International Journal of Advanced Research and Development
ISSN: 2455-4030, Impact Factor: RJIF 5.24
www.advancedjournal.com
Volume 1; Issue 12; December 2016; Page No. 01-04

Cytogenetic analysis of salivary gland chromosomes of *Culex Quinquefasciatus* (Diptera: Culicidae)

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Abstract
Dipteran contains polytene chromosomes which serve as an excellent tool for understanding in species complexes, as well as structural and functional cytogenetic analysis. Salivary gland chromosomes preparation in Culex species is quite tidies. The numbers of techniques are available but only few are reproducible. The problems arise because the culex polytene chromosome is long and fragile, invested with ectopic pairings and sticky ends. In this paper we have followed a simple and reproducible method which gives good polytene chromosomes preparation from *Culex quinquefasciatus*. The preparation results shows banded polytene chromosome with five arms attached at a common point called chromocentre. We also observe several special features like asynapsis, heterochromatin body etc.

Keywords: polytene chromosome, chromocentre, culex quinquefasciatus, cytogenetic

1. Introduction
Mosquito-borne infectious diseases pose unacceptable risks to public health and welfare (Tolle, 2009) [25]. Among mosquitoes, species of the genus Culex are the most taxonomically diverse and geographically widespread (Reddy, 2012 and Vinogradova, 2000) [20, 28]. *Culex quinquefasciatus* is principal vector for filariasis. The chromosome maps are fundamental tools to provide good cytogenetic analyses of these mosquitoes, which is of medical and economic importance. Mapping and sequencing of the genomes for three major mosquito genera namely *Anopheles gambiae* (Holt *et al*., 2002) [11], Aedes aegypti (Nene *et al.*, 2007) [19] and *Culex Quinquefasciatus* (Arensburger *et al.*, 2010) [2] provides important facts about genetic diversity of mosquitoes and evolution of the mosquito- pathogen relations (Bartholomay *et al*., 2010) [4]. But when compared to other mosquitoes, *Culex Quinquefasciatus* has the most fragmented genome. The cytogenetic and molecular biology of the Culicidae can be studied by analyzing the structure and function of their polytene chromosome. Salivary gland polytene chromosomes have been used in cytogenetic analyses of this complex (Sutton 1942, Kitzmiller & Clark 1952, Kitzmiller & Keppler 1961) [23, 14, 15]. Its salivary gland harbour polytene chromosome whose preparation in our laboratory is our target. Sutton (1942) described the salivary gland chromosomes of *Culex pipiens* L. as being three pairs, synapsed along most of their lengths. Kitzmiller and Clark (1952) and Kitzmiller and Keppler (1961) explained the preparation of the salivary gland chromosomes for the subspecies pipiens. Number of scientists described and mapped the salivary gland chromosomes of the subspecies *quinquefasciatus* (Dennhöfer 1968, Kanda 1970, Tewfik & Barr 1974, Verma *et al.*, 1987) [8, 13, 24, 27].

The problems encountered in the preparation of these chromosomes have also been recorded by almost everybody who has worked with Culicine species. Kanda (1970) found it was very rare to see free ends of the chromosomes, and Tewfik and Barr (1974) felt that good preparations in this system, with 3 completely separated chromosomes, were difficult to obtain. Verma *et al*., (1987) [27] also reinforced technical difficulties in the preparation of polytene chromosomes in Culex. Certain promising methods were tried by different persons several times, with many modifications. Recently, a simple and reproducible technique for obtaining well-spread and readable polytene chromosomes from the malpighian tubules of *Culex Quinquefasciatus* was developed (Achary., 1994) [1].

Polytene chromosomes are very important for the study of multiple aspects of the organization of the chromosomes at interphase and hence the genome (Zhimulev, 2001) [30].These are also an excellent tool to study phylogenetic relationships among closely related species and are used to distinguish members of a complex species group (García-Martinez *et al.*, 2009) [9].

