OBSERVATIONS

A. Salivary Gland Chromosomes

1. Anopheles tesselatus

Anopheles tesselatus Theobald is very commonly distributed in India and Malayan orient to Java, Philippines, Thailand and Yunnan. The salivary gland chromosomes of this species show several homologies with other members of the subgenus Cellia, A. gambiae and A. stephensi.

The X chromosome is the shortest and measures 57 μ in length. Chromosome 3 has arms of unequal length; 2R measures 178 μ and 2L, 168 μ. Chromosome 3R measures 225 μ and 3L, 140 μ. All these chromosomes are attached at the chromocenter which has been observed in many slides (Fig. 1). More commonly, individual chromosomes spread separately with both the arms joined together by the centromere (Fig. 2). In some preparations the chromocenter is difficult to locate due to its weak nature. As these chromosomes bear a considerable resemblance to those of A. gambiae and A. stephensi, the same zone numbers for the arms have been used. The X-chromosome is divided into zones 1-6; 2R, zones 7-19; 2L, zones 20-28; 3R, zones 29-37; and 3L, zones 38-46 (Fig. 3).

Diagnostic Features of the X-Chromosome

The X chromosome may be readily recognized as the shortest. At its free end are a pair of thin dark bands and two widely separated characteristic bands in the adjoining region of 1A
Figure 1. Salivary chromosomes of Anopheles tesselatus. 2R = Right arm of chromosome 2; 2L = Left arm of chromosome 2; 3R = Right arm of chromosome 3; 3L = Left arm of chromosome 3; C = chromocentre.
Figure 2. Salivary chromosomes of Anopheles tesselatus. Individual chromosomes 2 and 3 are separated, each with both arms joined together by the centromere.
Figure 3. *Anopheles tesselatus*. Salivary chromosome map.
and 1B, often marked by a constriction. A series of three very prominent dark bands occurs in 1C. Two heavy dark bands in 2C, followed by two doubles and a single dark band in 3B constitute a very diagnostic feature of the X chromosome. A prominent puff, variable in size in 4B with two sharp bands in 4A and two doubles in 5A are typical of the central region. The centromeric end is marked by the presence of a series of three very dark doublets in 6B and 6C.

Detailed Description of the X-Chromosome

The free end of the X-chromosome is characterized by two heavy dark bands, which are followed by a dark, two light ones, and then by another dark band at a narrow constriction in 1B. The three heavy dark bands are very consistent and is a diagnostic feature of region 1C. Preceding these dark bands are two light dotted bands in region 1B.

A very dark thin band which is sometimes twisted or shortened is always present at the end of 2A and is preceded by a series of light thin bands of different intensities as shown in the map. A dark thin dotted band is characteristic at the end of 2B, in front of which are a few light bands. Two thick deeply stained bands mark the end of 2C.

A series of three light doubles are characteristic of region 3A. Two prominent dark doublets and one heavy dark band, sometimes widely separated, constitute the most characteristic area of 3B. Regions 3C and 3D are mostly composed of a series of light thin bands. The diagnostic puff with a pair of dark dotted bands in region 4B is preceded by a pair of
widely separated dark heavy bands in 4A and is followed by 2 moderately staining bands in 4C. The rest of the bands in 4B are very light and dotted. They are even indistinct in most of the preparations.

A series of two dark doubles with a light band in the middle, in zone 5A is followed by a light area in 5B and 5C. This region contains numerous thin variable bands. A thin sharp band at the beginning of zone 5B and the somewhat lighter band at the beginning of 5C are always present; 5B is almost always twisted. Two dark heavy bands, separated by three lighter ones occur in 5C and 6A. 5C is always involved in a small puff.

The deeply staining band at the beginning of 6A is followed by a series of two doubles situated in a puffed region. The three dark doublets in 6B and 6C constitute a diagnostic land-mark of the centromeric region. A flared broken double dotted dark band marks the end of 6C. 6D is variable. In some preparations it is detached from the rest of the chromosomes. In others it is attached by a very small euchromatic connection. In some it is so much embedded in the nucleolar region as to obscure the detailed banding pattern. Most of the preparations reveal the band pattern as shown in the map. Tentatively, the region 6D is treated as a very short arm of an almost acrocentric X-chromosome. This is not certain. There never is a clear, unmistakable association with the X, rather at best, some weak, darkly staining connections. (Fig. 4)
Figure 4. X chromosome of Anopheles tesselatus.
C = centromere region. 6D = short arm of the X-chromosome
Diagnostic Features of 2R

A flared tip consisting of a few lightly-stained and dark bands which are followed by four sharp bands at the end of 7A and a large puff in region 7B are characteristically recognizable. Three heavy bands, two of which are doubles are typical of 8A. The next most prominent region is composed of two groups of three sharp, dark and closely spaced bands in a small puffed region of 10C and 10D. A series of heavily stained bands in a 1:2:1 doublet:2 doublet order constitute a landmark in 11D and 12A.

An area consisting of very heavy dark bands, in three groups of 3 bands each, occurs in regions 14C, 14D and 15A. Some bands in the area are not so heavily stained, but in a general view they all look very dark. The centromeric end can easily be recognized by the presence of a series of lightly stained bands in region 16C and 17A. Two very dark and thick bands which have three medium stained bands between them are typically found in 18B and 18C.

Detailed Description of 2R

At the free end of this arm the characteristic pattern consists of the flared tip, followed by a large and a small puff. The two thick dark bands at the tip and two thick dark bands sometimes appearing as a doublet in a constriction in 7A always stand out. The large puff in 7B is for the most part lightly stained, except for two dark bands at the end of 7B. The small puff in 7C has nine light and medium
staining bands as shown in the map. In most of the prepara-
tions three distinct doubles, mostly dark and dotted, stand
out in the middle of the small puff.

A series of bands in 8A which form a 2:1:2 pattern and
a group of four dark close bands at the beginning of 9A are
diagnostic. Most of region 8B is light and indistinct. 8C
contains one dark band followed by two light bands at the
beginning, followed by a series of four bands of which the
second always appears the darkest. One thin sharp band
flanked by two light ones occurs in the puff in 9A. Following
is a series of thin light bands in 9B. 9C, which is always
situated in a constriction, has four sharp bands, of which
the first three are thin and the last quite heavy. The large
puff in 9D and 10A is twisted in most of the preparations.
The band pattern shows a series of evenly spaced light and
medium bands, but the two bands in 9D and one in the beginning
of 10A serve as a recognition area.

Both 10C and 10D each contain a group of three sharp,
dark and closely approximated bands and constitute one of
the landmarks of the anterior part of the arm. 10B is a light
diffuse area except for one medium band in the beginning and
two light bands at the end. The next good recognition area
is comprised of two wide, thin dark bands in the middle of
a puff in 11C and a series of nine dark prominent bands in 11D
and 12A. The six dark bands in 12A occur as three doublets.
This area may in most cases be used as a starting point for
comparing the banding pattern for the rest of the arm. 11A
and 11B contain mostly light bands.
12B and 12C are also light except for two thin dark bands at the end of 12B and one sharp band at the end of 12C, which along with two darks bands in the beginning of 12D always stand out as a group of three closely associated dark bands. These are followed by a pair of dark bands which often appear as a doublet in the middle of 12D.

The next prominent landmark is situated at approximately one third the distance from the centrometic end and is marked by the presence of a group of three distinct bands in 14C, four bands in 14D and three bands in 15A. However, this group of a total of ten bands appears as two dark areas with bands in a 2:3 pattern in most of the preparations. The details are shown in the map.

Four bands in two pairs, of which the third is the darkest are flanked on either side by many thin light dotted bands in 13D and 14B. A single heavy band at the end of 13A, followed by a series of three groups of two bands each in 13B and 13C, always stands out. A few bands at the end of 12D and the beginning of 13A appear as an indistinct dark area.

One thin dark band in the middle of 15C followed by a group of three closely approximated dark bands at the end of this region is typical. This region is often twisted. The end of 15 B is involved in a constriction which appears twisted in some preparations. The detail is shown in the map. 15D has three evenly spaced bands in the middle and two dark bands at its end.
Region 16B often appears as an elongated puff bearing six bands in three groups of which the two middle bands are comparatively darkly stained. 16A is a light area except for one heavy narrow double band in the middle. 16C and 17A likewise have indistinct variable bands except for two medium bands in each. A single thick dark band at the beginning of 17B followed by a group of three distinct bands also in 17B and one heavy dark band in the beginning of 17D always stand out.

The typical pattern of one doublet at the end of 18B followed by two medium dotted bands, one thin medium band and a dark heavy band in 18C constitutes one of the excellent landmarks of the centromeric region. The rest of 18B and most of 18A are lightly stained areas. However, one dark doublet at the beginning of 18A preceded by three evenly spaced bands is always present.

The centomeric end of this arm is marked by a thick dark band at the tip in 19C, two dark bands at the end of 19B and two medium bands in 19A. The rest of region 19 appears as a light area.

