CHROMOSOME STUDIES OF ORIENTAL ANOPHELINES

III. The Salivary Gland Chromosomes of *Anopheles subpictus*

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Anopheles subpictus, belonging to the series pseudomyzomyia of the subgenus *Cellia* has a diploid chromosome number of 6 which is typical of the anopheline species. The heterochromosomes from the female larval brains are interesting. The salivary chromosome complement consists of five elements representing the three pairs of chromosomes; a short telocentric X chromosome and two pairs of longer metacentric autosomes with arms of distinguishable lengths, weakly attached in the region of the centromere. Some significant similarities in the X chromosomes of *A. subpictus* and *A. gambiae* have been observed. In addition, the autosomal arms of *A. subpictus* are compared with those of the other species of the subgenus *Cellia* thus far studied. Based on some important landmarks a 'standard' chromosome map is proposed for *A. subpictus.*

Introduction

Most of our knowledge of the cytogenetics of mosquitoes comes from studies on species belonging to the subgenus *Anopheles,* in particular species of the maculipennis group (see Kitzmiller *et al.* 1967). On the other hand, only few species of the subgenera *Nyssorhynchus* and *Cellia* which include the most important malaria vectors in the tropics have been studied in this regard. Prior to 1967 some preliminary reports appeared on the cytogenetics of *A. albimanus, A. aquasalis, A. stephensi* and *A. gambiae* (see Kitzmiller 1967). More recent work carried out on *Cellia* species is represented in a series of significant and stimulating papers on the *gambiae* complex (Coluzzi and Sabatini 1967, 1968, 1969); *A. pulcherrimus* (Baker *et al.* 1968); *A. stephensi* (Sharma *et al.* 1969); *A. superpictus* (Coluzzi *et al.* 1970) and *A. farauti* (Bryan and Coluzzi 1971). This paper, which presents a standard salivary chromosome map of *A. subpictus,* is the third in a projected series for the oriental species of the subgenus *Cellia.*

Materials and Methods

Larvae of *Anopheles subpictus* were collected from rice fields and rain water collections within seven miles of Bangalore, South India. The females caught in the wild were identified and then placed individually into vials for egg deposition. The larvae of each female were reared in separate pans. Both the larvae and adults were again checked for species identification.

The salivary gland chromosomes were prepared from the fourth instar larvae by essentially the same method as that adopted by Coluzzi *et al.* (1970). The mitotic chromosomes were prepared by the standard method for anophelines (French *et al.* 1962). The maps were prepared by photographing suitable chromosomes at 1000X and printed at a standard magnification. While measurements of the chromosomes and location of the principal bands were taken from the photographs, the details of banding pattern were made by direct observations of the chromosomes at 1000X oil immersion using an Amplival Zeiss Photomicroscope.

Description of the Chromosomes

The mitotic karyotype of *A. subpictus* showed a diploid number of six which is typical of the anophelines (Fig. 1). However, the sex-chromosomes in the female (XX) were found to lack the usual distinct knob-like terminal portions of the "maculipennis" type (Avirachan *et al.* 1969).

The salivary chromosome complement consists of five elements of synapsed polytene chromosomes appearing as five arms weakly attached in the region of the centromere—a short telocentric X chromosome and two pairs of longer metacentric autosomes with arms of distinguishable lengths (Fig. 2). The autosomal arms are designated as 2R, 2L, 3R and 3L. The X chromosome is the shortest, measuring 130 micra. The average measurements of the autosomal arms are : 2R, 350 micra; 2L, 180 micra; 3R, 264 micra and 3L, 284 micra. The autosomal arms were identified and divided into number of subdivisions following Frizzi and Holstein (1956). Thus, the X chromosome contains zones 1–6; 2R, zones 7–19; 2L, zones 20–28; 3R, zones 29–37 and 3L, zones 38–46. However, the lettering of the subdivisions within each zone is arbitrary (Fig. 3).

