The Salivary Gland Chromosomes of *Anopheles jamesi*

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Mosquitoes have become the key attraction for cytological analysis due to their economic importance in human life. Much has been done and still more cytogenetical studies are being carried out on these insects. Because of the considerable interest in the possibility of using the cytogenetical studies on mosquitoes in future programmes of their genetic control, the construction of salivary chromosome maps for more of the mosquito species seems to be essential. Reviews of the pertinent literature on the genetics and cytogenetics of mosquitoes have been presented by Kitzmiller 1953, 1963 and 1967. In India, the work on the tropical mosquitoes still remains in its preliminary stages. The chromosomal maps for only a few of the mosquitoes are available. The present paper provides a standard salivary chromosome map of *Anopheles (Cellia) jamesi*.

The female-adult mosquitoes were collected from the cattle-sheds in the deep forests of Khasi and Jaintia hills of north-east India, Assam and these were reared under laboratory conditions. The cytological preparations out of the well-fed and active 4th instar larvae were made following the procedure adopted by French *et al.* 1962.

Observations

The diploid set of six chromosomes of *Anopheles (Cellia) jamesi* appears in the salivary preparations as 5 euchromatic strands, radiating from a common point, the chromocentre which involves the centromere of each chromosome. The chromocentre is, however, not well defined in this species. The homologues synapse to form 3 chromosomes, a short X and 2 long pairs of autosomes. In the salivary gland nuclei of *Anopheles jamesi* (Figs. 1, 3) the metacentric small sex chromosome appears as X, while the submetacentric and metacentric autosomes appear as chromosome 2 and chromosome 3 respectively.

Owing to certain obvious similarities in the free ends and the banding patterns of the various chromosomes of *A. jamesi* with those of *A. gambiae* (Coluzzi and Sabatini 1967), *A. stephensi* (Sharma *et al.* 1969), *A. pulcherrimus* (Baker *et al.* 1968) and *A. maculatus* (Narang *et al.* 1973), the designations of the chromosomes and their future classification into zones and sections have been done according to the method adopted by them. It has been kept in view that the regions with almost similar banding pattern are assigned the same numbers and letters for a easy comparison with other species and the remaining regions where no similarity could be noticed, have been marked arbitrarily.

The chromosomes have been marked as X, 2R, 2L, 3R and 3L. The entire complement has been divided into 46 zones, X containing zones 1-6, 2R, 7-19, 2L, 20-28, 3R, 29-37, and 3L, 38-46.

Figs. 1-2. 1, a salivary gland chromosome complement of *Anopheles jamesi*. 2, X-chromosome (X) and right arm of chromosome 2 (2R) of *Anopheles jamesi*.

Description of chromosomes (Fig. 3)

X-Chromosome (Average length 105.2μ), 1-6 (Fig. 2)

This is the shortest chromosome of the complement and is quite distinct on account of certain prominent recognizable bands and areas which are specific to

Fig. 3. Salivary gland chromosome map of *Anopheles jamesi*.

it. A single strong band stands out in the middle of the free end which is followed by a dark band in 1A in the constriction. A sequence of 2-2-2 dark bands is exhibited by 2B and 2C, while 2D stains light. Region 3A through 4A is characterized by a series of prominent intensely stained bands, thus, serving as an excellent landmark in the structure of X-chromosome. A remarkably big swelling in 4B with 1-2-1 dark bands combines with another swelling placed at a distance to it in 5A with a band pattern of one pair of dark, a pair of moderate and a characteristic doublet, to constitute another diagnostic feature. A chained dark band can be very easily figured out in 5C. Zone 6 marks the other end of the X chromosome.

Chromosome 2

Right arm (Average length 232.3 μ), 7-19

A regular swelling (7A) with 2 prominent dark bands in its middle along with an extension carrying a pair of dark bands represents the free end of 2R. Two swellings in 7C and one in 8A are remarkably studded with a pair of prominent bands in their middle. Zone 11 is long with 5 swellings exhibiting bands of varying thickness and staining capacity. Zones 14 and 15 are characterized by the presence of certain prominent swellings and bands and serve as landmark for 2R. An unusually long double swelling included in 16D and 17A with certain dark heavy and light bands is quite prominent. 18A comes out with a swelling carrying a solitary thick dark band in the middle. Zone 19 marks the end of chromosome 2R.

Left arm (Average length 160.4 μ), 20-28

The free end of 2L is rounded and is marked by 2 dark bands in 28A. Three swellings constitute 28CD. A characteristic swelling with an alternate sequence of moderate and dark bands is included in 27A. Two equal-sized, small swellings constitute 27B. The first of these has a faint band bounded on both sides by dark bands, while in the other is evident a moderate band which is also guarded by dark bands on both the sides. Areas 26AB and 25A comprise 3 swellings characterized by a majority of light and moderate bands placed distantly. 25BCD is strikingly prominent with certain diagnostic bands. Zone 21 stains lightly except for a prominent doublet at the commencement of the region and another at the end. Zone 20 marks the end of 2L.