Diagnostic Features of 2L

Three bands of medium intensity in region 28A of the usually spatulate free end and a wide dark doublet followed by a single dark band at the end of 28C and very prominent. The next recognizable area occurs in 27A and 27B and consists of two pairs of heavy double bands, between which are one band of medium intensity and several lighter ones.
A puff in region 26C and 26D containing a wide thin dark band in the middle is characteristic. Two groups of heavy dark bands, each consisting of five and four bands respectively in regions 25B and 25C are diagnostic. Regions 24B and 24C are characteristically the narrowest part of the arm.

Two thick dark bands with two light bands between them are characteristic of 24B. Three dark bands occur in a puff in 23B. Following is a typical narrow area containing five dark thin bands of which the center at one is always the lightest. Region 22 contains many bands of which the most prominent is a wide double band in 22H. Region 21 is marked by a series of dark bands in 21B and four dark bands in 21D. Between these two groups of dark bands is a characteristic series of five thin light bands.

Detailed Description of 2L

The free end of 2L is semi-flared with two closely approximated dark thin bands and three medium stained bands in region 28A. A series of thin light bands in 28B and one very thin dark doublet at the beginning of 28C are involved in a constriction. A very prominent doublet followed by a single dark band at the end of 28C and a series of lighter bands in 28D are typical. The latter part of 28D and the beginning of 28E show a constriction in most of the preparations. This area contains several evenly spaced bands of medium intensity. Most of regions 28E and 27A are expanded into a puff, with two dark bands at the end of 28E forming an always recognizable area.
The end of 27A and the whole of 27B are involved in a very narrow characteristic constriction in which two dark bands each in 27A and 27B always stand out. 27C contains four evenly spaced thin sharp bands with light indistinct bands interspersed among them as shown in the map.

The next prominent recognizable area is a swelling in 26C and 26D. A dark doublet at the end of 26D clearly stands out. 26A is a lightly staining area with three bands of medium intensity at the end. 26B contains a single heavily staining band flanked by a series of lighter bands on either side. The characteristic puff in 26C and 26D contains one dark band in its centre and another thin sharp band at the beginning of 26C with a few light dotted bands in between. Two widely separated bands of medium intensity precede the diagnostic doublet in 26D. Region 25 is an excellent recognition area containing four groups of prominent dark bands in each of regions 25B, 25C, 25D and 25E. Two groups of widely spaced doubles, the last of which is darkest constitute a landmark in 25A. The dark area of 25B contains one sharp band, two dark doublets followed by a light and a dark prominent band. A group of four closely approximated thin dark bands in 25C are followed by a few light bands with a single heavy dark band at the end. Towards the end of 25D are situated four evenly separated bands of which the second and fourth are the most darkly stained. Similarly, 25E is marked by the presence of three widely separated dark heavy bands, with three lighter bands at its end.
Region 24A appears twisted most of the time obscuring a puff which is evident in a few preparations. Two dark bands at the beginning of the puff are always present. This is followed by a very narrow area in 24B and 24C. In 24B are two dark bands which most of the time appear indistinct and broken. The rest of the area is lightly stained. A single sharp band each marks either end of 24D. 24E has two dark bands with alternate light bands.

The next easily recognized area is a small puff with a series of three very prominent bands in 23B followed by a narrow constriction in 23C containing evenly spaced thin, dark bands in a 2:1:2 pattern. 23A is for the most part lightly stained except for three thin sharp bands of which the last two are closely approximated doublets. Region 23D along with the whole of region 22 and 21A is marked by a series of dark and light bands, making this diagnostic for the centromeric end of the chromosome.

A single dark band at the beginning of 23D followed by a few light bands and a series of three doublets at the end almost always stand out. A pair of thin sharp bands situated in the center of a constriction in 22B flanked by light bands on both sides and a single prominent band in the beginning of 22C are also typical. Situated in the center of a puff in 22C and 22D are four dark bands in two groups of two each. A small puff in 22H contains two closely approximated dark heavy bands. One doublet in 22G and a group of four dark bands in 21A are as shown in the map.
22E has two widely separated dark bands followed by a lightly staining area of 22F. Region 21B contains a 1-3 patterns of dark bands followed by five thin light bands which in turn are followed in 21D by four dark bands of medium intensity. The centromeric end of 2L is marked by seven clear bands in the order of 1:2:3:1 in 21E, a few light bands in 20A and 20B and one dark band at the end of 20B. One thin sharp band at the end of 20C along with a dark one at the tip of the chromosome in 20D always stands out. The rest of the region in 20C and 20D is lightly staining.

Diagnostic Features of 3R

The free end of this arm can be easily recognized by its semiflared tip with two dark bands in 29A. The posterior end of 29A is almost always stretched so that the puff in 29B and 29C appears comparatively more conspicuous. The next easily recognizable area is comprised of three series of dark bands in 29C, 29D and 30A. These often appear as 2:4:2:4 series of dark bands, widely spaced, but the detailed pattern is as shown in the map. A prominent series of three closely associated bands with lighter bands between them usually giving the appearance of a granular area, always stands out in 30D. The three sharp bands at the end of 31G followed by the five lighter bands in 332A and three closely approximated bands at the end of 32A can be reliably used to identify this section of the chromosome. A series of dark prominent bands in 32D, 32E, and 32F constitute a landmark for this region. These five bands in 32E and 32F almost always appear as three dark, one medium, then a final dark one. Detailed
study shows that the first two are doublets as shown. The next most easily recognizable area is composed of three thin sharp close bands in 34D followed by a series of thin light bands and about five prominent bands in 34E.

The centromeric end is conspicuously marked by several diagnostic areas. In 36A and 36B is a prominent puff preceded by four dark bands, two sharp bands in the centre of the puff followed by two dark bands at its end. Another prominent puff occurs in 36C with a series of three doubles. A dark diffuse area in 36D and 36E is composed of 2 dark bands, three lighter ones and 3 dark ones. A prominent dark band in the middle of a puff in 36E and 36F always stands out. The narrow dark patch composed of one dark band at the end of 36F and three dark bands at the end of 36G cannot be missed. The centromeric tip likewise is conspicuous by the presence of three dark bands in 37D and a continuous series of many sharp bands in 37F, 37G and 37H.

Detailed Description of 3R

The diagnostic free end in 29D and 30A is followed by a very light area in 30B and a small puff in 30C with a series of dark bands as shown in the map. The granular area in 30D is followed by one dark band at the end of 30D and by two dark bands in 31A. Regions 31B, 31C and 31D contain many thin, light, sometimes dotted bands with a few dark bands in 31D. A pair of dark thin bands in 31F precede the three conspicuous bands in 31G.
32A, 32B and 32C contain three small puffs spaced between the diagnostic areas of 31G and 32A on one side and 32D, 32E and 32F on the other side. The two bands in 32B and a single dark band in 32C often appear as doubles. The region from 32G to 33C is almost always twisted. However, the detailed pattern of the bands is as shown on the map. A dark double at the end of 33D followed by a light area in 33E, another dark double in 34A and two dark bands in the middle of 34B are always constant.

The diagnostic areas of 34D and 34E are followed by a series of five evenly spaced thin sharp bands in 34F. Regions 36 and 37 contain many small and large puffs. The thin doublet at the beginning of 35A usually appears as one thick band, while the four bands at the end of 35A usually are seen as two heavy bands.

Region 35C contains five heavy bands of which the first two and the last one often appear as doublets. Regions 36D, 36E, 36F and 36G contain well-marked heavy and dark bands as shown in the map. The characteristic puffs with diagnostic bands in the whole of the region 36 contain, in addition, many light bands as shown on the map. 37A appears as a small puff with light dotted bands in the middle and a single sharp band at the end. One dark band in 37B is followed by lighter bands in 37B and 37C. The thin light bands in 37E and 37F form a light patch in an otherwise densely banded centromeric end of this arm.
Diagnostic Features of 3L

The diagnostic features of 3L include a prominent sharp band at the tip of 46A, a pair of sharp bands in a small puff in 46C, and a large puff in 45D and 44A which contains a characteristic band of variable shape in 45D. This band appears in different preparations as a broken, dotted band, S-or cross shaped. The characteristic puff in 44D contains light bands. A set of four thin dark bands in 43B constitutes the landmark for this region. The series of dark bands in 43C is followed by a set of three light doubles in 43D and 42A, situated on two consecutive puffs. This is one of the most characteristic regions of the arm. 41D constitutes the narrowest region of the arm. Two singles followed by a double in 39A, followed by a light constricted area in 39B are typical.

Detailed Description of 3L

The prominent sharp band at the tip of 40A is followed by a series of light and dotted bands. A series of four closely approximated bands in 46B precedes the characteristically dark but somewhat diffuse area in 46C. A single dark band followed by two doubles in 46D is bounded by a series of lighter bands on either side. Region 45 is typical with a series of dark and medium staining bands preceding the large diagnostic puff in 45D and 44A.

