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# SALIVARY GLAND CHROMOSOME MAP OF *DROSOPHILA AURARIA* (INSECTA: DIPTERA)

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**Abstract:** The strain *of Drosophila auraria* used in the present study collected by putting rotten fruit baits was cultured on ripe banana media. The life cycle is approximately 12 days from egg to egg at 28°C. Third instar larvae were selected to dissect for salivary gland chromosomes, after *2%* acetolactic orcein for 15 minutes cover slip pressed, a giant chromosome map was prepared by measuring: the widths and lengths of the bands and puffs working from free end to centromeric end. The salivary gland chromosome consists of 5 long arms and a Miiall arm. No inversions were recorded. In puff DNA unfolds and actively synthesizes mRNA. The bands represent regions where DNA is more tightly coiled and in the interbands the DNA fibers are less folded. Comparison *of D. auraria* with other species reveals presence of a number of differences.
**Key words:** Polytene chromosomes, life cycle, metaphase plate, DNA.

## INTRODUCTION

It is difficult to determine the phylogenetic and evolutionary relationships among animals. We usually apply morphological and physiological comparisons for this purpose. Secretory tissue salivary gland chromosomes with their characteristic banding pattern offer a unique approach to evolutionary studies. Chromosomes were independently discovered by Oscar Hertwig (1876) and Strasburger (1877). According to them, the nucleus carries physical bases of heredity. The gigantic chromosomes found in the nucleus of salivary gland of dipterous fly are very important and useful for studying phylogeny and divergence, particularly of closely related forms. Balbiani (1881) first describe them as large banded structure in the larvae of genus *Chironomus camoy* (1884) made further morphological observations. Bolsius (1911) came to conclusion that salivary gland chromosomes are marked by the presence of darkly stained bands. The individual bands in the salivary gland

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chromosomes differ greatly among themselves, so they can recognized and identified and it is possible to construct a cytological map in which the appearance and position of each band is indicated.

***The chromosomal complex***

The whole chromosomal complex forms a sensitively balanced system. Gene activity in this system can be determined by its chemical constitution, by its position within the whole system and by the constitution of the whole system; in certain cells at certain stages of their life cycle, special type of giant chromosomes can be determined which are of enormous size (Demerec, 1938). These are named as polytene chromosomes particularly in salivary glands and are formed in Dipterous larvae (Painter, 1933; Koller, 1935; Cooper, 1938; White, 1946; Melland, 1942). All the detailed study of polytene chromosomes of flies began from 1933. Kostoff (1930) indicated the apparent similarity of the banded salivary gland chromosomes were first used by Painter (1933, 1934a, 1934b, 1934c and 1935) for cytological verification of genetic data already established by several geneticists. Almost immediately Bridges (1935) U.S.A., Fujii (1936) Japan and Bauer (1936) Germany adopted acetocarmine technique. A major contribution of Bridges was series of carefully prepared chromosomes maps of *Drosophila melanogaster.*

***Active genes***

The system of numbering the bands was originally introduced by Bridges in 1935; and had been adopted with minor modifications by Dobzhansky (1936); Beermann (1961); Khan (1966); Nasir and Aslam Khan (1968). Beermann (1952) interpreted the puffing of polytene chromosomes as an expression of gene activity. In *Drosophila* more than one gene may be present in a single band. Certain elements in the chromosomes of *Drosophila* maintain their identity from species to species through undergoing various changes in their relations to each other and in their internal structure (Sturtevant, 1921).

***Volume of nucleus***

The enormous size of giant chromosomes characterized by the increase in volume of the nucleus and the cell. In certain Dipteran larvae tissues such as salivary glands, gut, fat body cells and malpigian tubules chromosomes are strikingly different from somatic chromosomes of the same organism. In *Drosophila melanogaster* the volume of polytene

chromosomes is about thousand times greater than that of somatic chromosomes.

***Salivary gland nuclei have greater amount of DNA***

When a chromosome became polytenic, the DNA replicates by endomitosis and the resulting daughter chromatids do not separate and remain aligned side by side. The DNA of some special puffs DNA puffs is replicated more than the rest of the chromosome, providing are of the few examples of gene amplification. Polytene cells are unable to undergo mitosis and are associated to cell death. Another characteristic of polytene chromosomes is that the homologous pairs are closely associated as in meiotic prophase. This phenomenon is called somatic pairing and the chromosomes are considered to be in permanent interphase. Along the length of chromosomes a series of dark bands alternates with clear zones called interbands. The bands represents (Fig. 1) regions where the DNA is more tightly coiled and in the interbands the DNA fibers are less folded (Gilbert and Muller, 1967). It is easy to construct, form a giant chromosome, topographic maps of the bands and interbands and to verify any disarrangement or alteration in the order of their linear sir structure. Genetic studies indicate that there are about 5001 genes in *Drosophila.*

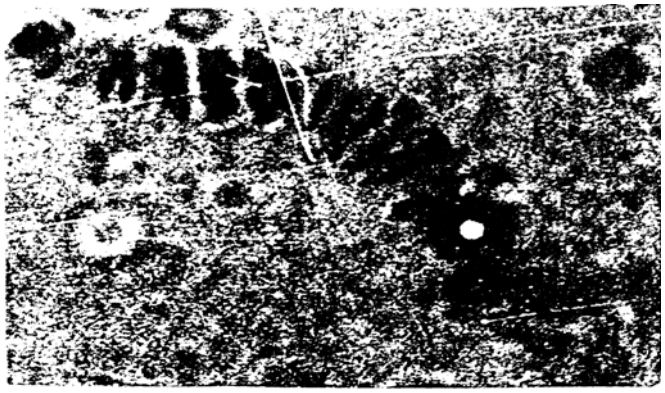

**Fig. 1 The bands represents regions where the DNA is more tightly coiled and in the interbands the DNA fibers are less folded.**

