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# Cytological aspects of *Culex (Culex) pipiens* Linn., from Manipur India

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### Abstract

The cytological studies on mosquito is a much to ascertain the systematic conflicts and as well as to adopt the materials in the regular practical classes in educational institutes. The chromosomal compliments, salivary gland and polytene chromosomes are yet to study for *Culex (Culex) pipiens* Linn., the larvae were collected on 27<sup>th</sup> June, 2021 from the locality and identity was compared with available literatures and published papers for adult, pupa and larvae. The larvae were dissected to harvest the salivary gland and imaginal discs (IDS) in hypotonic solution. The salivary gland consists of 8 (eight) small lobules. The salivary glands were fixed in Carnoy's Fluid for 10 minute and stained in 2% Acetocarmine solution and observed under microscope. The three elongated polytene chromosomes were observed. The IDS chromosomes comprises of one pair of submetacentric and two acrocentric pairs. The number of  $2n=6$  and three elongated polytene chromosomes are quite in accordance with published works but the types of the chromosomes are quite different that might be due to chromosomal variation or might be other reasons. In future, more inclusions of habitats and more materials will be required to ascertain the validity of the species *C. (C.) pipiens* from Manipur.

**Keywords:** Manipur, salivary, *Culex (Culex) pipiens*, polytene chromosomes

### 1. Introduction

The cytogenetics and molecular biology of Culicidae can be studied by analyzing their polytene chromosome [1] *Culex pipiens* is a species complex [2, 3] that includes *Cx. pipiens* Linnaeus, 1758. In molecular cytogenetic studies of *Culex*, salivary gland chromosomes have been used to locate the genes related to insecticide resistance [4, 5].

The cytological studies of mosquito will help to solve the systematic conflicts and adopt the larvae as the practical materials to study the salivary and the polytene chromosomes and more precisely the chromosomal aberrations both structural and numerical? *Culex (Culex) pipiens* Linn., was recorded from the state and cytological works were pending. *Culex pipiens* complex is considered as cosmopolitan species. At present the *Cx. pipiens* complex includes five species: *Cx. (Cx.) pipiens* Linnaeus, *Cx. (Cx.) quinquefasciatus* Say, *Cx. (Cx.) pipiens pallens* Coquillett, *Cx. (Cx.) australicus* Dobrotworsky and Drummond and *Cx. (Cx.) globocoxitus* (Dobrotworsky and Drummond) in New Zealand but the complex consists of *Cx. (Cx.) quinquefasciatus* Say, *Cx. (Cx.) pipiens pallens* Coquillett and *Cx. (Cx.) pipiens molestus* in Japan [6]. While in the Americas, it is composed of two main species, *Culex (Culex) quinquefasciatus* Say, which is adapted to tropical zones and *Cx. pipiens* L., which is found in temperate zones. The present reports of *Cx. (Cx.) pipiens* is contradictory to the reports of *Cx. (Cx.) quinquefasciatus* Say by other researchers [7, 8, 9]. In order to differentiate the *Cx. pipiens* from the *Cx. (Cx.) quinquefasciatus*, the cytogenetic study is a much at this juncture. The three elongated polytene chromosomes were observed. The IDS chromosomes comprises of one pair of submetacentric and two acrocentric pairs. The intense further works with comparison among the genus and between the genera to find out the evolutionary relationship will tremendously increase the cytological perspectives of the insects.

### 2. Materials and Methods

Larval stages of mosquito were collected from three breeding ground of Imphal-Keishamthong and DM College Campus during 27<sup>th</sup> June, 2021. The immature larval stages were reared with appropriate foods in beakers till the emergence of adults and identification of