A dark double at the end of 44A is typical. A small puff at the beginning of 44B is followed by a pair of medium and various lightly staining bands. The light banded puff of 44D is preceded by a series of five sharp bands in 44C and
followed by 3 doubles of medium intensity in 43A. A set of four bands in 43B and a set of six in a 1:3:2 pattern at the end of 43C are separated by four widely spaced bands of which the first two are closely approximated. The diagnostic puff in 43D and 42A and B appears more stretched than shown in some preparations. The last doublet in 42A often appears darker than the other two which precede it. This puff is followed by a long region composed of 42C through 40A, which in most preparations is twisted or asynaptic. However, the diagnostic band sequence can be easily made out. In the last part of 41A and the first part of 41B is a small puff which contains two sets of thin dark bands. The first of these is a doublet, but often appears as a thick, heavy single band. The second set consist of one single band plus a doublet which sometimes is seen as two thick single bands.

41C, 41D and 40A constitute mostly a light area except for a few thin sharp bands as shown in the map. 40B is almost always asynaptic or twisted and is a light area bounded by one sharp band on either end. 40C and 40D contain numerous dark bands most of which are in the form of doubles. A prominent double in the middle of 40C and three thin sharp closely approximated bands at the end of 40D and a prominent double at the end of 39A can easily be recognized. The rest of the sequence of light bands is as shown on the map.

The centomeric end of the arm is characterized by the presence of a dark, heavy band in the center of a small puff in 39B followed by a pair of narrow dark bands mostly involved
in a constriction in 39C. A wide prominent puff in 38B bears light bands in its center and a series of dark bands on either side. The tip of the centomeric end is marked by three sharp bands which often appear as doubles. Three dark bands at the beginning of 38D are followed by a narrow sharp one in the middle and a dark band at the extreme tip.
2. *Anopheles maculatus*

*Anopheles maculatus* Theobald is widely distributed in Southeast Asia. The salivary chromosomal complement (Fig. 5) is composed of a short X and two pairs of longer autosomes. The X is subtelocentric with a subterminal centromere, while the other two chromosomes are metacentric. Chromosome 2 has unequal arms and 2R is the longest arm of the complement. These chromosome arms are attached to one another by centromeres which are then joined together to form a weak chromosome center (Fig. 6). The X chromosome measures about 90 micra; 2R, 240 micra; 2L, 123 micra; 3R, 180 micra and 3L, 212 micra. As there are many similarities of the subgenus *Cellia*, the same numbering system has been used. The X contains zones 1-6; 2R, zones 7-19; 2L, zones 20-28; 3R, zones 29-37; 3L, zones 38-46 (Fig. 7).

Diagnostic Features of the X-Chromosome:

The X-chromosome may be easily recognized as the shortest member of the complement. At the free end in 1B is a set of three closely approximated dark bands followed by a sharp prominent band at the beginning of 1C. A dotted curved doublet at the beginning of 2A with thin light bands on either side is usually characteristic. A prominent puff in 2C with three equally distributed sharp bands is typical of this region. A diagnostic Balbiani ring configuration stands out in 5A. The centromeric end is marked by the presence of a diagnostic dark, thick and vacuolated band in 5B followed by three dark
Figure 5. Salivary chromosomes of *Anopheles maculatus*. 
2R = Right arm of chromosome 2; 2L = Left arm of chromosome 2; 3R = Right arm of chromosome 3; 3L = Left arm of chromosome 3.
Figure 6. Salivary chromosomes of Anopheles maculatus
Figure 7. *Anopheles maculatus* salivary chromosome map
and sharp bands in 5C. A small puffed region in 6B is characterized by the presence of a series of five dark bands in a 1:2:1 doublet:1 fashion.

Detailed Description of the X-Chromosome:

The free end of the X-chromosome is characterized by two thin dark bands with a lighter dotted band between them, in 1A. This is followed by a single sharp band in a mild constriction. The three dark diagnostic closely associated bands in 1B are followed by one sharp band at the beginning of 1C and a dark doublet at the end of this region. The two dark bands at the end of 1C are followed by many variable light bands in 1D and 2A, except for the diagnostic curved, often twisted double band in the middle of a light area as shown in the map. The dark narrow band at the beginning of 2B is followed by a few lighter bands. A somewhat elongated puff in 2C typically contains three dark thin evenly spaced bands with some very light bands between them as shown in the map. A series of 3-4 small puffs with dark bands beginning in 3A and extending up to 4B are easily recognized. A curved thick dark band in a puff in 3A always stands out. However, this area appears twisted in most of the preparations and may contain a small inversion of a few bands in this puffed region. The three evenly spaced dark bands in a small puff in 3B and 3C can always be picked up after the twisted region of 3A. A characteristically intertwined doublet in 3D is followed by a pair of thin medium-stained bands and is preceded by one dark and one light band. A pair of dark bands in 4A, with the first
lighter, and one dark band in 4B stand out among several variable light dotted bands in 4A, 4B and 4C. This part of the chromosome tapers at the end of 4C to a narrow constriction which contains two dark thin bands with two light bands between them.

5A is marked by the presence of a very characteristic Balbiani ring, which often appears as a large diffused puff with thread like chromatin outgrowths emanating on either side of the periphery of a central dark and somewhat compact band (Fig. 8). However, in some preparations, only the remnants of the ring can be recognized and the dark thick band is very clear (Fig. 9). Due to the presence of this ring the bands following this are usually not as sharp and clear as shown in the map. A few dotted bands followed by one sharp band in 5B marks the end of the Balbiani region. The heavy vacuolated dark band in 5B has one distinct dark band on either side. This is followed by a pair of closely approximated dark bands in 5C. A series of lighter bands is present on both sides of a pair of close dark bands in the middle of 6A. The characteristic puff situated near the centromeric end in 6B is made up of five dark thick bands. This is followed by three light and two dark thick bands as shown in the map in 6C.

Region 6D is always separated from the rest of the chromosome and is composed of a series of light bands of variable intensities. These few bands sometimes show a small thin connection with the rest of the arm and may represent a very short arm of a subtelocentric X-chromosome (Fig. 9).
Figure 8. X chromosome of *Anopheles maculatus*

C = centromere region. B.R. = Balbiani ring
Figure 9. X chromosome of *Anopheles maculatus*

C = centromere region  B.R. = Balbiani ring
6D = short arm of the X-chromosome
Diagnostic Features of 2R

This can easily be recognized as the longest arm of the complement. Its free end, revealing a pattern similar to that of several other species of the subgenus Cellia, consists of a flared tip with two dark bands at the beginning of 7A. These are followed by two more sharp bands at the end of 7A, the first one of them being a doublet. The puff in 7B has two dotted medium-stained bands in its center and one light dotted band on either side. The next diagnostic area is a puff in 7D, having one thick doublet and three single, heavy, evenly spaced dark bands.

A very wide puff in 8C, 9A and 9B consists of six distinct dark heavy bands, one of which at the beginning of 9A and another at the beginning of 9B are variegated and thicker. This prominent area can be used as a starting point to study the arm. Another puff in 11A, with three distinct bands in its middle and two sharp bands at the end is always present.

A series of three light, dotted double bands followed by five dark distinct doubles is diagnostic of 14D and 15A. Immediately following is another landmark in 15B, 15C and 16A. It consists of a series of 10 distinct bands in 2:3:1:4 sequence. The first two narrow bands situated in a slight constriction always stand out. The other bands in 15C and 16A are dark, distinct and somewhat widely separated, but spacing of the bands is often variable.
The centromeric end is characterized by the presence of a single sharp band at the tip followed by a dark and thick band in 19E. Preceding the thick band is a series of six or seven sharp bands. A small puff in 19D contains a diagnostic sharp band in the middle.

Detailed Description of 2R

An easily recognizable puff in 7B is characterized by the presence of four dotted bands, of which the two middle ones are of a slightly darker intensity. This puff is preceded by two dark thin bands at the tip and two more thick dark bands at the end of 7A. At the end of the puff there are two dark bands of which the first one is double. Two light dotted bands followed by a thin sharp band and two faint bands occur in 7C. The whole area of 7D is diagnostic. There are two sets of doubles in a mild constriction, followed by three evenly spaced distinct dark bands in a puff in 7D. This puff is followed by a series of mostly light dotted bands in 8A and 8B, except for two dark bands at the end of 8B.

Two distinct dark bands in 8C, followed by two dark, wide bands in 9A precede the wide diagnostic band in 9B. The first of the two bands in 9A appears vacuolated in most preparations. The wide puff in 8C, 9A and 9B tapers down to a narrow bridge-like constriction and is marked by a series of closely approximated dark doubles as shown in the map. Regions 9C and 9D are almost always twisted and consist of a series of dark and medium-stained bands in a small puff as shown in the map.
A very typical puff of dotted bands, two of which are heavier in 10A is diagnostic. Two groups of thick bands, one each in 10B and 10C are separated by a wide space with apparently no band. The first group contains one doublet, one very thick dark band and one narrower dark one. The second group in 10C consists of two dark prominent bands and four medium stained bands between them.

