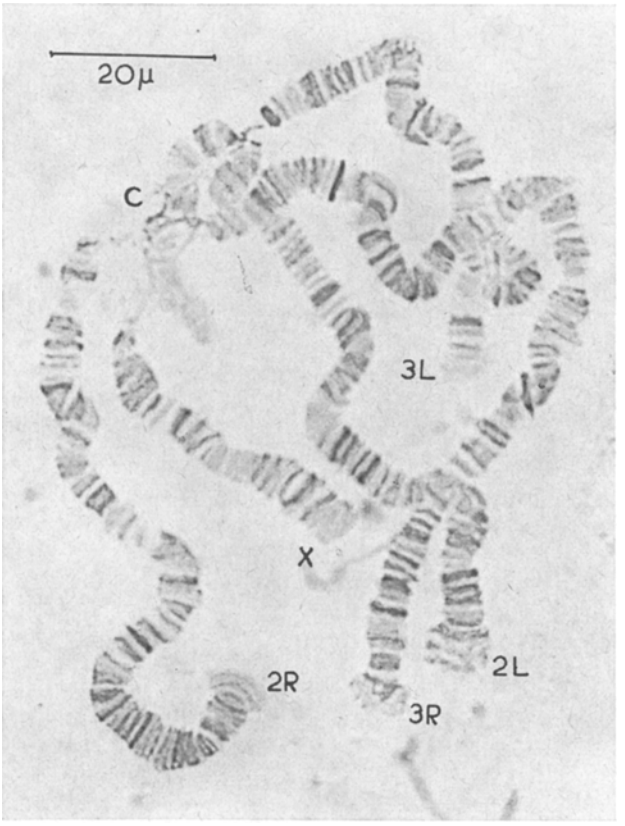

Fig. 2. *Anopheles barbirostris*. Entire salivary gland chromosome complement.

8A and a pair of twisted heavy bands in 8C. A series of dark bands in regions 10 through 12 serve to identify the middle portion of this arm of which those in 10A, 11C and 12A are excellent recognition areas. Region 13 contains 2 easily recognizable areas: 4 dark bands in 13A and a small puff in 13C with a 'bird's eye' at the beginning of the area. Region 14 is invariably barrel-shaped which is often stretched and variable. A heavy triplet in 14B may be regarded as a constant and very useful landmark for the centromere end of the chromosome.

Chromosome 2, left arm. This arm is characterized by the series of clear puffs distributed throughout its length. The distal end is easily recognized by its typical puff in which 2 dark bands stand out, one on either side of a diffuse area in 21A. 4 dark bands at the end of 21B and a pair of heavy bands in a constriction separating 21B and 20A are consistent features in all preparations. The puff in 19A is very distinct with a pair of thin curved bands. The heavy bands in the region 19B through 18C are typical. Region 17 as a whole is a landmark. The series of dark bands in 16B and 15A are also diagnostic. The centromere end of the arm which is slightly broad, is unmistakably recognized by the presence of a pair of dark curved double bands in 15C.

Chromosome 3, right arm. As in several other species, this arm is the longest of the complement and contains a number of areas which help in its recognition. The flared diffuse end with its 2 heavy curved bands and the 3 characteristic puffs that follow compare well with the end of 3R in other species of the subgenus Anopheles. 2 pairs of heavy bands in 24B, a small distinctive puff with a series of 1-2-1 heavy bands in 24D form an excellent landmark. The large puff with a pair of thin curved bands in 25A and 3 curved heavy bands in 26B are always recognizable. A very narrow lightly staining constriction found almost in the middle of the arm and separating 27A from 27B is consistently seen. A pair of heavy bands in 31D followed by a series of evenly-spaced thin dark bands in 31E are usually sharply defined and are typical of the proximal end of the arm. A single heavy band in the constriction at the beginning of 32A is remarkably constant. Region 32 with 2 small distinct spindle-shaped puffs each with a series of dark bands may be used to recognize the centromere end of the chromosome.

