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Variability in polytene chromosome in *aedes*, *anopheles*, *culex,* and *chironomus* species: A review

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Abstract
Mosquito borne diseases (MBDs) are an intolerable risk to public health globally. It is estimated that about 1300 million people in 83 countries all over the tropics and sub-tropics of South America, Asia, the Western Pacific, Africa, and parts of the Caribbean are at risk of this disease. *Aedes aegypti* is the main vector, which is responsible for Dengue virus, *Anopheles stephensi* for malaria and *Culex quinquefasciatus* for lymphatic filariasis. Different cytogenetic studies have provided us with a necessary background understanding about the different behavioral changes of these mosquito vectors, so that it can help us to control them. The chromosomal maps of different mosquito vectors are vital tools which provide us with good cytogenetic evaluation of these mosquitoes, which is of great economical and medical importance. In the present study, we provide a detailed review of literature on the variability in polytene chromosome in Aedes, Anopheles, Culex, and Chironomus species. The study will be one of the pioneer studies to provide a complete comparative database and baseline for future work.

Keywords: Mosquito borne diseases, *aedes aegypti, anopheles stephensi, culex quinquefasciatus,* polytene chromosome

Introduction

Mosquito borne diseases are an intolerable risk to public health globally. Among different mosquito vectors, species belonging to genus Culex are the most diversified and most prevalent geographically (Reddy *et al.,* 2012 and Vinogradova, 2000) [35, 47]. One of the species of Culex, i.e. *Culex quinquefasciatus* is the chief vector that causes lymphatic filariasis. It is estimated that about 1300 million people in 83 countries all over the tropics and sub-tropics of South America, Asia, the Western Pacific, Africa, and parts of the Caribbean are at risk of this disease (WHO, 2012). *Culex quinquefasciatus* possesses the most fragmented genomic sequences as compared to other species of mosquitoes (Parveen *et al.*, 2016). Polytene chromosomes of the salivary glands of *Culex quinquefasciatus* have been used in cytogenetic analyses (Sutton 1942, Kitzmiller & Clark 1952, Kitzmiller & Keppler 1961) [20, 45].

Among the different species of Aedes mosquitoes, *Aedes aegypti* is the main vector which is responsible for Dengue virus (Cecilia, 2014) [7]. According to NVBDCP (2014), dengue fever has been endemic in 16 states of India from the beginning. These states are Andhra Pradesh, Rajasthan, Puducherry, Goa, Delhi, Gujarat, Chandigarh, Haryana, West Bengal, Karnataka, Kerala, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Maharashtra and Punjab. Between 2010 and 2012, it invaded into the remaining other states also.

Among the different species of Anopheline mosquitoes, *Anopheles stephensi* is the main vector that is responsible for malaria in South Iran and the Persian Gulf (Manouchehri *et al.*, 1976) [23], in the urban areas of India (Pant *et al*., 1981) [30], and in rural areas of East Afghanistan and North Pakistan (Rowland *et al.*, 2002) [36]. Malaria is a critical public health problem worldwide. It is estimated that malaria is responsible for approximately 110 million proven cases and almost around 300,000 deaths per year (Parveen *et al.*, 2017).

WHO (2010) also gave its priority to control these vectors as only it can give us a sustainable solution to control and to completely eradicate these major vectors borne diseases in many tropical countries like India and Africa. Different cytogenetic studies have provided us with a necessary background understanding about the different behavioral changes of these mosquito vectors, so that it can help us to control them (Parveen *et al.*, 2017). The chromosomal maps of different mosquito vectors are vital tools which provide us with good cytogenetic evaluation of these mosquitoes, which is of great economical and medical importance (Parveen *et al.*, 2016).

In genetics, polytene chromosomes are defined as giant cable like chromosomes. These chromosomes consist of many other identical chromosomes, which are closely associated along their length. When the normal somatic chromosomes undergo multiple rounds of DNA replication followed by nuclear division, they give rise to polytene chromosomes. As a result, the cells so formed are polyploid, but their chromatids remain associated laterally with each other to produce the organization of polytene chromosomes. The study of these polytene chromosomes had been extensively used to clarify species complexes (Aju-Ameh *et al.* 1998) [1].