A large prominent puff in regions 10D and 11A has one narrow dark band at the beginning of 10D, three wide distinct bands in the middle of the puff, followed by two dark bands at the end. The rest of the bands in the puff are light. 11B is characterized by a series of light and dotted bands. Another wide puff in 11C and 12A is diagnostic and appears twisted in most of the preparations. Nevertheless, three dark distinct bands (one doublet and two singles) in the beginning of 11C and two wide bands in 12A are always present.

The middle portion of this arm consisting of the whole of regions 12, 13 and a part of 14 appears twisted in most preparations and it is very difficult to locate all the bands. However, by picking up a few diagnostic features, the area can be identified. A small but prominent puff in 13A has two dark bands in the middle followed by three additional dark bands at the end. This puff is preceded by a stretched section with a thick vacuolated heavy band and two heavy bands in region 12C. Another puff in 12B is characterized by two dark bands in the middle and one at the end. All the other bands in 12C and 12D are mostly light. The rest of the region 13B,
13C, 13D, 14A and 14B consists of a series of dark bands in small puffs, as shown in the map. Three dotted bands in a small puff of 14C can usually be recognized. This puff is followed by two sharp bands, the last one of which is darker. The whole of region 14D, 15 and 16 is quite diagnostic. A stretched puff in 14D and 15A consists of three light doublets and three dark and heavy doublets. This puff is followed by three dark and thick bands at the end of 15A.

Another elongated puff involving 15B, 15C and 16A can easily be recognized by a series of dark bands in a 2:2:3:1:4 sequence. The two middle ones at the end of 15C and beginning of 16A are the heaviest and widest of the series. 16B consists of a series of closely approximated bands. All the bands except one thick and heavy in the middle of 16B are narrow and sharp. This group of bands in 16B is separated by a wide space from another group of both light and dark bands in 16C and 16D as shown in the map.

A single prominent band in the beginning of 17A in a small puff is followed by several light dotted bands and a group of evenly spaced dark bands in 17B. A thick band in 17C in a constricted region, is followed by a thin dark band. A series of light dotted bands in 17C is followed by four sharp prominent bands in a 2:1:1 pattern in 17D. Most of the region 18A and 18B is light except for two light dotted and sharp bands in 18A.
The centromeric end is usually twisted and is often asynaptic. However, a series of heavy dark bands in an order of 2:2:1:1 doublet in region 18B and 18C can easily be identified. Region 19A is also composed of a series of light bands except for one which is dark thick and characteristic. The puff in 19B and 19C contains a varying number of both thick and thin bands of which two widely separated bands in 19C are diagnostic. A small puff in 19D is characterized by the presence of a heavy dark band in its center, followed by a series of nine bands of varying intensities in 19E. The last three bands are heavy, the middle one of which is thick and oval-shaped.

Diagnostic Features of 2L

This is the shortest autosomal arm. The free end is diagnostic with two prominent dark bands in 28A, flanking two dark dotted bands. Three bands in a 1:2 sequence stand out in a small puff in 27A and 27B. Four thin sharp bands in groups of two in 26C constitute the landmark of this arm. In about the middle of the arm, in 24A, there occur three characteristic heavy bands, the first one of which always appears to be a doublet. Another diagnostic area of this arm is formed by two heavy dark doublets in 22B. These are immediately followed by a series of three thin sharp bands in 22C, then two widely spaced doublets one each in 22C and 21A.

The centromeric end can be easily recognized by the presence of a series of dark heavy bands throughout the region 20B, 20C and 20D.
Detailed Description of 2L

The extreme tip of this arm consists of a light area which sometimes appears granular. The two diagnostic dark bands of 28A are followed by a single thin distinct band. A set of three thin dotted sharp bands situated in a small puff in 28B is followed by a series of thin light dotted bands. The light area in 28B and the beginning of 28C is preceded by the dark areas in 28A and 28B and followed by a long dark area in 28C, 27 and 26, and as such constitutes a diagnostic feature of the free end of the arm. Two dark prominent bands at the end of 28C are followed by a light area at the beginning of 27A. At the end of a small puff with three diagnostic bands in 27A and 27B occurs a series of dark bands in a 1:2:2:1 doublet order. These are followed by a large prominent puff bearing a thin sharp band with a few dotted bands on either side in the middle of 26A. A very heavy band situated at the end of 26A is always distinct in most of the preparations. This is followed by two thin dark bands in 26B. Following the four thin diagnostic bands in 26C is a small puff in 25A with a few light bands in its middle followed by a set of four narrow bands in a 2:2 fashion in 25B. This area mostly appears as a constriction between the puff in 25A and the region of this arm in 25C. A set of four evenly spaced dark prominent bands in 25B and 25C constitute an outstanding recognition area. The rest of the bands in this area are light and dotted as shown on the map. Again situated in a small constriction in 24A is a doublet, followed by two dark heavy bands. Another
doublet occurs at the end of 24B, preceded by five to six thin, light, dotted bands situated in a very small puff.

In about the middle of a small puff in 24C, there occurs a very heavy dark variegated doublet followed by a narrower doublet and a single sharp band. At the end of 24C is another narrow peculiar variegated band. The puff in 23A and 23B contains a prominent dark band in its middle followed by a doublet and a thin sharp band. These are flanked on either side by light dotted bands. At the end of this puff in 23B, occur two distinct bands followed by an extremely small characteristic puff in 23C with a set of light bands. At the end of this small puff is a narrow heavy band. A series of four dark bands in 23D of which the last two appear double in most of the preparations, are typical. Following these bands, in 22A, there is a series of three narrow sharp bands with dotted bands between them. In some preparations this area shows asynapsis. A dark area in 22B and 22C is composed of two dotted bands followed by two extremely heavy doublets in 22B, then three thin sharp bands followed by another doublet in 22C. The whole area from 21A through 21D almost always appears twisted. It is very difficult to make out details of the bands in this region. However, good preparations reveal a continuous series of thick and thin, light and sharp bands as shown in the map. Two heavy doublets with two thin and dotted bands between them occur in 20A. 20B contains a single thin sharp band followed by some thin dotted bands, then a wide heavy double at its end. In 20C and 20D is a group of five distinct sharp bands with many thin dotted bands between them as shown in the map.
Diagnostic Features of 3R

The free end can be recognized by the presence of a pair of dark bands at the beginning of 29A, another two characteristic bands at the beginning of 29B and a single thick prominent band at the beginning of 29C. A set of four evenly spaced bands in 29D of which the second is less intense and a pair of dark prominent bands followed by two thin and one thick dark band in 30A always stand out. The characteristic set of eight bands with three dark bands each in 30D and 31A and two dark bands between them is the most prominent landmark of this end of the arm. The bulbous puff in 31B with two prominent dark bands is also diagnostic. Another long, flattened puff in 32B, with a single dark band and no or very few light bands in its middle is typical. Immediately following this puff, situated in a narrow constriction, is a pair of very narrow sharp bands. These are followed by another characteristic band in 32C often bearing papillae like projections. A set of three dark bands in 33A of which the last two are doublets can be easily seen, and in actual practice the characteristic puff in 32B and the dark area in 33A can be used as a starting point to study this portion of the arm. The next easily recognizable area of this arm is a small puff with a single prominent band in the middle of 34B, followed by a few thin dotted bands on a constriction.

A set of three prominent thick dark bands in 34C is followed by three more in 34D. A series of heavy bands chiefly in 35D is usually recognizable. The four characteristic heavy bands
of 36C are also typical. The centromeric end may be recognized by the presence of a series of almost evenly spaced thick dark and sometimes dotted bands in 37C and 37D.

Detailed Description of 3R

The free end of this arm appears comparatively dilated with characteristic bands in the beginning of 29A, 29B and 29C. The rest of the region is sparsely banded. A single narrow band at the beginning of 29D marks the beginning of a comparatively narrower region which extends up to the bulbous puff of 31B. Following the single distinct band in 29D, is a set of light dotted bands. The set of three evenly spaced bands in 29D is followed by a sharp dotted band, then a characteristic series of five bands in a 2:3 sequence in 30A. These are followed by three sets of two light bands each in 30B. The four closely approximated dark bands at the beginning of 30C can be easily seen. Two thin sharp bands with a light band between them mark the end of 30D. The set of three prominent bands in 31A is followed by two thin sharp and two light dotted bands. The characteristic puff in 31B contains two widely separated dark bands. Following this puff is a continuous series of about a dozen bands, all light and thin except for a single narrow dark band in the middle of 31C. Ahead of the characteristic puff in 32B, there occur three prominent bands in 31D and a set of 6 sharp bands in 32A. Region 32B almost always appears as asynaptic and broken with hardly any details of the banding pattern; it is very likely that a small inversion may exist in this area. Immediately
following the diagnostic "branched" thick band in 32C, are a few light dotted bands, then a series of four almost evenly spaced sharp bands followed by a single and two doublets. One pair of dark prominent bands each in 33B, 33C, 33D and 34A are typical. The rest of the bands in this area are thin, light and dotted as shown in the map. The small puff with the characteristic band in the middle in 34B is preceded by two dark bands in 34A. This puff is followed by a light area with a few dotted bands at the end of 34B and the beginning of 34C. Two groups of three thick dark bands each in 34C and 34D always stand out.