X Chromosome

The short X chromosome which generally appeared isolated in squash preparations could be readily recognized by its length and characteristic banding pattern. The free end of the chromosome is slightly flared and marked by two moderately stained curved bands followed by two thin bands in region 1A. The three dark bands in a constriction in 1B mark the first landmark followed by two heavy bands in 1C. A characteristic sequence of four heavy bands in 2A and another of two at the beginning of 2C are constantly present. Sections 3A, 3E and 4A are mostly diffuse with a series of light bands. The five dark bands in 3C of which two are very heavy form an excellent second landmark followed by another series of equally distinct dark bands in 3D. Region 4 as a whole is a good recognition area. The small puff in 4A appears almost empty. The two heavy dark bands at the beginning of 4B and a dark beaded curved band in the middle of the puff are always present. The next sequence is characterized by three widely spaced dark bands in 4C and a series of four thin bands in 4D. Region 5 is often stretched. The six dark bands, three at the end of 5B and three at the beginning of 5C are very distinct. Region 6 is again characterized by the presence of three very thick bands in 6B and a very large puff with a series of thin broken and twisted bands forming an excellent landmark for the centromeric end of the chromosome.

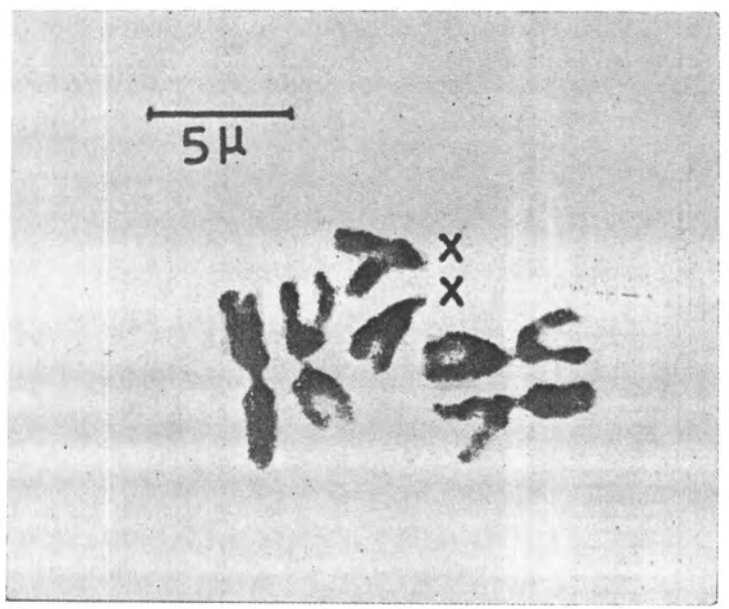

FIG 1. Mitotic chromosomes of brain from fourth instar female larva.