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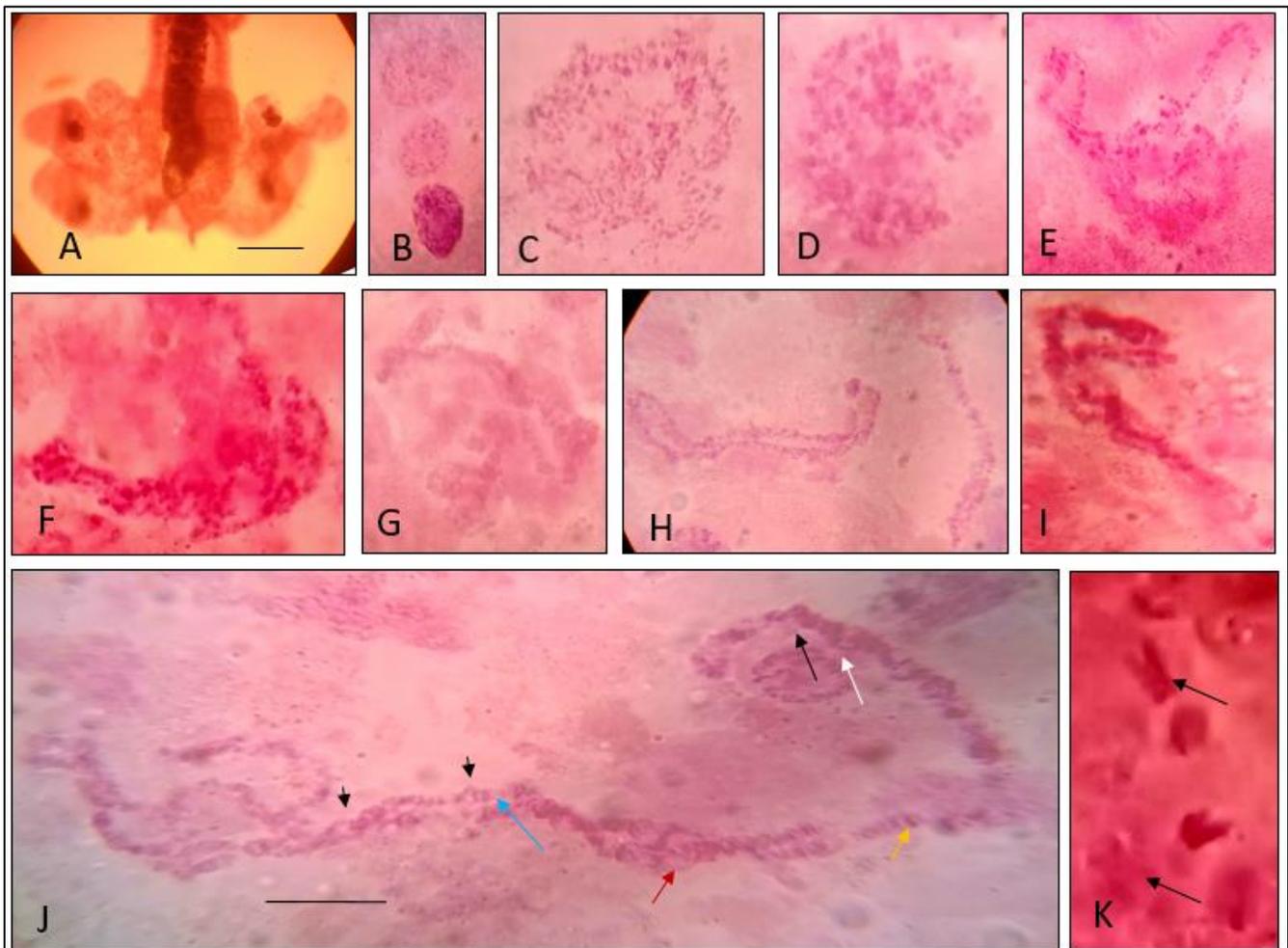
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the species were done from larvae and adults of both the male and female individuals. The taxonomic keys followed were Harbach [10] Walter and Harbach [10] and Tyagi *et al.* [6]. The salivary glands were isolated in 0.57 M KCl by pulling the head of the larvae and fixed in Carnoy's fluid for 2 days or the larvae was killed by pulling the head and put in the fixative in whole in a 1.5 ml centrifuged tube for 48 hrs. Then the gland was put in another tube with 2% acetocarmine solution for 24 hrs and finally slides was made with 0.5% lactic acid and pressed the slide in between filter paper and observed under microscope.

### 3. Results

The polytenic nuclei were begun with highly condensed heterochromatic structure (Fig. 1 B bottom), as in the case of

G1, the turned elongated mass with pigmented arranged all over the nuclei (Fig. 1 C and D). The duplicated chromosomes started pairing like synapsis (Fig. 1 E, F, G, H and I). The characteristic features of polytene chromosomes were could be seen in the final well spread salivary gland cells in mosquito. The black arrows represents the puff, white NOR, yellow the band and blue interbands and dark red the Balbiani ring (Fig. 1 J). The colchicized – hypotonic treated cells displayed 6 metaphase chromosomes comprising f two large sub metacentric chromosomes and two pairs of acrocentric chromosomes (Fig. 1 K). The chromosomes could not be individually distinguished. The quality of the polytene were very thin and elongated. The NOR bearing chromosome seem to be shortest.