The puff in 35A bears a series of dotted and thin sharp bands, followed by a group of a few dark bands at the end in 35B. A single thick dark band followed by three closely approximated thin sharp bands and a widely separated single sharp band in 35C is typical of this region. This area is followed by an almost continuous series of 7 dark prominent bands in a 3:3:1 pattern in 35D and 36A as shown in the map. The diagnostic set of four heavy dark bands in 36C is followed by a light area with many faint bands, then the typical, heavily banded area in 36E, 37A and 37B. This area is almost always twisted, with varying degrees of asynapsis. This region may very likely contain a minor inversion. However, in good preparations a continuous series of both dark heavy and dotted bands can be made out as shown in the map. A continuous series of both thick, dark and light dotted bands in 37C and 37D mark the centromeric end of this arm as shown in the map.
Diagnostic Features of 3E

This arm is characterized by the absence of any dark prominent bands at the free end. The first easily recognizable area consists of a very heavy dark, variegated band, possibly composed of three closely approximated bands in 45C. Following this dark region is a diagnostic puff in 45E and 45F, preceding which there are four evenly spaced sharp bands in 45D. Towards the end of the puff in 45F, there is a set of three characteristic dark bands. The middle of the puff contains three sets of two light dotted bands each. The two variegated dark heavy doublets in 44E are always prominent.

Following these doubles, the four evenly spaced dark prominent bands in 43A always stand out. Two very heavy somewhat diffused bands with a thick distinct band between them in the middle of 41B are diagnostic. The characteristic series of bands in 40D and 40E situated in two consecutive puffs always stand out. The centromeric end likewise contains mostly light bands. Region 38E, in most of the preparations, is detached from the rest of the arm at the junction of a single sharp prominent band in 38D.

Detailed Description of 3L

This arm is characteristic in having both free and centromeric end devoid of any prominent diagnostic landmarks. Regions 46A and 46B consist of a series of thin light dotted bands except for two sharp bands each at the end of 46B and 46C. 46D and 46E appear mostly as light areas. Two dark bands occur
at either end of 46F. A characteristic set of four bands in 45B is preceded by three dotted and light bands in 45A. The diagnostic heavy dark vacuolated (variegated) triplet in 45C is followed by a pair of light, dotted bands and a series of four sharp bands in 45D. These are followed by the characteristic puff in 45E and 45F. Widely separated from the heavy bands in 45F is a set of four narrow light dotted bands, which are followed by two dark bands in 44A and a series of light bands in 44B. The narrow dark band at the end of 44B is conspicuous. In most of the preparations, regions 44A and 44B show varying degrees of asynapsis. A set of three dotted sharp bands in 44C, followed by a single sharp band and a few dotted bands in 44D precede the two heavy diagnostic bands in 44E.

Four almost evenly spaced dark bands in 43A, a pair of sharp bands at the end of 43B and another widely separated pair of bands at the end of 43C are characteristic. The rest of the bands of this region are thin, light and dotted as shown in the map. A small light puff in 42A is flanked by dark prominent bands on each side. 42C contains a series of thin, light and dotted bands. A single wide, prominent band in the middle of the puff in 42D is typical. This puff is preceded by a single dark band and followed by a pair of thin sharp bands.

The two very heavy diffused bands with a heavy band between them mark the end of 41B. The rest of the bands of 41A and 41B are mostly thin, light and dotted except for a
series of 3 sharp bands at the end of 41A. A series of light bands in 41C and 41D is followed by a 1:1:1 doublet:1 sequence in 41D and 40A. This area in most of the preparations is asynaptic and stretched to varying degrees.

Regions 40 and 39 are marked by the presence of a series of five consecutive puffs with many dark prominent bands. The three wide dark bands in the center of a puff in 40B always stand out. A series of four comparatively light doubles in 40C followed by a set of three dark bands in 40D along with a series of heavy bands in a puff in 40E are typical. Two widely spaced sharp bands with many light bands in 39A are followed by another wide puff in 39A, which has a few light dotted doubles in its middle. The characteristic pair of dark bands at the end of the puff in 39B along with a series of dark prominent bands in 1:1 doublet:1:1 doublet:1 fashion in 39C can easily be identified. A pair of narrow bands in 39D marks the end of the puff in 39C and 39D. Most of the bands in 39E, 38A and 38B are light and dotted except for three thin sharp bands in 39E, two groups of two bands each in 38A and a set of three sharp bands in 38B. The centromeric end likewise consists mostly of thin, light and dotted bands except for a few thin sharp bands in 38C and 38D. The centromeric tip is characterized by the presence of two widely spaced thin sharp bands with two light bands in the middle. 38E in most of the preparations appears as detached from the rest of the arm except for a darkly-staining thread like connective at the junction of the sharp prominent and wide band at the end of 38D.
3. Anopheles subpictus

Anopheles subpictus Grassi has a wide distribution from Iran and Afghanistan on the east, west through India and Pakistan, part of China, eastward throughout the Malay Peninsula, the Philippines, the Pacific Islands and south to New Guinea.

The salivary gland chromosome complement consists of three pairs of chromosomes, a short x and two pairs of longer autosomes (Fig. 10). The X-chromosome has a terminal centromere or one very close to the end, while the autosomes are metacentric. The salivary chromosomes of this species also show autosomal homologies with the other members of the subgenus Cellia. The short X chromosome averages 80 micra; 2R, 220 micra; 2L, 195 micra; 3R, 180 micra; and 3L, 120 micra. 2R is the longest arm in the complement. A chromocentre can easily be recognized. It occurs where all the arms are joined together (Figs. 10, 11). Because of the apparent homologies, the numbering used is the same as for the other members of the subgenus Cellia. The X-chromosome contains zones 1-6, beginning at the free and ending at the centromere; 2R from 7-19. 2L contains zones 20-28, beginning at the centromere and ending at the free end. Similarly 3R has zones from 29 to 37 and 3L from 38-46 (Fig. 12).

Diagnostic Features of the X-Chromosome.

The free end of the X-chromosome can easily be recognized by two sharp distinct bands separated from each other by another distinct dotted band at the end of 1A. The puff in 2A and 2B with three dark bands in a 2:1 pattern is diagnostic. Another small puff in 2C with a series of 5 thin dark, distinct bands
Figure 10. Salivary chromosomes of *Anopheles subpictus*. 2R = Right arm of 2; 2L = Left arm of 2; 3H = Right arm of 3; 3L = Left arm of 3.
Figure 11. Salivary chromosomes of Anopheles subpictus
is typical. The last band, situated at the centre of the puff, is the widest, usually double, and always stands out. The heavy, dark band apparently of a vacuolated appearance, at the end of 3B is distinctive and can be used as a starting point in studying the X-chromosome. Another landmark is the heavy, narrow, dark band at the end of 3C, followed by a few bands and a conspicuous light area in a medium puff in 4A.

A Balbiani ring comprises segment 6A, almost always containing a heavy dark band and varying degrees of lateral expansion. Following the Balbiani ring is a series of three dark doublets, of varying intensities in 6B, and then a huge puff in 6C. This puff, perhaps a nucleolar region is very wide, distended, lightly-staining and with no consistent structure. Typically it contains a variable network of darkly-staining thread-like interconnected structure. Following this in 6D are 6 bands of medium to dark intensities which may represent a very short arm of the X. (Figs. 13, 14)

Detailed Description of the X-Chromosome

Two light bands with a medium dotted band between them at the tip of the free end in 1A, followed by one or two light bands precede the dark diagnostic bands at the end of 1A. 1B consists of mostly a light area except for one, dark, distinct band at the end. The thin, sharp band at the beginning of 1C is followed by one dark, dotted and and four bands of medium intensities. The puff in 2A, with a series of dark bands, is diagnostic. The two distinct dark bands in the centre of this puff always stand out. These are followed by a single, medium staining band at the end of 2A. The single thin, dark band at
Figure 12. *Anopheles subpictus*. Salivary chromosome map
Figure 13. X-chromosome of *Anopheles subpictus*. FE = Free end, C = centromere region.
Figure 14. Centromeric region of the X-chromosome of *A. subpictus*

6D = Small portion of the X-chromosome
the end of the puff, in the beginning of 2B, is followed by a light area in most of the preparations. Five dark bands precede the wide diagnostic doublet in the centre of 2C. The two widely separated dark bands in 3A, with about four thin, light, dotted bands between them are always present. The wide dark, apparently variegated band at the end of 3B is flanked on either side by two thin, sharp bands in 3B and 3C respectively. A narrow, dark band marks the end of 3C. The rest of the area in 3B and 3C contains light bands as shown on the map.