Chromosome 3

Right arm (Average length 141.6 μ), 29-37

Free end of 3R is broad with 3 (2+1) prominent dark bands in 29A. Except for a single dark band in 30A, the region 29C through 30B stains light. Three swellings constitute 32AB, whereas 32CD includes a long swelling with bands in an order of 2-2-1 dark, one light and a moderate. Two strong doublets in 33C along with another pair of prominent heavy bands in 34B serve as diagnostic feature of 3R. Zone 37 marks the proximal end of chromosome 3R.

Left arm (Average length 170.8 μ), 38-46

A single characteristic band followed by a dark doublet and a single dark band (46A) characterizes the free end of 3L. Regions 46BC and 45AB are prominent with a crowd of dark bands. A series of 4-5 double bands marks the region 45C through 44A, while 44B-D stains light. Two curved moderate and a light band are seen in 41C, while a swelling with 1-1-2 dark bands (41D) marks the end of zone 41.

Nearly 5 swellings are included in zone 40. Of these, first three (40AB) are small, whereas the last two (40CD) are bigger in size and are separated by a doublet in the constriction. In the middle of the last swelling (40D) are evident 2 closely approximated dark bands. Zone 38 marks the proximal end of 3L.

Discussion

The banding pattern in the salivary chromosomes of various species at the subgeneric level has been observed to show many blocks of genes common to them. The chromosome map of the present species, *A. jamesi* has been compared with those of the other tropical anopheline species of the subgenus *Cellia* and an attempt has been to determine the relationship of these species.

In the general pattern, the salivary gland chromosomes present an identical picture in all the anopheline species irrespective of the subgenus. The X-chromosome is the shortest of the complement, while the two autosomes are longer. The species of the subgenus *Anopheles*, however, differ drastically in their banding pattern from those of the subgenera, *Cellia* and *Nyssorhynchus* and do not show any resemblance with regard to the free ends or centromeric end of their chromosomes. The similarity in the subgeneric pattern is, nevertheless, consistent.

The chromosomal affinities are confined to the members of the same subgenus and they clearly demonstrate a chromosomal relationship. There are several areas within the autosomes in all the species of the same subgenus which are homologous. The X-chromosome, however, shows considerable differentiation of the banding pattern.

Thus far, the salivary chromosome map for only a few anopheline species of the subgenus *Cellia*, namely *A. gambiae* (Frizzi and Holstein 1956, Coluzzi and Sabatini 1967), *A. stephensi* (Rishikesh 1959, Sharma *et al.* 1969), *A. pulcherrimus* (Baker *et al.* 1968) and *A. maculatus* (Narang *et al.* 1973) are on record.

An interesting feature in the study of chromosomes of the subgenus *Cellia* is the designation of their arms. There seems to be a difference in the designation of 2L for 3R in *gambiae* compared with *jamesi*. Chromosome 2L of *jamesi* corresponds to 3R of *gambiae*, whereas 3R of *jamesi* is similar to 2L of *gambiae*. The possible explanation can be a reciprocal translocation of whole arms involving 2L and 3R. A similar situation has previously been reported by Sharma *et al.* (1969) and Baker *et al.* (1968) in *stephensi* and *pulcherrimus* respectively where too 2L corresponds to 3R of *gambiae* and vice versa.

Baker *et al.* (1968) and Sharma *et al.* (1969) gave an explanation that either the translocation of an arm has occurred in *gambiae* or in *pulcherrimus* and *stephensi*. Now it seems probable from the present studies that possibly the translocation might have occurred in case of *gambiae* rather than in *stephensi*, *pulcherrimus* or *jamesi*, for all these species have the same autosomal structure. The designation of arms in different anopheline species of the subgenus *Cellia* has been given in Table 1.

A comparison of the salivary gland chromosomes of *A. jamesi* with those of other species of the subgenus *Cellia* reveals certain regions of remarkable similarities. The regions of similarities in the chromosomes of various anopheline species of the

subgenus *Cellia* under discussion, have been presented in a comparative form in Table 2.

The X-chromosome of *jamesi* and *gambiae* is homologous in many areas. A dark double band occurs in 5A of *jamesi* and *pulcherrimus*, while a prominent dark

Table 1. Designation of arms

<i>jamesi</i> (Present work)	<i>gambiae</i> (Coluzzi and Sabatini 1967)	<i>pulcherrimus</i> (Baker <i>et al.</i> 1968)	<i>stephensi</i> (Sharma <i>et al.</i> 1969)	<i>maculatus</i> (Narang <i>et al.</i> 1973)
2R	2R	2R	2R	2R
2L	3R	2L	2L	2L
3R	2L	3R	3R	3R
3L	3L	3L	3L	3L

Table 2. An approximate comparison of the regions in the various anopheline species of the subgenus *Cellia*

