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Chromosomal Aberrations in Polytene Chromosome of *Anopheles Stephensi*

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Abstract:
The present studies deals with effect of environment on the vectoral status and genotype of Anopheles stephensi (Diptera: Culicidae). Comparison of chromosomal polymorphism in the urban and rural population of the species. A large number of different populations of A. stephensi may have to be examined cytologically for knowing the differences in chromosomal variability which might exist between vector and non-vector populations & those that are susceptible and resistant to insecticides, because vectorial capacity and insecticide resistance are pre-adaptive genetic phenomena with a base in the chromosomes. A well spread salivary chromosomes complement of A. stephensi is comprised of three synansped polytenes elements, attaché at the region of their centromeres, forming a heterochromatic chromocenter. They include a short telocenric/ areocentric X – chromosome is the shortest and appears isolated in the squash preparations, whereas 2R and 2L show attenuation between them. The average measurements of the chromosomes are X- chromosome - 65µ, 2R-270 µ and 2L-170 µ, 3R-220µ and 3L-230µ. While mapping the chromosomes, entire complement has been divided into 46 zones in which each zone can be identified by characteristics bands or puffs. In the present study a total of 20 cases of various aberrations were observed out of which 18 were inversions; 8 ectopic pairing, 1 asynapses and 2 cases of breakage. Inversions are the most common of all the aberrations. The X-chromosome had only one inversion chromosomal arms 2R had 3 and 2L had 8, 3R had 3 and 3L had 4 inversions. All the inversions accept one were restricted to autosomes, while the X-chromosome seems resistant to structural changes. This shows the genetic balance that has been achieved by the (sex. chromosome) is involved, the effect is more deleterious as it causes sex ratio distortions and specificity in the natural populations. In the present investigations all the inversions fail in the category of "floating inversions" none was encountered more than once in about 100 preparations studied for the purpose. Some intrachromosomal and interchromosomal ectopic pairings were observed between different chromosomes. The phenomenon of synapses was observed in 2L, where effected subzones were 15A-15C.

1. Introduction

Polytene chromosomes provided the first evidence that eukaryotic gene activity is regulated at the level of RNA synthesis. When dipteran chromosomes become polytenic, the DNA replicates by endomitosis, and the resulting daughter chromatids remain aligned side by side.There chromatids are visible during interphase and have a characteristic morphology of dark bands and alternating interbands. Within these chromosomes it is possible to observe the genetic activity of specific loci at local enlargements called puffs, which represent DNA undergoining intense gene transcription. Puff distribution varies from one tissue to another and can be induced experimentally, indicating the cell specialization results from variable gene transcription. Polytene chromosomes constitute a valuable material for the study of gene regulation because their gene transcription can be visualized directly in the microscope.An additional characteristic of polytene chromosomes is that the maternal and paternal homologue remain associated side by side, in what is called 'somatic pairing'. This permits the identification of deletions, inversions, duplication as regions looped out of the chromosomes. The pericentromeric heterochromatin of all the *Drosophila* chromosomes coalesces in a chromocenter, where the chromosomes are joined together. The satellite DNAs of the chromocenter are under replicated with respect to the rest of the chromosome, (i.e. they undergo fewer rounds of replication

In general, the chromosomes of most of the organisms are too numerous to be considered a good subjects for cytological investigations. Therefore, for a long time, cytogenticists had been in search of an experimental organism, in which the chromosomes are large enough to facilitate the observations of the qualitative difference along their length, corresponding to the genes. The answer was found in the classic genetic studies, carried out on certain traditional materials like *Chironomous* and *Prosophila* (Lemonier, 1973; Zhimulev, 1974; Gurzdev, 1975; Zhimuleve and Belyaeva, 1975' Kiknadze et al., 1976; Perov et al. 1976; Sorsa, Vikko, 1976 and Basile, 1977).

These insects were considered important for genetic studies, not only from the point of view of their lower diploid chromosome number (2n=8) and short life cycle, but also for the special type of giant sized chromosomes in their salivary gland nuclei. They are well suited for understanding the various problems related to the behaviour of genes, puffing activity and DNA regulatory mechanism.