Balbiani first identified these chromosomes in Chironomus larvae (Balbiani, 1881) [3], but got proper authentication 50 years later by Heitz & Bauer (Heitz and Bauer, 1933) [15]. The study on morphology and development of salivary glands and their chromosome was done by Moreira *et al.* (1999) [26].

Polytene chromosomes are found naturally in the Dipteran nurse cells (Stalker, 1954) [44]. They are produced because of repetitive DNA replications without cell division, and are characterized by their extra-large size, banding patterns (Zhimulëv, 1996) [52], specific areas of heterochromatin and diffused pufflike structures (Parveen and Prasad, 2016). Specifically, in Anopheline mosquitoes, they are found with

excellent morphology (Coluzzi *et al.*, 1970) [9] and they also provide us an excellent opportunity to develop physical maps of high resolutions (Parveen and Prasad, 2016). Not only in salivary glands, but these chromosomes are also found in different tissues like gut, Malpighian tubules and ovarian nurse cells (Parveen and Prasad, 2016). Generally, banding patterns are found to be consistent within a species, but exceptionally, they could also be somewhat consistent between closely related species (Parveen and Prasad, 2016). The pupal polytene chromosomes separated from Malpighian tubules also show visible structural characteristic features which are suitable for their use in locating resistance and vector competence genes (Campos *et al.*, 2003) [6].

The cytogenetic and molecular studies of Anopheline mosquitoes can also be performed by their polytene chromosome structure analysis (Campos *et al.,* 2003) [6]. Studies regarding preparation of these polytene chromosomes had proved to be complicated in Culex mosquitoes and the techniques available are also not very reliable (Campos *et al.,* 2003) [6]. Although, polytene chromosomes from Malpighian tubules are considered as an excellent material for detailed studies in the cytogenetic investigation of *Culex quinquefasciatus* (Gaona and Campos, 2002) [5]. But such investigations had remained difficult for certain species of mosquitoes such as *Aedes aegypti* (Gaona and Campos, 2002) [5]. The methodological complications in the preparation of polytene chromosomes of Aedes mosquitoes are so obvious (Sharma *et al.*, 1986) [42] and they are revealed in the lack of papers dealing with this material. *Chironomus larvae* have a small number (2*n*=8) of polytene chromosomes with many bands (Michailova *et al.,* 2003) [25].

Polytene chromosomes also offer a distinctive advantage in generating and integrating their physical and genetic maps (Severson *et al.* 2001) [38]. Chromosome polytene physical maps are the fundamental tools which provide a good cytogenetic evaluation of these mosquitoes, which are of great economic and medical importance (Campos *et al.,* 2003) [6]. With the help of fluorescence in situ hybridization (FISH) techniques, chromosome polytene physical maps associated with genetic linkage maps could be developed. This had been done for the metaphasic chromosomes of *Aedes aegypti* (Brown *et al.*, 2001) [4].

A number of difficulties have been proposed as contributing factors for the poor quality of polytene chromosome preparations in Aedes and Culex mosquitoes : (1) The chromosome arm length (Kitzmiller, 1963) [18, 19] also influence chromatic interactions; (2) Sutton (1942) [45] proposed the presence of weak points, also known as heterochromatic areas, where the chromosomes could break effortlessly (Semeshin *et al.,* 2001) [37]; (3) Asynapsis witnessed in polytene chromosomes (Zambetaki *et al.,* 1998) [51]; and (4) Ectopic pairings (inter and intra chromosomal connections) (French, 1962, Verma *et al.* 1987) [11, 46] arising from highly repetitive DNA regions (Rai & Black IV 1999, Severson *et al.,* 2001) [34, 38].

Here in this paper, we provide a detailed review of literature on the variability in polytene chromosome in Aedes, Anopheles, Culex, and Chironomus species. The study will provide a complete comparative database and baseline for future work.

Materials and Methods

For the study, we extracted crucial data related to our research using the terms 'Polytene Chromosomes', 'Giant Chromosomes' along with 'Anopheles Mosquitoes', 'Culex Mosquitoes', 'Aedes Mosquitoes', and 'Chironomus Larva' from the bibliographic database Google Scholar, Scopus, Springer, Taylor and Francis, and other standard search engines. Our search was carried out in September 2023, encompassing all research published up to that date.

