Chapter - I

Observations
Discussion
OBSERVATIONS

Salivary gland chromosomes

The following text includes the results of general observations on the salivary gland chromosomes of five anopheline species whose banding pattern has been described in detail. A salivary chromosome map of each species has also been constructed and the pictures of the various chromosomal elements are given. So far, only the polytene chromosome preparations have been studied from the salivary glands, but in recent years the ovarian nurse cells from the freshly hatched females (almost 30 hours old) have also been found to have an equally well-banded chromosomes. But there are limitations to the study of chromosomes from the ovarian nurse cells because it requires proper maintenance of the colony which is not possible for those species which are not habitual to the laboratory conditions or for whom the rearing procedures are not yet available. It is because of these factors that the present studies have been restricted to the salivary glands which have provided satisfactory results for the purpose of mapping. In addition, another important reason has been that in many cases the salivary chromosome preparations are made
right on the collection spot especially for those species whose larvae are available with difficulty and cannot survive for a long time in captivity and during transit over long distances.

Stalker (1967) and Chaudhry (1972) noticed the influence of periodicity on the standard of the salivary chromosome preparations. During the course of the present investigations, the same was noticed to a limited extent only because the quality of preparations did not differ such during the 24-hour cycle of the day but was considerably influenced by the seasonal changes. In the case of *Aedes lindesayi* which is prevalent in the colder environment of the hills no preparation could be secured when the dissections were made after bringing the larval collection down to the place of work. Whatever photographs of this species have been reproduced in this thesis are the results of those slides which were made at the collection spot.

The diploid number in all the species is uniformly six which is represented by a haploid number of three giant polytene elements in the salivary gland cells. The diploid metaphase karyotype consists of a very small pair of sex chromosomes and two large pairs of autosomes. The sex pair comprises acrocentric chromosomes (*XX,XY*), whereas the autosomes are metacentric with slightly
submetacentric condition of one of the autosomes. In
the salivary chromosome complement, the sex pair (♀)
corresponds to a small X-chromosome, whereas the two
autosomes are represented by a submetacentric and a
metacentric banded elements.

A well spread and brightly stained chromosome
complement consists of three intimately synapsed
homologues which are attached by their centromeres to
form a chromocentre. Unlike Drosophila, a stable chromo-
centre is of rare occurrence in the anophelean species.
However, when it survives or remains intact in the
preparations, the three elements are seen attached at a
common point from which five unequal arms are seen to
radiate out, because of the acrocentric nature of the
smallest element whose short arm is not easily visible.
This picture is common to all the species. When the
chromocentre disintegrates, the three chromosomes lie
freely in the cell.

In addition to a detailed description of the normal
banding pattern of each species given below, chromosomal
polymorphism has also been described wherever available.
Description of the chromosomes

Anopheles lindesayi
(Figs. 2-10)

Description of the X-chromosome (Figs. 6-7)

Due to the presence of a very weak chromocentre, the X-chromosome was always seen isolated from the other autosomal arms in the complement. It is easily identified by its short length (70 μ) and the presence of two closely approximated dark bands at the tip of its free end in subsome 1A, which is flared in some preparations. Each of the following subsomes 1B and 1C, contains four closely placed dark bands and two sharply stained thick and curved bands in a moderate swelling. The next zone 2 is marked by a large puff that ends in a single deeply stained band in subsone 2A which is a constant feature of the X-chromosome. Following it, there is a set of five to six prominent bands which are invariably located in subsone 2B. Similarly zone 3 also starts with a light area in 3A which is followed by seven prominent dark bands covering 3B and 3C. The constant occurrence of two pairs of dark bands in 4A and a triplet each in 4B and 4C are also important for the ready recognition
of the X-chromosome. A thick band at the beginning of
subzone 5A and a light area interrupted by thin broken
bands is a characteristic of zone 5 which ends with
three dark bands in 5C.

Description of chromosome 28 (Figs. 8, 9)

The right arm of chromosome 2 measures about
148 μ and is recognized by its dome-shaped free end
which invariably possesses a broken band at the tip
and two dark bands immediately behind in subzone 6A.
A light area with three dark and well spaced bands in
6B are also constantly seen. In the following zone the
diffused area with a series of several light bands in
7A, a pair of doublets in 7B and a set of 2+2+2 thin but
dark bands in a swelling in 7C are further helpful in
determining this end of 2B. Zone 8 is distinct in
having an elongated swelling with four dark bands on
its either side, whereas the following zone 9 has two
pairs of dark bands in its beginning in 9A and a pair
in its middle in subzone 9B. Because of the presence
of several deeply stained bands, zones 10 and 11 stand
in sharp contrast to the following zone 12 in which the
distinct bands are available only in subzone 12A, 12C
and 12D, which alternate with several light bands. Two
prominent pairs of deeply stained bands in subzones 13A
and 13B and a light area in subzone 13D are characteristic of zone 13 whose termination is marked by a set of three bands. The last zone 14 which falls towards the centromeric end of 2A is conspicuous by the presence of a pair of dark bands in 14A, three curved bands in 14B, and a set of five closely associated bands plus a pair of dark bands at the centromeric tip in 14C.

**Description of chromosome 2L (Figs. 8, 9)**

This arm of chromosome 2 is almost of the same length as that of 2A and measures about 155 μ. The oval free end is constituted by two curved bands at the beginning of a swelling which forms the free end in subzone 21A. This swelling is further occupied by two pairs of deeply stained bands. The shape of the free end shown in the map is of frequent occurrence, but in some preparations the margin at the tip is clearly defined to form a blunt free end. In the following segment a pair in 20A, two curved pairs facing each other in 20B, a large puff with a band in its middle in 20C and three distinct but moderately stained bands in 20D are characteristic of zone 20. In the next zone 19 two small puffs in 19A and 19B continuing into large puffs in 19C and 19D form an important landmark locating zone 19 and recognising 2L which is further recognised by a pair of heavy bands.
in 198. A distinct barrel-shaped swelling occupied by 3 + 2 very prominent dark bands, curving in the same direction in 18C provides an important character for identification of the left arm of chromosome 2. Alternating dark and light areas mark the individuality of zone 17 in which a very sharply stained set of seven bands in subzone 17C and two pairs of distinct bands in 17D further help in determining this arm. In the remaining part of 24, zone 16 marks a stretched out narrow region in which zone 16D is important in possessing a set of eight dark bands of constant occurrence. Similarly the centromeric zone 15 is also formed by a narrow region devoid of any recognizable swelling. Two pairs of dark bands in 15A, three pairs of the same intensity in 15B and a triplet in 15C can be easily located in the map.

Description of chromosome 38 (Fig. 10)

This is the longest arm in the complement which is about 202 µ in length. Its free end is somewhat blunt and squarish and as shown in the map is marked by 2 + 3 dark bands of constant occurrence in subzone 22A. A light puff in 22B with a pair of curved bands appearing as one thick dark band in most of the preparations and two equal medium-sized swellings with three pairs of widely spaced bands in the former and three dark curved bands in the latter are the diagnostic features of the
are in this end. Zone 23, in its turn, is almost lightly stained except four thick dark bands in 23A whereas the presence of a series of very deeply stained bands in the following region is a permanent feature of zone 24 which serves as an excellent landmark for identification of 3R. Two more or less equal-sized large puffs with a constriction in between are important in zone 25 in which three to five dark bands in 25A and 25B are also available. The remaining part of 3R (zones 26-29) is marked by the presence of light bands whereby the dark bands wherever present become more distinct. On the other hand, zone 26 can be easily recognized in having a light area consisting of a few dotted bands, whereas zone 27 also contains a lone band in its middle. Similarly a light and diffuse area often marks zone 28 which ends with a set of three curved bands. Almost in all the preparations zone 29 remains lightly stained, but has four or five thick dark bands in 29B. Strikingly visible banding sequence of over six dark bands in zone 30 which constitutes a diagnostic feature for identification of this arm is further identified by widely spaced dotted dark bands in 31A and three richly stained bands in a swelling in 31B. This chromosomal arm ends with zone 32 which has a pair of dark bands in 32A and two pairs each in 32B and 32C.
Description of chromosome 3L (Fig.10)

It is the shortest among all the autosomal arms in the complement and is about 128 μ in length. The funnel-shaped free end with a pair of broken bands at the periphery in subzone 39A is the characteristic feature of arm 3L. It is followed by a small swelling containing four curved bands in 39B and a very prominent dark doublet in 39C. The following zone 38 is marked by two large puffs enclosing a small swelling in between them. It is further characterised by two curved bands in 38A and a set of four pairs of deeply stained bands in 38B. A single large swelling possessed by a few complete and broken dark bands is a diagnostic feature of zone 37. Excepting a single thick band in 36A and a doublet in 36B, the whole of zone 36 is a light area. Zone 35 is of the same consistency as zone 36 and can be readily recognised in this arm by four sharply stained bands in 35C. The existence of two consecutive swellings with two unequal pairs of dark bands in the latter swelling are typical of zone 34. The last zone of arm 3L, which constitutes the centromeric end can be easily identified by the occurrence of a pair of heavy bands in 33A and widely spaced dark bands in 33B.
Salivary chromosome polymorphisms in the natural population of A. lindessavi

It has been revealed after the examination of a large number of preparations that the present material of A. lindessavi possesses a number of the various types of chromosomal rearrangements in the natural populations. Especially, the X-chromosome has been found to be rich in these aberrations in comparison to other arms in the complement. In all about 16 aberrations have been encountered, which include, inversions, deletions and translocations. These have been individually analysed below with respect to their nature, affected zones and the configurations which they form.

Inversions (Figs. 11-26)

Inversion in a salivary chromosome is the one which can be more easily detected as compared to deletions and translocations. It is the outcome of a chromosomal segment that has been turned around 180° so that its position in the normal banding sequence is reversed. Single inversions of this type result from a rearrangement which requires two break points. When both the breaks are present on the same side of the centromere they constitute a paracentric inversion; whereas if the broken segment carries a centromere it
will result into a pericentric inversion. Further, if this break has been suffered by both the synapsed homologues, the inversion will be in a homozygous state which can be detected with difficulty only by carefully comparing the resulting banding pattern with the normal pattern from which it will differ in being in the reversed order. In case the breaks have been suffered by one of the participating elements in the synapsed homologues, the inversion is in the heterozygous condition and forms a characteristic loop. It is the heterozygous inversion which is detected easily in the polytene chromosomes.

In the present observations of *A. lindessyi* a total of 13 inversions have been recorded which are distributed as 2 in *X*, 1 in 2*R*, 3 in 2*L*, 6 in 3*R* and 1 involving 3*R* and 3*L*.

The *X*-chromosome inversion is of constant occurrence and lies in its middle (Figs. 12-19). It invariably forms a clear inversion loop which generally gets coiled to form a figure of eight (Figs. 12-18). However, it depends upon the plane in which it spreads in the squash preparation. As far as its break points are concerned, this inversion begins with a pair of two intensely stained thick bands in subzone 1*C* and terminates
at the other break point in subzone 4C, which is recognized by the constant occurrence of a light area just before a puff in Zone 5. However, marked variations have been noticed in the location of the two breakpoints which shift slightly in Zone 1, on the one hand and Zone 5 on the other. All these variations are indicated in Table 3. In Figure 11, the X-chromosome exhibits a small inversion near the right free end which, as usual, begins from subzone 1C but terminates immediately in 2C involving only a small segment of this chromosome.