Bridges in 1935 made extensive and detailed investigations of the salivary gland chromosomes of *Drosophila melnogaster*. He published a detailed salivary gland chromosome map of *Drosophila melanogaster* and described the relation of the bands to the genes. This was a significant discovery which opened up new fields in cytogenetical research. The banding patterns of the polytene chromosomes have been of particulars value in genetic studies.

Most of the work, on the chromosomal polymorphism has been done on dipteron insects in which the genera *Drosophila* (Goldschmidt, 1956 ; Richmond and Dobzhansky, 1976), Sciara (Casron, 1946) and *Chrionomous* (Action 1956, Beerman, 1956, 1971, Brady et al 1977, Martin, 1979) have been investigated more extensively than any other specues. Dobzhansky (1944, 1947b, 1948, 1950, 1957, 1970), Ranganath and Krishnamurthy (1981) and Clyde (1982) have done extensive work on the evolution of various species groups of the genus *Dropsophila* i. e. *D. pseudoobscura, D. paulistorum, D. willistoni.*

For a long time, the mosquito workers had been taking keen interest in studies on the various aspects of mosquito behaviour and morphology because of the role played by these insects in disease transmission and human economy all over the world. During the last three decades, the work on culicine cytogentics has grown to an international level an the scientists from the various research centers of the world have contributed significantly towards our understanding of their basic biology, evolution, speciation and insecticide resistance, linked with the hereditary process (Davidson, 1956, 1957, 1959, 1979; D' ; Mosna et al. 1958; Brown, 1958, 1959, 19611967; Mason and Bron, 1996; Mariani et al, 1964' Singh and Mohan, 1965; WHO 1970; Coluzzi and Kitzmiler, 1975).

In mosquitoes, work of similar nature was carried out in several species of the family Culicidae in which details of standing banding pattern of polytene chromosomes have been taken into account in solving the problems of speciation and taxonomy in the genus *Anopheles* (Firzzi,1947 a, b,c, 1949-5a, b 1954, 1958 a, b, c Frizziand De Carli, 1954, Frizzi and Holstein, 1956, Frizzi and Kitzmiller, 1959, Baker and kitzmiler, 1963 a, b kitzmiller, 1966, Coluzzi and Sabatini, 1967, Coluzi et al, 1970 wattal andkalra, 1967, Rioux et al, 1959, Puri 1960, Rabanni and Kitzmiller, 1964 Chowdiah et al, 1971, Chaudhary 1971, 1973, 1975, 1979, 1985, Kreutzer et al, 1970, Belcheva and Mikhailona, 1970, 1972, Brown and Pal, 1971, Kabanova et, al, 1973, Green, 1972a, 1982, 1983, Green and Miles, 1980, Green et al, 1985, Smithson, 1972, Mehmood and Sakai 1984, 1985, Coetzee, 1983, 1984).

Inversions and translocations is an important source in insect evolution and speciation (White, 1957, 1973, Baker and Kitzmiller 1961, 1963b, Coluzzi & Sabtaini 1967, Coluzzi Di Deco and Cancrini, 1970, Dobzhansky, 1970, Kreutzer 1972a, b, 1973, Narang *et al*. 1973, Kretuzer and Kitzmiler 1971a, Narang et al, 1973, Kitzmille *et al* .1973, a,b, 1976, Green 1982, Sharma et, al, 1968b, 1976, 1982, Sharma & Chaudhary, 1972, 1973, 1979.

It has two geographical races viz. type form, which is an urban inhabitant and an active vector of malaria, and variety mysorensis, a poor vector of malaria, and variety mysorensis, a poor vector of malaria. The two forms were identified on the basis of the number of the ridges on the egg. Examination of several *A. stephensi* strains in the laboratory revealed that there were three variants instead of two, with reference to ridge numbers i.e. type form, variety mysorensis and an intermediate form.

In the recent years it has been strongly emphasized that cytogenetic studied involving chromosomal polymorphism in the natural populations of mosquito vectors of malaria should form an integral part of research progrmmes on vector cytogenetics. Based on these recommendations the present piece of research work eneitled "cytogenetic investigations on an urban and rural population of *Anopheles stephensi* was undertaken.*Anopheles stephensi* is a well known urban vector of malaria in India.

