CHAPTER II
OBSERVATIONS

Culex bitaeniorhynchus

The giant chromosomes in the genus Culex have generally been found to be comparatively longer than those of the genera Anopheles and Mansonia. They exhibit an elaborate phenomenon of asynapsis. In certain preparations, asynapsis has been observed to such an extent that it involves a great length of the chromosomes. Even the already asynapsed halves have been observed to exhibit further asynapsis, thus, confirming further the polytene nature of the chromosomes. No chromocentre has been observed in this species and the chromosomes, thus, lie separately. The sex chromosome is not distinguishable. The diploid number of chromosomes is 6 and all the three pairs of chromosomes are metacentric (Fig.5). The chromosomes have been designated 1, 2 and 3 on the basis of their length, chromosome 1 being the shortest and chromosome 3 the longest. The right and left arms of the chromosomes have been marked by dividing the chromosomes at their centromeres. The centromere in each chromosome is differentiated by a double swelling with some indistinct bands.

For the division of the chromosomes into zones, the method adopted by Dennhofer (1968) for Culex(p.) pipiens
has been adopted. The whole complement has been divided into 70 zones. Chromosome 1R bears zones 1-9, 1L, 10-16, 2R, 17-29, 2L, 30-41, 3R, 42-58 and 3L, 59-70.

DESCRIPTION OF THE CHROMOSOMES
(Fig.10, Ph.10)

Centromere-I (Average length 10 μ)

The centromere is present nearly in the middle of the chromosome I and it is constituted by two swellings of unequal size, carrying some thick dark bands and also there are present a few thin and weak light bands. The two swellings of the centromere at their constriction have 2 darkly stained strong bands forming a sort of waist of the centromere. The centromere lies in zone 9.

Centromere-II (Average length 7.5 μ)

The centromere of chromosome 2 is again made up of two unequal swellings with a few light and thin bands. Of the two swellings, the one towards the right arm of the chromosome, is comparatively smaller in size with faint bands, while the other towards the left arm, on the contrary, is bigger in size, with 3 faint bands in the middle and 2 light thin bands near the constriction towards the left arm. The centromere lies in zone 30.
Centromere-III (Average length 10 μ)

Centromere of chromosome 3 is restricted only to two big swellings, looking alike in their size and band pattern. The centromere lies in zone 58.

Chromosome-1

It is the shortest of the chromosomes. The two arms right and left of this chromosome are demarcated at a point where the centromere is differentiated. The centromere is present nearly in the middle of the chromosome.

Right arm (Average length 55 μ), 1-S

The telomere (free end) of the right arm of this chromosome is typically fan-shaped with a flared tip. 1A is characterized by the presence of 6 dark bands in a succession and following them is a small swelling exhibiting two light bands. In 2C and 3B there are present thick intensely stained single bands. However, a majority of the bands of the swelling in 3A, B, C are heavily stained. Regions 4A, B, C possess a series of dark thick bands present in a big swelling which can well be considered as an excellent landmark for this arm. A series of 5 dark bands in the area 6C-7B and a thick double band with a high staining capacity present in 8B are quite prominent.
Left arm (Average length 52 μ), 10-16

The club-shaped free end of this arm is characterized by the presence of a thick darkly stained band in 16B which is followed by 3 broad heavily stained bands in the same region. The telomere exhibits a heavy thick dark band and a faint band in 16C. The following region 16A reveals 2 moderate straight bands. The two dark bands in 15C and a characteristic doublet in 15A are the diagnostic features of this arm. The following area 14C, B has got some moderately and lightly stained bands. Again at a distance from it a series of lightly stained bands is evident in sections 13B, 13A and 12C. The two dark bands present in the region 12B mark the constriction between the two swellings, one each in 12C and 12A. A small swelling with 2 dark and a moderate band in 11C, another swelling of similar size with centrally placed thick, heavily stained band in 11B together with a comparatively big swelling rich in bands present in a sequence of a dark double- a moderate- a dark straight- a light and 2 dark bands in 11A, form a prominent landmark in the construction of this arm. In continuation of this richly banded area can be seen a small swelling in 10C, which is marked by the presence of 5 straight thin darkly stained bands.

**Chromosome 2**

Considering the length of the chromosomes, chromosome 2 is comparatively much longer than chromosome 1.
but smaller than chromosome 3. The two arms of this chromosome are somewhat equal in length.