The chromosome 2R carries only one inversion which also involves a small segment from subzone 7C-8B (Figs. 8, 20) and forms a ring configuration without indicating any points of bifurcation of the two synapsed homologues at the breakpoints. Three inversions are present on the left arm of the chromosome 2. Starting from the free end, the first inversion extends from subzone 21A-20B whereas the second and third extend from 20B-19C and 15A-15C, respectively (Figs. 8, 21-23). The first inversion starts immediately behind the free end (Fig. 21) and forms a small inversion loop. The second, which lies from subzone 20B-19C (Fig. 22) falls approximately in the middle of 2L and forms a medium sized loop. The third one involves the centromeric
zone and forms a clear inverted loop in which asynapsis of the homologues at the two breakpoints is very clear (Fig. 23). Although inversions first and second lie side by side they are not fixed (non-random) and are never available together.

In chromosome 3 there are seven inversions in all, out of which six are paracentric and the remaining one is pericentric. The former lies in 3A alone, whereas the latter also involves the centromeric zone of 3L. Beginning with the free end of 3A the six paracentric inversions (Figs. 24-27) involve subzones 23B-24A (Fig. 24), 24B-24C (Fig. 25), 25B-27B and 28A-29A (Fig. 26), 28B-28C and 28C-31B (Fig. 27). Out of these, the inversions with breakpoints 25B-27B and 28A-29A lie side by side with a gap of normal banding pattern consisting of five distinct dark bands of subzone 27C-D (Fig. 26). Similarly the inversion falling in between 28B-28C and 28C-31B have a gap of three dark bands of subzone 28C (Fig. 27). Each of the above mentioned inversions affects only a small segment of the chromosome. Therefore, they do not form the typical inversion loops. The most prominent inversion in chromosome 3 is the pericentric inversion which forms a large coiled loop involving as many as seven subzones with breakpoints from 26C-33B (Figs. 10, 28).
Translocations (Fig. 29)

In the present material translocations have been rare, but in one instance a translocation in the X-chromosome has been noticed. In figure 29 an interstitial translocation is visible which has resulted by the insertion of a part of a different X-chromosome into the normal X-chromosome. In order to achieve this configuration two X-chromosomes are involved, a donor and a receiver. The inserted segment involves zones 1 and 2 and the point of insertion lies inbetween subzone 2C and 3A. As the broken segment involves zone 1 and 2, it causes a terminal deletion in the donor X-chromosome and an interstitial translocation in the receiver X-chromosome.

Deletion (Deficiency) (Figs. 30-31)

A deletion can result from the loss of a part of the chromosome. A terminal deletion is produced by a single break near the end of the chromosome whereas an interstitial deficiency is produced by two breaks anywhere between the two ends of a chromosome. Since there is an observable alteration in the banding pattern, the deletions are readily detectable in the polytene chromosomes. In this respect a heterozygous deficiency is more readily seen because in a homozygous condition
the deletion loop will not be forced. In the salivary
gland chromosomes of *Anopheles lindesayi*, two interstitial
deficiencies have been found in a heterozygous condition
in the chromosomal arm 2A. One is deficient in the
banding pattern from 11B-12D (Fig. 30) while the second
is deficient for the segment 13A-14B (Fig. 31).

All the above described aberrations have been
summarized in Table-3.

**Table-3**

Record of the various chromosomal aberrations with their
breakpoints in the natural population of *A. lindesayi*

<table>
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<tr>
<th>Sr. No.</th>
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<th>Chromosome Involved</th>
<th>Zones Involved</th>
<th>Figure</th>
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Anopheles sundicus
(Figs. 32-41)

Description of the X-chromosome (Figs. 34, 35-36)

It is the shortest in the complement, approximately 86 µ in length. Though it usually remains isolated from the two autosomes, its attachment to the chromocentre along with the other elements has been observed in some preparations (Fig. 35). It shows a distinct banding arrangement and can be easily identified by its narrow free end with a small thin band at the tip regularly followed by a distinct dark band in the middle of a small swelling in 1A, three deeply stained bands in 1B and an invariable dark doublet in 1C. The following zone 2, containing a fixed puff in 2A and a drum-like structure with three intensely stained bands in 2B is the most important landmark to recognize the X-chromosome. Besides this, a consistently thick doublet in 3A, and three sharply stained, widely spaced bands in 3B are diagnostic for zone 3. Except a thin dark doublet in 4B, the whole of zone 4 is impregnated with dark broken bands. Zone 5 clearly stands out with an empty puff interrupted by two very thick bands curving in opposite directions. The next zone 6 is very characteristic and in some preparations it is marked by a typical
Balbiani ring (Fig. 36) in subsone 6A. The size of the Balbiani ring depends upon the age of the larva. Usually, the subsone 6A possesses two to three prominent dark bands. The remaining part of zone 6 has a group of about five closely placed thin dark bands in 6B and a large diffused puff in 6C. This puff serves as another important landmark for identifying the X-chromosome from the centromeric end.

Description of chromosome 28 (Figs. 34, 37)

This arm, with a length of 290 μ, is the longest in the complement and is readily recognizable by its fan-shaped free end with 2-3 broken bands at the tip and a pair of dark bands at the base in 7A. This is followed by subsone 7B and 7C each of which contains a light swelling and a pair of dark bands. Similarly, zone 8 is also occupied by a swelling each in 8A and 8B, but with five dark bands in the former and four bands of medium staining intensity in the latter. These characters of this zone together with details of the free end are taken into consideration at the time of identifying 28. Zone 9 is also conspicuous and always contains one pair of dark bands each in subsone 9A, 9B and 9C. The following zone 10 maintains its individuality due to the constant occurrence of
two pairs of closely placed curved dark bands in its middle, whereas zone 11 which is slightly stretched out in some preparations appears almost dark due to the presence of two unequal pairs of dark bands in 11A, three to six closely placed curved bands in 11B and three dark bands in 11C. The frequent occurrence of three heavy bands in 12A and three curved bands in 12B is a salient feature of zone 12. Another important landmark for the recognition of 2B is provided by zone 13 which is constituted by four variegated bands in 13A, three curved bands in 13B, a series of about nine deeply stained bands in 13C and four sharp bands in 13D. The adjoining segment, comprising zone 14 is almost in conformity with zone 13 with regard to the number and staining intensity of bands. The following zones 15 and 16 can be easily made out in this area due to the presence of three spindle-shaped heavy bands in 15A and 16B, whereas a group of 2+2+2 dark bands in 17A, five closely placed dark bands in 17C and a doublet in 17D is a diagnostic feature of zone 17. A somewhat stretched area interspersed with thin dark bands identifies zone 18. It is continued into the centromeric zone 19 which is marked by the presence of a pair of dark bands in 19A, five compactly
arranged thin bands in 19B, three heavy bands in 19C, five dark bands in 19D and a dark doublet at the termination of the centromeric end in 19E.

Description of chromosome 2L (Figs. 34, 38)

The left arm of chromosome 2 is the second largest element in the complement as is evident from its length (218 μ). A thin dark band at the flower-vase-type free end in 28A and a number of thin light bands in a diffused area in the remaining part of zone 28 easily identify the arm 2L. Two widely spaced thick bands followed by two pairs of thin bands in 27A mark the beginning of zone 27. It is further characterized by a swollen area occupied by a thick curved band at its commencement in 27B and a curved band of the same thickness and staining intensity at its end in 27C. It is followed by a constriction containing three thin bands in 26A and a dark doublet in a swelling in subsone 26B. These two characters serve as useful landmarks for zone 26. The next zone 25 has a large puff in its middle flanked by a small swelling on either side. Three moderately stained bands in this puff and a pair of bands in each swelling maintain their constancy in a large number of preparations. The following zones 24 and 23 mostly remain lightly
stained, except the presence of three heavy bands in 24A, two curved bands in 23B, and two wavy bands in 23C. A thick band in 22A, two curved bands in 22B and a group of four regularly arranged dark bands in 22C are a common feature of some 22. The regular occurrence of three consecutive swellings in some 21 is an excellent recognition area of 2L. The first swelling contains six dark bands and the following swellings have two and four bands respectively. A group of three pairs of evenly distributed dark bands along with a deeply stained band at the termination of some 20 marks the centromeric end of 2L.

**Description of chromosome 3** (Figs. 34, 39, 41)

The right arm of chromosome 3 measures about 195 μ in length. It can be identified in the complement by the presence of a balloon-shaped swelling in the free end of some 29. At its beginning this swelling has 2-3 thin dark bands in 29A and a constant dark doublet in 29B. The next some 30 has two thin and three thick dark bands in a swelling in 30A, a light puff in 30B and 4-2 dark bands in 30C. An arrangement of two pairs of deeply stained bands in 31A and three smaller bands of the same staining intensity in 31B is a regular feature of some 31, whereas a series of
several thin but dark bands in 32A followed by three widely spaced dark bands in the remaining part of zone 32 is commonly observed. The following zone 33 always begins with a series of four sharply stained curved bands in 33A and ends in two parallel bands in 33D. This zone constitutes the most important landmark to recognize the right arm of chromosome 3. Except two pairs of dark bands in 34B, three dark bands in 34D and a group of five heavy bands in 35D, both the zones 34 and 35 remain differentially stained and occupied by broken bands. A large puff with two very prominent dark doublets forming a figure of X in 36B and two dark curved bands in 36C is the characteristic identification mark for zone 36. The centromeric zone 37 is an elongated area with three widely spaced bands in 37B and a pair of prominent bands in 37D.

**Description of chromosome 3L (Figs. 34, 40, 41)**

It is the shortest autosomal arm with an optimum length of about 169 μ. A squarish free end with four dark and three moderate bands in 46A followed by a light area ending in two dark curved bands in 46D serves as the best character for recognition of this free end. Chromosome 3L can be further recognized
by the constant occurrence of about nine very sharp bands in zone 45. A serrated and deeply stained band in the middle of a swelling in 44A is a useful landmark in zone 44, whereas a group of five distinct bands in the middle of zone 43 and a 2+3 arrangement of closely approximated thin dark bands in 42B are also diagnostic for zones 43 and 42. A group of six pairs of thin dark and curved bands in zone 41 and a pair of deeply stained bands in a light puff in subzone 40C can be easily located in the preparations. In the remaining part of 3b a pair of dark bands in 39A and a group of three darkly stained bands in 39C and D are present in zone 39 whereas zone 38 is always marked by two pairs of dark bands in 38B and four sharply stained bands near the centromeric end in 38C.

Salivary chromosome polymorphism in the natural population of *A. mundaicus*

Though a fairly high rate of chromosomal polymorphism has been observed in *A. mundaicus*, only the inversions were encountered and these too have been found only in chromosome 2. The X-chromosome and the autosome 3 are completely devoid of any type of rearrangement in their normal banding pattern. In spite of the fact that a large number of preparations
I

were thoroughly scanned.