The formation of a polytene chromosome is related with the exclution of the whole process of mitosis after each DNA duplication. As a result, the cell cycle consists only of two periods: synthesis and inter-synthesis, at the end of which, the sister chromatids do not segregate, but remain paired one with each other in different degrees (Koltzoff, 1934 and Bauer, 1935) [16, 5]. Hence the purpose of the present study is to try to reconcile the various chromosomal preparations which have been published**.**

2. Materials and Methods
2.1 Mosquito rearing
Pure culture of *Culex quinquefasciatus* mosquitoes were reared in laboratory (Insect Microbial and herbal control laboratory, Department of Zoology, MLSU, Udaipur, Rajasthan). Field collected larva reared to adult and identified for species and then pure culture is maintained at temperature 28±2 and with relative humidity between 70-80% in the laboratory. The larvae fed with dog biscuits and yeast powder in the ratio 3:1. 10% Sucrose solution and blood meal were used for adult. Larvae were reared one egg raft to a pan about 29 x 17 x 4 cm.

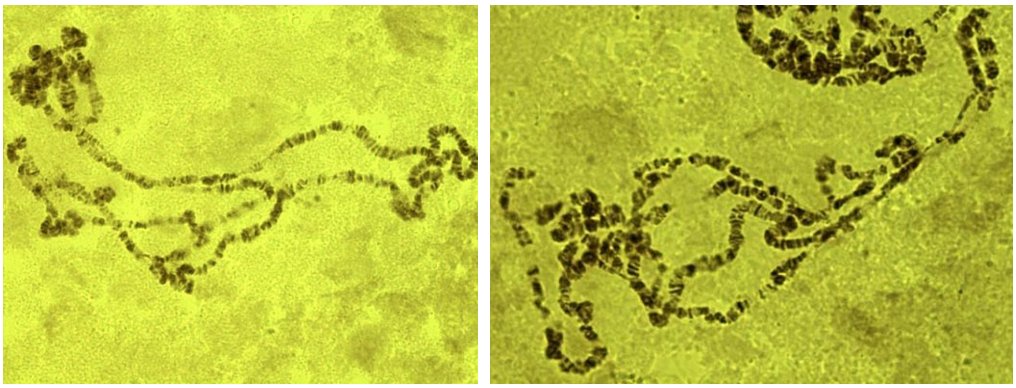
2.2 Mosquito salivary gland dissection and polytene chromosome preparation.

D.V.Jensen (1955) [12] has explained number of methods for larval dissection. Simultaneously Russel, West, and Manwell (1946) [21] and Trembley (1955) [26] cited good literature about it. And a larva was placed with dorsal side up in a drop of 5% propionic acid. The best preparations were of salivary glands from last 4th-instar larvae which are generally large and just ready to pupate. The glands were fixed in carnoys fixative for 1 min. Placed in 50 % propionic acid for 4 minutes and stained for 5 minutes in lacto-aceto-orcein which was made by dissolving 2 gm of orcein in 100 cc of a 1: 1: 1 solution of distilled water, glacial acetic acid, and lactic acid. After staining the glands were mounted in 50% propionic acid. For proper spreading of chromosomal arms, proper squash technique was applied. A cover slip was placed and light mechanical force was created by using the tapping device specially prepared in our laboratory. The force of tapping was applied to the entire surface for proper spreading and to express the chromosomes from the nuclei. Extra propionic acid present on slide was removed by placing slides between the filter paper. The prepared slide then put on hot plate for 1 min so that excess liquid may evaporate and chromosome becomes clear. These temporary preparations sealed with clear nail polish so that air bubbles may not destroy the preparation. Prepared slides are preferred to store in a refrigerator

