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Molecules and Cells

G-, R- and C-Band Patterns of Goral (*Nemorhaedus caudatus*) and Comparison to Goat (*Capra hircus*)

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The karyotype of goral (*Nemorhaedus caudatus*, 2n = 56) was prepared using lymphocytes and its chromosomal band patterns were compared with those of goat (*Capra hircus*, 2n = 60) by CBG-, GTG- and RBG-banding techniques. The standard karyotype of goral was composed of 54 acrocentric autosomes, submetacentric X chromosome, and acrocentric Y chromosome. C-bands were prominent in all autosomes except the X chromosome. G- and R-band patterns of goral were dissimilar to those of goat. The data support the idea that the goral did not originate from a common ancestor of bovid, or that there were numerous complicated chromosomal interchanges during goral evolution, in contrast to other bovids.

INTRODUCTION

There is an increasing interest in phylogenetic research on wild mammals including goral (*Nemorhaedus caudatus*) and Japanese serow (*Capricornis crispus*) (Huang et al., 2005). *N. caudatus* is an endangered species belonging to the Bovidae family, which includes four species in the genus Nemorhaedus; Himalayan goral (*N. goral*), long-tailed goral (*N. caudatus*), red goral (*N. baileyi*), and Chinese goral (*N. griseus*) (Grubb et al., 2005). The goral habitat is restricted to the rugged and wooded mountains of Asia (Jim, 1989). A core set of microsatellite markers was used to measure the genetic diversity of goral in Korea (An et al., 2010). The karyotype of female goral was initially reported to comprise 55 chromosomes, which was believed to be the result of autosomal translocation (Wurster, 1972). It was subsequently determined that the female goral has 56 chromosomes composed of 54 acrocentric autosomes of similar size and shape, and an acrocentric X chromosome (Huang et al., 2005; Soma et al., 1980). There is no metacentric or submetacentric autosome, which contrasts with other Bovidae. Both Wurster and Soma et al. described only the karyotype of the female goral, and the Previous karyotype analyses (Soma et al., 1980; Wurster, 1972) utilized the low band resolution Giemsa conventional staining - the G-band technique. The technique does not reveal Y chromosomes, therefore, the Y chromosome types of goral have never been observed. This makes it impossible to compare the karyotype of goral with the standard karyotypes of other bovids.

The standard karyotypes of cattle, sheep and goat, which are the main domestic animals in the Bovidae family, have been investigated by many workers using high resolution G- and R-banding (Di Berardina et al., 1987; 1990; Hayes et al., 1993; Iannuzzi et al., 1995; Kaftanovskaya and Serov, 1994), reflecting their economic importance. Recently, karyotypes of wild bovid have been reported. Their biarmed chromosomes are the result of Robertsonian translocation (Claro et al., 1994; 1995; 1996; Cribiu et al., 1990; Robinson et al., 1996). In Korea, the goral has been classified as *N. caudatus* based on morphological characteristics and geographical distribution (Groves and Grubb, 1985). However, the intraspecific genetic variation between Korean goral and Chinese goral is considerable, or comparable to the interspecific variation within the closely-related genus, *Capricornis* (Min et al., 2004). The domestic goat (*Capra hircus*), which is the closest species to the Korean goral, is one of the most common species of Caprinae in Korea (Kim et al., 2004). Therefore, a comparison of goral and goat karyotypes may be potentially important in exploiting unidentified samples.

In this study, we investigated the standard karyotype of the Korean goral and compared it to the karyotype of the domestic goat, to clarify the evolutionary relationship between the goat and goral.

MATERIALS AND METHODS

Goral (*N. caudatus*) were captured in Mt. Sorak, Korea, in 1978,

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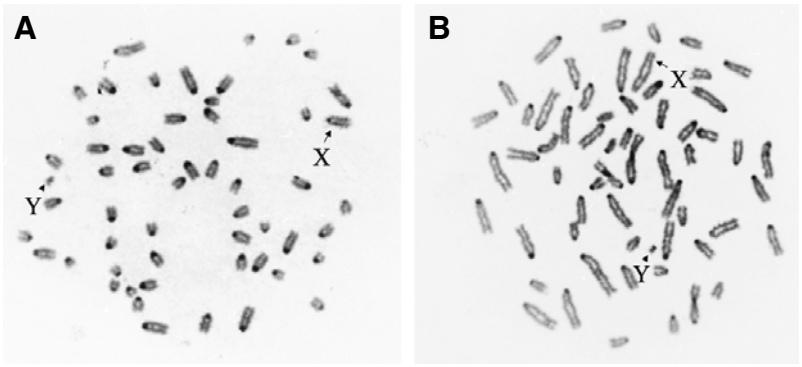

Fig. 1. CBG-banded metaphase of a male goral (A) and a male goat (B). The arrow indicates X chromosome and the arrow head indicates Y chromosome.

and were bred artificially in the Samsung Everland Zoo, Korea. An additional animal was captured on Mt. Worak, Korea, in 1998. Three male and two female gorals from the captive zoo population were sampled in order to carry out these investigations.