The puff in 4A is conspicuous in having a wide light area preceded by a series of a few light bands and one sharp band. A very distinct doublet marks the end of the light area of the puff in 4B. This band is followed by a thin band, a few dotted ones and one very distinct wide band at the end of 4B. Most of the area in 4C and 5A is light except for two thin, dark bands at the end of 4C and another two dark bands in the middle of 5A. 5B contains two closely approximated thin, sharp bands followed by a thin light and a single dark, distinct band. One prominent dark doublet at the end of 5B, along with the three evenly spaced doublets in 5C, are always present. The rest of the area in 5C has light dotted bands.

The Balbiani ring in 6A appears diffused and branched except for a single heavy, dark band at the beginning of 6A (Figs. 12, 13). Four doublets of which the last one is the widest and curved in 6B, constitute a landmark. The bulbous puff in 6C shows no clear bands and usually appears as shown on the map. The tip of the centromeric end contains a dotted band, one sharp band, two dotted bands and two wide thin sharp closely
approximated bands in 6D. These six bands at the tip may be a very short arm of an almost acrocentric X-chromosome, but not certainly so. In this diffuse area, the exact location of the centromere is in doubt.

Diagnostic Features of 2R

2R is the longest arm in the complement. The free end is diagnosed by the presence of a semiflared tip, with about three closely-approximated, dark, dotted bands. Two dark doublets with a light band between them in 7A, followed by a light, banded puff in 7B constitute the landmark of the free end. The puff in 8B followed by a series of dark sharp bands, is diagnostic. Two dark bands in 9A and one wide doublet in 9B always stand out. A big puff with three bands in 9C and two closely approximated dark bands at the end of 10A can usually be recognized. Most of the area in the centre of the puff in 10A appears clear with a few or no bands in most of the preparations.

The four approximately evenly spaced, distinct, sharp bands in 13C constitute the landmark of this area. Two series of three and four thin, sharp, closely associated bands each, the former at the beginning of and the latter in the middle of a puff in 13A, may be used as a starting point for the study of the middle region of this arm.

Towards the centromeric end of this arm, a diagnostic landmark is constituted by the presence of four dark bands and three heavy doublets in 17B and 17C. The centromeric end can easily be recognized by the presence of two wide, dark bands, one each in the puffs in 19B and 19C. A thick dark doublet and a dotted
band at the tip can easily be seen in 19E.

Detailed Description of 2R

A series of 3 or 4 closely approximated, dotted bands at the tip of the free end is followed by 1 sharp and 1 light dotted band. The two dark doublets with a medium band between them in 7A precedes a wide, light puff in 7B. Two sets of two light dotted bands each, occur in the centre of the puff. A single thin sharp band marks the end of the puff. This is followed by four thin light bands and two dark distinct bands in 7C. 7D is an area with mostly thin bands of a medium intensity. 8A contains a series of three sharp bands in a 1:2 sequence, followed by a small puff with three dotted double bands. The wide, dark band in the middle of the diagnostic puff in 8B and 8C is preceded by a series of five dark bands in 8B. A single thin sharp band followed by three sets of dotted bands each and a single narrow sharp band occur in 8C. 9A and 9B are characterized by the presence of a series of prominent bands. A single medium band at the beginning of 9A is followed by three light bands and two closely set dark bands. The dark single band in the beginning of 9B appears broken in the middle in most of the slides. This broken band is followed by two dotted bands, a thin sharp one, another dotted one and a thick dark doublet near the middle of 9B. Two dark bands near the end of 9B precede a narrow neck-like region of 9C. The puff in 10A, with no band in the middle, is diagnostic. Three dark bands in 9C and two curved sharp bands at the end of 10A always stand out. The puff is followed by a comparatively narrow region of this arm. A single narrow dark band with one
dotted band on either side in 10B is followed by two pairs of dotted and light bands and by a single light and another dark sharp band at the end of 10C.

A series of four sharp bands situated in a minor puff marks the region 11A. The rest of the area of 11A, 11B, and part of 11C contains light, thin and dotted bands. A pair of closely approximated sharp bands followed by a single sharp band in 11C is separated from a series of four dark bands in 12A by a narrow bridge-like region. A series of three thin sharp bands in 12C is almost always present. 12B contains a series of thin and light dotted bands as shown on the map. The two series of three and four thin sharp diagnostic bands each in 13A are always distinct. In some slides, however, this area is extremely twisted. 13B is sparsely banded except for a pair of narrow, sharp bands and a few light bands as shown in the map. Four evenly-spaced dark bands in 13C are followed by a series of dotted and light bands in 14A.

A series of four thin, sharp bands in a 2:2 pattern followed by two light bands and three closely set, thin, sharp bands in 14B can easily be identified. This area is followed by a series of closely approximated thin, mostly light bands in a region which is usually narrow. The three narrow dark bands at the end of 14C, along with two narrow dark bands in the beginning of 15A, often gives the appearance of a dark diffused area. The area from 15A to 16A contains numerous bands, mostly thin, light and dotted, except for one thin sharp band in the middle of 15A, three narrow dark bands in the beginning of 15B and four dark bands in 1:1:1:1 doublet fashion in 15C. The puff
in 16B and the narrow region in 17A is profusely banded. The whole region often appears twisted and broken and hence it becomes very difficult to trace all the bands in a given preparation. A detailed study of many slides shows a series of eight dark distinct bands with many thin, light and dotted bands as shown in the map. 17B and 17C appear as a dark area with a set of four dark singles, two doubles in 17B and another double in 17C. The rest of 17C and the beginning of 18A contain numerous closely set thin, light and dotted bands. A pair of closely approximated bands, in the centre of the puff in 18A preceded by a dotted and a single thin sharp band can always be recognized. One sharp band at the beginning of 18B is followed by thin light bands and two dark bands. A single dark band can sometimes be seen in the middle of two dark bands at the end of 18B. 18C and 19A contain a series of light bands except for three dark bands at the beginning of 18C.

The two dark prominent bands in 19B and 19C are separated by an usually constricted region with light bands. A narrow thick dark band at the end of 19C is followed by a series of light bands and a set of three prominent bands at the end of 19D. The centromeric end is mostly indistinct fuzzy and heterochromatic. The last segment shown as 19E in the map sometimes is detached from the rest of the arm except for a very narrow neck-like connection. A detailed study of many preparations, however, shows that there is a single dotted band preceded by a thick dark band at the tip. There occur about six bands in 1:2:2:1 fashion preceding the dark band at the tip in 19E as shown in the map. It is not certain that
this region actually is part of 2R. The centromeric region may actually occur at the end of 19D.

Diagnostic Features of 2L

The free end of this arm can easily be recognized by the absence of any dark band at the tip in 28A. The first diagnostic area consists of four dark pair of bands, almost evenly spaced in 27B. These are followed by a series of bands in one doublet: 3:4 fashion at the end of 27C and 26A, which always stands out. A single sharp dark band at the start of 26B is followed by a long area with progressively light staining bands. The three dark bands, in 26C are always distinct. The two prominent doublets with a pair of sharp bands between them in 25B are typical. Near the middle of 25C is one dotted band followed by two dark bands and two more dotted bands. This area can be observed in almost all the slides. A very thick dark doublet at the end of 25C, immediately followed by a pair of dark bands in the centre of 24A, always stands out. This area can in fact be used as a starting point for studying this arm. A puff in 24B and C bearing two doubles in the center and another two dark bands towards the end is diagnostic. Towards the centromeric end of the arm in 22C is a landmark consisting of a group of 6 bands which gives the appearance of a vacuolated dark area. This group is followed by a light area with a single dark prominent band in 22C. An extremely thick, dark vacuolated band in a puff in 21C is typical. A single dark band at the extreme tip of the centromeric end of 20E is always present.
Detailed Description of 2L

Two pairs of closely approximated dotted bands mark the tip of this arm in 28A. A series of five prominent dotted bands in a 1:2:1:1 pattern in 28B is interspersed with many thin light dotted bands. Five moderately stained bands associated with a small puff in 28C are mostly involved in a twisted region which is sometimes asynaptic. In most of the preparations, the whole of the region 28C, except for the two dark prominent bands at the end, is hard to follow and appears as a light, twisted region. However, in some good slides the light bands can be made out as shown on the map. A single sharp band followed by a few light bands in a constriction precede the puff in 27A. Two pairs of dark bands with a pair of light dotted bands between them follow the puff. These two pairs, along with another two pairs in 27B, comprise the landmark of this region. Three widely separated sharp bands occur in 27C. The rest of the region 27C contains thin light and dotted bands except for three sharp bands and a dark doublet at the end. Seven bands in a 3-4 series are followed by a single dark prominent band at the beginning of 26B. The remaining portion of 26B contains a series of thin light and dotted bands. This light area stretched between the series of dark diagnostic bands in 26A and the two thick dark bands in 26C can be easily recognized as a starting point for following the band pattern in this area of the arm. A small puff in 25A with two thin prominent bands in its middle is preceded by a series of closely approximated light dotted bands. A set of two dark heavy doublets with two dark bands between them in 25B is followed by two light dotted and a single sharp band at the beginning of 25C. A pair
of dark prominent bands with one sharp dotted band preceding and two dotted bands following them in 25C, constitutes another landmark of this area. This area is immediately followed by a very thick dark doublet at the end of 25C. Except for the thick dark doublets in the centre and one doublet at the end, 24A is a light area. A diagnostic puffed region of 24B and C contains a series of dark bands. Two dark bands at the beginning in 24B are followed by two pairs of dotted bands. Then come two pairs of dark closely approximated bands in the centre of the puff, then in 24C, one dotted band followed by 2 prominent bands and 2 dotted bands.