2. Material and Methods

A natural population of *Anopheles stephensi* was used for the present investigation. The adult of this species is a medium sized mosquito which is characterized by speckling on all the three pairs of legs and equal sized apical and pre-apical pale bands on the palpi. This species has peak prevalence in the monsoon and post monsoon months when the highest number is available from September to December and the lowest number from February to July. Collection of the various developmental stages were made mostly from the breeding places it and around Chandigarh such as villages Dhanas and Khudda Lahora.

The larvae in the various stages of development were collected with the help of strainer, petridish and long nosed wide mouthed dropper and were transferred to a plastic jar with perforated laid for proper aeration. The collection was brought back to the laboratory where it was transferred to different bowls, according to the age of the larvae. The 4^{th} instars larvae were dissected immediately while younger ones were allowed to grow up to the fourth instar stages for preparing the chromosomal squashes. A feed consisting of finely powdered yeast and dog biscuits (Singh *et al.* 1974, 1975) served as an excellent nourishing medium for the larvae which are surface feeder. The water in the bowls was changed to avoid scum formation. A few larvae were allowed to mature up to and adults for the purpose of identification of the species.

3. Squash Preparation Of Salivary Gland Chromosomes

Preparation of the polytene chromosomes was made from the salivary gland of well developed fourth instar larvae. Polytene chromosomes can be obtained from salivary glands, hepatic caecae, gut epithelium and Malphigian tubules. In the adults, polytene chromosomes can be obtained from ovarian nurse cells. However, only the salivary glands were used as they have the best developed polytene chromosome which have a good staining and spreading capacity and distinct banding pattern. The fourth instar iarval stage is recognized by the largest larval size and the much expanded thorax. Both male and female larvae were taken as there is not much difference in the organization of the polytene chromosomes, except there a chromosome, which is thinner in the males.

The dissections were carried out in a cavity slide containing a drop of 0.67% aquous saline solution. In the summer months, the larvae were kept at a temperature f 15^0 18^0 C, at least for a night before the day of the dissections. This enables better development of the salivary glands and chromosomes and also prevents the pupation of the larvae. The dissections were carried out under the Bausch and Lomb dissecting binocular microscope using fine dissecting needles. First of all, head was separated from the the body. The salivary glands were removed from the dissecting medium and transferred to a drop of IN HCI for one or two minutes. The tissue was hydrolysed and then all excess traces of HCL were removed by means of methods used for chromosomal preparations have been given by Darlington and Lacour (1960) and Niciletti (1959). French, Baker and Kitzmiller (1962)

developed a separate cytological technique for the study of mosquito. In the present study chromosomes preparations were made following the procedure of French *et al.* (1962).
For suitable staining of the salivary chromosomes, 2% Lacto-Acto-Orcein stain; (French *et al.* 1962). Deep staining provided a better contrast and gave finer details of the bands in black and white photography. These preparations were immediately examined under low power of the microscope and then studied and photographed using 100x objective under oil immersion and 15x eye pieces to achieve maximum magnification. For photocopy, slow, 35 mm ORWD black and white film of 125 or 200 ASA was used.

4. Preparation Of Permanent Slides

The temporary slides were made permanent. The temporary slides were kept in the freezer of a refrigerator for overnight after which an attempt was made to make them permanent (Bhaduri and Ghosh 1954). The nail enamel was removed using acetone and then slides were inverted over a petridish containing a mixture of 1:1 butanol and glacial acetic acid. The cover ship separates in about 5-10 minutes while the squashed tissue remains either on the slides or on the slides or coverslip or on both. Both the slide and the coverslip were dehydrated separately for about 10 minutes in N butyl alcohol. The slide and the cover slip were mounted separately in euparol and dried at room temperature.
For making permanent slides, the following procedure was also tried out. After screening, the slides were not sealed but were kept upward in a dish containing Butanol.
Next morning, after lifting the slides out, the excess of butanol was blotted out and a drop of euparol was released being the edge of the coverslip. The required amount sensate under the covership and the slides are allowed to at room temperature. The slides were then screened and the chromosomal arms were identified by means of the important diagnostic features such as the shape and banding patterns of free ends, centromeric ends and the combination of district bands and puffs. Sometimes the salivary chromosomes complement of *Anopheles stephenesi* is comprised of five synapse polytene chromosomal arms, which are weakly attached by them centromeric ends to form a chromocentre.