**Inversions (Figs. 42-53)**

A total of about seven inversions have been found on chromosome 2. Out of these, five are distributed over its right arm (2R), whereas the remaining two are confined to the left arm (2L). All the seven inversions are heterozygous and paracentric.

Starting from the free end zone 7 of 2R the first inversion extends from 11A-15C (Fig. 42) and involves about four zones. Its breakpoints are not clearly visible and it does not form a typical figure of eight but appears like a locked loop. The second inversion involves subzones 12C-14A (Fig. 43). It forms a ring-like configuration and the breakpoints are clearly visible because they show asynaptic light areas at these points. The third inversion lies almost in the middle of 2R and is seen with a high frequency in a large number of preparations. It starts from subzone 13A and extends up to 15C. However, with slight variation in its breakpoints which shift within zone 13 on the one hand and up to the end of zone 16 towards the centromeric end (Table-4). In some cases it forms a usual figure of eight (Figs. 45-49), whereas in others it just
forms a simple loop. The next two inversions i.e., inversion fourth and fifth are present side by side in which it is difficult to identify the intervening part of the normal banding. But as they lie independent of each other, they cannot be considered as overlapping inversions. Both of them affect the segment from subzone 15C-19C (Fig. 50). The last inversion which extends from subzone 16B-18B (Fig. 43) always occurs together with inversion second (Fig. 43). It forms a clear inversion loop but in the figure 43 this loop falls on the chromosome whereby it forms a looked loop-like configuration.

The left arm of chromosome 2 is heterogeneous for two inversions. The first inversion occurs near the centromeric end and extends from 228-20B (Fig. 51), while the second extends from 24C-23A (Fig. 53) and occupies the middle part of 2L. The former inversion sometimes extends from 22C-20A in which it starts from a pair of heavy bands in subzone 20A and extends up to a lone band at 22C (Fig. 52) towards the free end. The latter inversion includes only a few subzones and appears like a knot without any clear ring-like figure or breakpoints because it involves only a small segment from 24C to 23A. A complete list of all the above explained inversions is given in Table-4.
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<th>Chromosome involved</th>
<th>Zone(s) involved</th>
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<td>24C-23A</td>
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Table: Record of the various chromosomal aberrations with their breakpoints in the natural population of *A. sundaisiau*.
Anopheles annularis

(Figs. 5a-6d)

Description of the X-chromosome (Figs. 5c, 5f, 5h)

The X-chromosome is approximately 79 μ in length. It is easily traceable in preparations due to its short length and a characteristic free end which is slightly expanded with two thin dark bands in it, in subzone 1A, whereas four moderately stained bands in subzone 1B constitute the remaining portion of zone 1. A thick dark doublet in the beginning and a thin doublet at the end of a swelling in 2C are diagnostic features. The next zone 3 also has a swelling marked by a dark doublet. The following zone 4 with a pair of swellings having alternating complete and broken bands serves as an important recognition area for this chromosome which is further identified by the constancy of an S-shaped dark band in zone 5. The last zone 6, marking the centromeric end is often puffed and sometimes shows asynapsis. It has two dark bands in 6A, three dark bands in the middle of 6B and three curved dark bands at its termination in subzone 6C.
Description of chromosome 2R (Fig. 56)

As usual, the right arm of chromosome 2 is the longest of all the autosomal arms and measures about 28.4 μ in length. It is represented throughout its course by constrictions and swellings of varying sizes. The expanded free end with two complete or broken bands at its tip and a pair at the end in 7A, can help in recognizing this end of 2R. In the remaining part of zone 7 a large puff with a pair of broken bands in 7B and a triplet in 7C can also be observed. The following zone 8 is possessed by a set of three swellings which have an arrangement of 2+2+2 bands in them. The next zone 9 is again prominent in having a small swelling followed by a large sized puff. The swelling is occupied by three thin bands whereas two sets of unequal dark bands characterize the puff. Similarly a large sized puff in 10B with five very sharp bands in front in 10A and a triplet of the same intensity behind in subzone 10C is a salient feature of zone 10. The adjoining zone 11 has five deeply stained bands distributed as two in 11A, and three in 11B, while a sharp S-shaped band in 12A, three intense bands in 12B and a pair each in 12C and 12D mark the individuality of zone 12. The next zone 13 manifests itself with a light puff guarded
by two pairs of darkly stained bands on its either side. Excepting the presence of three dark bands in 16B, three heavy bands in a swelling occupying 15A and 15B, and two wavy bands in 15C, the rest of the zones 14 and 15 are full of light broken bands which give them a faint appearance in a majority of the preparations. Zone 16 always appears dark due to the presence of several sharply stained broken bands and a group of five compactly arranged dark bands. The following zone 17 has a large swelling with alternating thick and thin bands in 17A and 17B, and a deeply stained triplet in 17D, whereas zone 18 is always distinguished by the presence of 2+3+2 dark bands which extend from 18A to 18C. At the centromeric end of 28 three dark bands in 19A, one in 19B and two pairs in 19C can be easily located in the chromosome as well as in the map.

Description of chromosome 2L (Figs. 56, 59)

Apart from its short length (18+1), it can be easily made out in the complement by its distinct banding pattern. A group of four thin bands covered over by a broken band in 28A, and another group of five darkly stained bands in 28C characterize the free end zone 28. A set of three dark bands at the end of all the swellings
in some 27 always stands out clearly in many preparations. The next zone 26 appears almost light except the presence of a dark band in a swelling in subzone 26A and three widely placed bands in the remaining part. Two dark bands in subzone 25A followed by a series of complete and broken bands in 25B and 25C mark the identity of zone 25. Zone 24 comprises two medium sized swellings, one with six dark dotted bands and the other with three darkly stained bands separated by a thick band in the constriction between them. This is an important character for the identification of 2L. The following zones 23 and 22 appear almost alike in the consistency of bands as shown in the map. Further, each zone is composed of alternating light and dark bands of a variable number. The constant occurrence of four closely placed dark bands in 21A and a series of six to nine thin dark bands in the remaining subzones of zone 21 is peculiar. The centromeric end of 2L can be easily identified by the presence of 2+3 curved dark bands in a swelling in 20C and a group of five thin dark bands at the end in subzone 20D.

Description of chromosome 22 (Fig. 56)

This arm measures about 20\% μ and is immediately located by the constant existence of nine deeply stained
bands in 29A always followed by a light puff in 29B. Along with this a group of four evenly spaced dark bands of varying intensity in 30A and six dark bands in 30B constitute a significant feature of this end of the arm 3A. A row of very thin deeply stained bands in 31B and 31C preceded by three pairs of curved dark bands in 31A is a salient feature of zone 31, whereas two pairs of dark bands enclosing a band in between in 32B and a doublet at the beginning and three curved bands at the end of subzone 32C form a unique character of zone 32. Excepting two distinct curved bands in 33B, the whole of zone 33 appears as a mixture of complete and broken bands. The next zone 34 can be readily identified by a group of six, very sharp, dark bands in 34A. The following zones 35 and 36 appear heavily stained due to the constant occurrence of a large number of closely placed dark bands. A deeply stained doublet in 37A followed by three bands of the same staining intensity in 37B and four thin bands in 37C characterize the centromeric zone 37.

Description of chromosome 3L (Fig. 56)

In addition to its length of 187 μ, the left arm of chromosome 3 has a set pattern of alternating light and dark areas throughout its length. The
elliptical and lightly stained free end in 46A is
followed by a large puff in 46B which further has three
dark bands at its base and serves as the best recognition
area for 3A. The three unequal sized swellings
constituting zone 45 and with a curved band in 45A
and a doublet each in 45B and 45C, collectively form a
distinguishing character of this end of 3A. Similarly
the constant occurrence of five dark bands in 44A,
four heavy bands in 44C and a light area in 44D are
also prominent features of zone 44. The next zone 43
appears dark due to the regular presence of a series
of about twelve dark bands throughout its length.
Except two parallel bands in 42A and three very prominent
dark bands in 42B, the rest of the zone 42 is constituted
by a group of broken bands. Like zone 43, zone 41 is
also a dark area containing a large number of bands of
varying shapes and staining intensities. The next two
zones 40 and 39 falling towards the centromeric end
are almost lightly stained except two dark bands in 40A
and five sharp bands in 39A. The centromeric zone 38
possesses a barrel-shaped puff which begins with a thin
doublet in 38A and terminates at the centromere with
four dark bands in between.
Salivary chromosomes polymorphism in the natural populations of *A. annularia*

The present material of *A. annularia* showed a moderate amount of polymorphism in its natural population. The right arm of chromosome 3 shows more susceptibility to breaks causing heterozygous inversions as compared to the other elements in the complement. The X-chromosome and 3L show no polymorphism. Except inversions, rearrangements like translocations, duplications and deletions etc. have not been encountered.

**Inversions** (Figs. 60-64)

Out of a total of five inversions three are present on 3R, whereas one each is present on 2R and 2L. With the complete absence of any aberration in the X-chromosome, the first inversion is available on 2R wherein it affects the subzones 13B-15D (Fig. 60). As usual, it shows asynapsis near the points of breaks and forms a typical inversion loop.

The second inversion is on 2L in which it starts only two zones away from the free end and extends from 27B-26B (Fig. 61). Its break points are not clearly visible because of a small compact loop.
The remaining three inversions located on 3R involve subzones 29C-32A (Fig. 62), 32A-35A (Fig. 63) and 33A-34B (Fig. 64) respectively. The first inversion forms a hook-like configuration and starts from a light area behind a prominent band in 29C and ends at two broken bands in subzone 32A. In some preparations the basal breakpoint of this inversion serves as the breakpoint for the origin of the second inversion which is coiled and after involving about three zones ends at 35A. The third inversion which involves 33A-34B (Fig. 64) starts with asynapsis of the homologies at a dark band in 33A and terminates at a puff in 34B. All the above mentioned inversions are heterozygous and paracentric and have been summarized in Table-5.

**Table-5**

<table>
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<th>Sr. No.</th>
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<th>Zones involved</th>
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</tr>
<tr>
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<td>&quot;</td>
<td>&quot;</td>
<td>33A-34B</td>
<td>64</td>
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</table>
Anopheles splendidus
(Figs. 65-70)

Description of the X-chromosome (Figs. 67, 68)

In addition to its being only 80 μ in length, it can be traced in the complement due to its characteristic free end which is slightly flared and is marked by three broken bands in 1A, two very closely associated dark bands in 1B and a large puff containing three thin and curved bands in the beginning along with a few dark broken bands in 1C. A highly puffed zone 2 with two very sharp triplets in its neck in 2A and two dark bands at its base in 2C is always seen in the X-chromosome.