Lymphocytes separated from the peripheral blood were cultured at 37°C in RPMI 1640 medium (Di Berardino et al., 1987). CBG-banding was performed with the some modifications as previously described (Sumner, 1972). Briefly, the sample slides were immersed in 3% $Ba(OH)_2$ at 65°C for 30 min without prior treatment with hydrochloric acid, followed by renaturation in 2 × SSC at 65°C for 30 min, rinsing in tap water, and staining with 10% Giemsa in McIlvaine buffer for 8 min. Also, GTG-banding was obtained according to a modification of a previous method (Seabright, 1971; Soma et al., 1980). The slides were incubated in Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate buffered saline (PBS) at 4°C for 2 min, treated with 0.025% trypsin in PBS at 4°C for 1.5-2 min, rinsed in running water, and stained with 2% Giemsa (pH 6.8) for 6-7 min. The RBG-banding technique was a slight modification of a previously-described procedure (Di Berardino et al., 1987).

The chromosomes of goral were arranged in order of decreasing length as recommended by the Reading conference, and sub-bands were numbered in the manner described for the human karyotype (Ford et al., 1980; ISCN, 1985). Bromodeoxyuridine incorporation was adopted after cell cycle synchronization using a thymidine block to establish standard karyotype of goral for high resolution of the banding.

RESULTS AND DISCUSSION

An important karyotypic difference between bovine and ovine species is the different shape of the X chromosomes. While those of cattle are submetacentric, X chromosomes in goat and sheep are acrocentric with short arms (Huang et al., 2005).

The male goral (*N. caudatus*) karyotype contains 56 chromosomes, with 27 pairs of acrocentric autosomes, a submetacentric X chromosome and an acrocentric Y chromosome (Fig. 1A). The numbers of autosomal arms (NAA) of goral were determined to be 54 and the NAA of the goat (*C. hircus*) to be 58, confirming previous results (Di Berardino et al., 1987; Soma et al., 1980). The goal karyotype was first reported as 55 (Wurster, 1972); however, the single submetacentric and one unpaired chromosome were believed to be the result of Robertsonian translocation. This form of translocation is thought to have caused the interspecies karyotype differences in Bovidae (Buckland and Evans, 1978a; Gallagher and Womack, 1992). It is well-known that the NAA is generally constant and in the range 56-58, for most species of Bovidae, although their chromosome numbers are not equal (Buckland and Evans, 1978a)

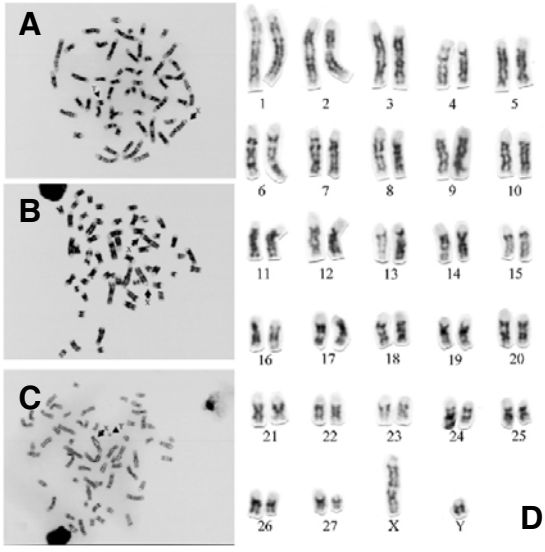

Fig. 2. GTG-banded metaphases of a male goral (A), a female goral (B), and a male goat (C). The chromosomes of a male goral are karyotyped with a single metaphase (D). The arrow indicates X chromosome and the arrow head indicates Y chromosome.