Polytene chromosomes of different mosquito species

Culex Mosquitoes

The nuclei of salivary gland of *Culex quinquefasciatus* possess long polytene chromosomes with bands, which are connected at a common point called as chromocenter (Fig.1). Various structural characteristics among various arms were observed such as asynapsis (Fig 2.a, b) and heterochromatin bodies (Fig 2.c) in several regions of some chromosome arms. The specific feature of these chromosomes is that their arms show a distinctive banding pattern. Different characteristic features of these chromosomes are the extended length of chromosomal arms, ectopic pairings and telomere contacts. The polytene chromosome complement in *Culex quinquefasciatus* comprises of three synapsed and long chromosomal arms (2n = 6) which generally tend to adhere to each other forming a compact mass. It is because failure of pairing was observed in these regions. This failure in pairing is due to the structural heterozygosity found in these areas (Parveen *et al.,* 2016).

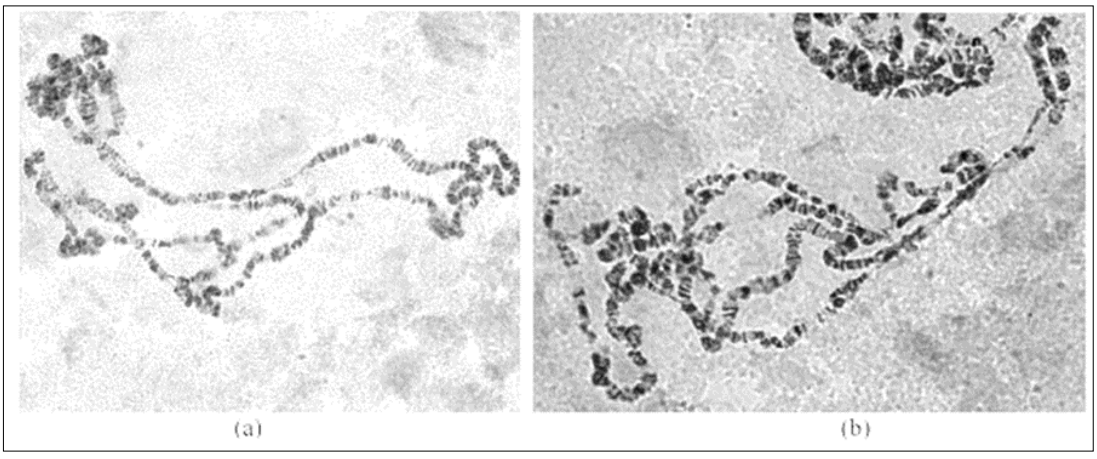

Fig 1: Polytene chromosome of *Culex quinquefasciatus* (Source: Parveen *et al.,* 2016).