This and another very large puff containing a beaded band at its commencement in 3A and a group of 3+2 deeply stained bands in 3B together make an important landmark for further identifying the X-chromosome. Two subequal pairs of dark bands in 4A, three sharp bands each in 4B, 4C and 4D are a regular feature of zone 4. The next zone 5, starting with a light puff in 5A, has five to six dark bands in 5B and 5C. Their number is, however, never constant. The centromeric end with five closely placed dark bands in 6B and a set of 2+3 dark bands in 6C serves as another useful landmark for locating the centromeric end of the X-chromosome.
In comparison to other autosomal arms in the complement, it is the longest and measures about 250 μ in length. A funnel-shaped free end with three closely approximated dark bands at its tip and a similar set of three bands at its base in subzone 7A is a diagnostic feature of 2R. Immediately behind, a 3+1 arrangement of bands in subzone 7B and a pair in 7D are also helpful in determining the free end. Zone 8 and 9 often remain differentially stained and the number of bands is also not constant in them. However, a pair in 8B, five dark bands in 8C, two heavy bands in 9A and a lone band in 9B are uniform in their existence. Excepting five very sharp bands in 10A and a triplet in 10C, the rest of zone 10 is occupied by lightly stained puffs, whereas zone 11 with a series of several dark bands is comparatively darker in staining capacity. Likewise zone 12 is also dark because of the presence of 4+5+6 deeply stained bands extending from subzone 12A-12E. In spite of the presence of a crescent-shaped band at the end of a puff in 13A and two heavily stained bands in 13B, zone 13 appears light because of several light bands in between, whereas zone 14 distinguishes itself with a dumb-bell-shaped region which is rich in having a large...
number of closely placed dark bands. Except a large puff at the end, the front region of zone 15 is occupied by four medium-sized distinct swellings. A group of about five dark bands in 15B, a very band in 15C and a 2+1 arrangement of thick bands in 15K can be located in this zone. A very conspicuous series of widely separated heavy bands interrupted by broken bands is a feature of zone 16, while a group of about ten, very closely placed dark bands in a swelling in 17A and two groups of six bands in 17C constitute an additional character for the identification of 2R. The striking character of zone 18 is the constant occurrence of a set of three well spaced dark bands in 18A, 3+2 thick bands in 18B, a series of deeply stained dark bands in 18C and two dark doublets in 18E. Further, five to seven curved dark bands in 19B and about six parallel bands at the end in 19D denote the prominence of the centromeric region of this element.

Description of chromosome 2k (Figs. 67, 69)

It is about half the length of its counterpart 2R because of the submetacentric condition of the chromosome 2. Measuring about 125 μ, it is recognized by the specific banding pattern of the free end zone 2k which is composed of a very prominent dark band followed by three thin bands in 2k and seven bands of different staining intensities, in the remaining part. The following
zone 27, with a pair of heavy bands in 27A and a row of about eight dark bands of uniform intensity in 27B are also taken into consideration at the time of determining this free end. A pair of deeply stained curved bands in the middle of 26A and another pair of the same intensity in 26B are clearly visible in zone 26. In zone 25, a biconvex dark doublet in the beginning of 25B and a variable number of slender bands in the remaining part of this zone are seen in many preparations. Two dark bands in 24A, a pair in 24B, a light swelling in 24C and three very distinct bands in 24B are also considered important in identifying the middle part of 24. About six thin bands in 23B, a large puff with eight bands in 23A and a small swelling occupied by four sharp dark bands in 22B are contrasting in the segment marked by zone 23 and 22. In the remaining two zones towards the centromere, three medium sized swellings possessing a varying number of dark but curved bands are constantly present in zone 21, whereas a wavy and a straight band in 20B and three very deeply stained bands along with a very prominent dark band at the end in 20C are present in zone 20.

Description of chromosome 3B (Fig. 67)

The right arm of chromosome 3 measures about
188 μ in length. The most important character of its expanded free end is the constant occurrence of three thin bands in 29A, a large puff in 29C, and three distantly placed bands in 29D. A small swelling in 30A, three very close deeply stained bands in a constriction in 30B and four wavy bands in subzone 30E are diagnostic for the subterminal zone 30. Except a thick band at the end of a lightly stained puff in 31A, the remaining part of zone 31 remains somewhat stretched and is made up of thin and broken bands. The next zone 32 can be readily recognised by the presence of two pairs of dark bands in 32A and two darkly stained bands in a constriction in 32C. An elongated swelling with five well spaced bands in 33C is a constant feature of zone 33. The regular occurrence of a pair of dark bands in 34A and a triplet each in subzones 34B and 34C mark the individuality of zone 34. The adjoining zone 35 is formed of a series of alternating light and dark bands as exhibited in the standard map. Five equally spaced thick dark bands, filling up a large puff in 36C, constitute an important landmark for identifying zone 36. Two subequal pairs of dark bands in a large swelling in 37A, a pair in the middle of a swelling in 37B and an irregular dark band at the end of subzone 37D mark the extremity of the arm 38.
Description of chromosome 3H (Figs. 67, 70)

In an optimum stretching, it is about 174 μ in length. A thin dark doublet always covered by a broken dark band in 46A identifies the free end. It is immediately followed by a light diffused area containing three evenly spaced bands in 46B. The next zone 45 manifests itself by two dark bands in 45A, a curved band in the middle of a light puff in 45B, and a set of three bands of the same staining intensity in 45C. Each of the subzones 45A and 45D carries a set of three dark bands along with two widely spaced bands in zone 44, whereas zone 43 remains almost lightly stained except a prominent band in subzone 43A and a 2+1+2 dark band arrangement in 43B. Five equidistant dark bands in 42A indicate the beginning of zone 42, whereas a series of differentially stained bands is available in its remaining part. The following zone 41 clearly stands out with three well spaced bands in 41A, three closely associated dark bands in 41B, and a continuous row of narrowly arranged dark thick bands in 41D. The existence of three pairs of dark bands in 40C and the same number of moderate bands in 40D are the salient feature of this segment. The next zone 39 distinguishes itself by having three pairs of sharply stained bands in 39A and
five bands in 39D, whereas the centromeric zone 38 begins with a light puff and ends with a dark band. In addition, its middle part is composed of fourteen dark bands distributed as seven in 38B, 2+3 in 38C, and two in 38A.
Anopheles cementus
(Figs. 71-74)

Description of the X-chromosome (Figs. 72, 73)

The X-chromosome of Anopheles cementus averages about 50 μ in length and is the shortest in the complement. The free end in subzone 1A is funnel-shaped and has a set of five dark bands in it. The first three bands are thin, whereas the remaining two are thick and curve in the same direction. In most preparations, depending upon the age of the larva, the former set of three slender bands, occupying the tip of the free end, do not show their constancy in shape and staining capacity because this area tends to get flared. In that condition the latter two bands which are constant are used for identification of this free end. The remaining subzones 1B and 1C are prominent in having a moderate swelling with two dark bands curving away from each other and separated by four light dotted bands. The following zone 2 has two small swellings with three and two dark bands respectively. Zones 3 and 4 have some of the most prominent bands and a swelling in them which make this region an important landmark for identification of the X-chromosome. This identification is further aided by
a large puff in zone 4 containing a set of four bands which show their constancy in almost all the preparations. Similarly a triplet in 3C and a pair in 4C are also constant. The next zone 5 begins with a comparatively lighter area in 5A which is characteristic in having an identical pair of bands curving in an opposite direction and separated by a dark brown band, whereas 5B and 5C carry a set of three thick dark, four thin dark and two thick dark bands. The following zone 6 which is also comparable to the left arm of the chromosome in some species, is composed of a pair of equal swellings with a set of three heavy bands in each of them.

**Description of chromosome 2R (Fig. 72)**

In comparison to other autosomal elements, it is the longest (210 μ) and has many excellent landmarks for its identification. The free end in zone 7 has two dark bands at the tip and two dotted light bands in 7A, whereas 7B has a very large puff having five wavy bands. This zone ends at a clearly defined subzone 7C which contains a closely placed set of four deeply stained bands. The area behind this region, covered by zones 8 and 9 has got almost similar puffs and sets of bands held by it in the various subzones. The most prominent of them is a puff in 8B-C and 9C. The
succeeding zones 10, 11 and 12 are darker in appearance due to the richness of banding in almost all the subzones, especially 10 to 12B. Three puffs, one each in 10C, 11C and 12B are prominent and can be easily located because of their enlarged size in those preparations which are made from the early fourth instar larvae. A second series of closely placed dark bands extends from subzone 13B to 14D which is separated from the former series, from 10B to 12C, by a pair of light puffs having just a band or two in 12D. This pair of puffs is important because it marks the limitations of two series of bands to its left and right. Zone 15 is characterized by a swelling in its beginning and at the end. The constant bands are those that are present in 15B, whereas the rest of the dark bands (three in 15B and four in 15D) are generally seen as dark broken bands with several dotted bands present in between. The next zones 16 and 17 are constituted by equally spaced pair of bands in 16A and 16D, whereas a large puff in 17B with a set of six dark bands guarded on either side by identical lightly stained swellings, is unique for this zone. A comparatively stretched area with a row of about twenty five recognizable dark bands in it is characteristic for zones 18 and 19. The latter also marks the centromeric end of 28, which is identified by a set of four dark bands each
in 19A and 19B plus a group of five to seven moderate bands.

Description of chromosome 2L (Fig. 72)

Due to its short length of 120 μ, it can be readily differentiated from its counterpart 2R as well as from the other elements of the complement. A highly flared and fan-shaped free end with a very prominent pair of dark bands further helps in the identification of 2L. A set of three small swellings, one each in 28B, C and D and four dark bands in 28C are also important to recognize the free end. The following zone 27 is marked by the presence of two large puffs in subzones 27B and 27C, whereas the next zone 26 is comparatively narrow and straight with several constant bands in almost all the subzones of these two zones. Another characteristic feature for the ready recognition of 2L is the constancy of a large puff with dark bands in it at the end of zone 24. This puff is also seen readily in the preparation because of the absence of any such puff in the neighbouring subzones 25B to 24B and 23A to 22C which lack any such swellings. Several sets of dark bands can be located in the map in subzones 25A, 25B, 24B, 24C, 23A and 22B. The zone 21 once again provides a landmark due to a row of several dark bands extending from 21A to 21C, whereas
zone 20, marking the centromeric end of 2L, is important in possessing a large puff in 20B and a pair of bands in 20B and 20C.

Description of chromosome 3B (Fig. 72)

The right arm of chromosome 3 approximately measures about 100 μ. A funnel-shaped free end with a group of three dark bands at its tip is characteristic of 3B. It is invariably followed by a set of 4+2 dark bands in subzones 29B and 29C respectively. The next zone 30 starts with a small swelling and four thin bands curving in unison. A set of seven dark bands in 30B is readily recognised while identifying the free end of 3A. The following zone 31 is unique in having a double swelling with an identical pair of bands having opposing curvatures. Beyond this region zones 32, 33 and 34 are constituted by moderate swellings and almost equally spaced series of several dark thick and dark thin bands. A very heavy band in 35A marks the end of these series. The most prominent bands can be located in subzones 32C, 33B, 34B, 34C and 35A. The subzones 35B, C and D have a slightly overstretched region which is generally provided with a variable number of detectable dark bands and irregularly distributed light dotted bands. Zone 36, in its turn, is unique in having the characteristic
"S"-shaped configuration of sharply staining bands in which the horizontal connection between the doublet is not a constant feature. The centromeric end of 3A is marked by zone 37 which possesses three pairs of clearly defined bands in 37A and 37B. A set of five dark bands in 37C marks the end of 3A.