**Right arm (Average length 102.5 μ), 17-29**

The free end of this arm resembling a kite in shape, is marked by the presence of a series of 8 straight bands of varying staining capacity. The small swelling present in 17B following the telomere has again another series of straight dark bands. The two big equal swellings present one in each 18A, B are separated by 2 dark bands forming a bracket at the constriction. The swelling in 18A has a single thick heavily-stained band on either side of which are visible dotted and light bands. The two darkly stained thick bands are prominent in the swelling present in 18B. On the contrary, the swelling in region of 18C possesses three light bands. A doublet with dark stain in 19C with a dark band on either side towards the free end of 2R and a moderately stained band towards the centromeric end collectively constitute a swelling which is further followed by a prominent region of dark bands made up of two swellings of different sizes. Out of these, the smaller one located in 19C has 3 dark bands in a row. The following big swelling is separated from the swelling on its either side, by two dark bands present in the contractions. In the big swelling, occur a curved dark thick, a light(20A),
a dark pair (20B) and a moderate band (20C). A small swelling in 21A carries only a single light band, while in the following swelling in 21B occurs a thick dark band. 21C is prominent on account of the presence of a pair of dotted bands. Section 22A with a dark band and a faint band in its swelling is followed by 2 small swellings, each with 2 dark bands representing 22B and 22C respectively. 22D depicts a single light band. In zone 23 occurs a chain of bands in the order of a dark- a dotted- a doublet in the constriction- 2 light and a dark band in the constriction. A typical and diagnostic band forming a Z present in the middle of a big spindle-shaped swelling with a light and a dark band at the constriction in zone 24, is a landmark for this arm. The swelling next to it is again characterized by the presence of a pair of prominent dark, broad, curved bands in its middle in 25A. 25B and 25C carry 2 bands each. Zone 26 is very small with 2 dark bands in 26A and a dark, a light in 26B. Zone 27 stands with some dark bands. A characteristic thick pair of dark bands in 27A is followed by one light and 2 dark bands in 27B. The two dark bands along with a very thick and prominent dark band represent 27C. Two swellings with a sequence of 2 moderate, a dark, a light, a thick dark and a moderate band constitute zone 28. Zone 29 is dark in its appearance due to the occurrence of closely placed dark bands of varying thickness and make it a prominent area
present near the centromere. 29A is characterized by 3 closely placed dark bands and a parenthesis doublet. 29B carries a typical pair of dark broad bands and 29C exhibits a single dark band.

Left arm (Average length 91.7 μ), 31-41

The free end of 2L falls under section 41C. It is marked by the presence of a light and 2 dark bands and is club-shaped. The swelling included in 41B carries in its middle evenly placed 2 dark bands. In 41A occur 3 pairs of dark, light and dark bands and an additional moderate one. Zone 40 is small with a centrally placed dark doublet having 2 moderate bands on one side and 2 light ones on the other. Zone 39 is typical in having 4 pairs of bands, of which the one in 39A is lightly-stained, while the others are dark. A swelling in 38D and 38C shows a pattern of a moderate- a light dotted- a straight light- a moderate dotted and a dark band. 38B, A are marked by the presence of a series of 6 dark transverse bands. The typical swelling in 37C carries a single light band in the middle with dark bands on either side. 37B and 37A collectively have 1 dark band on either side of a centrally placed thick dark band. A single dark, broad band in 36D and a striking dagger-shaped dark doublet in 36B with a dark band in the centre of 36C constitute a prominent landmark for this arm. 36A is constituted by a
pair of dark bands. Zone 35 is short with 3 moderate evenly placed bands. Zone 34 is made up of a series of dark bands distributed as 2 dark in 34C, 2 dark and a bracket-shaped dark doublet in 34B and a light and a broad dark in 34A. A row of bands with varying staining capacities occurs in zone 33 in the sequence of a thin dark doublet- a thin medium in 33C, a light- a thin dark in 33B and 2 light in 33A. Zone 32 is well marked and can be recognized by the presence of some diagnostic thick dark bands. A big swelling with centrally placed 4 broad characteristic bands with a set of 2 light bands on either side is worth considering as a landmark for this arm. The following bands in 32A give a picture of a thick doublet and 2 prominent dark bands. Zone 31 is comparatively long and includes 6 swellings with some dark bands. A single swelling in 31C has a series of 3 light and 4 (1+2+1) dark bands. In 31B are present 2 swellings carrying a moderate, a light and 3 dark bands. All the bands in 31A are darkly stained with varying breadth except 2 bands which are light and are present in the last swelling.

**Chromosome 3**

It is the longest chromosome of the complement with centromere placed approximately in the middle. Certain
prominent bands and regions in this chromosome serve as landmarks for its easy identification. The two arms, right and left are marked from a point where the centromere is present.