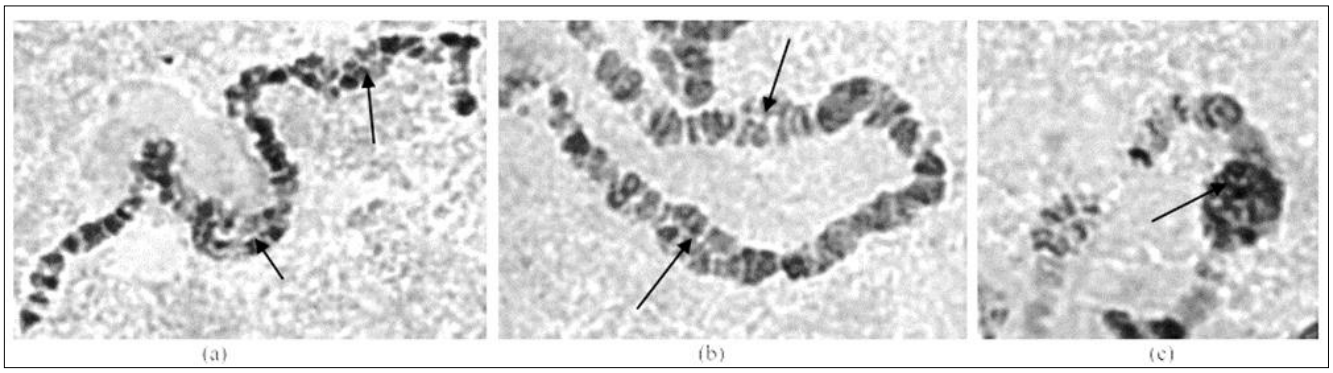

Fig 2: Microphotographs of polytene chromosome showing various structural features: a. and b-asynapsis, c-heterochromatin body (Source: Parveen *et al.,* 2016).

Aedes Mosquitoes

Aedes aegypti mosquitoes are polymorphic mosquitoes which comprise of at least three morphological forms. These mosquitoes are identified based on physiological, morphological and behavioral differences. This identification indicates the variations in their genetic load (Spielman and Kitzmiller, 1967) [43]. These variations are observed due to morphological dissimilarities among different chromosomes. Such variations could be some inversions, from which certain other inversion effects get repressed, as result of which irregular gene lengths are observed among different chromosomes (Gale and Crampton, 1989) [12].

Polytene chromosomes in salivary glands of *Aedes aegypti* had poor banding patterns. Their banded structure was although clear at some places, but their pattern of banding was not followed by individual arms of chromosome, as they are interrupted by "weak spots". At these places, chromosomes get fragmented during smearing, and different parts of same or different chromosomes stick in these regions due to which continuity of chromosome arm is disturbed. Due to these reasons, they are not suitable for their physical mapping (Sutton, 1942) [45].

Based on the above outcomes, it is concluded that the genomic size, protein configuration and the repetitive DNA - single DNA patterns (interspersion patterns) are the major aspects that can affect the spreading of chromosomes (Campos *et al.,* 2003) [6]. *Culex quinquefasciatus*, has a moderate genomic size and moderate to short interspersion patterns, and on the other hand, *Aedes aegypti* had larger genomic size and relatively short interspersion patterns (Severson *et al*., 2001) [38]. The proportion of repetitive DNA of *Culex quinquefasciatus* (80%) is larger as compared to *Aedes aegypti* (60%) (Warren & Crampton 1991, Knudson *et al.* 1996, Brown *et al.* 2001) [4, 21]. Therefore, the declaration of Severson *et al.,* (2001) [38] which states that the difficulty in the spreading of polytene chromosomes is caused by the high inter and intra chromosomal connections (which occur at high repetitive DNA areas), is partially unjustified; as there is relatively better spreading of polytene chromosomes in *Culex quinquefasciatus* as compared to *Aedes aegypti* (Campos *et al.,* 2003) [6].

Chromosome arm conservation is common in Dipterans of higher taxa. Comparison between the linkage maps of *Culex pipiens* and *Aedes aegypti* showed that chromosome 1 in both above-mentioned mosquitoes is highly conserved. Also, the arms of chromosome 2 and chromosome 3 of the above-mentioned mosquitoes possess certain homologous loci (Mori *et al.*, 1999) [27]. Overall, culicine complexes seem to be fewer and their identification is based on their morphology (Aju-Ameh *et al.,* 1998) [1].

Anopheles mosquitoes

In literature related to Anopheles mosquitoes, the arms of polytene chromosome are classified on the basis of two types of designations: (i) According to the old nomenclature for polytene chromosome arm designation, the two arms of a chromosome are termed as left (L) and right (R) arms respectively; this nomenclature system is followed by many Anopheline cytogeneticists and also by Drosophila cytogeneticists; and (ii) according to the new nomenclature for polytene chromosome arm designation (Green and Hunt, 1980) [13], each autosomal arm is designated with a different number i.e. 2, 3, 4 and 5 and the euchromatic arm of the X chromosome in the polytene chromosome is designated as X (Parveen and Prasad, 2016).

Table 1: Conventional and alternative nomenclature of the polytene chromosomes in the genus *Anopheles* (Praveen and Prasad 2016).