Two prominent doublets at the end of 23A and one pair of dark bands in the middle region of 23B are typical. The remainder of the area contains light, thin and dotted bands as shown in the map. The puff in 23C bears two pairs of distinct dotted bands in the middle, preceded by a pair of sharp bands at the beginning of 23C. Near the end of the puff are four evenly spaced dark bands in 23C and 23D. A series of six bands in a 1:2:3 fashion occurs in a constriction in 22A. A light area with only a single variegated dark band in the middle marks the elongated puff in 22B. Towards the end of the puff in 22C there is a group of six dark diffused bands giving the appearance of a dark vacuolated continuous area. This constitutes the landmark of this region. With different light sources it appears as six dark thin, close bands. A single dark band in a small puff in 22C followed by a group of five prominent bands, of which the last two are doublets is characteristic of 22C.
A profusely banded puff often involved in a twisted and broken region occurs in 21A, 21B and 21C. In most of the preparations only a dark area consisting of a series of sharp bands in a 3:2:1 pattern in 21B can be made out. The rest of the details of this region can be seen only in very good slides. This is followed by a heavy dark vacuolated band at the end of 21C. A single thick narrow doublet followed by three dotted and two dark doublets in 21D is always present. The centromeric end of the arm is marked by the presence of a single dark band in 20E. The whole of the region 20C, 20D and 20E often appears to consist of light, thin and dotted bands, except for a single dark band in the middle of 20C, two sharp bands in 20D and one dark band in the beginning of 20E. A pair of thick, medium bands in 20A is followed by two light dotted and a narrow dark doublet. This doublet is often situated in a small constriction and is followed by a series of four dark bands in a 2:2 series. The rest of the region has light bands as shown in the map.

Diagnostic Features of 3R

The free end of this arm appears mostly as a puff with two dark bands near the tip in 29A, three closely approximated dark bands in the middle of the puff followed by a thick narrow band in a small constriction in 29C. A small puff with two dark bands at the end of 29D, followed by a few dotted and two prominent bands and a narrow doublet in the middle of 30A, form a landmark for this end of the arm. A narrow dark vacuolated band followed by a prominent wide dark band in 30C can almost always be promptly identified. The characteristic thick, sometimes curved band situated in the middle of the dark puff in 32A is typical. A diagnostic puff with two dark apparently vacuolated
doubles with a single prominent band between them in 33A always stand out. This area may easily be identified in almost all the slides and can serve as a good starting point in the study of a detailed band pattern for the central region of the arm. A series of three evenly spaced bands of which the first two are closely approximated doubles in 34A is diagnostic. The centromeric end can easily be recognized from its characteristic morphology with four closely situated small puffs. The majority of the area of each of these puffs is light except for 1 or 2 dark bands in each.

Detailed Description of 3R

The tip of the free end at 29A is extremely variable. The two bands at the tip may sometimes be missing or may show asynapsis making the details uncertain. The three dark closely approximated bands in 29B are flanked on either side by a pair of thin light dotted bands. Three dotted bands follow the narrow dark band in 29C. A prominent, dark dotted band followed by three lighter dotted bands and a pair of dark bands in 29D can always be seen. These two dark bands, along with another two dark bands and a narrow doublet in 30A, are reliable identification areas. The puff in 30B is profusely banded as shown in the map. The two dark doubles in the middle of 30C are flanked on either side by a pair of dotted bands. Two dark doubles on either side of a single thin sharp band in the middle of a puff are found in 30D and 31A.

A series of small puffs in 31C, 31D and 32A contain mostly light thin dotted bands except for one sharp band at the beginning of 31C, three dark dotted bands in the middle and 2 dark
bands at the beginning of 31D, and a doublet followed by a curved band in 32A. The two thick dark bands at the beginning of 32B are followed by a characteristic thick, apparently vacuolated band. Except for two thin sharp bands in 32D, 32C and 32D are generously furnished with thin light bands. The puff with the three thick diagnostic bands in 33A always stands out. A single sharp band in 33B is followed by two dark dotted and one heavy variegated band. A dark doublet and one sharp band flanked on both sides by the light dotted bands is found in 33C. A series of three dark bands in 33C, a heavy doublet followed by a dark vacuolated band in 33D, and a group of three evenly spaced dark bands in 34A can always be recognized. The three bands in 34A are doubles and are often closer together than shown, and may sometimes give the appearance of a dark area. Two sharp bands in 34B and two dark bands in 34C are typical. Two narrow dark bands of which the latter is usually in the form of a doublet occur in a small constriction in 34D.

The three characteristic dark doublets in 35A, two of which are variegated, are always diagnostic. Two prominent bands in 35B and a group of six sharp bands in 35C constitute one of the diagnostic areas of the centromeric end of the arm. One thin dark doublet at the end of 35C is followed by about three light dotted bands and two thin sharp bands. A 3:2 series of thin sharp dark bands in 35D, followed by a second series of 3:3:2:2 sharp bands in 36A, are typical. The next to the last band in this series is the thickest. Another series of eight bands in a 2:2:1:3 sequence is always present in 36B as shown in the map. A single dotted band followed by two dark bands at the end of 36C sometimes occurs in a group to give the appearance of a
dark area. Four thin dark bands of which the last two are closely approximated are found in 36D. The centromeric end of the arm is marked by the presence of a series of small puffs in 37A, 37B, 37C and 37D. A single dark prominent band in the centre of the puff in 37A and B is flanked on both sides by a series of light dotted bands. The two widely spaced dark bands in a puff in 37B and 37C are typical. The puff in 37C and 37D is profusely banded. Most of the bands are light except for two dark bands in 37C and two sometimes broken bands, in 37D. Regions 37C and 37D are often asynaptic, giving the bands, the appearance of being broken.

Diagnostic Features of 3L

The free end of this arm can easily be recognized by a series of 5 dark prominent bands at the tip in 46A. The first thick dark band is followed by two thin sharp bands and one dark doublet. A small puff in 45C with three dark prominent doublets always stands out. The area between the tip in 46A and the puff in 45C appears twisted in most of the preparations. Another pair of dark doublets in 44C is typical. A set of two doublets and one sharp band in 41A is diagnostic. In 41D a broad area containing a series of dark bands is followed by a lighter stretched area, then three or four closely set, apparently vacuolated bands in 40B. This area may well be used as an initial reference point from which to study the arm. The centromeric end of the arm is involved, in all our specimens, in a U-shaped loop, which appears to contain a reverse repeat and a deletion. This loop is always attached to both the centromere and to the distal portion of the arm by thin, darkly-staining strands.
Detailed Description of 3L

A pair of light bands precede the five diagnostic bands at the tip in 46A. A series of thin, light and dotted bands occurs in 46B. This light area almost always is twisted, making the detailed pattern obscure. The two thick medium-stained dotted bands at the end of 46B followed by two closely approximated dark bands in 46C can easily be picked up. These two dark bands are followed by four lighter dotted bands, then two small puffs, one each in 45B and C, which contain a series of both dark and dotted bands. The three doubles in a small puff in 45C are characteristic. Another puff in 44A is typically light and with only a few lightly staining bands. At the end of this puff, three prominent bands are always present usually in a constriction. The two dark doubles followed by a series of thin, light and dotted bands are always visible. A series of three thin, sharp, closely-approximated bands is situated in the centre of a light puff in 43C. Preceding these three bands is a series of four almost evenly spaced thin sharp bands. The remainder of the regions 43A and 43B is light containing thin dotted bands.

A characteristic series of dark bands begins in 42A and continues through 41B. The principal bands are as follows: three thin dark bands in 42A; a single dark band at the beginning and a doublet in the centre of 42C; a doublet and three wider thin bands in 41A; and three dark bands in a constriction in 41B. A characteristic pair of narrow bands in 41C marks the start of a wide area of the chromosome which contains a wide vacuolated band and three thin wide bands in 41D. The rest
of the region 41 and all of 40A is a light area usually stretched and attenuated. The characteristic dark vacuolated area consisting of four closely set bands in 40B always stands out. This is followed by two sharp bands and a series of thin light dotted bands. Another series of six bands in 3:1:2 fashion in 40C and D follow the dark area of 40B. The characteristic thick, apparently vacuolated, band at the end of 39A is preceded by five thin but very sharp bands in a 2:3 pattern. Immediately following this dark band is a series of about 12 bands of variable staining intensities, but usually dark, on a wide but elongated puff in 39B and 39C. This series, although variable, can in most cases be separated into two groups of 5 and 7 bands each. At the end of the elongated puff in 39D, there are two thick dark narrow bands attached to the centromeric loop. The centromeric loop in the region 38, perhaps represents a reverse repeat and a deletion. The banding pattern consisting of 7 dark bands in a 2:5 fashion in a small puff in 38A is always constant.

