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Chromosomal profile of Deccani sheep*

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ABSTRACT

The present study was conducted to standardize the karyotype profiles and various morphological and morphometric characters of chromosomes of Deccani sheep. The diploid chromosome number in Deccani sheep was 54. All the autosomes were acrocentric except the first three pairs, which were sub-metacentric. The X chromosome was the longest acrocentric, while Y-chromosome was the smallest bi-armed chromosome. Sex of the animal had a nonsignificant influence on the morphometric measurements of all the chromosomes, while the differences among the chromosomes were significant. The mean relative length of autosomes varied from 1.78 to 9.35%. The X- and Y- chromosomes contributed 5.05 and 1.70% of the total genome, respectively. The means for the arm ratio, centromeric index and morphological index of the sub-metacentric autosomes 1, 2 and 3 varied from 1.17 to 1.20, 0.54 to 0.55 and 9.40 to 11.57, respectively. The G-banding pattern of the chromosomes in Deccani sheep more or less matched with the standard karyotypes. All the autosomes revealed centromeric C-bands. The nucleolar organizer regions were detected on chromosomes 1, 2, 3, 4 and 25.

Key words: Banding, Chromosomal profile, Cytogenetics, Morphometric characteristics, Sheep

Screening of animals before breeding will minimize the economic losses in rearing animals with chromosomal abnormalities and enables detection and culling of such individuals. Cytogenetic characterization also forms a basis for molecular genetic characterization and techniques like fluorescent *in situ* hybridization. Ultimately, this information on chromosome structure and organization may lead to a more thorough understanding of gene expression, which may in turn result in the development of novel methods to control patterns of growth and development. Therefore, the present study was conducted with the objectives of characterization of Deccani sheep cytogenetically.

MATERIALS AND METHODS

Purebred Deccani sheep (15 rams and 15 ewes), 1 to 3 year-old were utilized for the present study. Blood samples (2 ml/animal) were collected aseptically from external jugular vein into sterile heparinized vacutainers. Short-term lymphocyte culture technique described by Moorehead *et al.* (1960) was followed with minor modifications.

Banding techniques

The GTG-banding was done as per Seabright (1971) with slight modification. One-week old slides were subjected to trypsin treatment for about 40 sec, rinsed in normal saline and stained with Giemsa solution for 2 min. The C-banding protocol described by Sumner *et al.* (1971) was followed with minor modifications. About 3–day-old slides were incubated in 0.2 M hydrochloric acid for 1 h at room temperature in 1% barium hydroxide solution for 15 to 20 min at 50° C and then in 2 × SSC buffer for 1 h at 60° C. The slides were rinsed with distilled water after each incubation. The slides were stained with 4% Giemsa solution for 9 min. The method of Howell and Black (1980) was adopted for NOR-banding with slight modifications. Four drops of colloidal developer and 8 drops of 50% silver nitrate were dropped and mixed on the slides which were kept on a slide warmer at 55° C until the colour of the solution changed from golden yellow to light brown. The slides were washed, dried and counter stained in 2% Giemsa for 20 to 30 sec. The metaphase chromosomes were photographed and individual chromosomes were cut and pasted on a format, as per the International System for the Cytogenetic Nomenclature of Domestic Animals (ISCNDA 1990).

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Morphometric measurements

The morphometric measurements of relative length, arm ratio, centromeric index and morphological index were computed as per Bhatia and Shanker (1991). The relative lengths were measured in percentage of the total genome length and were transformed into angles as per the procedure given at www.helios.bto.ed.ac.uk for conducting further statistical analysis. The t-test was conducted to test the differences between the sexes for all the morphometric measurements. Analysis of variance was conducted on pooled data for all the morphometric measurements to test for the significant difference among the chromosomes (Snedecor and Cochran 1987).

RESULTS AND DISCUSSION

Chromosome number and morphology

The diploid chromosome number was 54 consisting of 26 pairs of autosomes and one pair of sex chromosomes. The first 3 pairs of autosomes were sub-metacentric whereas remaining 23 pairs of autosomes were acrocentric. The X-chromosome was the largest acrocentric and Y-chromosome was the smallest bi-armed. These findings were in agreement with the reports made on several Indian breeds of sheep like Mandya (Umrikar and Narayankhedkar 1997), Lohi (Intizar *et al.* 1999) and Nellore (Amareswari *et al.* 2005). However, metacentric nature of the first 3 autosomes and telocentric nature of the remaining 23 pairs of autosomes was observed in indigenous sheep breeds of Muzaffarnagri (Benjamin and Bhat 1978) and Malpura (Gupta and Gupta 1995).

Morphometric measurements

Sex had no significant effect on all the morphometric measurements studied and also as reported by Bhatia and Shanker (1996, 1999) and Amareswari *et al.* (2005).

Relative length

The mean relative length ranged from 1.78 to 9.35% for the autosomes while the mean relative lengths of X- and Y-chromosomes were 5.05 and 1.70%, respectively. Similar mean relative lengths were also reported in other breeds such as Magra (Bhatia and Shankar 1996), Patanwadi (Bhatia and Shanker 1999) and Nellore sheep (Amareswari *et al.* 2005).