**Description of chromosome 3L (Figs. 72, 74)**

The left arm of chromosome 3, which measures about 120 µ in length, has a squarish free end which generally has a fused mass of chromatin granules and three dark bands in 46A. A large puff with very heavy and sharply staining dark bands in 46B helps in easy recognition and confirmation of this free end. In overall consistency 3L is probably the richest with respect to the availability of dark bands in the various zones. The major landmarks are provided by the large puffs in zones 42 and 41, whereas zones 40 and 39 are characteristic in having a row of several closely placed dark bands extending from 40A to 39B. The other prominent characteristics are a set of eight dark bands curving in the same direction in 44B, three heavy bands in 44D and four to six bands in 43A. The centromeric zone 3B is slightly thinner because of a little overstretching.
It begins with a pair in 38A followed by a doublet in 38B, a group of 5+1 dark bands in 38C and three dark bands in 38D.
DISCUSSION

Salivary gland chromosomes

This study of the salivary gland chromosomes has contributed tremendously to our understanding of the trends in evolution of the species. Much of our present knowledge is the result of remarkable researches carried on Drosophila which reveals the best developed and well banded giant chromosomes (Dobzhansky, 1950, 1951; Patterson, 1952).

The phylogeny or evolution of the species belonging to the order Diptera has been worked out through comparative studies of the chromosome banding pattern. During the last twenty years, cytogenetic studies on mosquitoes were made exactly on the same lines as had been carried earlier for Drosophila and Chironomus. Although most of the studies were repetitive, they had their own significance due to the fact that mosquitoes have a direct relation to human economy all over the world. As many species are serious vectors of diseases of man their cytogenetic studies paved the way for developing methods for their control through genetic means (Pal and White, 1974). It was also found necessary to understand their exact taxonomic status and bionomics, because these aspects were important for their vectorial role and control.
The approach of comparative cytogenetics solved many problems connected with their cytotaxonomy and interspecific relationships (Colussi, 1970; Kitsmiller, 1967; Kitsmiller et al., 1973). The process of speciation, interrelationship of the species and the evolutionary trends in the family Culicidae have been studied by the various workers using banding pattern of the salivary gland chromosomes. Based on a comparison of the salivary chromosome maps, the phylogenetic kinship among the various species of the genus *Anopheles*, studied so far, is now well understood (Schreiber, 1963; Kitsmiller, 1967, 1976; WHO Scientific Group, 1964, 1967, 1968; Chowdiah et al., 1971; Kanda and Ogusa, 1972a, b). Many anophelines form species groups in which it is possible to distinguish the various species only by their chromosome studies. This has been particularly true for *Anopheles gambiae* complex (Colussi, 1964a, b, 1966; Colussi and Sabatini, 1967, 1968; Davidson, 1962, 1964a, b; Davidson et al., 1967). The practical importance of cytogenetical studies in the insect vector of disease is now undisputed.

In the oriental region, the genus *Anopheles* is represented by about 45 species, whereas the salivary chromosome maps are available only for about a dozen of them. This has provided sufficient scope for the study
of those species in which nothing is known about their salivary chromosome make up. Presently, the salivary chromosomes have been described in *Anopheles lindesavi*, *A. sundaisus*, *A. annularis*, *A. splendidus* and *A. aconitus*. The first species belongs to the subgenus *Anopheles*, while the remaining four belong to the subgenus *Cellia* (Christophers, 1933; Knight and Stone, 1973). In addition to the detailed descriptions of the banding pattern of the chromosomes and the production of their maps, each of them has also been compared with its morphologically and taxonomically close relative. Such an approach is important in tracing the interrelationships among the closely related species of the region covered by the Indian subcontinent. Many species falling in the same series or a group of the subgenus are so alike in their morphological characteristics that their banding pattern homologies and differences provide additional information with regard to the extent of their relationship and exact taxonomic status. For these obvious reasons the present five species of the genus *Anopheles* have been compared with their allied species as follows:-

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<td><em>Anopheles</em></td>
<td><em>A. nigerrimus</em> and <em>A. barbirostris</em></td>
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</table>
While carrying out comparisons between the species listed above, the banding pattern similarities have been traced by band to band comparisons between the maps which are already available in literature.

Comparison of *A. lindesayi* with *A. nigerrimus* and *A. harbirostris*

*A. lindesayi* is a representative of the subgenus *aphorodes*, which has 13 species in all in the Indian region, whereas the salivary chromosome maps are available for only three of them viz., *A. hyrcanus* var. *sinensis* (Sharma, 1971), *A. hyrcanus* var. *nigerrimus* (Seetharam and Chowdiah, 1971), and *A. harbirostris* var. *ahmed* (Pasaher, 1974; Chowdaiah et al., 1970). The present map of *A. lindesayi*, when compared with their maps, has shown very little homologies with *A. hyrcanus* var. *sinensis*, whereas sufficient similarities were noted with the maps of the other two species. It is for this reason that comparison of *A. lindesayi* with *A. hyrcanus*...
var. nigerrimus and A. barbicornis var. shomi has been carried out in detail.

Comparison with A. hyrcanus var. nigerrimus

The X-chromosome of lindesavi is quite distinct in its banding pattern like the X-chromosome of the other snophaline species, but shows only negligible homologies with the X-chromosome of nigerrimus because only the shape of the free end and some light bands along their lengths seem similar to some extent. When the chromosome 2 is compared the right arm of chromosome 2(2n) of lindesavi appears to show many similarities with that of nigerrimus. The shape of the free and the centromeric ends is quite alike in the two species. Despite this, a single dark band at the tip of the free end in 6A and a set of eight sharply stained bands in subzone 8C can be clearly made out in lindesavi and nigerrimus. Zone 9 is completely different in the two because the richness of dark bands in lindesavi is lacking in nigerrimus which has a row of light bands instead. But the existence of two dark bands at the end of subzone 10B can be easily paired in both the species. Except a little difference in the staining intensities of bands, subzones 11C-D and 12A of lindesavi are identical with subzones 11D and 12A
of *nigerrimus*. The following subzones 12C, 13B and 14A are again alike in the two species. In the remaining part of zone 14, a group of about five thin dark bands in 14C of *lindesavi* resembles a similar group in 14B of *nigerrimus*.

Striking homologies are exhibited when the left arm of chromosome 2 of *lindesavi* is compared with that of *nigerrimus*. Zone 21 and subzone 20A and 20B are almost alike in both of them. A group of 3+2 dark bands covering subzones 19D-18A of *lindesavi* are further comparable to those which are present in subzone 19B of *nigerrimus*, whereas subzone 19C of *lindesavi* resembles 18B of *nigerrimus*. Excepting the presence of a few similar bands in 17A and 15C of *lindesavi* and *nigerrimus*, the remaining part of 2L is quite different in them.

In chromosome 3 of *lindesavi* and *nigerrimus* large scale homologies have been encountered. These homologies are more in 3A than in 3B which is probably due to the fact that 3A is the longest of all the autosomal arms. Even otherwise, most of the banding pattern seems unaltered in the two species. As far as the shape and banding pattern of the free end (zone 22) are concerned only the banding is the same because the tip is highly flared and fan-shaped in *nigerrimus*. 
Immediately behind 22A, in which four dark bands are identical in the two species, the following subzones 22B, 22C, 23B, the whole of zone 24 and subzones 25A and 25B of *lindessyi* carry dark bands which can be easily homologised with those present in the same numbered zones and lettered subzones of *nigerrimus*. Similarly a set of four heavy bands in 27A, 27D and a triplet in 28A of *lindessyi* are remarkably identical to those available in the same segments of *nigerrimus*. In the remaining section of 3A the similarities are mainly with respect to the dark and heavy bands and puffs, whereas the light band arrangements show variation in number and staining intensity. The more closely comparable zones are as follows:

*lindessyi* 29B 30A 30B-C 31A 32A-B  
*nigerrimus* 29B 30A 30B-C 31A 32A-B

With respect to the left arm of chromosome 3, the two species do not show sufficient homologies to warrant close banding pattern relationships. However, the shape of the free ends along with a group of four dark bands in subzone 39B is identical in the two. The other comparable bands in the remaining zones are encountered in subzones 38B-C, 36A-B and 34 of *lindessyi* and 38B-C, 36A and zone 34 of *nigerrimus* respectively.
Comparison with *A. barbirostris*

The salivary gland chromosomes of *A. lindesavi* when compared with those of *A. barbirostris* show less homologies in comparison to those encountered in *nigerrimus*. As usual, the X-chromosome of *lindesavi* shows sufficient independence in its banding arrangement because only a dark doublet at the tip of the free end in subzone 1A and three curved bands at the centromeric end in 5C of *lindesavi* are comparable to those present in the same subzones of *barbirostris*. The rest of the zones are quite different in the two species.

In chromosome 2 of *lindesavi* and *barbirostris* moderate amount of resemblances are available. Although there is a considerable difference in the length and banding pattern of this arm in the two species yet identical banding can be seen in the following regions.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>2A</th>
<th>11D</th>
<th>12A</th>
<th>12C</th>
<th>13B</th>
<th>14A</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>lindesavi</em></td>
<td>2R</td>
<td>11D</td>
<td>12A</td>
<td>12C</td>
<td>13B</td>
<td>14A</td>
</tr>
<tr>
<td><em>barbirostris</em></td>
<td>2R</td>
<td>11D</td>
<td>12A</td>
<td>12C</td>
<td>13B</td>
<td>14A</td>
</tr>
<tr>
<td><em>lindesavi</em></td>
<td>2L</td>
<td>21A</td>
<td>20D</td>
<td>19A</td>
<td>17D</td>
<td></td>
</tr>
<tr>
<td><em>barbirostris</em></td>
<td>2L</td>
<td>21B</td>
<td>20A-b</td>
<td>19A</td>
<td>15B</td>
<td></td>
</tr>
</tbody>
</table>

When the right arm of chromosome 3 of *lindesavi* is compared with that of *barbirostris*, some homologies are apparent. In the free end a set of four bands in
22A and 6-7 bands in 23A-B of *lindesayi* are identical to the four bands in 22A-B and 6-7 bands in 22C-23A of *barbirostris*. Beyond this region, a limited correspondence of bands can be made out in zones 25, 30, 31 and 32, in the two species. The left arms 3L of these two species are quite independent in their banding sequence and do not show homologies at all.

As mentioned earlier, the subgenus *Anopheles* is represented by 13 species, out of which 8 belong to the series *Anopheles*, one to series *Lophoscelomyia* and four to series *Mysorrhynchus* (Christophers, 1933). The series *Anopheles*, to which *A. lindesayi* belongs and also the series *Lophoscelomyia* are yet unexplored so far as their chromosomal analysis is concerned. However, in the series *Mysorrhynchus*, the chromosome maps are available for *A. abisi*, of the *Barbirostris* group (Raid, 1962) of species and *picerrius* and *sinensis* of the *Hyrcanus* group (Raid, 1953).