Chromosome arm	Conventional nomenclature	Alternative nomenclature
X	**X**	**X**
Right arm of chromosome 2	2R	2
Left arm of chromosome 2	2L	3
Right arm of chromosome 3	3R	4
Left arm of chromosome 3	3L	5

The identification to recognize different chromosomal arms are as below

- **Chromosome X:** It is the shortest chromosome. It can be easily identified by a significantly stained dark band at telomeric region of chromosome (Fig.3). This chromosome consists of a characteristically large puff at centromeric end.

- **Chromosome 2(2R):** A strong identification for this arm is a distinguished granulated large dark band with small puff at centromeric region. The right arm of chromosome 2 is the longest among the 4 autosomal arms (Fig.4).

- **Chromosome 3(2L):** The distinguishing feature of this arm is the presence of doublets of dark bands just before centromeric end. It has a very light telomeric end and very a smaller number of dark bands. This chromosome is shorter than chromosome 2 (Fig.5).

- **Chromosome 4(3R):** This chromosome is characterized by the presence of numerous puffs near the centromere. This chromosome is shorter than chromosome 2 and chromosome 3 (Fig.6).
- **Chromosome 5(3L):** Telomeric end has less bands, but numerous dark bands are concentrated at the center. Centromeric end possess small puffs. It is larger than X chromosome but shorter than all others (Fig.7).