5. Results

Active, well fed, fourth instars larvae were found to be the most suitable material for the study of salivary gland chromosomes. All the members of family culciade have only six chromosomes in their genial metphase karyotype with XX (Female) and XY (Male) sex determining chromosomes. In the salivary glands, this gets reduced to a haploid number of three long an banded elements due to the phenomenon of somatic pairing in which the homologues are synapsed intimately.
A well stained and well spread salivary chromosome complement is comprised of three synapsed polytene chromosomes which are weakly attached at the region of their respective centromere and to form a chromocentre. The chromocentre is usually fragile and break under pressure during the squash preparations. Consequently, the individual arms or chromosomes lie separately; the individual arms or chromosomes lie separately in the cell. A complement consists of a short areocentric X chromosme and 2 longer autosomes. The longest of the two autosomes is designated as chromosomes 2 and this is submetacentric, while the other autosomes is designated as chromosome 3 and it is little smaller than the 2 an is metacentric.
In the males, the X-chromosomes is without its counterpart, therefore it is poorly developed and thin element. They chromosome is heterochromatic an is included.
in the formation of the chromocentre which sometimes may also involve the short arm of X-chromosome. As per the scheme followed for the species of the sub-genus *Cellia* all the chromosomes have been mapped into zones an subzone. While preparing a chromosome map, the free ends of the arms are placed to the left, while the centromeric ends are put towards the right. The right and left arms are designated by the abbreviations R&L respectively. Each arm is divided into a number of zones which are further divided into subzones. The zones are represented by the numbers 1,2,3--- 46 and in all, there are 46 zones. The subzones are designated by the alphabets A,B.C.D.---etc. fig.1-12. Each zone can be identified by clearly recognizable puffs or bands. The identified by clearly recognizable puffs or bands. The distribution of zones on the chromosomes according to the scheme for sub genus *Cellia* .

6. Diagnostic Featues Of The Salivary Gland Polytene Chromosomes Of *Anopheles Stephensi*

6.1. X-CHROMOSOME

The x- chromosome, being the shortest element in the whole complement, measures only about 65um with an optimum stretching. It is characterized by the presence of two large puffs, one is in the sub-zones 2B and 2C while the other is almost in the middle of sub-zone 3D. The latter is sometimes seen as a Balbiani ring (BR) from which radiating RNA fibers can be seen. Zone 6 is also characterized by the presence of 6 dark bands.

6.2. CHROMOSOME-2

It is the largest element of the complement and is characterized by the attenuation of its right and left arms. The centromeric and of its left arm is distinguishable by a loop like configuration in zone 20. The loop is formed by ectopic pairing of the first and last band of zones 20. This loop of zone 20 is a good marker for the identification of both the chromosomes and the species.

6.3. RIGHT ARM–2R

It is the longest in the complement with a length of 270 um in an optimum stretching. It is about 1-1/2 times longer than 2L. The free end, i.e. zone 7 is characterized by 2 sets of dark bands and a puff in subzone 7B. The first set of dark bands consists of three

identical band, while the second set has a thin dark bank flanked on either side by dark bands. Zone 19 has a pair of dark bands in subzone 19B and three dark bands in 19C. 19A is characterized by a puff.

6.4. LEFT ARM-2L

It has an optimal length of 120um and it is the shortest autosomal arm. Zone 20 always has a loop formed by ectopic pairing. The free end of these arms is slightly flaired with two light bands. 28B has a small puff with thick bands in the middle of the puff. Zone 26 is also puffed and zone 25 is elongated.

6.5. CHROMOSOME-3

The two arms are approximately equal is size and are usually spread individually.

