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GIEMSA BANDING IN MALE RAT (*RATTUS RATTUS*)

Ashok J. Patil

Department of Zoology, Pratap College, Amalner – 425401, (M.S.), India

E-mail: yashapat@yahoo.in

ABSTRACT

G-banding analysis was carried out on specimens of house rats. The diploid chromosome number of *Rattus rattus* is 2n=38 and the karyotype formula is 16m + 4sm+ 16a + 1X+1Y. The bands produced were specific for each chromosome pair in this animal. The karyotype by G-banding was prepared for male rat including 36 autosomes contains 16 metacentric, 4 sub-metacentric and 16 acrocentric chromosomes which were arranged in decreasing order of each group. The male contain 2 different sex chromosomes the X which is sub-metacentric and Y chromosome is acrocentric which have arranged separately.

Key Words: G-banding, Karyotype, *Rattus rattus*, Chromosome, Trypsin

INTRODUCTION

Pairing of homologous chromosomes had for a long time being done by visual observation of chromosomes in photographs and sometimes it becomes difficult to identify chromosomes with certainty. This can be corrected by using different banding techniques. Different patterns, usually observed at specific regions on particular chromosomes are being increasingly used for the identification of human chromosomes. These bands are made visible through low and high intensity regions under the fluorescence microscope or as differently stained areas under the light microscope.

The giemsa banding patterns of human chromosomes have been describe by (Pardue and Gall, 1970).The earlier methods depended on alkaline denaturation followed by renaturation in an adequate buffer. The same procedure was used by Aggrighi and Hsu (1971) to show these regions probably represented repetitive DNA. Matsui and Sasaki (1975) suggested that the macromolecules like DNA and proteins are lost in G-banding which causes an uneven distribution of chromatin.

In present investigations the chromosomes of *Rattus rattus* analyses and prepared male karyotype by arranging the homologous chromosomes. These homologous chromosomes were observed by G-banding technique.

MATERIALS AND METHODS

Rattus rattus were traped from godowns of cereals in the city of Amalner (M.S.), India. The taxonomic identification of animal was done by consulting standard manuals and publications (Ellerman, 1961; Prater, 1971). The somatic metaphase chromosomes in bone marrow of rat were used for G-banding. The air dried chromosome spread slides were prepared and kept for 2-3 days at 4^0C. Before treating the slides were prewarmed at 60^0C on a hot plate.

After one hour slides were immersed in a phosphate buffer saline for 5 minutes.

The freshly prepared Trypsin +EDTA (50mg of trypsin powder and 20 mg of EDTA dissolved in PBS) solution in phosphate buffer saline (PBS) was used. To get the proper banding different timing is set for each slide to treat them with trypsin. To get the proper binding different timings started from 12 seconds – 30 seconds is taken for different slides. At 20 seconds the proper banding was found to prepare the karyotype by arranging the exact homologous chromosomes.

After treating the slides with trypsin by giving the different timings, the slides were immediately transferd to PBS to stop the further treatment. From the buffer the slides were transferred to alcoholic grades (70, 90 and 100%). In each grade the slides were treated for 3 minutes.

After alcoholic treatment the slides were completely air dried and stained with 2% Giemsa solution. After 10 minutes of stain, slides were taken out and washed thoroughly with distilled water for differentiation.

After mounting in DPX, the slides were observed for G-banding. Each slide was screened thoroughly, nearly 25 metaphase plates were examined to ascertain the diploid number and these were photomicrographed for preparing the karyogram for G-banding.

RESULTS AND DISCUSSION

In mammals, particularly in human beings different banding procedures prove an authentic identification of individual chromosome segments (Pearson and Van Egmond – Cowan, 1976).It was reported by Comings (1973) that intercalary heterochromatin appeared in G-bands and the G-band positive areas were rich in non repetitive DNA. Miller (1973) contended that interaction with DNA is an important factor in Giemsa – banding. DNA molecules have the banding sites for Giemsa which are revealed by protein and proteolytic enzymes.