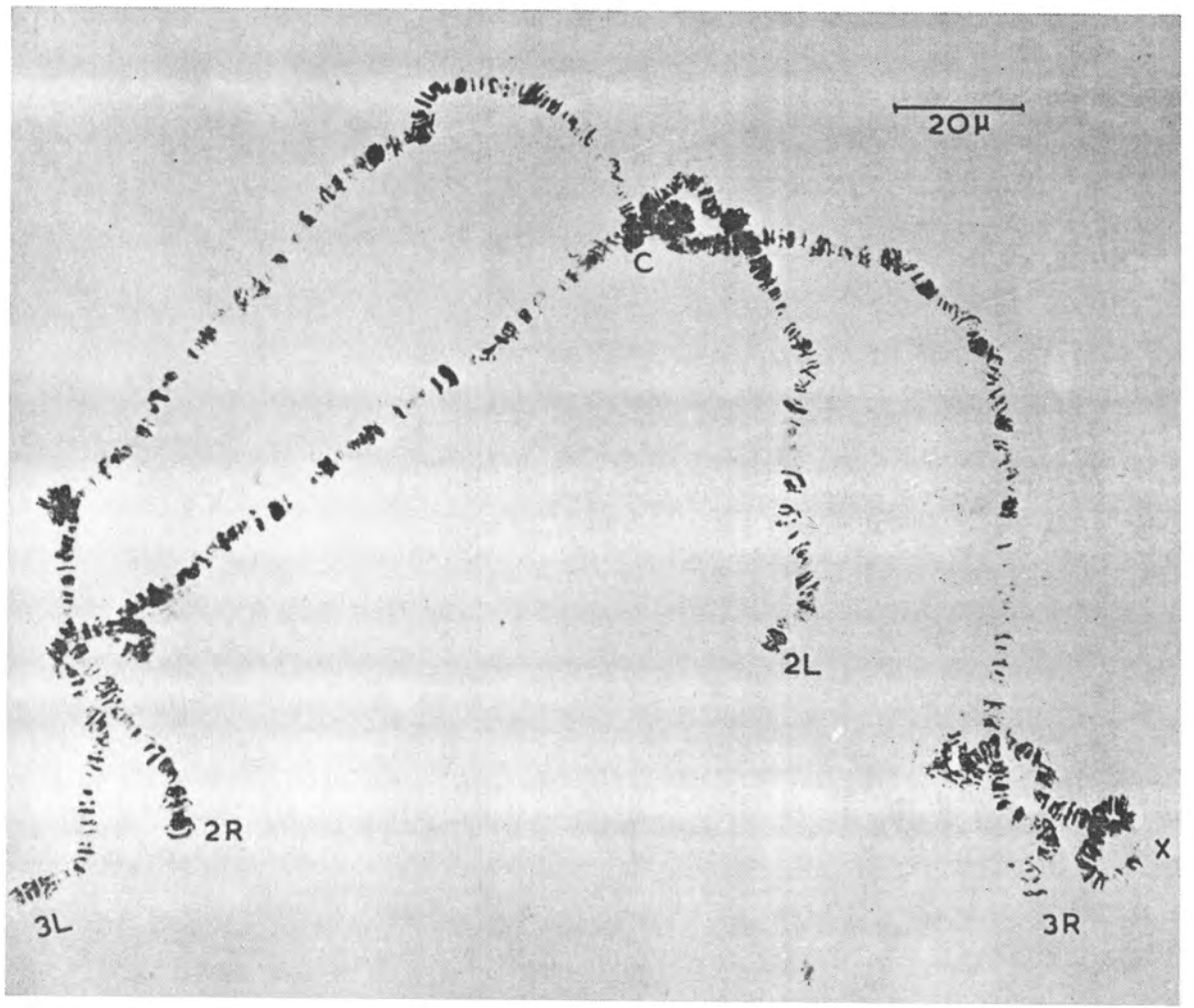

FIG. 2. Salivary chromosomal complement of *Anopheles subpictus*. C, Centromere. X, X Chromosome. 2R and 2L, right and left arms of chromosome 2. 3R and 3L, right and left arms of chromosome 3.