Region 38, in my preparations, is invariably in the form of a loop. The first two bands in 38A are always paired, and appear continuous with the last two bands in 38C. The latter half of 38B and all of 38C often appear narrower, and may in fact be only one chromosome. The last five heavy bands in 38A and the first dark band in 38B appear to be a reverse repeat. The centromere appears to be at the juncture between 39D and 38A.
B. Karyological Studies

1. A. tesselatus Theobald

a. Mitosis

The nuclei of the resting spermatogonia are either oval or somewhat rounded in shape. These are loaded with irregular small chromatin granules (Plate I, Fig. I). A darkly stained dot has been observed in the nuclei of all the cells. This may represent the sex-chromosomes. The prophase configuration (Plate I, Fig. 2) is recognized by the presence of thin thread-like chromosomes filling up the entire nuclear cavity. These threads are studded with chromatin granules. No definite character distinguishes the sex-chromosomes from the autosomes.

The diploid complement (Plate I, Figs. 3, 4) consists of six chromosomes which arrange themselves at the equator of the spindle and are fully condensed. The first pair of chromosomes is the smallest and subtelocentric. This pair represents the sex-chromosome. X is slightly larger than Y. X measures about 3.5 \(\mu\) (longer arm 3 \(\mu\) and shorter arm 0.5 \(\mu\)). Y measures 3 \(\mu\) (longer arm = 2.5 \(\mu\) and shorter arm = 0.5 \(\mu\)). The shape of the sex-chromosomes is frequently rod-shaped and may simulate a terminal centromere in some of the preparations. The short arm is difficult to demonstrate, being only about 0.5 \(\mu\) in length. No positive or negative heteropycnotic behaviour of the sex-chromosomes has been observed. The second pair of the chromosomes is metacentric. Both of their arms measure 2.5 \(\mu\) each. The third pair is slightly submetacentric. Their two arms measure 3 \(\mu\) and 2 \(\mu\) respectively. The second and third
PLATE 1
ANOPHELES TESSELATUS (♂)
MITOTIC AND MEIOTIC CHROMOSOMES
pair of chromosomes are the autosomes.

The mitotic anaphase shows the synchronous movement of both the autosomes and sex-chromosomes. During late anaphase and telophase, all the chromosomes condense to form diffused masses on either poles. This is followed by cytokinesis and results in the formation of two daughter primary spermatocytes.

b. Meiosis I.

The resting spermatocytes (Plate I, Fig. 5) are characteristically rounded with eccentrically placed nuclei. A darkly stained heteromorphic mass can be distinguished along with a network of chromatin granules. No clear thread like structures are observed.

No leptotene or zygotene phase has been encountered despite the study of a large number of preparations. This clearly indicates a very short duration of these stages. The pachytene chromosomes (Plate I, Fig. 6) make their appearance as very long but thick bivalents. They are studded with small granules throughout their length. The sex-bivalent which is positively heteropycnotic is also smaller than both of the autosomes.

During the late pachytene or diplotene, the nucleolus disappears altogether. In diplotene (Plate I, Figs. 7, 8) the bivalents are condensed and the homologues of the chromosomes remain attached at the points of chiasmata. The number of chiasmata varies from 1 to 4. The sex-chromosomes usually form the smallest bivalent. No heteropycnotic behaviour characterizes the sex-bivalent.
Diakinesis (Plate I, Figs. 9, 10) represents the termination of the chiasmata. Usually two rings and one cross-shaped bivalent are formed. The rings represent the autosomes whereas the cross configuration is the sex-bivalent.

During metaphase I (Plate I, Fig. 11, 12), the three bivalents, which are fully condensed, arrange themselves at the equator of the spindle. This phase seems to be the longest. The sex-bivalent is the smallest. No heteropycnotic behaviour has been noticed. Sex-bivalent clearly shows the union of X and Y in some of the preparations.

Anaphase I (Plate I, Fig. 13) shows the separation of the chromosomes towards the poles of the spindle. The movement of the chromosomes is synchronous both with respect to the autosomes as well as the sex-chromosomes. The sex-chromosomes do not show any heteropycnotic behaviour. As the autosomes and sex-chromosomes divide reductionally there are three univents on either side. The chromosomes are now smaller and thinner than those of the mitotic anaphase. These chromosomes come nearer to each other and form thickly stained masses on each pole during the telophase. After this, there develops a cytoplasmic membrane thus forming two separate cells, each having a deeply stained mass.

c. Meiosis II.

The thin light stained, cross-shaped prophase II (Plate I, Fig. 14) chromosomes make their appearance. These are in the form of two cross-shaped and one v-type configurations with their chromatids separated. These threads become condensed
during metaphase II (Plate I, Figs. 15, 16) which shows clearly the configurations of metacentric autosomes and subcentric sex-chromosomes.

At anaphase II these chromosomes divide equationally and produce two types of spermatids (Plate I, Fig. 17).

2. *A. subpictus* Grassi

a. Mitosis

The chromosomal morphology, behaviour and nature of the autosomes as well as the sex-chromosomes are quite similar to those of *A. tesselatus*. The diploid number is 6 (Plate II, Figs. 1, 2 and 3). The first pair consists of the smallest chromosomes i.e. the sex-chromosomes. The centromere is subterminal in position in both X and Y. The X chromosome is larger than Y. The longer portion of X is slightly larger than that of Y. The smaller knob-like arm is equal in both X and Y. In this species two arms of the X chromosome measure 2.5 \( \mu \) and 0.5 \( \mu \) respectively while Y has the two arms of 2 \( \mu \) and 0.5 \( \mu \) length. The second pair of chromosomes is again metacentric having a clear central centromere. Both the arms measure 2.5 \( \mu \) each. The third pair of chromosomes is slightly submetacentric and the two arms measure 2.5 \( \mu \) and 2 \( \mu \) respectively.

No special behaviour of the sex-chromosomes has been observed during the late mitotic anaphase (Plate II, Fig. 4) and telophase. The chromosomes separate synchronously and no bridges or laggards have been noticed.
PLATE II

14  ANOPHELES SUBPICTUS (♂)

MITOTIC AND MEIOTIC CHROMOSOMES
b. Meiosis I:

As in the other anophelines, no leptotene or zygotene stages have been observed, but clear pachytene threads make their appearance (Plate II, Fig. 5). The sex-bivalent shows positive heteropycnosis in about 60% of the preparations studied. The chromosomes start condensing and during the diplotene (Plate II, Fig. 6), three clear bivalents are observed. The number of chiasmata varies from 1-6. Again no heteropycnotic behaviour of the sex-chromosomes has been observed during this stage. The sex-chromosomes are the smallest and small knob-like portion of both X and Y can be recognized clearly.

Usually very clear ring-like configurations, during terminalization of the chiasmata, mark diakinesis (Plate II, Figs. 7 and 8). The sex-bivalent is again the smallest and cross-shaped. All the autosomes and the sex-chromosomes stain uniformly. Metaphase I (Plate II, Figs. 9, 10) presents three fully condensed bivalents. The sex-bivalent is the smallest. During early anaphase I (Plate II, Fig. 11), the sex-bivalent shows prococious movement as it separates later than the autosomal bivalents. But during the late anaphase or telophase, no such character has been observed.

c. Meiosis II:

Two kinds of prophase II (Plate II, Fig. 12) cells have been noticed. Some of them are with X, and the others are with Y. The chromosomes are thinner and thread-like, but they are smaller than those of A. tesselatus. Each autosome has the shape of a double v to give an appearance of X, as the chromatids
remain separate. The X and Y show subterminal position of the centromeres. It is very difficult to make out the smallest arm in both X and Y in most of the preparations. These chromosomes become more condensed during metaphase II (Plate II, Figs. 13, 14). At late anaphase II (Plate II, Fig. 15), the chromosomes divide equationally and synchronously.

3. *A. culicifacies* Giles

a. Mitosis:

The resting spermatogonium in this species is the smallest in size as compared to those in the other species studied. The size of the chromosomes is also very small. The karyotype (Plate III, Figs. 1, 2) consists of six chromosomes. Out of these two pairs are the autosomes and the third pair is the sex-chromosomes. The pair of sex-chromosomes is clearly submetacentric. The two arms of X measures 2.5 μ and 1.5 μ respectively, whereas those of Y measure 2.5 μ and 1 μ respectively. In this species the longer arms of both X and Y are equal in length, but the smaller arm is slightly longer in X than in Y. All the chromosomes have the same staining intensity. Mitotic anaphase and telophase are just similar to those of *A. tesselatus* and *A. subpictus*.

b. Meiosis 1

Very clear dark sex-bivalent shows its positive heteropycnotic behaviour during the pachytene (Plate III, Fig. 3). The autosomal bivalents are long thread-like double strands, whereas the sex-bivalent is just a small condensed darkly stained body.