During trypsinisation the two phase action of trypsin takes place. Firstly it denatures the protein and secondly hydrolysis occurs. This results in differential staining with Giemsa (Chiarelli, 1973)

In *Rattus rattus* the total number of chromosomes was 38 (18 autosomal and 1 pair of sex chromosomes). Somatic metaphase chromosomes of male house rat (*Rattus rattus*) showing G-banding in fig.1 The karyotype by G-banding was prepared for male rat, autosomes contain 16 metacentric, 4 sub-metacentric and 16 acrocentric chromosomes which were arranged in decreasing order of each group. The male contain two different sex chromosomes the X which is sub-metacentric and Y chromosome is acrocentric which have arranged separately shown in fig.2

Badenhorst *et al.,* (2009) collected 18 rodents from different localities of Thiland and prepared karyotypes using G-banding and found that chromosome morphology differs slightly among animals of same species. The present study reveals that the number of bands on each chromosome was definite, irrespective of the length of the chromatids.

Figure-1. Somatic metaphase chromosomes of male house rat (*Rattus rattus*) showing G-banding.

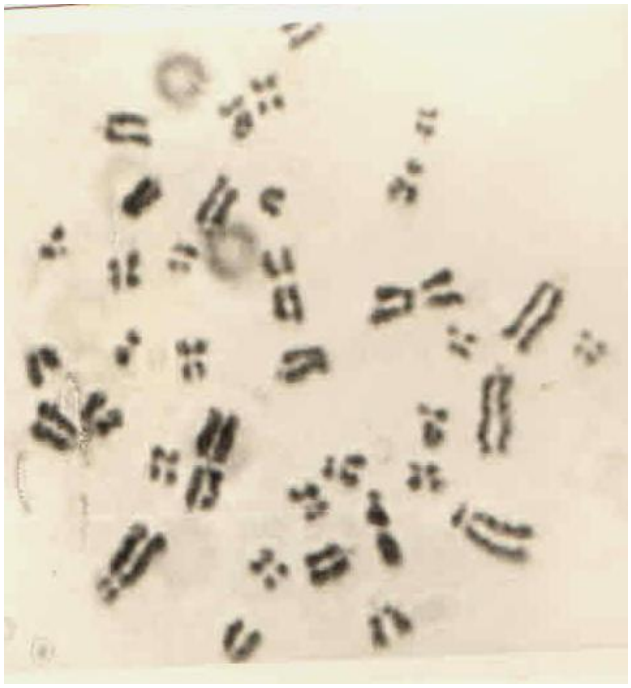
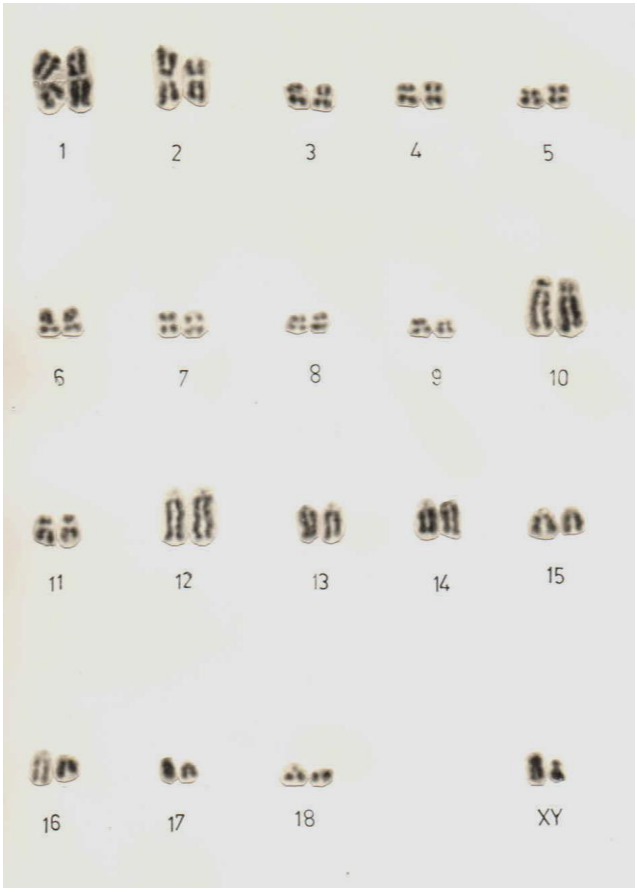


Figure-2. Karyotype of somatic metaphase chromosomes of male house rat (*Rattus rattus*) showing G-banding.



CONCLUSION

The present findings are in conformity with the findings of Zatsepina *et al.*, (1989). The G-bands appeared as densely packed regions due to uneven distribution of DNP fibrils along chromatids. It is concluded that similarity exists between the autosomes and the sex chromosomes of house rat. Therefore, the present work can be referred to as a confirmatory effort in G- banding of the *Rattus rattus*.

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