Chromosome	<i>jamesi</i>	<i>pulcherrimus</i>	<i>stephensi</i>	<i>maculatus</i>	<i>gambiae</i>
X-	1C, 2A, 5B				1C, 2A, 5B
	6A	—	—	—	6A
	2B	2B	2B	—	—
	3B	—	3B	—	—
	5A	5A	—	—	—
	5C	—	5C	5B	5C
	6B	6B	6B	—	—
2R	Free end	—	—	—	Free end
	18D, 16B	—	—	—	18D, 16B
	18B, 19BC	—	—	—	18B, 19BC
	14A	14A	—	—	14A
	8A, 15A	—	—	—	8A, 15A
	16D—17A	—	17	—	16D—17A
	12A, 18A	—	—	—	12A, 18A
	8C	—	8C	—	—
	15B—D	15A	16AB	—	—
	19D	—	19C	—	—
2L	25B	25B	—	—	—
	20B, 22	—	—	20B, 22	—
	27B—27D	—	—	27B—27D	—
3R	Free end	—	Free end	—	—
	31A, 32A	—	31A, 32A	—	—
	35A	—	34C	—	—
	30C, 33B	30C, 33B	—	—	—
	30A	—	—	29C	—
3L	38A—D	—	—	—	38A—D
	44A—D	—	—	—	44A—D
	46A, 40D	—	46A, 40D	—	—
	38C, 42A	—	38C, 42A	—	—

band followed by a few light ones is exhibited in 5B of *jamesi* and *gambiae*. A prominent characteristic chain-like band seen in 5C of *jamesi* is also found in *gambiae* (5C) and *maculatus* (5B), while in *stephensi* a pair of dark bands is found instead in

5C. A dark double is common in 6A of *jamesi* and *gambiae*, while 6B of *jamesi* and *pulcherrimus* shows another similar doublet but in *stephensi* a pair of strong bands is exhibited instead.

Chromosome 2R is the most conservative arm in the complement with very few changes from species to species. 2R of *jamesi* shows a close affinity with *gambiae*. The shape of free end of 2R of *jamesi* and *gambiae* is very similar. 16B, 18B and 19BC are homologous in *jamesi* and *gambiae*. Further, the area 16D through 17A is quite identical in *jamesi* and *gambiae*, whereas a similar region is represented in 17 of *stephensi*.

A big swelling in 15B–D of *jamesi* corresponds to the one in 15A of *pulcherrimus* and 16AB of *stephensi*. A sequence of 2+1 dark bands is found in 19D of *jamesi*, whereas such a pattern is witnessed in 19C of *stephensi*.

Chromosome 2L of *jamesi* shows a considerable differentiation of banding pattern from the other species. No striking similarities have been encountered between 2L of *jamesi* and that of *stephensi*. A pair of dark bands in the middle of a swelling in 25B is common in *jamesi* and *pulcherrimus*. The areas 22 and 27B–27D in both *jamesi* and *maculatus* are identical in carrying some dark doublets.

The free end of 3R of *jamesi* resembles that of *stephensi* in carrying a pair of dark bands at the tip and further by the presence of some dark bands in 29B of both. In *jamesi* and *stephensi* sections 31A and 32A are almost alike, while 35A of *jamesi* corresponds to 34C of *stephensi*. In *jamesi* and *pulcherrimus* 30C and 33B are similar. A thick dark band is seen in 30A of *jamesi* and 29C of *maculatus*.

Chromosome 3L of *jamesi* resembles that of *pulcherrimus* in some details of its banding pattern. Areas 38A–D, 44A–D compare reasonably well in both *jamesi* and *gambiae*. Besides a few doublets in 46A, 40D and three dark bands in 38C, a swelling with a prominent dark band in 42A exist in both *jamesi* and *stephensi*.

A few of the above mentioned homologies in prominent bands and band arrangement of certain regions in the chromosomes of all the species compared suggest that these members of the subgenus *Cellia* have at least the same evolutionary line. This study indicates that these different species might have been evolved from a single root and with the inbreeding and cross breeding in nature, a change in their genetical make up has come to exist, thus, furnishing each species with its cytological individuality carrying specific banding pattern in their salivary chromosomes. In the pattern of the morphological grouping of the anopheline species, their cytological grouping is the need of the present. One of the means to determine their exact cytological grouping and then find out the degree of relationship among various species is through hybridization. Certainly the concept of reproductive isolation is the basic one in a study of the process of speciation. It would be desirable to describe definitive phylogenies of the various species within the subgenus *Cellia* to ascertain the relationship and to estimate the relative genetic relationships among all the oriental species. Such a complete picture is, of course, not yet available, but the evidence does indicate the step to be taken in this direction.

Abstract

This paper presents the standard banding pattern in the salivary gland chromosomes of *Anopheles jamesi*, a member of the subgenus *Cellia*. Mosquitoes were collected from the cattle-sheds in the thick forests of Khasi and Jaintia hills of north-east India (Assam). Attempts have been made to compare the banding pattern of the salivary chromosomes of the present species with those of other species belonging to the subgenus *Cellia* and to draw a cytological relationship among them. The observations reveal that there are several areas in the autosomes of these species which are homologous. The X-chromosome, however, shows considerable differentiation in its banding pattern.

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