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Giemsa and Centromere Banding Patterns in the Chromosomes of Indian Donkey (*Equus asinus*)

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Chromosome banding techniques have helped identification of individual choromosomes of domestic animals and have been used in comparative chromosome banding studies among different species to establish their homologies or differences. The standardization of chromosome nomenclature based on different banding techniques stands updated for cattle, sheep, goat and horse (ISCNH, 1997; ISCNDB, 2000) but for donkey it has not yet been completed and hence this study was attempted.

Materials and Methods

Indigenous domestic donkeys inhabiting in four districts of Tamil Nadu viz., Salem, Erode, Perambalur and The Nilgiris were involved in the present experiment. 20 donkeys consisting of 5 males and 15 females were utilized for this study. The blood samples were collected from jugular vein of donkeys and the cultures were set up with RPMI 1640 culture medium, pokeweed mitogen (PWM) or Phytohaemagglutinin (PHA) and, plasma and buffy coat. The cultures were incubated at 37.5°C for 72 h and arrested with colchicine, 1.5 h prior to harvest. This was followed by hypotonic treatment (0.075M Kcl) and fixed in methanol: acetic acid fixative. Air dried slides prepared were stained with 4 per cent Giemsa. Differential count of about 200 cells was made for each culture to estimate the mitotic drive and mitotic index. For G-banding, four to five days aged slides were immersed in 0.25 per cent trypsin solution for 10 to 15 sec with gentle shaking. The slides were dried and stained with 4 per cent Giemsa for 10 min and rinsed thoroughly in distilled water (Seabright, 1971).

For C-banding the air-dried preparations were allowed to age for 3 days at room temperature and were treated with 0.2 N hydrochloric acid for one hour at room temperature. The slides were then rinsed in distilled water and immersed into 2.5 per cent solution of barium hydroxide maintained at 50°C in a water bath for 15 to 20 min. The slides were then incubated in 2x SSC (0.3 M sodium chloride, 0.03 M sodium citrate, pH 7.0) for one hour at 60°C and then thoroughly rinsed with distilled water. The slides were then stained with 4 per cent Giemsa for 90 min (Sumner, 1972). The karyotypes were prepared as per the method followed by Eldridge and Blazak (1976) for donkey chromosomes.

Results and Discussion

Of the 100 mitotic spreads screened, the male and female donkey chromosome complements consisted of a diploid number of 62, XY and 62, XX respectively. Among the autosomes, 23 pairs were submetacentric / metacentric / subtelocentric and 7 pairs were acrocentric in nature. Of the 23 pairs of submetacentric / metacentric / subtelocentric chromosomes, the first two chromosomes were largest submetacentric, the third chromosome was also submetacentric and the fourth chromosome was subtelocentric,

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and chromosomes 5 to 17 comprised both submetacentric and metacentric chromosomes. The next 6 chromosomes were smallest metacentric. Among the seven acrocentrics, chromosomes 24, 25, and 26 were larger in size. The X chromosome was the fourth largest submetacentric and the Y chromosome was found to be the smallest acrocentric of the karyotype.

G-banded metaphase spread for male and karyotype of male donkey indicated that the autosomes were arranged into two groups, the first one was non-acrocentric (23 pairs of submetacentric / metacentric / subtelocentric) and the second group was acrocentric (7 pairs of acrocentric). The sex chromosomes were placed next to acrocentric chromosomes. The G-banded chromosomes nomenclature was described based on the International System for Cytogenetic Nomenclature of the Domestic Horse (ISCNH, 1997). A total of 342 G-bands were recorded in haploid set of chromosomes on Indian domestic donkey.

G-banding pattern obtained in the present study was similar to those obtained by Ryder *et al.* (1978) and Raudsepp *et al.* (2000) and helped in identifying almost all the chromosomes and permitted grouping of homologous pairs. Based on G-banded morphology, Trommershausen-Bowling and Millon (1988), demonstrated centric fission in donkey; Ryder and Chemnick (1990) identified Robertsonian translocation in Asiatic wild asses and Alaoui *et al.* (2004) reported a centric fission in Spanish breeds of donkey. However, in the present study no such structural chromosomal rearrangements were identified in any of the samples screened. Raudsepp *et al.* (2000) identified 350 G-bands in diploid chromosomes of a male donkey. Di Meo *et al.* (2006) reported an improved G-banded karyotype at about 450 band level whereas in the present study a total of 342 G-bands were recorded. The variation in number of bands may be attributed to the different stages of chromosomes arrested during the culture, which leads to fusing of two or three bands.

