THE POLYTENE CHROMOSOMAL
PATTERN OF ANOPHELES
STEPHENSI MYSORENSIS OF
KAZEROON (IRAN)**

Z. Sahabi*
J. D. Amirkhanian*
E. Shahgoudian*

ABSTRACT
The Anopheles stephensi mysorensis originating from Kazeroon area of Iran, which had been maintained at the laboratory conditions of 27°C at 70% relative humidity for almost 150 generations, were subjected to cytogenetical analyses. The 'instant' landmarks in the squash preparations of the salivary gland chromosomes, are indicative of cytotoxic characteristics of the species. The characteristic features of the banding patterns are as follows: Darkly-stained oblique bands at zones 4A and 4B, also the terminal ringed bands at zone 6C; The asynaptic loop at zone 20 and its point of attachment to 2R chromosome; the puffs at zones 21, 24, 27 & 28 of 2L chromosome; the well defined puff at zone 19 of 2R chromosome, with its characteristic terminal endings; darkly-stained thick bands at zones 32, 33 & 36; weekly-stained zone 37 and funnel-shaped terminal ending of 3L chromosome at zone 46.

INTRODUCTION
The pattern of the salivary gland chromosomes of Anopheles could be used as a convenient tool for differentiating malaria vectors, previously recognized only by crossing experiments.

The salivary gland chromosomes of A. steph. mysorens. of Iran was first described by Zohre Sahabi (1969) according to the

**This study was supported partly by the Institute of Public Health Research, University of Tehran and in part from the funds of The Ministry of Health and Plan Organization.
*Department of Environmental Biology, School of Public Health & Institute of Public Health Research, University of Tehran, Iran.
method of Rishikesh (1959). In the present paper a revision is made of the chromosomal pattern of the same mosquito with the objective of determining the most characteristic ‘instant’ landmarks for the identification of the species, following the methods of Baker & Kitzmiller (1965) and Coluzzi et al. (1970).

**MATERIALS AND METHODS**

The stock of the *A. steph. mysorensis*, originated from Kazeroon area of Iran, had been reared in our insectarium for about 150 generations by mass inbreeding techniques, before being used for cytogenetical studies. It is a vigorous laboratory colony, with easy mating behavior in the cages of about 40 cub. cm. The mosquitoes were kept at 27°C and 70% relative humidity. The larvae were reared in enamelled wash-basins (40 x 25 x 5cm.) filled with drinking water to a depth of 4cm. The larval density was adjusted to the quantity of food (Bemax’ cereal plus yeast) in each tray, in order to avoid putrefaction of water or fungal growth, which would otherwise prevent the development of salivary gland cells. Adult mosquitoes were fed on 10% sugar and the females were bloodfed on guinea pigs, prior to oviposition.

The salivary gland chromosomes were dissected out in 0.65% NaCl on a slide, fixed and hydrolysed according to the technique for mosquito chromosome spreads (Amirkhanian 1968). Photomicrographs taken from the temporary slides were subjected to the analysis of the banding pattern of the chromosomes. Endeavour was made to note as far as possible, the whole chromosome complement together, in order to differentiate the point of attachment of the chromosomal arms to the centromeric regions.

**RESULTS**

*A. stephensi mysorensis* of Kazeroon has a typical Anopheles karyotype, 2n= 6 or five, banded polytenic chromosomes. The short X-chromosome, which is telocentric, averages 36.6 in length. Average measurements for the autosomal arms are: 2R, 130 ; 2L, 100 ; 3R, 166 ; 3L, 136 . The right arm of the chromosome two is longer than the left, and the two arms of the third chromosome have an average of 30 difference in length.

The numbering of the zones on the chromosomes, which has been followed after Coluzzi, et al, 1970, are as follows: The chromosome X=1, contains zones 1-6; 2R, zones 7-19; 2L, zones 20-28; 3R, zones 29-37; and 3L, zones 38-46.

The paired chromosomes are all asynaptic, except the zone 20 of 2L chromosome, which has a characteristic asynaptic loop.

The most characteristic ‘instant’ landmarks are easily
noticeable on the first and the second chromosomes.

**X-chromosome**: Generally appears isolated from the other chromosomes in squash preparations, but it could be seen attached to the centromeric region of the other, if the time for fixation-hydrolysis procedure is reduced to almost two minutes. The zone 1A of X-chromosome starts with weakly staining region, then followed by two thick bands and a thin one. 1B, has one thick band in the middle and two thin ones at both sides. Zones 2A & 2B, weakly staining regions, contain one puff each. 3A, has diffusely staining regions and 3B has one dotted band in the middle. Zone 4A has a thick, oblique and darkly staining band followed by another darkly staining band at 4B, which are the most characteristic landmarks of the species. 4C with a diffused weakly staining band is followed by a small puff. Zone 5A consists of a thick band in the middle. Zone 5B starts with three closely attached bands, followed by four slightly staining bands. 5C starts and ends by two dark bands. Zone 6A starts with four weakly staining bands followed by 6B, with two diffusely staining bands. 6C is most characteristic, in starting with a thin band, then dotted area, followed by darkly staining double bands, then a dotted band. Zone C terminates by two darkly staining rings at the terminal end of the chromosome X.

**2R-chromosome**: The most striking feature of this arm is series of puffs at zones 7, 11, and 19.

**3R-chromosome**: Consists of a series of darkly staining bands, at zones 32, 33 & 36, with slightly staining dotted regions at zone 37 and a puff at zone 34.

**2L-chromosome**: Consists of puffs at zones 21, 24, 27 & and a most characteristic synaptic loop at zone 20. This arm is connected to the right arm of 2R, from the darkly staining band at the base of the loop (the centromeric connective).

**3L-chromosome**: Contains darkly-stained bands at zones 40, 41 & 42, puffs at zones 43 and 46, also a funnel-shaped terminal ending at zone 46.

**REFERENCES**


Amirkhanian, J.D., 1968. A combined HCl-Acetic-Alcohol Fixation & Hydrolysis followed by Cresyl violet staining for mosquito chromosome spreads, Stain Technol. 43, 167-170.

ACKNOWLEDGEMENTS

Our sincere thanks are due to Prof. M.A. Faghih, the Dean, and Dr. A. Nadim the Vice-Dean of the School of Public Health and Institute of Public Health Research, for their attention and encouragement. We are also thankful to Prof. A. Mesghali, Head of the Department of Environmental Health, for providing all facilities and advice.

Fig. 1 Entire polytene chromosomes complement of *Anopheles stephensi mysorensis* of Iran, from the salivary gland of fourth instar larva. The chromosomal zones are presented by numbers.
Fig. 2 The X chromosome of Anopheles stephensi mysorensis, further enlarged to show clearly the characteristic landmarks.