**Fig 1:** The represents the salivary gland (A Bar represents 3 mm) extracted from the larva of *Culex pipiens pipiens*, and various stages of formation of polytene chromosomes from the nucleus to three polytene chromosomes (J Bar represents 3 micron meter) and the six metaphase chromosomes from colchicized imaginal discs (IDS) cells of the larva. The black arrows represents the puff, white NOR, yellow the band and blue interbands and dark red the Balbiani ring

### 4. Discussion

Recent reports of presence of fifty-five species of mosquitoes under ten genera from Manipur totaling the species composition of the state eighty-three under thirteen genera. Dutta *et al.*, [7] reports 17 *Culex* species under sub genus *Culex* and *Cx. (Cx.) quinquefasciatus* Say was mentioned not the *Cx. (Cx.) pipiens*.

The *Culex pipiens* complex has been the subject of intensive study in Europe, America, Africa, and Australia as a result of which many interesting facts have come to be known which

throw light on the various aspects of its study [9]. At least three intraspecific forms of this subspecies are known to occur in the Ethiopian region [12]. The *Culex pipiens* species complex has many related species, ecotypes and hybrids which are situated in geographical introgression zones on multiple continents [13]. *Culex quinquefasciatus* was first described in 1823 by Thomas Say from a specimen collected along the Mississippi River in the southern United States. At that time, a number of similar species around the world like *Culex fatigans* [14] from the Old World tropics were used

synonymous to *Cx. quinquefasciatus* [15, 16]. Females of *Culex pipiens* and *Cx. quinquefasciatus* are morphologically indistinguishable and hybrid zones for the two species are well documented. Owing to this, *Culex quinquefasciatus* has been considered and designated as a subspecies of *Cx. pipiens* with the name *Culex pipiens quinquefasciatus* [17]. Studies have indicated that *Culex pipiens* and *Cx. quinquefasciatus* are two distinct sympatric mosquito populations [18] and they also exhibit a distinct and clear genetic difference [19] which led to the elevation of *Cx. quinquefasciatus* to a species status. According to Dehghan *et al.*, [20] the main distinguishing features of the two taxa included were shape of siphon: wide in middle and number of branches in seta 1a-S and 1b-S is between 4-12 and 3-10 in *Cx. quinquefasciatus* while it is 2-6 in *pipiens* in larval stage. In adult stage former had dorsal arms which were pointed nearly parallel; ventral arms were leaf shape (broad and long) but latter has the male genitalia showing broad dorsal arms truncate at the apex and narrow and sharply curved ventral arms.

The systematic relationships of *C. pipiens* complex species are still under debate, and the vectorial capacity of each species is under investigation. Speciation, in general terms, occurs not only by gene mutations, but also to chromosomal rearrangements, especially inversions. Thus, finding the location of genes in a genome helps to improve our understanding of genome organization, contributes towards deciphering how the genome functions, and provides possible landmarks of genome organization like inversions that may be associated with different taxa in the complex. Studying the *C. quinquefasciatus* genome organization is the first step towards clarification of *C. pipiens* complex relationships. Additionally, as a required framework against which phenotypes and genotypes can be associated in population studies, it can form an improved basis for the development of new vector control and disease prevention strategies.

The number of  $2n=6$  and three elongated polytene chromosomes are quite in accordance with published works [21] but the types of the chromosomes are quite different that might be due to chromosomal variation or might be other reasons. In future, more inclusions of habitats and more materials will be required to ascertain the validity of the species *C. (C).pipiens* from Manipur.

## 5. Conclusion

The present study deals on the cytology of *Culex (Culex) pipiens* Linn., from Manipur. The number of  $2n=6$  and three elongated polytene chromosomes are quite in accordance with published works but the types of the chromosomes are quite different that might be due to chromosomal variation or might be other reasons. In future, more inclusions of habitats and more materials will be required to ascertain the validity of the species *C. (C).pipiens* from Manipur.

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