The centromere regions of all the acrocentric chromosomes exhibited darkly stained C-bands. Twelve pairs of submetacentric / metacentric chromosomes appeared deficient with appreciable amount of C-band. Chromosomes 1, 2 and 6 possessed centromeric C-band. Chromosomes 1p, 3p, 6q, 8p, 10p, 11p, 12p, 13q, 16p, 17p, 18p, 20p, 22p, and 23p exhibited telomeric heterochromatin. The Y chromosome consisted entirely of constitutive heterochromatin. The X chromosome which is submetacentric lacked centromeric heterochromatin but had lightly stained interstitial C-band.

Ryder *et al.* (1978) reported darkly stained constitutive heterochromatin in 8 pairs

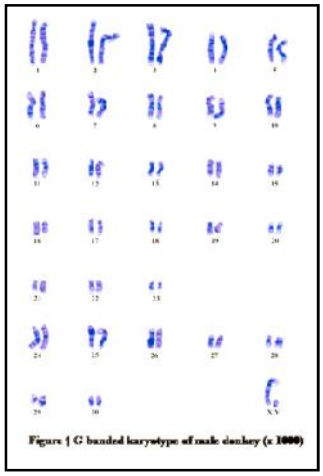

Fig 1. G banded karyotype of male donkey (x 1000)

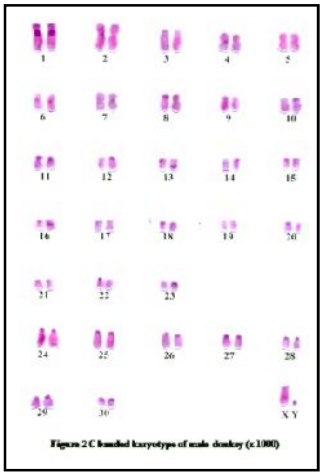

Fig 2. C banded karyotype of male donkey (x 1000)

of metacentric chromosomes in donkey and in chromosomes 4, 5 and 13 it was not located at the centromere. Gadi and Ryder (1983) observed large paracentromeric C-band only in metacentric chromosome 2; Kopp *et al.* (1988) identified prominent CMA3 positive centric heterochromatin bands on chromosomes 2, 8, 11, 12, 20 and 21; Alaoui *et al.* (*loc. cit*) detected C-bands in all chromosomes and C-band polymorphism in chromosome 1, 3 and 4, whereas in the present study centromeric C-bands was observed in chromosome 1, 2 and 6 metacentric / submetacenric chromosomes. However, the observation of darkly stained C-bands in all acrocentric chromosomes in this study confirms the findings of earlier workers. The number of telomeric heterochromatin varies among chromosomes. The telomeric heterochromatin was reported on chromosomes 1, 4, 5 and 13 in Sicilian donkey (Ryder *et al.*, *loc. cit*), on metacentric chromosomes 4, 7, 9, 10, 13, 14, 17, 18 and 19 in Asiatic wild ass (Gadi and Ryder, *loc.cit*), on 6, 9, 10, 11, 13, 17 and 19 chromosomes in Spanish donkey breed (Alaoui *et al.*, *loc. cit*). In contrast to the observation made in previous studies, the telomeric heterochromatin was observed on more number of chromosomes viz., 1p, 3p, 6q, 8p, 10p, 11p, 12p, 13q, 16p, 17p, 18p, 20p, 22p and 23p. In general, the centromere staining revealed additional morphological features and may facilitate the recognition of individual chromosomes and is useful in the identification of the sex chromosomes.

Summary

The individual chromosome morphology of Indian domestic donkeys was studied by karyotyping and banding techniques. Blood samples were collected from 20 domestic donkeys (5 males and 15 females) inhabited in four districts of Tamil Nadu, India. Chromosome preparations were made from short-term lymphocyte culture technique. A total of 342 G-bands were recorded in a haploid set of chromosomes. The C-banding profile revealed presence of darkly stained centromeric C-bands in chromosomes 1, 2 and 6 and in all acrocentric chromosomes. Chromosomes 1p, 3p, 6q, 8p, 10p, 11p, 12p, 13q, 16p, 17p, 18p, 20p, 22p, and 23p exhibited telomeric heterochromatin. The Y chromosome consisted entirely of constitutive heterochromatin and the X chromosome lacked centromeric heterochromatin but had an interstitial C-band.

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