6.6. RIGHT ARM-3R

The free end is usually flared and the optimal length is about 220um. Puffing in zone 29 B and 29C followed by two dark bands in zone 29A. Sub zone 31C is devoid of any significant band and is seen as an almost clear area between a series of dark bands. This serves as a useful identifying features. Subzones 33C and 36A have small puffs. At the centromeric end, zone 36 is characterized by the presence of three dark double in 37A-37C.

6.7. LEFT ARM 3L

This arm is about 230 um long. It is recognized by the free end (Zone 46) alongwith the entomic end zone 38 both of which have their own characteristic end zone 38 both of which have their own characteristic bands. An almost clear cylindrical area from zone 46A to 46D consisting of a number of light bands forms the free end.

The centromeric end has a puff in subzone 38C to 38E followed by two dark bands at the tip of the chromosome. The subzones 44B is always puffed and the puffs are also present in subzone 41A to 4C and 40C to 40A.

7. Chromosomal Polymorphism In *A. Stephensi*

In the present investigation, a moderate amount of chromosomal polymorphism has been recorded in the urban population. At least 4 different types of aberrations were recorded. The total number of aberrations was 20 which include about 18 cases of inversions, 1 asynapsis, two breakages, 2 cases of ectopic pairing.

7.1. Inversion

Inversions are the most common of all the aberrations encountered in the mosquitoes. They involve the complete rotation of a chromosomal segment through 180 resulting in the reversal of the normal banding pattern of the inverted. Inversions are classified into two categories. If both the breaks lie on the same side of the centromere then the inversion is said to be paracentric. If the breaks lie both sides of the centromre, the inversion is said to be pericentric. Further, an inversion is heterozygous if it involves only one of the synapsed homologues chromosomes. In such a case, either a loop is forms or a figure of eight is seen to be formed which makes the detection of the inversion segment in the effected homologue tends to pair with the normal segment of the other homologue. If both the homologues are involved are said to be “floating” if they are encountered briefly in a given population of species. Such Inversions may be of great help in the adaptive capacity of a population. On the other hand, an inversion is termed as a fixed inversion if it is found constantly as in all the individuals of a population.

In the present course of study, only the floating, paracentric, heterozygous were available. All the Inversions were distributed over the autosomal arms in which the maximum number of inversions was encountered in 2L, 4 in 3L, 3 in 3R, 3 in 2R and only 1 in X-chromosome.

7.2. Asynapsis

Asynapsis is seen when the two homologues of a polytene chromosome fail to synapse together over a small distance, or in some cases even over the whole length. The saynapsed region depicts the double nature of the polytene chromosome. The synopsis may be 1+1, 2+1, 3+1, or 1+1+1+1 type. The 1+1 and 2+2 are the terms used for the separation at homologues or chromatics respectively, because the two synapsed homologues are comprised of four synapsed chromatids. Accordingly, 1+3 involves the separation of a single chromatid from the rest of the chromosome. When all the four chromatids get separated, it leads to the last type of asynapsis i.e. 1+1+1+1. Asynapsis of the type 1+1 or 2+2 was seen in the subzones 6A-6C of the X-chromosome 2R from subzone. Due to limited amount of investigations the structural disorders like translocations and deletions were net encountered in the present material, fig.11.

8. Discussion

The study of chromosome morphology important for investigations of various aspects of vector genetics. The banding pattern is species specific and the occurrence of chromosomal polymorphism originating from inversions, translocations and deletions is important for adaptation evolution and speciation.

The bulk of the data on chromosomal on dipterans insects in which the genera Drosophila (Sturtevani and Beedle, 1936, Goldschmidt, 1956, Carson, 1946) and Chironomous (Acton, 1956, Beerman, 1956, Martin and Walker 1971 are investigated more extensively than any other species. Dobzhansky (1950, 1957, 1970). Dobzhansky and Spassky (1959), Rajeshwari and Krishnamurthy (1969), Dobzhansky and powell (1975), Knibb *et al*. (1981), Ranganath and krishnamurthy (1981), Clyde (1982) have done valuable work in the evolution of various species groups of *Drosophila*.