Morphologically *lindesayi* resembles *barbirostris* and *hyrcanus* only to the extent of colouration and body size, whereas on the basis of banding pattern homologies it is more close to *hyrcanus* than to *barbirostris*. But as the three species belong to
different series, the homologies seem to represent only the subgeneric relationship. Otherwise *lindesavi* has got its own banding pattern.

**Chromosomal polymorphism in *A. lindesavi***

The most striking feature of *A. lindesavi* is the occurrence of a high rate of chromosomal polymorphism in its salivary gland chromosomes. Though a few deletions and translocations have also been recorded, inversions are the commonest. A total of 13 heterozygous inversions, distributed as two in X, one in 2R, three in 2L, six in 3R and one involving the arms 3R and 3L, have been isolated. Out of these 13 inversions, only one involving the arms 3R and 3L is pericentric, whereas the remaining ones are all paracentric. Out of the 13 inversions recorded herein, only those present in the X-chromosome and 3R (those varying between zones 28-31) fall in the category of non-random or static inversions, whereas the remaining ones are "recurrent endemic" and therefore were missing from some of the preparations. For example, the four inversions in chromosome 2 are random because in about 50% of the preparations, the chromosome 2 was found to be normal. The maximum number of aberrations were available in those preparations which were made in the months of October and November, whereas those
made in the months of August and September had only the constant inversions. It indicates the marked influence of fluctuations in the meteorological conditions and an overall impact of the seasonal changes. Though much has not been done with regard to the impact of seasonal changes on the chromosomes of mosquitoes, Dubinin and Tinashova (1945) and Carson (1959) reported the effect of seasonal variations in the gene arrangement of Drosophila humbrica and D. robusta respectively. Similarly, Kabanova et al. (1973) found the effect of seasonal variations on chromosomal polymorphism in A. ersonal. As has been mentioned earlier, A. lindestayi inhabits the alpine environment with considerably lower temperatures. During the present collections of this species, it was found breeding in a spring with the temperature of water ranging from 10 to 15°C which is about 10°C below the normal temperature generally required by mosquitoes. Aronin (1968) and Fukutani (1976) also observed a marked influence of temperature changes on the giant chromosomes of D. flavinosa and D. lutescens respectively.

From the present investigations, it can be concluded that A. lindestayi is highly polymorphic and its chromosomes are greatly influenced by the various natural factors which is not generally the case in the wild populations. DaCunha et al. (1950), while working
on the adaptive polymorphism in *Drosophila willistoni*, stated that any species which inhabits a temperate or a cold climate finds itself exposed at different seasons to a succession of sharply different environments. In the light of these comments the inversion polymorphism (except fixed inversions) of *A. lindesayi* is the result of a tendency on the part of the species to adapt itself to the changing environment.

Comparison of *A. sundalis* with *A. subpictus* and *A. gambiae*

Morphologically *A. sundalis* is a close relative of *A. subpictus* whose salivary chromosome maps have been produced by Narang *et al.* (1973a) and Seetharan and Chowdiah (1974). As the banding pattern of the present species *A. sundalis* seems more near to the *subpictus* map of Narang *et al.*, the comparative account that follows relates to their map. Along with this, some comments are also added about its comparison with *gambiae* because of striking similarities of two inversions in chromosome 2, which are alike in the two species, and the general banding pattern as a whole.

Comparison with *subpictus*

The banding pattern of X-chromosome in *sundalis* and *subpictus* is almost unaltered whereby band to band
Homologies can be easily derived. The only difference present is in zones 2-4 in which the entire banding sequence from subsone 2A-4A of *sundaicus* has undergone a homologous inversion. This is indicated by the fact that a puff in 2A and four dark bands in 2B of *sundaicus* can be easily located in subsone 4A of *subnictus*. Likewise, bands in the region inbetween 2A-4A are also reversed. The banding sequence in X-chromosome of *sundaicus* is further complicated by the occurrence of an included inversion from 3A to 4A which has restored the normal order of bands which are present in subsones 2A-3A of *subnictus*. The exact derivation of *sundaicus* arrangement from *subnictus* may be seen in the table 5 below and figures 75, 76.

<table>
<thead>
<tr>
<th>Subnictus zones</th>
<th>1A</th>
<th>2A - 2C</th>
<th>3A</th>
<th>3C - 4A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1A</td>
<td>4A - 3C</td>
<td>3A</td>
<td>2C - 2A</td>
</tr>
</tbody>
</table>

**Final order**

| 1A | 4A | 3C | 2A | 2C | 3A |

| *Sundaicus* zones | 1A | 2A | 3A | 4A |

In chromosome 2, the shape of the free end and the banding arrangement in the two species are alike, but the following zone 8 has once again suffered a homologous inversion as a result of which the bands in this region are in the reverse order when compared with those of...
subpictus, because a puff having four bands in 8A and 
a doublet in 8C of subpictus are present as a doublet in 
8A and a puff with four bands in 8C of subpictus. In 
zones 9-12, the normal banding pattern follows in which 
the arrangement of bands in the regions 9,10,11C and 12B 
of subpictus corresponds to that of regions 10, 11A-C and 
12C of subpictus. In zone 13, there is again a homologous 
inversion which has reversed the order of bands in 
subpictus because in subpictus there is a 3+4 band 
arangement in subzone 13A and 13B respectively, whereas 
it is 4+3 in case of subpictus. The remaining parts 
of 2A from zones 14-19 of the two species are further 
homeologous because of the identical nature of several 
puffs and dark bands especially the series of dark bands 
from subzones 13C-14A, 16B-17A and 19A-19B. This is 
also the region which is prone to several paracentric 
inversions whose account has already been given earlier 
in the chapter on observations, whereas their impact on 
the species has been discussed later in this chapter.

The left arm, 2L is also morphologically similar 
in subpictus and subpictus. A comparatively light area 
interrupted by a few dark bands marking the centromeric 
end in zone 20 is also constant in them. The segments 
inbetween the free and the centromeric ends exhibit 
important similarities in which comparable zones are
One of the most important features of 2L of
sundaicus is the presence of a homzygous inversion
from 26A-26C in which the banding sequence is the reverse
of the band arrangement in subnictius, because a biconvex
doublet in 26B and a set of four dark bands in subzone
26C of sundaicus are present as a set of four bands in
26A and a biconvex doublet in 26C of subnictius.

As far as the comparisons between the autosomes
3 of sundaicus and subnictius are concerned, a close
 correspondence of bands has been revealed in the zones
extending from 30-37 in 3R. In this area remarkable
similarities have been encountered with respect to the
alternating series of dark and light bands in which a
puff in 30B, a set of four bands in 31A, a set of four
curved bands in 33A and a dark swelling in 35D of sundaicus
are comparable to the details of same numerical subzones
of subnictius. In its counterpart 3L, starting from
the free end zone 46, the same band arrangement is
available up to the subzone 43 of the two species.
A group of four dark bands in 46A and a light puff with
two dark bands in 46C-D are quite identical in *sundaicus* and *subnictus*. Apart from this, sections 40C and 39A-D of the present species are also comparable with 40B and 40D-39D of *subnictus*.

Comparison with *gambiae*

The X-chromosome in the two species *sundaicus* and *gambiae* is entirely dissimilar except for a puff in some 6C of *sundaicus* which is comparable to a similar puff in the XL of species A and B of *gambiae* (Colussi and Sabatini, 1967). As regard the four autosomal arms, the right arms of chromosome 2 are alike in the details of their major band sequences in the two species, whereas 2L, 3R and 3L of *sundaicus* resemble 3L, 2L and 3R of *gambiae* respectively. This situation is exactly similar to the one encountered in *A. subnictus* by Narang et al. (1973a) and also refers to the foot note on page 179 in Colussi and Sabatini’s paper of 1969 on the cytogenetic observations on the salt water species, *A. artemis* and *A. salae* of the *gambiae* complex. Keeping this in view, the homologies in the salivary gland chromosomes of *sundaicus* and *gambiae* are briefly below in Table-7, in which more conspicuous banding homologies are given in brackets:
Comparison between the chromosomes of *A. undalis* and *A. gambiense*

<table>
<thead>
<tr>
<th>Species</th>
<th>Chromosome</th>
<th>Comparable zones</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>undalis</em></td>
<td>X</td>
<td>3A 3B 6C (puff)</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>3A 4C XL (puff)</td>
</tr>
<tr>
<td><em>gambiense</em></td>
<td>2R</td>
<td>7 8A 9A 9C 11A 16B 17D 19E</td>
</tr>
<tr>
<td></td>
<td>2R</td>
<td>7 8A 8C 9C 10A 16D 17D 19D</td>
</tr>
<tr>
<td><em>undalis</em></td>
<td>2L</td>
<td>28 27B-26B 25A-25B 22C 20</td>
</tr>
<tr>
<td><em>gambiense</em></td>
<td>3L</td>
<td>46 44C-43B 45A-45C 39B 38 (inverted)</td>
</tr>
<tr>
<td></td>
<td>3R</td>
<td>29-30A (free end) 30C (dark bands and puff) 33</td>
</tr>
<tr>
<td></td>
<td>2L</td>
<td>28 (free end) 27D (dark bands and puff) 23</td>
</tr>
<tr>
<td><em>undalis</em></td>
<td>3L</td>
<td>46 45C-45B 42A-42C 38 (four bands)</td>
</tr>
<tr>
<td><em>gambiense</em></td>
<td>3R</td>
<td>29 31B-31D (inverted) 33 37C-D (four bands)</td>
</tr>
</tbody>
</table>
Chromosomal Polymorphism in *A. sundaisius*

The natural population of *sundaisius*, collected from the hot and humid environment of the coastal areas of the Eastern India has been found to possess an extraordinary amount of chromosomal polymorphism which has not been ordinarily seen to the same extent in the anopheline species studied so far, because the wild populations are normally less polymorphic (Hobbs, 1962) as compared to the laboratory bred strains (Coluzzi et al., 1973; Sharma et al., 1977). However, there are instances where a low to moderate amount of polymorphism has been reported in many species (Baker and Kitzmiller, 1964a; Chowdaiah et al., 1966, 1971; Kreutzer and Kitzmiller, 1970, 1971; Kreutzer et al., 1972, 1975). In the present population of *A. sundaisius*, a total of seven heterozygous inversions has been isolated in addition to the four homozygous inversions. In the number and distribution of these inversions, *sundaisius* has a remarkable similarity to *A. gambiae*. Further, the total area of chromosome 2 which suffers inversions is also the same in *sundaisius* and *gambiae*. Moreover, the subzones having the breakpoints of these inversions also coincide with those of *gambiae*.