Right arm (Average length 175 μ), 42-57

Zone 42 is prominent on account of the presence of a free end which carries 2 broad dark bands at the tip. A big remarkable puff-like swelling in 42B, C is quite evident to serve as a landmark in the structure of 3R. A strong band present at the neck separates the puff from the free end. The puff shows a diffused area with 2 distally placed bands and a dotted, a dark at its end. Four swellings constitute zone 43 and the one present in 43B possesses 4 (1+2+1) dark bands, two of which are closely placed. Zone 44, though it is short, is characterized by the presence of a series of 5 dark bands and a dotted one inbetween. The three prominent oval doublets darkly stained present in 45A, B, C together with two fairly big swellings in 45C, 46A, B with a chain of transverse dark bands, are sufficient for the recognition of this arm. Region 46C to 48B carries a series of small swellings. 46C stains light. A thick dark band in 47A is quite prominent and a similar band is present in 48B. 48A is characterized by the presence of three prominent dark bands. Except for a pair of darkly-stained bands in 49A, the area
from 48C-49C stains lightly with widely spaced bands. Zones 50 and 51 are quite short and are represented by a small and another big swelling bearing bands in the order of 2 dark- 3 light and 4 dark. The bands in zone 52 are compactly placed. 52A contains 2 light, and 2 dark bands which are repeated in 52B. 52C carries all the bands moderately stained. The two swellings of 53A and 53B, carrying a single band in their middle region, bear 2 light bands at their constriction. Zone 54 is long and stands out with prominent double bands each in 54A, C, D, while 54B is conspicuous by a few dark prominent bands. A doublet in 55A and 4 dark bands in 55B, C and 2 light, 2 dark in 55D constitute zone 55. A characteristic dark doublet at the constriction and a single dark in the middle of the swelling represent 56A. A chain of straight dark bands in 56B, C make the region easily recognizable. Zone 57 carries certain conspicuous dark bands serving as a good marker for the arm. A pair of one thick and one thin dark in 57A, a broad dark at the start of 57B and a similar dark band in 57D can easily be figured out.

Left arm (Average length 161.7 μ), 59-70

The free end of 3L gives an appearance of a flask with quite a thick dark band in the middle of the swelling in 703 and preceding it are a curved dark and 2 dark bands at the tip in 70C. The following region is narrow with small
swellings. A characteristic dark band in 69A is conspicuous. Rest of the zone 69 contains bands with moderate and light staining capacities. The bands in zone 68 are very closely placed and make it prominent as majority of them have a high staining capacity. 68C reveals 4 dark bands, while 68B has a straight dark and a pair of a straight and a curved dark. 68A, however, contains a single dark and 2 light bands followed again by a dark one. The bands in 67C, B are weak and usually break. A big swelling with lightly-stained bands is evident in 67A. The bands of varying staining intensity are distantly placed in zone 66. A long area constituted by zones 65, 64 and 63 serves as an excellent landmark for this arm. The bands in this area are characteristic and compactly placed. Zone 65 possesses 3 small swellings with 3 prominent dark and a moderate band in 65C, a straight dark accompanied by a characteristic dark and 2 moderate in 65B, and an oval prominent doublet, a curved dark in 65A. The bands in zone 64 are present in the order of 5 (2+2+1) dark - a light in 64D, 3 dark in 64C, a light- a dark in 64B and 3 dark in 64A. Zone 63 begins with a small swelling having a dotted band in its middle. It is followed by 2 prominent big swellings. The one in 63C bears at its either constriction thick distinct dark bands. Besides 2 thin dark and a light band, there is present a characteristic thick dark band in this swelling. 63B is marked by 3 light
and 2 dark bands. 63A is peculiar in the existence of one pair of light at the beginning and 5 pairs of dark bands, which are followed by 2 separately placed light bands. Two characteristic dark doublets in 62C, 4 prominent dark bands in 62B and a single curved thick dark in 62A are sufficient to make the zone 62, a diagnostic feature of this arm. Nearly all the bands stain heavily and are distantly placed in zone 61. Two dark bands at the beginning and the following dark band in 60C together with a small and another very big swelling in 60A with a pair of prominent dark bands in each, are evident. Zone 59 is characterized by a triangular doublet in 59C and a broad intense band in 59B. However, 59A contains a few dark and light bands.
DISCUSSION

Relationships within the genus Culex

So far the salivary chromosome maps have appeared for only two species of the genus Culex, viz., C. a. aipiens (Dennhofer, 1968) and C. a. fatigans (Sharma et al., 1969). Three more species, C. fuscocephalus, C. gelidus (Pasahan, Ph.D. thesis), C. vishnui (Chaudhry, Ph.D. thesis), have also been worked out in this laboratory. A comparison of the banding pattern in Culex bitaeniorhynchus has been made with that in the other species worked out so far.