This field of research also saw considerable developments in mosquitoes in which the details of the standard banding pattern of polytene chromosomes have taken into account while solving the problems of phylogenetic relationships and taxonomy in culicines (Frizzi, 1949-1953a, b, Frizzi and De carll, 1954, Frizzi and holsteinm, 1956, Frizzi and Kitzmiller, 1959, Baker and Kitzmiller, 1963a, b, Kitizniller, 1967, Coluzzi 1966, Coluzzi and Sabatini, 1967, Chowdaiah *et al.*, 1971, Chowdaiah and Venkatachaliah, 1986, Chowdaiah *et al*., 1967, Kerutzer *et al*., 1970, Seetharan and Chowdaiah, 1971 and Green, 1972a).

Chromosomal polymorphism in the form of tanslocations and inversions, being the most frequent are used to study the evolutionary relationship among species. In mosquitoes, this has been done mainly in the subgenera *Anopeles* and *cellia*. Coluzzi et al, (1973a, b) examined ten strains of *A. stephensi* form defferent geographical locations viz. delhi-two strains, Bangalore-one strain Karachi-two strains, Islamabad two strains, Shekupura-one strains, Iran-two strains and Iraq-one strains, Three typical inversions encountered in the Karachi strain were designated as the basic sequence for anopheles stephensi. One of the inversions was seen to involve the x-chromosome while three overlapping inversions were found as one in 2R, one in 2L and one in 3L. As a result of these investigations, the ten strains could be differentiated on the basic of inversions. Similarly, vector and non vector populations of *A. nuneztovani* ere distinguished on the basis

of a fixed homozygous inversion in the long arm of the X-chronosome by kitzmiller *et al.* 1973b.

Rishikesh (1955), Reid (1962), Baker and Kitzmiller (1963), White (1957, 1973), Coluzzi and Sabatini (1967), Coluzzi *et al*. (1970), Dobzhansky, (1970) Kreutzer (1972a,b), Kreutzer and Kitzmiller (1971a), Kitzmillr *et al*. (1973, 1976), Narang *et al*. (1973b) Chaudhry (1974, 1976, 1977, 1986), Chaudhry and Soni (1978), Sharma (1975, 1976), Sharma and Chaudhry (1976), Sharam, *et al*., (1968a, 1969, 1971, 1976, 1982), Sharma and Raina (1986), Rao (1981), Green (1982), Robert *et al*. (1989) have some of the most valuable contributions in the cytogenetic data related to structural alterations in the polytene chromosomes of the members of the genus Anopheles.

Many species of Anopheles from complexes in which the identification of the subspecies through morpho taxonomic characters is difficult. This was one of the major reasons which initiated researches in the cell division, metaphase karyotypes and polytene chromosomes of these insects. As an initial step, the polytene chromosome studies on *Anopheles gambiae* showed it to be a group of six closely related sibling species which differ only in chromosomal features but are similar in their outward appearance (Coluzzi and sabatini, 1967, 1968, Davidson 1964b, green, 1972). Similarly, *Anopheles maculipennis* was also found to be a group of several species identified on the basis of chromosomal polymorphism (Frizzi, 1947 a, b, c 1949, 1950, 1951, 1952, 1953a, b; Martin, 1979). As a result of present investigation on the chromosomal polymorphism of an urban population of *Anopheles stehensi*, several points of genetic differences between urban population and a rural populations worked out by other workers. Except for minor difference in the two, which were seen as the presence and absence of light bands and the size of puffs, the overall band arrangement in the X-chromosome and the autosomes is similar.

It was also seen that there was a certain level of periodically in obtaining good preparations. This has ben noticed by Stalker (1967) and Chaudhary (1972). Most of the preparations were obtained in the lean period of prevalence of the present species of mosquitoes i.e. from November to February. In the genus *Anopheles* subgenus *myzomyia* has 13 species and 1 variety in its group Neocellia (Christophers, 1933). In the Indian subcontinent, ten of them have been worked out cytogenetically but without any information on the chromosomal polymorphism to any satisfactory level. Some information about inversion polymorphism and translocations of evolutionary significance is available for *A. superpictus* (Coluzzi *et. al.* 1970), *A. stephensi* (Sharma *et al*., 1976), *A. maculates* (Narang *et al.*, 1973 b) and *A. pulcherrimus* (Baker *et al.*, 1968). From this data it is evident that much remains to be done before any valuable conclusions are drawn. Even otherwise larger number of different populations of *A. stephensi* need to be analyzed with positive role in malaria transmission and insecticide resistance. Because these two factors are operative in the species through genetic alterations.

