Asian J. Exp. Sci., Vol. 26, No. 1, 2012; 57-60

Giemsa Banding in Two Mammalian Species of India

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Abstract: Two species of Indian mammal house rat (male), *Rattus rattus rattus (Linn.),* and five striped squirrel (female) *Funambulus pennanti* (Wr.) were used for G-banding. The bands produced were specific for each chromosome pair in these animals. Correlation, dissimilarities and polymorphism have been recorded from both species. The G-bands were exhibited in densely packed regions of DNP fibrils along chromatids.

Key Words: G-banding, Polymorphism, DNP Fibrils, Chromatids, Chromosome

Introduction :

The banding techniques are based on the principle of *in situ* hybridisation of DNA developed (Pardue and Gall, 1970). The G-banding studies reveal the visualization of molecular sequences at the cellular stage i.e. microscopic level. Photomicrographic analysis of bands and understanding of basic mechanism of genetic regulation in animals is done. Matsui and Sasaki (1975) suggested that the macromolecules like DNA and proteins are lost in G-banding which cause an uneven distribution of chromatin. Thus G-bands show the thermostable chromatins consisting of smaller non histone protein molecules. Lampo-Tang and Daniel (1973) observed that in G-banding the staining of protein at the intercalary A-T rich sites of DNA takes place.

Zatsepina *et al.* (1989) suggested that hypotonic treatment results in differential decondensation of chromosomes which causes the uneven distribution of DNP fibrils among chromatids in the form of bands.

Material and Methods :

Funambulus pennanti were caged from the fields on the outskirts of Kanpur and *Rattus rattus rattus* were traped from godowns of cereals in the city of Kanpur. The taxonomic identification of animals were done by consulting standard manuals and publications (Ellerman,1961; Prater, 1971).

The somatic metphase chromosomes *in vivo* were prepared by using the techniques of Meighan and Stich (1961) after certain modifications. For *G-banding,* Seabright (1971) technique was employed with minor changes. Air dried slides were treated for 20 seconds in 0.35% trypsin solution (prepared in Hank's solution) at 20^0C. The slides were immersed in Hank's solution for 2 minutes and stained in 4.0% Giemsa solution for 10 minutes. After mounting in DPX, the slides were observed for G-banding. 30 metaphase plates of both species were examined to ascertain the diploid number and these were photomicrographed for preparing the karyogram for G-banding.

Results :

The chromosomes have been divided into regions correlatively numbered from centromeric to telomeric segments. In the present analysis, the major bands on chromosomes were delineated and counted but the sub-bands in each region were not included in countings. In some chromosomes some of the sub-bands fused to give the appearance of major bands but actually they were not the major bands to be counted.

Rattus rattua rattus (Male) :

The diploid number in this animal is 42 which consisted of 6 metacentrics,10 submetacentrics and 24 acrocentrics. The X is metacentric and Y is acrocentric (Figures 1, 2). The chromosomes exhibited bands with clear or obscured identity.

The 1st pair of chromosomes had 3 regions with one proximal, two median and one distal clear bands. The 2nd pair had 2 regions with inconspicuous bands. The 3rd pair had 2 regions with three bands of obscured identity. The 4th pair showed 3 regions with two median and one distal bands. The 5th and 6th pairs had 2 regions. Each region had one distal band of dull intensity. The 7th to 9th pairs showed indeterminate bands. The 10th pair had 2 regions with one proximal band. The 11th to 16th pairs showed bands of obscured identity. The 17th pair showed 2 regions with clear distal bands. The 18th to 20th pairs showed indeterminate bands.