Chromosome 3, left arm. This is the shortest autosomal arm. The free end is very similar to the comparable region of most species of the subgenus Anopheles. This arm is characterized by the presence of a series of large puffs. A single heavy band in a constriction separating 39A and 39B is very prominent. Perhaps the best landmark of the free end of the arm is the large puff in 38A which is characteristically empty. The expanded puff in 37B with 3-1-3 series of dark bands is seen fairly consistently. The puff in 34A with a pair of heavy curved wavy bands which is followed by another series of dark bands in 34C and 34D serves as a diagnostic area for the proximal part of the arm. The centromere end of the arm is characteristically bell-shaped with a single thin dark band at the beginning of 33A.

Even though it is too early to evaluate the importance of chromosomal analysis of tropical mosquitoes based on the study of a single species, the close morphological similarity of *Anopheles barbirostris* to other members of its own group which has an extensive distribution and the differential malarial transmission within the group make the study of the salivary gland chromosomes all the more interesting. A detailed cytogenetical investigation of a large number of neotropical and tropical anophelines

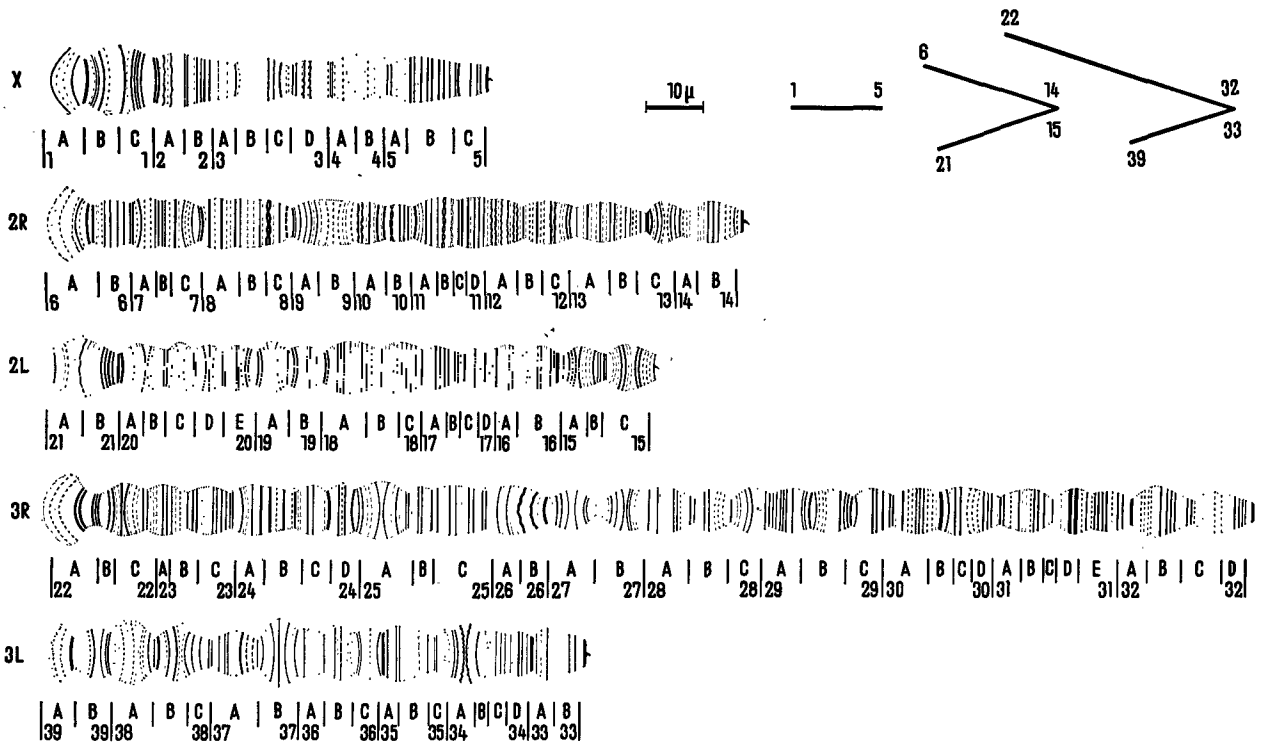

Fig. 3. Salivary gland chromosome map of *Anopheles barbirostris*. C, Centromere; X, *X*-chromosome; 2R and 2L, right and left arms of chromosome 2; 3R and 3L, right and left arms of chromosome 3.

is very much warranted to shed more light on the cytogenetic and phylogenetic relationships within the subgenus Anopheles[9].