9. Conclusion

From this data it is evident that much remains to be done before any valuable conclusions are drawn. Even otherwise larger number of different populations of *A. stephensi* need to be analyzed with positive role in malaria transmission and insecticide resistance. Because these two factors are operative in the species through genetic alterations. It is seen that the percentage frequency of aberration in the urban population of *A. Stephensi* 94% is Inversion, 40% ectopic pairing 10% breakage and in rural area it is 66.6% is Inversion 16% asynapsis 8.3% deletion and 8.3% translocation. Percentage frequency of in chromosome/arm in urban poulations of *A. Stephensi* is 40% in chromosome no. 2L, 15% in 2R and 3R and 20% in 3L and 10% in X- chromosome. Percentage frequency of in chromosome/arm in rural poulations of *A. Stephensi* is 12.5% in chromosome no. 2L, 50% in 2R and 3R and 2.5% in 3L and 12.5% in X- chromosome. It is found that inversion, ectopic pairing and breakage is more in urban populations of *A. Stephensi*.

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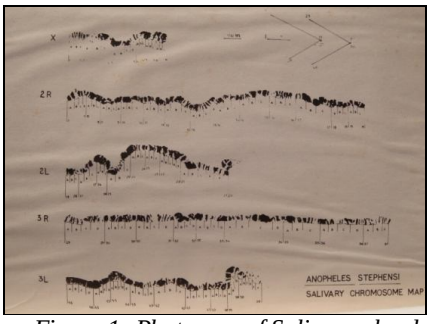


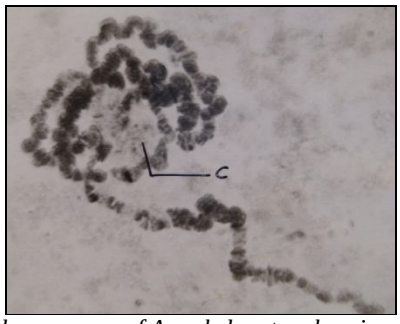
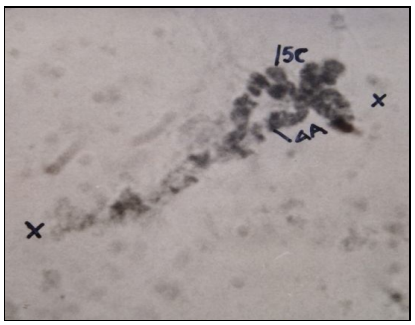


Figure 1: Photomap of Salivary gland chromosome of Anopheles stenphensi
Figure 2: Complete polytene chromosome complement with all arms of Chromosome attached to chromocentere



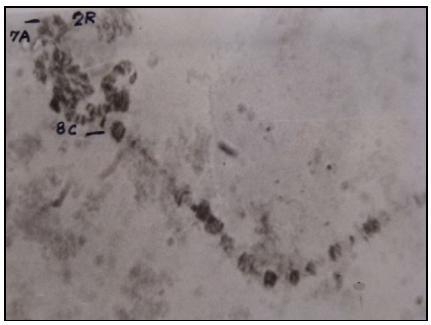
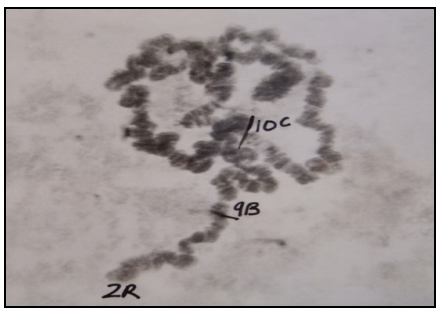


Figure 3: Inversion in X- Chromosome from subzones-4A and 5C
Figure 4: Inversion in Chromosomal arm2R from subzones 7A-8C