The sub-metacentric chromosomes together contributed 24.82 % of the genome length in males and 25.29 % in females. Gupta and Gupta (1995) observed that the largest biarmed chromosomes together accounted for 25.11 and 24.65 % of the total genome length in male and female Malpura sheep, respectively.

Among all the acrocentric chromosomes, the X-chromosome contributed highest to the total genome. Langhe *et al.* (1993), Gupta and Gupta (1995) and Bhatia and Shanker (1996) reported that in indigenous breeds, the X-chromosome contributed generally the highest among all the acrocentric chromosomes.

Arm ratio

The differences among the arm ratios of the 3 sub-metacentric chromosomes were significant ($P \leq 0.01$) indicating that the proportion of the chromosome arms was different in the 3 chromosomes. Thus, the arm ratio apart from the relative length may help in proper identification of the chromosomes. The mean arm ratio varied from 1.17 to 1.20. The arm ratio decreased from first to third autosome. These findings agreed well with the reports of Bhatia and Shanker (1994) in Munjal, Gupta and Gupta (1995) in Malpura, Bhatia and Shanker (1996) in Magra, Bhatia and Shanker (1999) in Patanwadi and Amareswari *et al.* (2005) in Nellore breeds of sheep. The magnitude of the arm ratios being greater than one indicated that the chromosomes 1, 2 and 3 were sub-metacentric in nature.

Centromeric index

The centromeric index of the 3 sub-metacentric chromosomes did not differ significantly. The mean centromeric indices ranged from 0.54 to 0.55 in the present study, which were in agreement with the reports of Bhatia and Shanker (1994 and 1996) and Amareswari *et al.* (2005). The mean centromeric indices reported by Langhe *et al.* (1993) in Bannur, Gupta and Gupta (1995) in Malpura and Bhatia and Shanker (1999) in Patanwadi sheep were slightly lower than those found in the present investigation in Deccani sheep. The mean centromeric indices, deviating from 0.5 for the biarmed autosomes in the present study confirmed the sub-metacentric nature of these autosomes in Deccani breed of sheep.

Morphological index

The morphological indices of the sub-metacentric chromosomes differed significantly ($P<0.01$) indicating the subtle differences in the morphology of the chromosomes despite all being sub-metacentric. The mean morphological indices of the first 3 autosomes were 11.57, 10.57 and 9.40, respectively. Langhe *et al.* (1993) reported much higher values for the morphological indices of Bannur sheep while Amareswari *et al.* (2005) reported lower values than the present finding.

G-banding

The centromeric regions of sub-metacentric and acrocentric autosomes had in general, negative G-bands while the centromeric region of X-chromosome stained faintly. The G-banding pattern of Y-chromosome was not clear in the present study, but indicated a dot like stained area. The G-banding patterns observed in the present study were almost similar to the standard karyotypes described at Reading Conference (Ford *et al.* 1980). Major bands were fairly recognizable while the clarity of the minor bands depended upon the level of contraction of the chromosomes. The banding pattern facilitated easy pairing and proper identification of the chromosomes. The autosomes did not stain at the centromeric regions.

C-banding

All the 26 pairs of autosomes revealed distinct and densely stained C-bands in their centromeric regions. Buckland and Evans (1978) reported that the constitutive heterochromatin bands of bovid chromosomes were almost exclusively centromerically located and there was great variation in quantity, even between closely related species. Evans *et al.* (1973) in Suffolk and Bruere *et al.* (1974) in Romney and Drysdale sheep found an appreciable amount of heterochromatin on a majority of autosomes, while Buckland and Evans (1978) found no centromeric heterochromatin in Barabara and Eland sheep. In Afghan wild sheep (*Ovis ammon cyclocerus*), all acrocentric chromosomes in the females possessed prominent centromeric C-bands, while metacentric pairs had a large area of diffused centromeric blocks as reported by Hsu and Arrighi (1971) in contrast to the findings of the present study and those of Bhatia and Shanker (1991) in Nali sheep.

In contrast to the autosomes, the acrocentric X-chromosome of Deccani sheep did not reveal any centromeric C-bands, which helped in easy identification of X-chromosome. Previous studies on sheep chromosomes showed that X-chromosome contained little, if any, centromeric C-band material (Buckland and Evans 1978). Bruere *et al.* (1974) reported slight C-banding in X-chromosomes of Romney and Drysdale breeds. The centromeric region of Y-chromosome stained similarly throughout the length of the chromosome in the present study, which was in agreement with the findings of Evans *et al.* (1973).

Nucleolar organizer region (NOR) banding

The NORs appeared as darkly stained spots on 5 pairs of chromosomes Viz. 1p, 2q and 3q and telomeric ends of chromosomes 4 and 25. These findings were in agreement with the reports of Ansari *et al.* (1993), Meo *et al.* (1993), Bhatia and Shanker (1995 and 1999), Lydia Dhanammal *et al.* (1999) and Ansari *et al.* (1999). The mean number of NORs present per metaphase varied between the animals indicating the existence of polymorphism for the number of NORs. This variation could be due to the state of the NORs. The average number of NORs present per metaphase ranged from 4.76 to 5.78 with an overall mean of 5.28 ± 0.11 per metaphase while Bhatia and Shanker (1995) reported a range of 3 to 10 NORs with a mean of 6.53 in Magra breed of sheep.

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