It is, therefore, apparent from the extent of homologies between *sundaisius* and *subpictus* and *sundaisius* and *gambiae* that the three species are closely related.
cytogenetically and show their ancestral affinities. It is surprising to note that Christophers (1933) carved out a separate group Pseudomysomyia out of the subgenus Callie (Knight and Stone, 1973) for the oriental species subpictus and mundicus. This group actually consists of three species viz., subpictus, varus and mundicus and it is presumed that varus which is very closely similar to subpictus in morphology might also reveal its kinship with mundicus.
Comparison of *A. annularis* with *A. stephensi*

*A. annularis* belongs to the group *Neocellia* of the subgenus *Cellia*. Though Chaudhry (1978) published a brief note on the salivary gland chromosomes of this species, he worked on a population from Guwahati, Assam (North-East, India). Its comparison with *stephensi* (Sharma et al., 1969; Mittal and Vas Dev, 1977) has been carried out on the basis of its obvious homologies with the banding pattern of the latter.

Although the X-chromosomes of *annularis* and *stephensi* are distinctive and species specific yet a few sections in both appear to reach close approximation. For example, four dark bands in a barrel-shaped swelling in subzone 1A, two thin bands in a constriction in 38, a group of about five deeply stained bands in 4A and a constant occurrence of three dark bands at the centromeric end in subzone 6C can be easily correlated in the two species.

Likewise, when the right arm of chromosome 2 of *annularis* is compared with the same of *stephensi* sufficient homologies are observed. A large puff at the free end with four dark bands in 7A and three dark bands in 7C are easily comparable in *annularis* and
Similarly a light puff in 8A, a doublet in 9C followed by four widely spaced dark bands in a swelling in subzone 10A of *annularis* can be easily paired with similar characteristics of subzones 8C-9A, 9B and 10B of *stephensi*. Further, a medium sized puff with 2+3 dark bands in 11A-B, a group of dark bands in 12A-B and a part of zone 17 of *annularis* also correspond to a light puff in 11A, a few dark bands in 12B-C and a part of zone 17 of *stephensi*. At the centromeric end, the whole of zone 19 is identical in the two species except for minor adjustments of the subzones. On the other side of the centromere, the left arm 2L also shares striking homologies. In this arm, the free ends are slightly similar but the following area comprising subzones 27B, 26A, 22C-D and 20C-D of *annularis* is very much identical with that of *stephensi* while 28C, 27B and 25B-24A of the former are comparable to the bands and puffs in subzones 27A, 27C and 25C-24B of the latter.

The right arm of chromosome 3 of *annularis* shows the least amount of comparable bands with *stephensi* except that a light swelling in free end zone 29 and a few dark bands in a puff in 32C of *annularis* correspond well with zone 29 and subzones 33C-D of *stephensi*. 
The remaining part of this arm shows an entirely different banding sequence in both the species. To the contrary, 3L of *annularia* shares more homologous areas with *stephensi*. A comparatively light area extending from the free end zone 46-45 is almost alike in them, whereas the differences are limited only to the distribution of a few dark bands in this region. Similarly in the following zones 44, 43, 41 and 40, striking homologies are visible in the two species wherein a light puff in zone 40, a group of bands in 43, and a series of alternating light and dark bands extending from zone 41 to subzone 43C are more prominent.

Group Neocellia is represented by fourteen species in the Indian region out of which the salivary chromosome maps are available for about five of them. These are: *pulcherrima* (Baker et al., 1968), *stephensi* (Sharma et al., 1969), *annulatus* (Sharang et al., 1973), *phillipinensis* (Sharma, 1977), and *rassavi* (Chaudhry, 1979). When compared with these, *annularia* seems to have inherited its banding pattern from *stephensi* or vice versa. But its more close relationship with *phillipinensis* cannot be ruled out because the two differ in their external morphology only by a minor difference in venation of the wing. But in the map of *phillipinensis* the X-chromosome
is extraordinarily long and the autosomal arms are also disproportionate when compared with those of *annularis*.

**Chromosomal polymorphism in *A. annularis***

As regards the chromosomal polymorphism, *A. annularis* shows a moderate amount of inversion polymorphism. In all, five inversions have been encountered in the present population of *annularis* which are all paracentric and heterozygous. Out of these, three inversions are located on 3R, whereas one each is present on 2R and 2L. None of them was constant because only 20% preparations had these inversions in one or more complements.

On the basis of these inversions, *annularis* and *stephensi* are only distantly related because none of the inversions in *annularis* is common to those found in *stephensi*. Again, the *stephensi* population which co-exists with *annularis* has been found to be completely free of inversion polymorphism (Sharma et al., 1978), whereas 24 different inversions were isolated from a laboratory bred strain of this species (Sharma et al., 1977).
Comparison of *A. splendidus* with *A. stephenai*

While comparing all the chromosomal elements of *splendidus* with *stephenai*, it has been revealed that the chromosomal arm 2 and 3A in both the species show more comparable areas than the X and 3A. As has been seen in other species of the genus, the X-chromosome shows a characteristic banding pattern. When compared with *stephenai*, the most striking similarity exists between the large puff in zone 3. In addition to this, three dark bands in 2A, four dark bands in 4A and the whole of zone 6 (except subzone 6A) are also very much identical in them.

The right arm of chromosome 2 of *splendidus* appears slightly longer than that of *stephenai*, but shows many banding pattern similarities with 2A of *stephenai*. Being longer, there are differences in the marking of zones and subzones for the same sets of bands. The free end zone 7 with a number of dark bands and a larger puff is exactly identical in *splendidus* and *stephenai*, whereas at the centromeric end, zone 19 of *splendidus* is very similar to the combined zone 18 and 19 of *stephenai* in the number and intensity of bands. Despite this, a dark and light puff in zone 10, a series of dark and light bands in zones 11, 12 and 14 are almost alike in *splendidus* and *stephenai*, whereas a group of about twelve deeply
stained bands occupying 16A-B and a series in 17C of splendidus can be easily homologised with the same number of bands in 15B-16A and 17A-B of stephensi. In chromosome 2L, the free end does not show any affinity in shape and number of bands with that of stephensi but zone 27, adjoining the free end zone 28, with a number of deeply stained bands clearly stands out in splendidus and stephensi. Unlike zone 27 a series of dark bands occupying the middle part of zone 25 of splendidus corresponds well with a similar series of dark bands in an area extending from 25C-24C of stephensi. Likewise, a dark triplet in 24B and four prominent bands in 22B of splendidus can be traced in 23B and 22C of stephensi. In addition to this the centromeric zone 20 is strikingly similar in both the species.

When a band to band comparison is made between the arm 3A of splendidus and stephensi it is seen that the shape of the free end is somewhat alike in the two species, but the central part and the centromeric end are very much divergent. Whatever little homologies are encountered, they differ with regard to the position of the zones and subzones. The comparable segments are clear from the following table:-

<table>
<thead>
<tr>
<th>splendidus</th>
<th>3A</th>
<th>29D</th>
<th>30E</th>
<th>32B</th>
<th>33C</th>
<th>34C</th>
<th>35C</th>
</tr>
</thead>
<tbody>
<tr>
<td>stephensi</td>
<td>3A</td>
<td>29D</td>
<td>31A</td>
<td>32A</td>
<td>33C-D</td>
<td>34C</td>
<td>35A</td>
</tr>
</tbody>
</table>
In contrast to arm 3H, the left arms 3L of *splendidus* and *stephensai* are similar to a greater extent. The free and the centromeric ends are also similar in them. Similarly, a light area extending from subzone 46A to 45 and the following dark area up to subzone 44A are exactly the same in the number and staining intensity of the bands in them. Excepting zone 43, 42, 41 and a part of 38 which show differences the remaining areas of this arm are again similar in *splendidus* and *stephensai*.

A. *splendidus* and A. *stephensai* belong to the same subgenus and have almost the same areas of distribution with a similar type of seasonal prevalence. For the present investigations, *splendidus* was collected from two localities in India, i.e., a semi-hill terrain around Chandigarh and the coastal areas of Bhubaneswar (Orissa, India). But it was collected along with *stephensai* only in the former place and the present results are also from this region. In this respect both are sympatric and bound to share a certain amount of genetic characteristics. From the above given comparison the two species are fairly related with the sharing of over 60% of the gene arrangements. This is further supported by the fact that almost the whole of 2R and 3L are similar in them with minor differences in the order of light bands. In addition to this, the two species have close morphological similarities because
both have speckled appendages and palpi. As for the characteristics of the palpi, these species and the variety willmoral of A. aculeatus are the only species of the subgenus Cellia of the Indian region which have speckled palpi. When compared further, the three species may prove to be having a similar genetic constitution. This may be further studied in greater detail through their hybridization. Another important point of phylogenetic relationship exists in the fact that the two species are also free from chromosomal polymorphism as is evident from the preliminary investigations carried out so far.

Comparison of A. ascinitus with A. fluviatilis

Because of its taxonomic status and close morphological resemblance with A. fluviatilis, its salivary chromosome banding pattern has been compared with the standard maps of this species. Surprisingly close homologies were encountered between these two species. These homologies are closer with the map produced by Chowdaiah and Seetharam (1975) than with the one produced by Sharma and Chaudhry (1976). This is probably due to the fact that the former map of A. fluviatilis has also been produced from a population prevalent in the same geographical limits from
which *A. aconitus* has been procured, whereas the latter
map of *A. fluviatilis* belongs to a wild population from
the North-western India.

On a closer comparison of the banding pattern
of the X-chromosome and the four autosomal arms, it has
been observed that with the exception of X-chromosome,
sufficient similarities are exhibited by the autosomes.
The X-chromosomes of *A. aconitus* and *A. fluviatilis* share
only a few homologies in the shape and banding sequence
at the free and centromeric ends in subzones 1A and 63
respectively. A set of five to six bands in subzone 53
of *A. aconitus* can also be homologised with the same set
of bands in 5C of *fluviatilis*.

When a comparison is made between the four autosomal
arms in the two species, the maximum similarities are
exhibited by 2A and 2L, while 3A and 3L do not show any
significant resemblances except for a few blocks of
bands in zones 45 and 46. In 2A, regions 7C, 9, 10A, 12A,
14C-D, 15, 18C and 18D and the entire zone 19 of *aconitus*
are readily comparable to the arrangement of bands in the
regions 7B-C, 8, 9C, 12C, 13B-C, 15, 16, 18 and 19 of *fluviatilis*.
On the other hand, in the left arm 2L, identical banding
can only be seen in zones 26, 27 and 28 of both the species.

From the extent of homologies encountered in
chromosome 2 of the two species, it is evident that the bonding pattern of this chromosome had been least affected during the course of evolution and provides an evidence for the closeness of species and their place in the group Musomyia. About 80% of the dark bands and puffs are represented alike in them. This condition is generally seen in the species of the subgenus Celia studied from the oriental region.