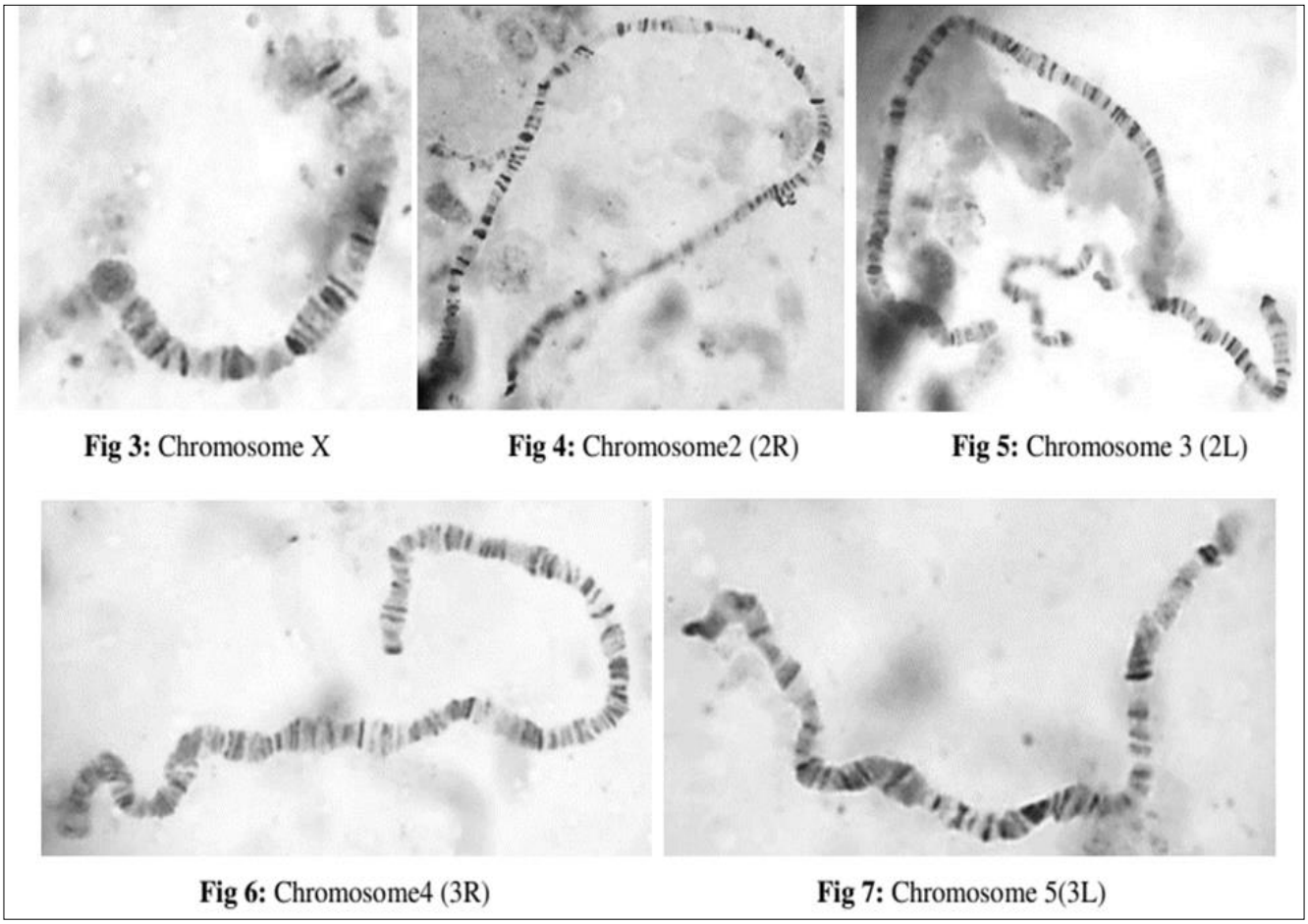

Fig 3: Chromosome X

Fig 4: Chromosome2 (2R)

Fig 5: Chromosome 3 (2L)

Fig 6: Chromosome4 (3R)

Fig 7: Chromosome 5(3L)

(Parveen and Prasad, 2016)

For ~50 different species of Anopheles mosquitoes, chromosomal maps have been established (Sharakhov & Sharakhova, 2008) [39]. During the last decade, cytogenetic photomaps of polytene chromosomes of *Anopheles albimanus* (Cornel & Collins, 2000) [10], *Anopheles funestus* (Sharakhov *et al*., 2001) and *Anopheles stephensi* (Sharakhova *et al.,* 2006) [40] were created. Also, in 2002, a good quality photomap of polytene chromosomes from ovarian nurse cells of Anopheles mosquitoes was published (Coluzzi *et al.,* 2002) [8].

As compared to autosomes, X chromosome of Anopheline mosquitoes has three times higher rate of fixed genomic inversions (Neafsey *et al.* 2015, Artemov *et al.* 2018) [2, 29]. In ovarian nurse cells, species of Anopheline mosquitoes show a wide range of nuclear organisation. *Anopheles funestus* and *Anopheles gambiae* possess well-formed polytene chromosomes in nuclei of nurse cells (Sharakhova *et al.* 2010) [41]. The left and right arm of each polytene chromosome in the species of Anopheline mosquitoes live in adjacent regions inside the nucleus, similar as that of normal chromosomes (Hochstrasser *et al.,* 1986) [16].

***Chironomus* larvae**

- **Chromosome 1:** It is the longest chromosome and carries a NOR (nucleolar organizing region) connected with a large puff in the 2D segment. Other important features of this chromosome include presence of small puff in 2A segment, three dark bands in the middle of 3A segment, one thick band between 3B and 3C segments, a constriction and a dark band at 3/4 and two thick bands in 5A segment.
- **Chromosome 2:** This chromosome carries a NOR (nucleolar organizing region) connected with a very large puff in the 9C segment. Other important features of this chromosome include one more or less expanded free tip which immediately narrow down in 7A segment, some closely packed thin bands and a small puff in 8A and 8B segments, a little swelling in 10D segment and a small spindle with characteristic dark bands in 11A segment.
- **Chromosome 3:** This chromosome is smaller than the above two chromosomes. Other important features of this chromosome include an extended free tip and a thick dark band in 12A segment, three lightly stained bands which had dispersed chromomeres in distal half and three darkly stained bands in the front half of 12C segment, a constriction at the connection of 14C/D and light bands in 15D segment.
- **Chromosome 4:** This is the shortest chromosome among all.