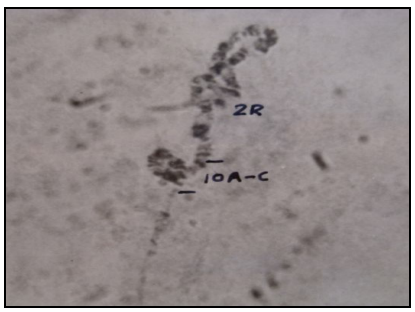
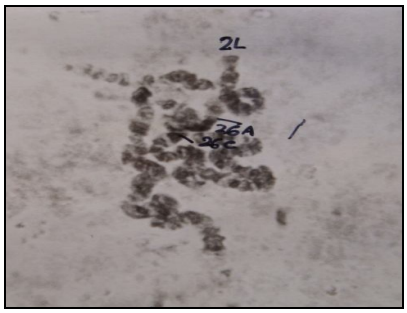


Figure 5: Chromosomal arm2R with inversion from subzones 9B-20
Figure 6: Chromosomal arm2R with inversion from subzones 9B-20



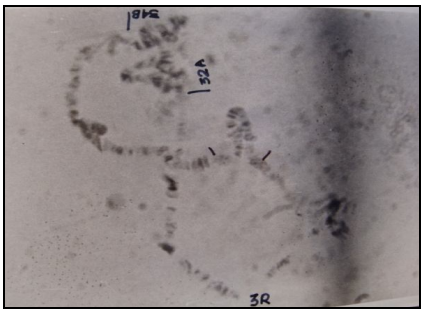
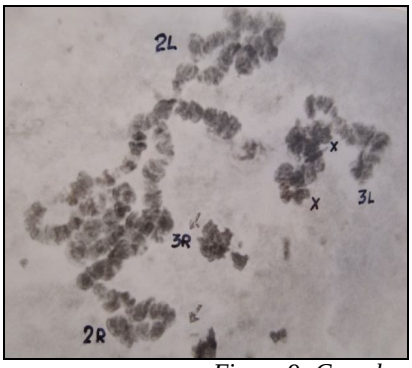


Figure 7:Chromosomal arm2L with inversion from subzones 26A-26C
Figure 8: Chromosomal arm 3R with inversion from subzones 32A-34B



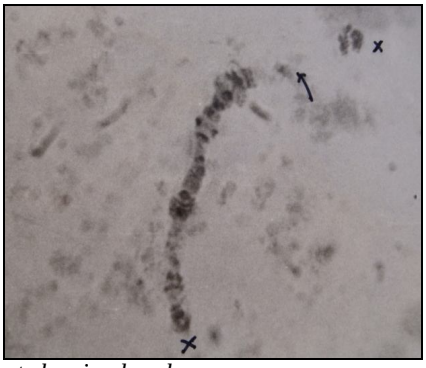
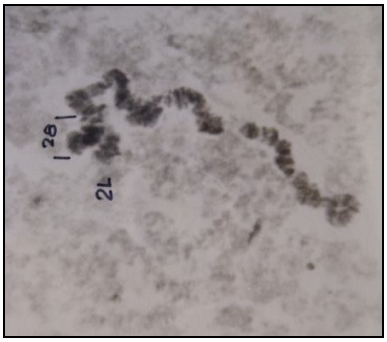


Figure 9: Complement showing breakage
Figure 10: Chromosome arm X –showing breakage



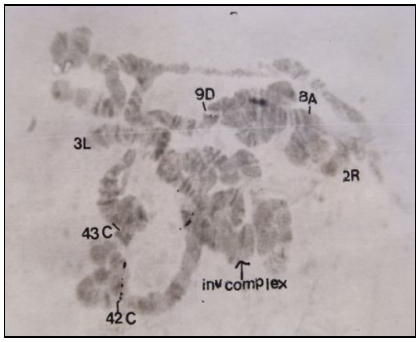


Figure 11: Chromosome arm showing asynapsis
Figure 12: Complement with multiple intra and inter chromosomal ectopic pairing also showing unidentified inversion complex