The chromosomes in Culex bitaeniorhynchus have been found to be longer as compared to those in the genera Anopheles and Mansonia.

All the species viz., Culex a. aipiens, C. a. fatigans, C. bitaeniorhynchus, C. fuscocephalus, C. gelidus and C. vishnui belong to the same subgenus Culex.

A comparison of the chromosome maps of these species shows a good deal of resemblance in their banding pattern. The differences in the chromosome lengths are shown in Table-III.

The centromere-I in Culex bitaeniorhynchus is constituted by two swellings which are unequal in their size. The swellings exhibit certain dark and light bands. A sort of waist is formed due to the presence of 2 strong bands at the
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**TABLE III**

Chromosome Lengths in Microns
constriction between the 2 swellings. A somewhat similar is the centromere-I of pipiens though in this case the swellings are long and seem extended on their longitudinal axis. The centromere-I in fatigans also is formed of 2 unequal swellings carrying 2 dark broad bands at the waist. Centromere-I in bitaeniorhynchus and fuscocephalus seems interestingly to be similar except for the reverse position of its two swellings. In fuscocephalus, out of the 2 swellings one towards 1R carries 2 dark bands, while a similar swelling of the centromere-I with 2 dark bands in bitaeniorhynchus is present towards 1L. Similarly, the other swelling with 2 light bands in the middle and a doublet in constriction (9C) in fuscocephalus is present towards 1L but in bitaeniorhynchus the similar swelling is seen towards 1R. Centromere-I of gelidus and vishnui too is constituted by 2 swellings which are marked by a few faint bands. Centromere-I is alike in all these species inasmuch as it is formed by 2 swellings.

The centromere-II in bitaeniorhynchus is again made up of 2 unequal swellings with a few light and thin bands. A similar type is met with in pipiens also. The two swellings of the centromere-II in pipiens are separated by a chord of fine relatively weakly stained bands, whereas, in bitaeniorhynchus there is only one light band. At the constriction of the two swellings of the centromere-II of
fatigans are present 2 darkly stained bands and the two swellings show almost a similar band pattern except for an additional dark band in the swelling towards 2R. Centromere-II in fuscoccephalus, gelidus and vishnui is made up of 3 swellings instead of 2 as is found in bitaeniorhynchus.

A wide dissimilarity is evident in the structure of the centromere-III of pipiens and bitaeniorhynchus. Centromere-III in pipiens is unusually long, being formed of nearly 8 swellings carrying some diffused bands. In gelidus also it is long and is formed by five swellings. On the contrary, in bitaeniorhynchus, it is restricted to only 2 big swellings looking alike in their size and band pattern. The condition in vishnui, is however, more close to that in bitaeniorhynchus inasmuch as it is formed of 2 swellings. In fatigans and fuscoccephalus an additional swelling towards 3L is also seen.

Chromosome 1 in bitaeniorhynchus shares the banding pattern with pipiens, fatigans and vishnui and to some extent it also resembles fuscoccephalus and gelidus. The presence of 2 dark bands in 12B is a common feature of all these species.

The free end of 1L of bitaeniorhynchus is identical to that of fatigans and gelidus. The free end of 1R in both bitaeniorhynchus and vishnui reveals similar bands.
1A\(B\) of *bitaeniorhynchus* corresponds to 1A-C of *vishnui*.

12C in *bitaeniorhynchus*, *fuscocephalus* and *gelidus* is homologous. Zone 13 stains light in *bitaeniorhynchus* and *gelidus*. *Bitaeniorhynchus* and *piniens* are related in carrying a similar band sequence in 14A, 8B, 16BA and 11CA. A small swelling in 6A of *bitaeniorhynchus* corresponds to a puff in 6A of *piniens*. 1C with 2-1-2 series of prominent dark bands in a swelling of *bitaeniorhynchus* correspondingly resembles to 4A of *fuscocephalus*. The bands in 16A and 15C of both *bitaeniorhynchus* and *faticans* are almost alike. Regions 2C and 4AB of *bitaeniorhynchus* correspond to 3C and 8B of *vishnui*. Besides a swelling (14C, 15A) with 2 moderate and one characteristic doublet in *bitaeniorhynchus* corresponds to a similar swelling in 14B of *vishnui*.