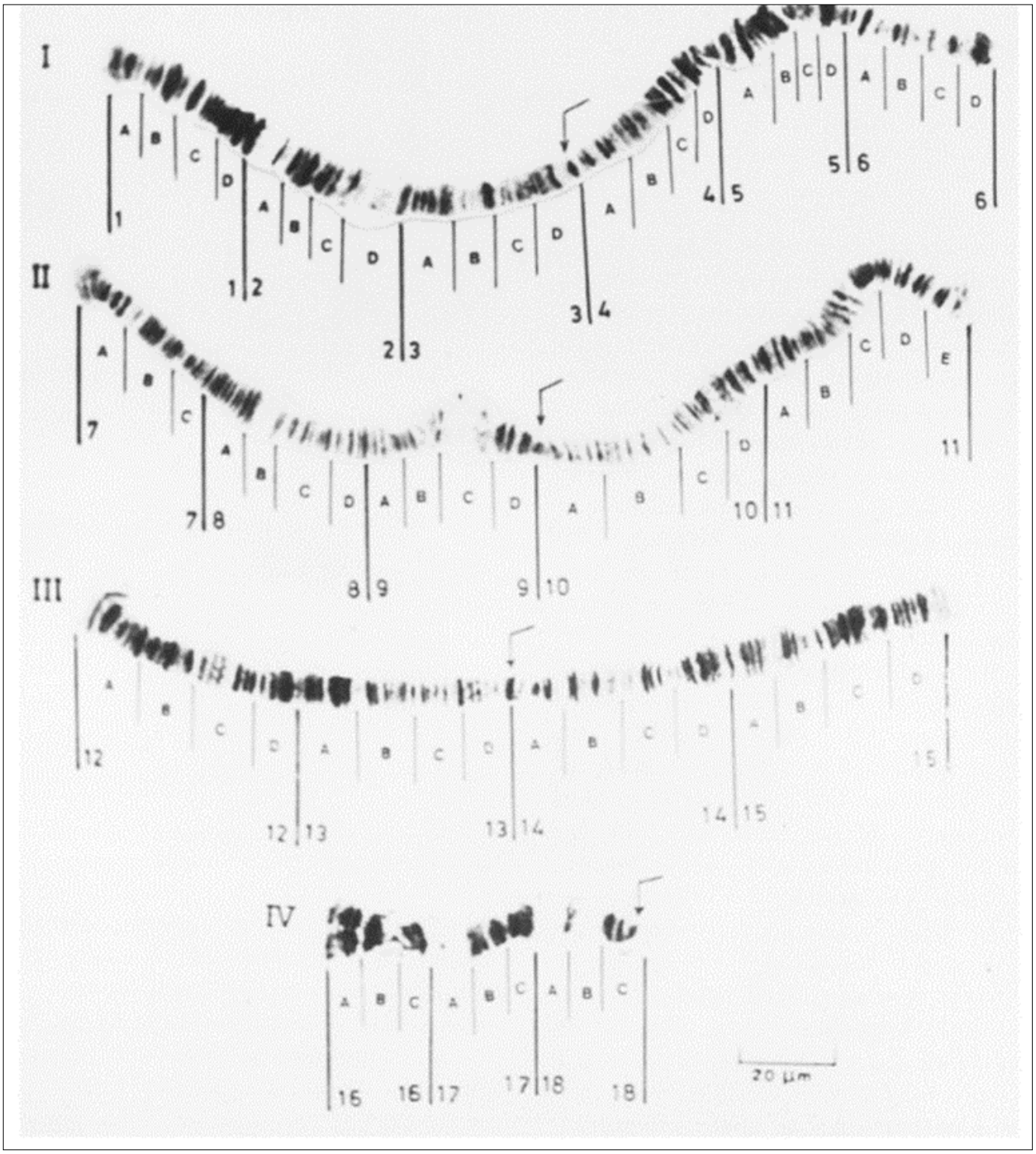

Fig 8: Photomap of the polytene chromosomes of *Chironomus circumdatus.* Arrow showing centromeric position in polytene chromosomes (Kumar and Gupta, 1990) [22].

Maps of the polytene chromosomes of *Chironomus riparius* (Hägele, 1970; Kiknadze *et al.,* 1991) [14, 17] were used to restrict chromosomal abnormalities. The set of polytene chromosomes of *Chironomus riparius* is 2n = 8 and its chromosome arm combinations are AB, EF, CD and G with a distinct characteristic centromeric region. Also, there occurred three Balbiani rings (BRa, BRb and BRc) and a nucleolar organizing region (NOR) in chromosome arm G (Michailova *et al.,* 2006) [26].

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