Zusammenfassung. Es werden verschiedene tropische Anophelesarten, besonders die orientalische, cytogenetisch untersucht. Es wird eine Standard-Chromosomenkarte von *Anopheles barbirostris* van der Wulp (Typ der Barbirostris-Gruppe einer orientalischen Region und zur Serie Myzorhynchus, der Untergattung Anopheles gehörend) als erste der Serie orientalischer Anophelesarten vorgeschlagen.

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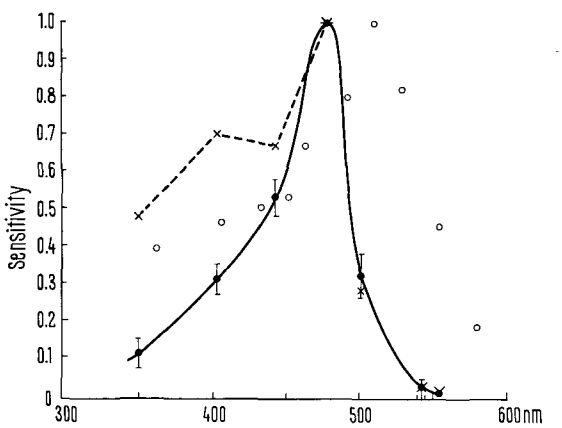
The Spectral Sensitivity of the Tail of *Urodacus*, a Scorpion

Recordings of spike activity in the nerve cord have revealed an extraocular light sense in an Australian scorpion, *Urodacus novae-hollandiae*[1]. It was shown to be sensitive to light in the range from long UV to green. The present investigation is an attempt to determine its spectral sensitivity quantitatively.

The recordings were made with hook electrodes from the mesosomal nerve cord, between the second and the third mesosomal ganglion. The cord was severed ahead of the recording site and the neural vessel was stripped from the connectives. Illumination was restricted to the metasoma (the 'tail'). The light source was a heat-filtered 150 W xenon arc whose intensity was controlled with a neutral density wedge or, in the UV, with metal sieves. Wavelengths were selected by means of Schott FIL interference filters in the visible range and a Schott UG2 (2 mm) filter in the UV; their transmission was calibrated with a thermopile and the readings divided by the wavelength in order to obtain energy values proportional to the number of quanta.

The preparations were kept in the dark except for the test flashes of about 4 sec duration. The intervals between flashes were sufficient to ensure complete recovery, as indicated by previous tests. Spikes were counted from filmed records for the third second after the onset of light. Preliminary experiments had shown that at the intensities used the spike frequency could be regarded as a linear function of log I. Consequently each wavelength was tested at a number of intensities, the spike frequencies were plotted against log I and a straight line drawn through the points. This resulted in a family of 7 lines, one for each wavelength. Spectral sensitivity curves, based on a standard response taken as one-half the maximum response of the preparation, were derived from this graph. The spectral response curves, based on equal energy stimuli, were very similar. 5 preparations were used with duplicate determinations in reverse order of stimuli.

The peak of the sensitivity curve (Figure) lies in the blue-green region at about 480 nm. The data for median eyes of a number of other scorpion species published by Machan[2] are plotted for comparison. The tail curve is rather narrower; it is in fact narrower than the typical retinene pigment curve[3]. A yellow filter, reducing the sensitivity in the blue and long UV-ranges, would account for the narrowness of the curve and specifically for the low values at short wavelengths. A tail segment can be described as a box with sclerotized edges and panels of unsclerotized cuticle. These translucent panels are yellowish in colour and determinations of their spectral transmission confirm that it drops steadily and markedly towards the shorter wavelengths. The transmission values were used in plotting the corrected curve. This curve is comparable with that of scorpion median eyes[2], although shifted towards the left. The shoulder is reminiscent of similar shoulders and secondary peaks reported from many invertebrate eyes[4].



Spectral sensitivity of the extraocular light sense of *Urodacus* (means of 5 duplicate determinations ± standard error). ×, values corrected for cuticular transmission; ○, values for scorpion median eye (Machan[2]).

Zusammenfassung. Die spektrale Empfindlichkeit des extraokularen Lichtsinnes des australischen Skorpions *Urodacus* wurde bestimmt. Das Maximum liegt im Bereich von 480 nm. Die Einberechnung der Kutikulartransmission verändert die Form der Kurve, besonders im UV, aber das Intensitätsmaximum bleibt dasselbe.

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