Chromosome 2R of *bitaeniorhynchus* contains many areas which have strong resemblances to similar areas in *vishnui* and *piniens*. A club-shaped free end of 2R in *bitaeniorhynchus* with a pair of dark bands in the middle (17A) and a few dark bands in (17B), resembles the free end 2R of *vishnui*. The bands in zone 20 of *bitaeniorhynchus* correspond to similar bands in 25 of *fuscocephalus* and 24A of *vishnui*. The bands in 2 swellings present in 18AB of *bitaeniorhynchus* are identical to 2 similar swellings present in 20BC-21A of *fuscocephalus* and in 19AB and 20A of *vishnui*. Sections 28C and 29B are homologous in *bitaeniorhynchus* and *piniens*. 
The banding pattern of 2L of *bitaeniorhynchus* shows many similarities to those of *vishnui* and *pypiens*. A strong double band in 40A of *bitaeniorhynchus* is also evident in *fatigans*. Section 32B is alike in banding pattern in *bitaeniorhynchus*, *pypiens* and *gelidus*. Regions 38BA and 33CB compare favourably in *bitaeniorhynchus* and *pypiens*.

41BA of *bitaeniorhynchus* possesses a banding pattern similar to the one in regions 40 and 39DC of *vishnui*. Area 34C-31C of *bitaeniorhynchus* remarkably corresponds to the area 36B-33C of *vishnui*.

The club-shaped free end of 3R in *bitaeniorhynchus* is identical to the free end of 3R in *fatigans* and *vishnui*. Darkly stained double bands in each of 52B, 54AC, and 56A are observed to occur in *bitaeniorhynchus*, *pypiens* and *fatigans*. Further, double bands are also seen in 57A of *bitaeniorhynchus* and *pypiens*. *Bitaeniorhynchus* resembles *pypiens* in carrying similar bands in 43B. 46AB is identical in *bitaeniorhynchus* and *gelidus*, whereas 49B of *bitaeniorhynchus* corresponds to 43C of *gelidus*.

Areas 50B and 51 of *bitaeniorhynchus* very well correspond to 46C of *vishnui*. Furthermore, a few double bands in 49A and 45 in *bitaeniorhynchus* are found to exist in regions 46A, 43C and 44 of *vishnui*.

The free end of 3L is very similar in *bitaeniorhynchus*, *pypiens*, *fuscocephalus* and *vishnui*. The existence of 2 dark
bands in 613 is a common feature for *bitaeniorhynchus*, *piciens* and *fatigans*. 2 dark bands exist in 67B of both *bitaeniorhynchus* and *fatigans*. A striking similarity is evident in 3L of *bitaeniorhynchus* and *piciens* in having a doublet in 60C and a prominent intensively stained broad band in 59B of both the species. Two dark bands with a light inbetween (66C), 2 dark (65C) and a swelling with four dark transverse bands (62B) in *bitaeniorhynchus* are repeated in similar regions of *vishnui* too. Further, 67A of *bitaeniorhynchus* corresponds to 69A of *vishnui*.

The phenomenon of asynapsis is quite common in *Culex bitaeniorhynchus*. It has been observed that asynapsis is more frequent in the chromosomes found in the hepatic caecae and the Malpighian tubules where it extends to a great length of the chromosomes. However, the chromosomes in the salivary glands exhibit comparatively less of asynaptic regions. Sutton (1942) mentioned that polytene chromosomes of the Malpighian tubules of *Culex piciens* lie completely apart. Later Kitzmiller and Clark (1952) too reported asynaptic regions in the salivary gland chromosomes of *Culex piciens*. These asynaptic regions are not always constant and are changeable. In well spread preparations, these gaps are rarely seen. These asynaptic regions are perhaps the result of a combined effect of more of pressure during squashing and the loose synapsis of the homologues.
The nature of the similarities suggests that *bitaeniorhynchus* is perhaps more closely related to *vishnui* than it is to the other oriental species of the subgenus *Culex*. Furthermore, *bitaeniorhynchus* and *vishnui* show a close morphological relationship too. A study of more and more species of the genus *Culex* will, therefore, help in proper grouping of the species.

**GERM CELLS**

The chromosomes of *Culex bitaeniorhynchus* are of the "pipiens" type. There is no evidence for the distinction of the heterosomes. The diploid number 6 and the chromosomal karyotypes of all the Culicine mosquitoes studied so far are surprisingly uniform. There are 3 pairs of metacentric chromosomes, 2 longer pairs of almost equal size and one pair considerably smaller. However, Baker and Aslamkhan (1969) reported new karyotypes for 3 *Culex* species viz., *C. fuscanus, C. raptor* and *C. gelidus*, in which chromosome 2 is considerably shorter and approximates chromosome 1.