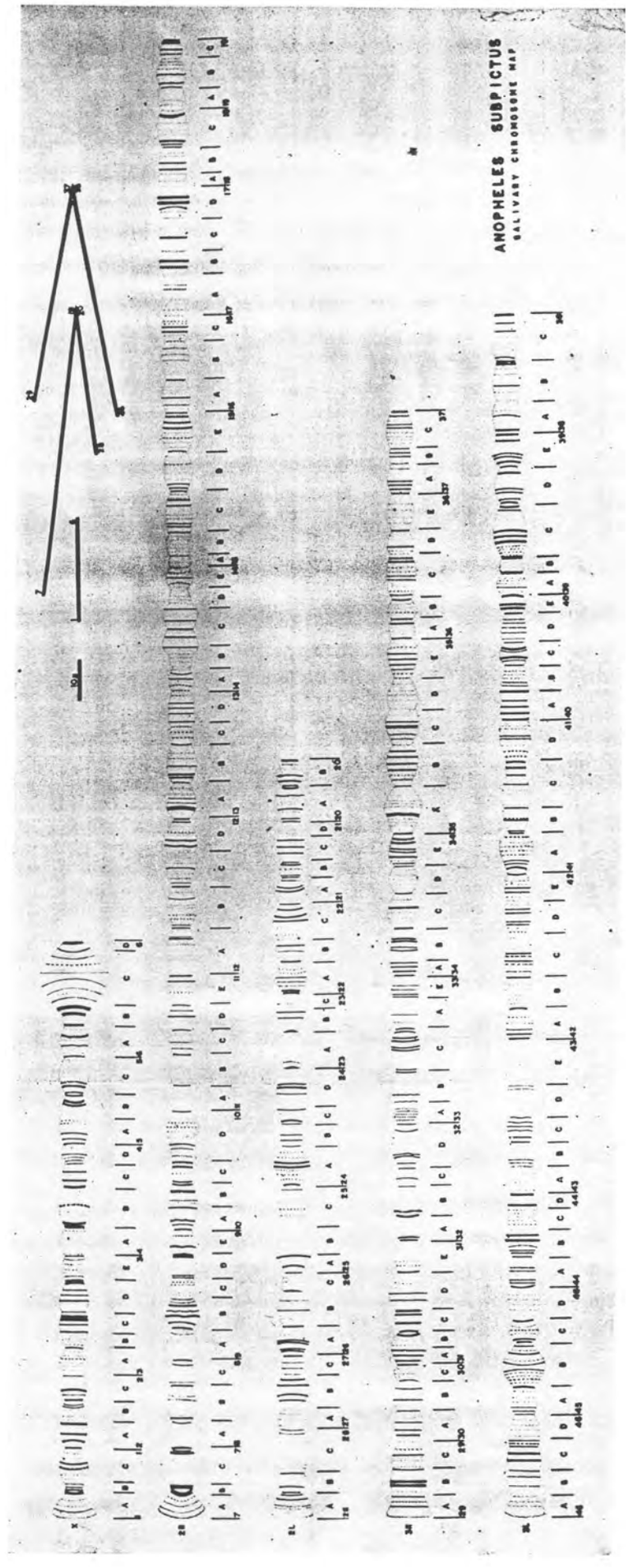

Fig. 3 Salivary chromosome map of *Anopheles subpictus*

Chromosome 2, Right arm

This arm is the longest in the complement. The free end is readily recognizable by the presence of four dark bands in a constriction at 7B together with a fairly expanded light puff in 7C. The curved bands in 7A are quite variable in their expression. Region 8A through 9A in general is a light area. The puff in 9B with a series of dark bands forms an excellent landmark. Region 10 through 12 is more or less uniform. Three dark bands at the beginning of 10A and two at the end of 10C are always seen as shown on the map. The two small puffs one in 11A and another in 11B are constantly very light. A pair of dark bands in the beginning of 12D followed by another pair in the end and a pair of closely approximated thick curved bands at the beginning of 13A form an excellent landmark for this area. The two dark bands in 13B and four in 13D are easily identifiable. Region 14 appears to include a duplication of 14A, 14B and a part of 14C. The puff in 15B is again very light and appears empty. A series of dark bands in 15C and another in 15E are constant. A pair of heavy bands in 16C is diagnostic. Region 17 is a landmark with a pair of heavy bands in 17B, a single dark band in 17C and another pair of heavy bands in 17D. A series of conspicuous dark bands present in regions 18 and 19 mark the centromeric end.