3. Results and Discussion

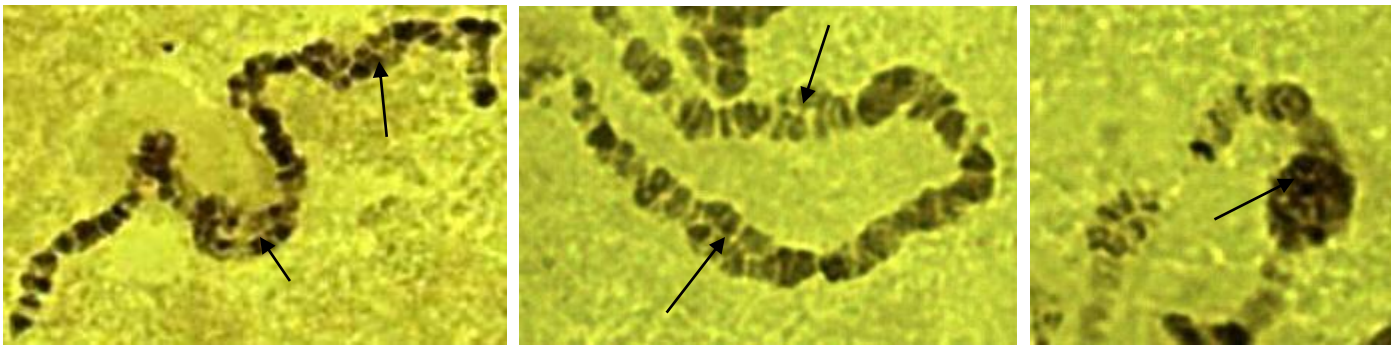
The salivary gland nuclei of *Culex quinquefasciatus* contain banded long polytene chromosomes attached to a common point known as chromocentre. (Fig.1).In our preparation we observed various structural characteristics like asynapsis among various arms (Fig 2.a, b).And also heterochromatin body in few places also (Fig 2.c). The arms shows characteristic banding pattern which is the peculiar feature of these chromosome. Good preparations with the 3 chromosomes completely separated are difficult because sometimes chromocenter is not visible. Special features of *Culex quinquefasciatus* polytene chromosomes such as ectopic pairings, telomere contacts and extended length of chromosomal arms prevent a high resolution of prepared slides. Thus polytene chromosome preparation here is not efficient on a large scale and also hinders whole-genome physical mapping using polytene chromosomes.

The salivary gland chromosome complement in *Culex quinquefasciatus*, like other Culicine species so far studied, consists of three long and synapsed chromosomal arms (2n = 6) which is generally tend to adhere to each other to form a compact mass. One of the most striking characteristics observed in *Culex quinquefasciatus* polytene chromosomes is the asynapsis observed in several regions of some chromosome arms.The most probable cause is the failure in pairing found in these regions of polytene chromosomes arms. The reason behind this is not due to mechanical cause, but rather due to the fact that structural heterozygosity may exist in these areas.



(a) (b)

Fig 1: Polytene chromosome of *Culex quinquefasciatus*



(a) (b) (c)

Fig.2: Microphotographs of polytene chromosome showing various structural features:
(a) and (b)- asynapsis (c)Heterochromatin body

Studies about cytogentics of mosquitoes are important to better understand their genome organization, function and pathogenicity. For *Anopheles gambiae* and *Aedes Aegypti* mosquito's chromosome work was performed back in the 1970s and 1980s but for the *Culex Quinquefasciatus* (Naumenko *et al.*, 2015) [18] for the first time described the details of morphology, length, and proportions for the mitotic chromosomes of *Culex quinquefasciatus*. A Wells spread chromosomes are undoubtedly much more difficult to obtain in Culex as compared to Anopheles. In the present work also the salivary gland chromosomes of *Culex quinquefasciatus* preparation and study is describe. In early studies the salivary gland chromosomes of *Culex pipens* L. are described and compared with previous descriptions of chromosomes of the *Culex pipiens* complex (Helmy,1974) [10]. Campos *et al* (2003) [7] described a technique for preparing polytene Chromosomes from *Aedes aegypti*. As told before Physical mapping in *Culex quinquefasciatus* is not easy because of the poor quality of the polytene chromosomes. Thus number of attempts to create a polytene chromosome preparation using *Culex Quinquefasciatus* has been made. Similarly the Malpighian tubule chromosome map for *Culex. pipiens* (Zambetaki *et al*., 1998) [29] and *Culex quinquefasciatus* (Campos *et al*., 2003) [7] and the salivary gland chromosome map for *Culex quinquefasciatus* were also developed by number of scientists. But no similarities between landmarks of different chromosome maps were found (McAbee *et al*., 2007) [17]. These problems occurred because of poor levels of polyteny, pairing of nonhomologous chromosome regions, high frequency of ectopic contacts and poor spreading of Chromosome, which we also observed in our preparations. These Chromosome maps are essential implements for good cytogenetic studies of this medically and economically important mosquito. Thus the present analysis of *Culex quinquefasciatus* the polytene chromosomes provides a cytogenetic basis for potential populational studies.

4. Acknowledgments

The authors are thankful to management of MLSU for providing necessary facilities and team of Insect herbal control laboratory for their cooperation.

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