Anopheles moquito is a member of the subgenus Celia group Musomyia which in the Indian subcontinent is represented by about ten species, but the salivary gland chromosome maps are available only for A. fluviatilis (Chowdaiah and Seetharam, 1975; Sharma and Chaudry, 1976) and A. culicifacies (Saifuddin et al., 1978). Studies of its salivary gland chromosomes are important because of its role in the transmission of malaria in recent times. Although it was considered to be a secondary vector of very limited vectorial capacity, yet lately natural infection in it has been occasionally seen slightly on the higher side, whereby considerable importance is being given to it now (Personal communication with the senior Entomologist Communicable Disease investigation cum training centre, Mandya, Karnataka). Though its exact vectorial status has not been firmly established in India, Atmoseadjono and Dennis (1977) have confirmed it as a malaria vector in Indonesia.
Phylogenetic relationship in the subgenus *Anopheles* and *Culiseta*

Phylogenetic and evolutionary relationships among animals are difficult to determine because one is normally restricted to morphological and physiological comparisons. However, in some species of the order Diptera, the salivary gland chromosomes are so well developed that their characteristic banding pattern has offered a unique approach to the evolutionary and cytotaxonomical studies. The classical examples are provided by the members of the family Drosophilidae, inasmuch as our present knowledge of genetic principles, population genetics and chromosomal polymorphism is the result of researches carried out on these flies (Dobzhansky, 1950, 51; Dobzhansky and Pavalosky, 1962; Patterson, 1952; Patterson and Stone, 1952; Carson, 1959; Carson and Stalker, 1960; Carson et al., 1967; Stone et al., 1960; Stalker, 1966; Erneic, 1970, 1976; Lambert, 1978).

But in the last few years the salivary gland chromosome studies have also been extended to the members of the genus *Anopheles* in an attempt to clarify the problems connected with their phylogeny and taxonomy. This is an interesting group because many species of mosquitoes are serious vectors of malaria and other...
vector borne diseases. Many form species complexes in which the various species could only be identified on the basis of their chromosomal characteristics (Prissi, 1947a, b, c, d, 1949, 1950, 1951, 1952, 1953; Davidson, 1962, 1964a, b; Davidson et al., 1967; Colussi, 1964a, b; 1966; Colussi and Sabatini, 1967, 1968, 1969; Kitzsiller et al., 1967; Chowdaiah et al., 1970; Kanda and Oguma, 1972a, b).

An examination of the salivary gland chromosomes of anophelines has shown that closely related species and even sibling species may sometimes be distinguished on the basis of the banding pattern of their polytene chromosomes (Baker and Kitzsiller, 1963a, 1965a, Klassen et al., 1965; Chowdaiah et al., 1966; Colussi and Sabatini, 1967, 1968, 1969; Kitzsiller et al., 1967). For example, Colussi and Sabatini (1967) demonstrated the practical value of comparative salivary gland chromosomal analysis as an important taxonomic tool in differentiating two cryptic species (A and B) of A. gambiae complex which are otherwise very difficult to distinguish by their morphological characters. Baker and Kitzsiller (1963b) stated that in closely related species or closely inhabiting species most of the chromosomal differences appear to be in the X-chromosome. This was actually seen by them in their studies on the identification of certain anophelines by means of salivary gland X-chromosomes. In those species
which are widely different such differences are also there in the autosomes (Kitzmiller, 1966; Baker and Chowdiah, 1966; Baker et al., 1965, 1966).

In the light of these achievements, the importance of the banding pattern comparisons which form an important aspect of comparative cytogenetics is undisputed. While carrying out comparisons in the presently worked out species viz., A. lindesayi, A. mundicus, A. annularis, A. splendicus and A. monnitus, it has been seen that species which are morphologically similar have close correspondence of bands and also the banding pattern is subgeneric in an overall arrangement with the exception of some regions which are species specific. It is in line with the phenomenon observed in the Nearctic species of the subgenus Hymenobatrachus (Kitzmiller et al., 1973a; Kreutzer, 1972; Kreutzer and Kitzmiller, 1978; Kreutzer et al., 1975). The X-chromosome is more conservative than the autosomal areas in which 2R and 3L have undergone least changes in the course of evolution. On the other hand, the percentage of comparable bands is lesser in 2L and 3R which perhaps have contributed towards the diversification of the species.

Although band to band comparisons of the salivary gland chromosome maps in morphologically closely related species or even in sibling species, have contributed a
lot in understanding the trends in the evolution of the
family Culicidae, more clarity in the chromosomal inter-
relationship is provided by the study of chromosomes of the
hybrid. There is no doubt that similarity of the banding
pattern certainly argues for close relationship but close
genetic affinity can be tested only by hybridisation
and the degree of synopsis of F1 chromosomes (Kreutzer
and Kitzmiller,1971a), because the mosquito species
which have similar banding pattern show varying degree
of synopsis when the salivary chromosomes of their
hybrids are studied (Baker and Kitzmiller,1963a,1965b;
Kreutzer and Kitzmiller,1970,1971b,1972a; White,1971; Oguma,
1976; Kanda and Oguma,1977a,b; Narang et al.,1972a).

However, hybridisation is possible in those
species only which can cross easily or through induced
copulation (McDaniel and Horsfall,1957; Baker and Kitzmiller,
1961; Baker et al.,1962) and show a wide range of adapt-
bility to the laboratory conditions. Furthermore, there
are limitations with regard to the availability of species
in sufficient numbers and also the species to be crossed
must be prevalent at the same time in nature. All the five
species, covered in the present investigations, have a
varied seasonal cycle. For example, A. lindesaini, is
a species of the colder environment of the high altitudes
where it is available in the months of September to November. On the other hand, *A. splendida* and *A. annularis* are the species of the planes and are available in the months of March to May respectively. *A. aconitus* is prevalent in the southern and north-eastern parts of India where it is prevalent in post-monsoon months of September and October, whereas *A. sundaeus* is a species of the coastal areas of the eastern India. There, it is present around the year with peaks of prevalence in September and October. Apart from these limitations in the hybridising experiments, various other factors like the body size, physiology and behaviour of a species also forbid the study of their hybrid chromosomes. However, many of these problems have been solved and in many species such experiments have been conducted successfully (Barr, 1954; Baker, 1956; Burgess, 1955; Kitzmiller et al., 1967; Colussi et al., 1971; Kreutzer and Kitzmiller, 1971b, 1972; Bryan, 1973; Davidson and Hunt, 1973; Ogusa, 1976; Kanda and Ogusa, 1977a,b).

Apart from comparative studies of the banding pattern and hybridization studies which play an important role in understanding some close genetic relationships a striking phenomenon of chromosomal polymorphism has emerged in species of the genus *Anopheles* which also helps in solving the evolutionary and phylogenetic kinship in mosquitoes. The chromosomal rearrangements, especially
the inversions, are significant in this respect (Kitsmiller and Baker, 1963b).

In *A. sundalae*, studied presently, the entire banding pattern is similar to its close ally *A. subnictus* but differs from it in having one homologous inversion in the X-chromosome and three in 2A. These are the only areas where *sundalae* differs from *subnictus*, because on reversing the arrangement of bands in the areas undergoing homologous inversions in *sundalae*, it was seen that the banding pattern in the two species becomes exactly alike. It is, therefore, a remarkable revelation which indicates that the *sundalae* arrangement has evolved from the *subnictus* or vice versa. If one goes by the extent of distribution of the species, then *subnictus* which is abundantly available and breeds in all sorts of water collections, especially rain water pools, is primitive and has given rise to *A. sundalae* through the said homologous inversions because *A. sundalae* has a restricted distribution and is secondarily adapted to both fresh and salt water media. The interrelationship of *sundalae* and *subnictus* provides an opportunity to extend these studies further and in doing so *A. varus* should also be included in such a programme. Because this is the only other species belonging to the group *Pseudomyxomatia* which is morphologically similar to *sundalae* and *subnictus*. 
and co-exists with them.

In this laboratory, a beginning has been made by studying the standard salivary chromosome banding pattern of *A. subpictus* (present investigations), *A. subpictus* (Narang et al., 1973a) and *A. vagus* (Chaudhry, 1971). Therefore, further studies of these species hold sufficient promise. This is also valuable because *A. subpictus* is the chief vector of malaria in the coastal areas of Orissa (India). On account of the apparent differences in the behaviour and vectorial capacity of *subpictus* in Orissa, it is believed that the species might really be a complex of two races or forms, one breeding in saline and the other in fresh water (Rao, 1955).

In addition to the role of these inversions in the evolution of the species, their influence on the vectorial capacity of the species cannot be ignored because the impact of such inversions in controlling the vectorial status of a species has already been established. Kreutzer et al. (1972) stated that the high rate of chromosomal polymorphism in *A. darlingi* may be linked with its vectorial capacity, whereas Kittmiller et al. (1973b) actually proved Kreutzer's presumption by their studies on vector and a non-vector populations of *A. munsikvari*. They found that the two populations differed by a homozygous inversion in the centre of the X-chromosome (subzone 2A to 3A). Here
it is interesting to add that the homozygous inversion in the present population of *A. sundialius* is also in the X-chromosome and involves nearly the same segment. Moreover, it is only the fresh water form which is a vector whereby the inversion in question, in addition to distinguishing it from *subpictus* may distinguish it from its own non-vector form breeding in the saline water.

The high rate of inversion polymorphism in *A. lindesayi* not only shows their evolutionary significance but also exhibits their adaptive value to the species, because, as stated earlier, *A. lindesayi* is an alpine form and inhabits sufficiently low temperature areas. Brncic (1966, 1968, 1972) clearly explained the effect of temperature, seasonal fluctuations and ecology on the polytene chromosomes of *Drosophila flavonigra* in which he drew the relationship of inversion polymorphism with certain ecological factors. *A. lindesayi* has also provided good examples of the genetic effects of such factors and the various inversions isolated in this species are attributed to the impact of its environment, whereas their evolutionary significance is related to its isolation from the remaining species of the planes. Colussi *et al.* (1970), while studying the polytene chromosomes of *A. supernius* and its relationship with *A. stephensi*, also assumed that "certain chromosomal regions have a
tendency towards high fitness when in the heterozygous state".

The inversions encountered in *A. annularis* are only random and seem to be seasonal. In fact, *A. annularis* also exists in the form of two varieties, a winter variety and a summer variety (Christophers, 1933; Rao, 1934). The present results are from a winter variety, whereas a few preparations made from a summer variety lacked these inversions.

On the other hand, *A. splendidus* and *A. eoonitus* have not been found to carry any type of inversion polymorphism in these which supports the view that the wild populations are less polymorphic than those populations which have been bred in the laboratory for a long time (Hobbs, 1962). But from the results of the other species the presence of aberrations cannot be ruled out completely because the salivary chromosomal studies on the present species are still in the initial stages which leave sufficient scope for further investigations.

In summation, it may be added that in nature different species and species populations are separated by one or more isolating mechanisms often including a combination of several ethological, ecological, geographical and reproductive barriers, out of which the last two are
of prime importance as they have a marked impact on altering
the gene arrangements and forming different populations
as close genetic systems in different demes in a given niche.
A. lindesayi and A. gambiae are the examples from the
present studies. Whatever differences have been recorded
as a result of the present attempts, they are related to
these factors. The cytogenetical studies on the oriental
fauna of the family Culicidae are still in their initial
stages because the oriental elements show great diversity
in prevalence and vectorial behaviour of the different
species. This is because of the marked variations in the
climatic and other ecological factors which also would
the genetic make up of the species. There may also be
present the various species complexes on the lines of
the *masulipennis* and *gambiae* complex. In this respect, the
*Barbirostris* group and *Nyssanus* group of species have
already been recognised.