Chromosome 2, Left arm

This is the shortest autosomal arm. The distal end is clearly recognized by its typical puff and a pair of distinct dark bands in the constriction between 28A and 28B and an equally distinct dark band on either side. The two puffs, one in 27B another in 26A, each with a series of heavy dark bands serve as excellent landmarks for this end of the chromosome. The small puff in 27C is variable. The two widely spaced dark bands at the beginning of 25A are equally distinct which are followed by diffuse areas in 25B and 25C. The paired dark heavy bands in 24A and 24D along with a triplet of thin dark bands in 24B serve as useful landmarks for the middle region of the chromosome. The light puff in 23A is again diffuse and almost always appears as shown. The paired heavy bands in 23B are consistently seen. Region 22 as a whole is an excellent landmark for this end of the arm. Region 21 with two curved dark bands in 21A and three widely spaced ones in 21C serve equally well to identify the proximal end of the arm. Region 20 with two distinct medium sized puffs with a few dark bands may be used to recognize the centromeric end of the chromosome.

Chromosome 3, Right arm

The triplet in 29A and the paired closely approximated heavy dark bands in 29B are diagnostic for the free end of the arm. The series of dark bands in regions 30 and 31 are equally diagnositc for the distal region of the arm. The dark bands at the beginning of 30A are consistently present. The puffs in 30B and 30C are variable. Several landmarks which serve to identify the middle region of the arm include mainly paired dark bands in 32A and 32B and a series of them in 33C, 34A, 34B and 34E. The small puff in 32C is often stretched and variable. The two wavy bands in 32D are constantly present. The small puff in 34D looks

almost empty with faintly staining thin bands. Another series of dark bands including two in 35A, four in 35B, two in 35C and three in 35E and an empty puff in 35D are good recognition areas. A series of paired dark bands including an empty barrel-shaped puff in the region 36 serve to identify the proximal region. Four distinct equidistant dark bands in 37A, three in 37B and another series of four in 37C mark the centromeric end.

Chromosome 3, *Left arm*

A diffuse expansion containing a series of light bands in 46A and 46B followed by a pair of heavy dark bands in 46C characterise the free end of this arm. Thin bands in 46D and 45A are fairly constant. Region 45 containing a large puff in 45B and a small puff in 45C with a 'Bird's eye' at the beginning serves to identify the distal end of the arm. The puff in 45D looks almost empty with pale bands. The paired heavy dark bands in 44B and 44D are always constant. Region 43 as a whole is a good recognition area with a series of wavy dark bands in 43C and diffuse puffs on either side. Region 42 is often stretched, however, the dark bands in 42A, 42C, 42D and 42E are always recognizable. Three curved dark bands followed by a series of thin light bands mark the puff in 41A. The narrow constriction between 41A and 41B is a useful landmark. A series of dark bands in regions 40 and 39 serve to identify the proximal region of this arm of which those in 40A, 40C and 40E are important. The small puff in 39B is stretched in many of the preparations. A triplet of heavy dark bands in 39C is another excellent landmark. The sequence of bands in 39D and 39E is fairly constant. The areas of 38A and 38B are light which are easily recognizable. The vase shaped puff in 38C terminating in a single heavy dark band marks the centromeric end.

Discussion

So far no chromosomal aberrations have been found in this species. However, the heterochromosomes from the female larval brains are interesting. They appear to lack the usual identifiable second arms which probably are very minute to be detected easily as described earlier (Avirachan *et al.* 1969).

Salivary chromosomes of *A. subpictus* are compared with those of *A. pulcherrimus*, *A. stephensi* and *A. gambiae*. It has been very difficult to homologize the X chromosomes among all the species thus far studied under the subgenus *Cellia*. We find some significant similarities in the X chromosomes of *A. subpictus* and *A. gambiae* species, both belonging to the series pseudomyzomyia. But the comparison of the X chromosomes of *A. subpictus* with those of *A. Pulcherrimus* and *A. stephensi* which belong to the series Neocellia is less secure. Unlike in case of species A and B of *gambiae* and *pulcherrimus* where the left arm of the X chromosome has been reported, the X chromosome in *subpictus* has no left arm. However, the whole of region 6 of *subpictus* containing a characteristic large puff compares more closely with the XL of species A and B of *gambiae* rather than that of *pulcherrimus*. Thus, region 6B of *subpictus* with a pair of dark heavy bands closely corresponds to the centromeric end of the XL of the *gambiae* species. In 2R, the large puff in region 7 is nearly identical in all the species. Region 10 of *subpictus*, 13 of *pulcherrimus* and 11 of *stephensi* appear similar. The dark bands