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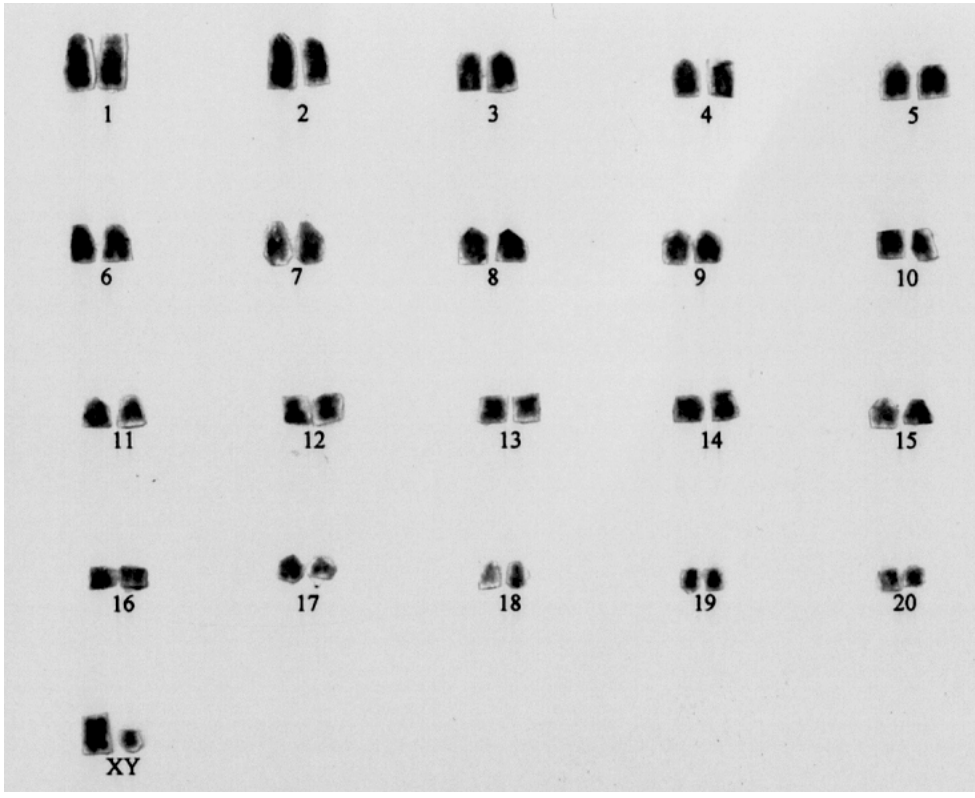

Fig 1. Karyotype of somatic metaphase chromosomes of male house rat (*Rattus rattus*) showing G-banding.

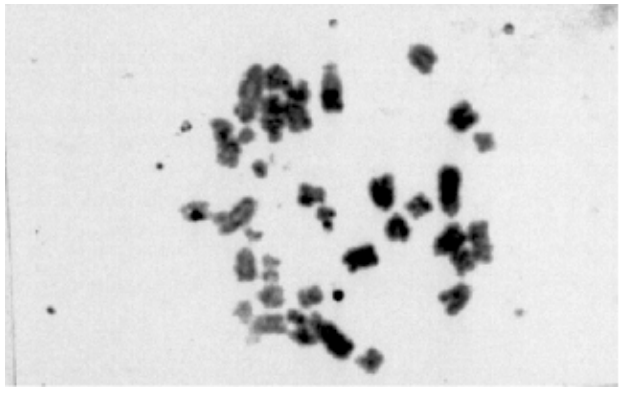

Fig 2. somatic metaphase chromosomes of male house rat (*Rattus rattus*) showing G-banding.

The X had 3 regions and three bands of hazy appearance. One band was on p arm while two bands were on q arm. The Y chromosome had inconspicuous bands.

Funambulus pennanti (Female)

The diploid chromosome number in this animal is 54, having 4 metacentrics, 12 submetacentrics and 36 acrocentrics. The X is submetacentric (Figures 3,4). Most of the chromosomes showed prominent bands except a few which did not show distinct appearance. The 1st pair of chromosomes had 3 regions having one prominent proximal, two median and one distal confluent bands. The 2nd pair had 2 regions with one proximal band on p arm and one clear median broad band on q arm. The 3rd pair had 2 regions. One proximal band on p arm while one median and one thin distal band on q arm. The 4th pair had 2 regions and three conspicuous bands. One dark band on p arm and two distinct bands on q arm. The 5th and 6th pairs showed indeterminate bands. The 7th pair had 2 regions with one proximal band of indistinct nature on p arm, one median and one distal band of distinct identity on q arm. The 8th pair had 2 regions with one proximal band on p arm and a distal band of confluent nature on q arm. The 9th pair had 2 regions having one proximal band on p arm. The 10th pair showed indeterminate bands. The 11th pair had 2 regions with two confluent bands, one on each arm. The 12th to 16th pairs showed indeterminate bands. The 17th pair had 2 regions. One proximal band on p arm and one median band of light intensity on q arm. The 18th to 26th pairs showed indeterminate bands. X had 3 regions having one proximal band on p arm and one on q arm. Besides these one median band and one distal band of distinct nature was present.