***A puff as a site of transcription***

One of the most remarkable characteristic of polytene chromosomes is that it is possible to visualize in them the genetic activity of specific

chromosomal sites. The morphological expression of such sites is represented by local enlargements of certain regions called Puff. A puff can be considered as a band in which the DNA unfolds into *open* loops that actively synthesize RNA, Beermann are the morphological expression of gene transcription and provide a unique opportunity to study the regulation of Eukaryotic gene. It provides evidence that in Eukaryotes gene activity is regulated at the level of RNA synthesis. Some puffs has been correlated with the production of specific proteins which are secreted in large amounts in larval saliva. Puffs can be induced by ecdysones and heat shock. Ashbumer *et al*. (1973). In the present study an attempt has been made to study the salivary gland chromosomes of *D. auraria of* this region and to construct a map so to aid in further studies on phylogenetic relationships.

## MATERIALS AND METHODS

The strain of *D. auraria* used in the present study was originally collected from New Campus. University of the punjab, Lahore by putting rotten fruit baits. *D. auraria* can tolerate low temperature and rare during summer.

***Culture and keeping fruit flies in lab***

The strain was cultured on ripe banana media with tissue paper toweling, transferred into the sterilized culture tubes capped with clean sterilized cotton plugs. The life cycle is approximately 12 days from egg to egg at 28°C,

***Tissue staining***

In salivary gland chromosomes preparation Strickberger's (1962) modified technique was used. For this purpose third instar larvae were selected and washed in distilled water and dissected in 45% acetic acid solution. Washed in distilled water, to remove the contents of food media on the body of larvae. During dissection a cut was made with the help of sharp needle in the head region. Another needle was placed about the middle of the body. The needles were dragged in opposite directions so as to expose the larval viscera. Salivary glands were present just behind the jaws. The fat bodies attached to the salivary glands were carefully and completely removed and the glands were transferred by a needle to a clean slide having *2%* acetolactic oreein and kept for 15 minutes and a cover slip

was placed on it. Chromosomal spreading follows this. A piece of blotting paper was laid on the coverslip and firmly pressed with the ball of the thumb. The blotting paper served to absorb all the extra stain. The pressure can also be applied by tapping on stroking the cover slip gently with the back of a rubber pencil. The slides thus sealed with nail polish were then studies under the microscope. By systematically photographing by using 35 mm Forte black and white film the chromosome map was prepared. The detailed banded patterns were drawn and thus the map was prepared by measuring the widths and lengths of the bands and puffs. Working form free end to centromeric end each chromosome is broken up into regions, each region begins with easily identifiable band spaced at such distances that within each region approximately six to seven bands are visible.

## RESULTS

The metaphase plate of *D. auraria* consists of one pair of rods, 2 pairs of V's and a pair of dots. The salivary gland chromosomes consists of 5 long arms and a small arm (Fig. 2). No inversions were recorded. As compared to *D. auraria,* metaphase plate in *D. simulans appears* to have 3 pairs of V's and a pair of dots, same apttern in *D. robusta* (Patterson and Stone, 1952). *D. auraria* resembles with the *D. melanogaster* in metaphase plate pattern; where also two pairs of V's a pair of rods and a pair of dots are recorded. The study of salivary gland chromosomes provide us various kinds of valuable information. These are unique in showing the history of relationship within a species and also between species. Salivary gland chromosomes gave useful information because of the fact that the differences in gene sequences can e readily localized. The chromosomes have played a major role in studying both intraspecific and interspecific differences in gene orders. Dubinin *et al.* (1973) using the salivary gland chromosomes checked for heterozygous inversions, from a large scale study of the free living populations of *Drosophila.*

***Description of chromosomes***

***Chromosome-X***

Free end of X-chromosome is marked by the thin broken bands. There are 3 equally spaced thin broken bands and 4 thick dark bands gradually increasing in size in 1A. Region 1B is characterized by a puff

which consists of nine thin broken bands. Region 2 starts at the end of region 1B.

***Chromosome 2-R***

Free

**Fig. 2 Secretary tissue (salivary gland) chromosomes of *Drosophila auraria consists* of 5 long arms and a small arm and have resemblance to *Drosophila melanogaster* in many regions.**

## DISCUSSION

*Drosophila simulans* mitotic figure also sharply differ from *D. auraria* where two pairs of Vs. 2 pairs of rods and a pair of dots and differs also *D. auraria* from *D. immigrans* which have two pairs of rods, a pair of V's and a pair of dots. In *D. repleta* a very stable gene sequence was found (Wharton, 1942). The studies *of* gene arrangement in *D. anannasae (*Kaufmann, 1936; Kikkawa, 1938) have shown the presence of inversions as well as heterozygous translocations. Moreover cytological analysis of *D. Algonquin* (Miller, 1939), *D. azteca* (Dobzhansky and Sokolov, 1939), *D. Miranda* (Koller, 1939b), *D. texana* (Patterson *et al.*, 1942), *D. melanica* and *D. paramelanica* (Griffen, 1942) have provided with the information that the gene arrangement of the chromosomes does not remain constant and is likely to show variability. Chromosome-2R of *D. auraria* have similarity in certain regions with *D. simulans* and some similarity with *D. melanogaster*, such as 22A of *D. melanogaster* and 21B of *D. auraria*, in 24A and 24B of *D. auraria* have one convex followed by three light broken bands and then two dark thick bands but in *D. melanogaster* in region 35B and 35C this sequence changed by one addition of thin broken band.

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