in 17B, 17C and 17D of *subpictus* are comparable to those in 19A and 19B of *stephensi*. In 2L, regions 28 and 27A are very similar in both *subpictus* and *pulcherrimus*. The puffs in 23A of *subpictus*, 23D of *pulcherrimus* and 23C and 23D of *gambiae* are also fairly similar. In 3R, a part of the region 29 of *subpictus* corresponds to the region 29 of *stephensi*. Areas 34E and 37B in *subpictus* are comparable to 35D and 37C of *pulcherrimus*. In 3L, the puffs in 45B of *subpictus* and *pulcherrimus* and those in 44B of *subpictus* and *stephensi* are comparable. The dark bands in 44B and 44D of *subpictus* compare with those in 43A and 43B of *gambiae*.

Any attempt to explain the evolutionary relationships based on the chromosomal comparison alone, that too of a few species would be arbitrary. Hence, more definitive information on intraspecific and interspecific crosses and cytogenetic studies of a large number of species in this group is essential. The pioneering works of Coluzzi and Sabatini (1967, 1968, 1969) and Davidson (1964 *a*, *b*) bear testimony to the practical value of such studies in the subgenus *Cellia*.

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REFERENCES

Avirachan, T. T., Seetharam, P. L., and Chowdaiah, B. N. (1969). Karyotype studies in oriental Anophelines I. *Cytologia.*, **34**, 418–422.

Baker, R. H., Nasir, A. S., and Aslamkhan, M. (1968). The salivary gland chromosomes of *Anopheles pulcherrimus* Theobald. *Parassitologia.*, **10**, 167–177.

Bryan, J. H., and Coluzzi, M. (1971). Cytogenetic observations on *Anopheles farauti* Laveran. *Bull. Wld Hlth Org.*, **45**, 266–267.

Coluzzi, M., and Sabatini, A. (1967). Cytogenetic observations on species A and B of the *Anopheles gambiae* complex. *Parassitologia.*, **9**, 73–88.

————(1968). Cytogenetic observations on species C of the *Anopheles gambiae* complex. *Parassitologia*, **10**, 155–166.

————(1969). Cytogenetic observations on the salt water species *Anopheles merus* and *Anopheles melas* of the *gambiae* complex. *Parassitologia*, **11**, 177–184.

Coluzzi, M., Cancrini, G., and Di Deco, M.(1970). The polytene chromosomes of *Anopheles super pictus* and relationships with *Anopheles stephensi*. *Parassitologia.*, **12**, 101–112.

Davidson, G. (1964*a*). *Anopheles gambiae*, a complex of species. *Bull. Wld Hlth Org.*, **31**, 625–634.

————(1964 *b*). The five mating types in the *Anopheles gambiae* complex. *Rev. Mal.*, **43**, 167–183.

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

Frizzi, G., and Holstein, M. (1956). Etude Cytogenetique d'*Anopheles gambiae*. *Bull. Wld Hlth Org.*, **15**, 425–435.

Kitzmiller, J. B. (1967). Mosquito cytogenetics. *In* : Genetics of Insect Vectors of Disease, *ed. by* Wright and Pal Chapter 4, 133–150, Elsevier Publ. Co., Amsterdam.

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, *ed. by* Wright and Pal Chapter 5, 151–210, Elsevier Publ. Co., Amsterdam.

Sharma, G. P., Ramparshad, Narang, S. L., and Kitzmiller, J. B. (1968). The salivary chromosomes of *Anopheles stephensi*. *J. Med. Ent.*, **6**, 68–71.