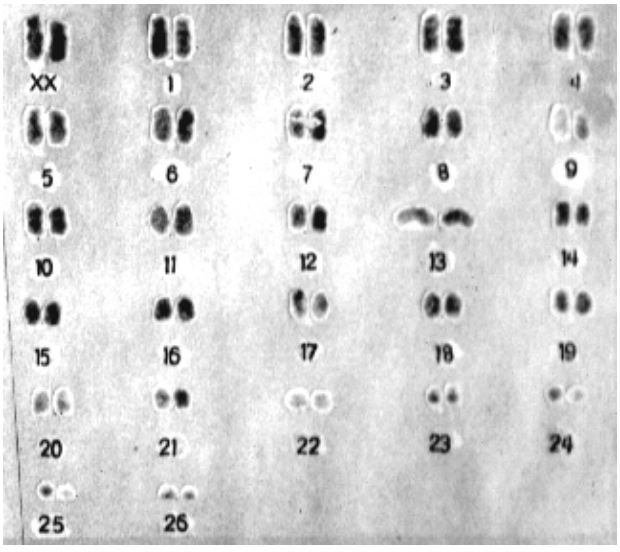

Fig 3. Karyotype of somatic metaphase chromosomes of female Indian five striped squirrel. (*Funambulus pennanti*) showing G-banding.

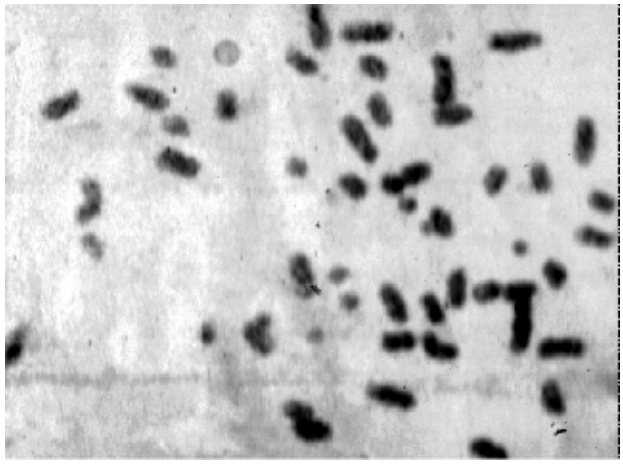

Fig 4. Somatic metaphase chromosomes of female. *Funambulus pennanti* showing G-banding.

Discussion :

Different banding procedures prove an authentic identification of individual chromosome segments in mammals, particularly in human beings (Pearson and Van Egmond – Cowan, 1976). Millar (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. 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. Marki and Osterhoff (1985) reported that identification of individual chromosome in cattle could be done with accuracy by banding techniques. Zatsepina *et al.* (1989) recorded that the hypotonic treatment to mitotic chromosomes of Chinese hamster resulted in differential decondensation of chromosomes. The densely packed regions appeared due to uneven distribution of DNP fibrils along chromatids. 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 (Chiarelle, 1973) and the optimum action was obtained at moderate temperature (20^0 C). In *Rattus tanezumi, Rattus norvegicus, Rattus exulans* and *Rattus muelleri* the karyotypes and banding patterns were similar with slight differences (Tosihide *et al.,* 2004).

Comparison of G- and R-banding in 28 cases of different animals of mammalia group were studied. Verma *et al.* (2008) found that R- and G- banding proved worthwhile in localization of breaks and abnormalities.

In present investigations it was found that the maximum number of bands were four in both the animals. Some bands were well determined and divided into many sub-bands. A few sub-bands were very clear in some chromosomes in these species, while others were indeterminate. Due to indeterminate nature of sub-bands they were not counted with major bands. Each pair of chromosome showed a specific band pattern. Both the species showed clear, obscured, dark, faint, dull confluent and indeterminate bands. The present results suggest that a correlation exists between the bands of two different species studied. This correlation is attributed to depend upon the base composition of DNA and protein constituents, as also reported by Dutrillaux *et al*. (1972).

The present findings are in conformity with the findings of Zatsepina *et al. (1989),* as 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 and five striped squirrel. There were only a few differences in the banding pattern of these two animals. The polymorphism of bands has also been recorded between homologous pairs in both species (1^{st} pair of rat and 1^{st} pair of squirrel).

The G-banding in two species of Indian mammals i.e. *Rattus rattus rattus* and *Funambulus pennanti* shows correlation, dissimilarities and polymorphism. This correlation is attributed to depend upon the base composition of DNA and protein constituents. The G-bands appeared as densely packed regions of DNP fibrils along chromatids.

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.

Karyotypes of somatic chromosomes using G-banding of *Mastomys erythroleucus* (more than 270 individuals) from different localities (about 50) were prepared and studied. It was observed that there were widespread polymorphysm among the karyotypes of four chromosome pairs (Dobigny *et al.*, 2010).

The present study reveals that the number of bands on each chromosome were definite, irrespective of the length of the chromatids. The results indicate that the G-banding pattern is rather variable in different mammals and similarity in banding patterns showed inter-relationship between the two animals studied.
It is concluded that the phylogenetic relationship exists between species having similar banding.

Acknowledgements :

The authors are thankful to the Principal, D.A.V. College for providing necessary facilities.

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