The C-band pattern of female goral has revealed that all the chromosomes including X chromosome are acrocentric (Soma et al., 1980; Wurster, 1972). The present results revealed the submetacentric nature of the X chromosome, and the distinct C-banding positive spotting (Fig. 1A) conflicts with that normally associated with the goat. The Y chromosome of goral did not display any prominent sign of CBG-band. Both the X and Y chromosomes of the goat, on the other hand, did not display CBG-banding (Fig. 1B). The ambiguous X chromosome may be due to its short p arm or to damage incurred during the course of the CBG-banding technique, as presently happened.

The GTG-banded metaphase of the examined male and female gorals presented different chromosome lengths (Figs. 2A and 2B). The autosomes of the goral were arranged according to decreasing size from pair 1 to pair 27, and the karyotype was made with a single metaphase (Fig. 2D). The last small chromosomes (number 24, 25, 26 and 27) were difficult to identify because of their similar band patterns. As these band patterns differed depending on chromosome length, it was difficult to find an overall band similarity between goral and goat. Studies have established the high degree of similarity in the banding patterns of Bovidae species (Buckland and Evans, 1978a; Gallagher and Womack; 1992; Hayes et al., 1991). Furthermore,

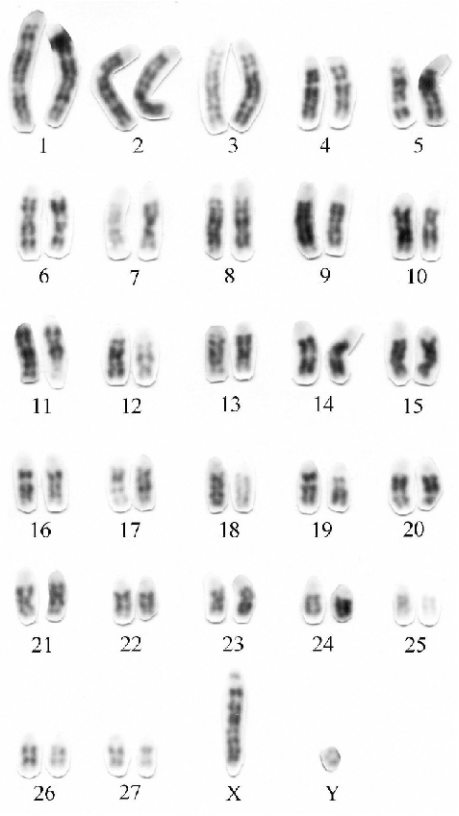

Fig. 3. RBG-banded metaphase of a male goral, which was used as the basis for the proposed ideogram.

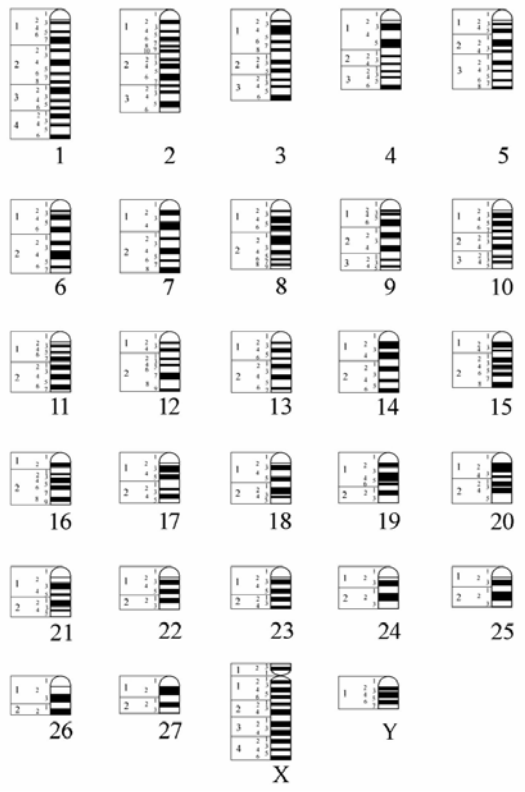

Fig. 4. GTG-banded ideogram of a male goral.

many genes have been localized in homologous regions (Chowhary et al., 1991; Hayes et al., 1993; Iannuzzi et al., 1991). The conservation of band patterns indicates that karyotype evolution has occurred by centric fusion (Buckland and Evans, 1978a; 1978b; Chowdhary et al., 1991; Hayes et al., 1991; Iannuzzi et al., 1991). In Bovidae, it was reported that chromosomes with Robertsonian translocation lost centric heterochromatin during the course of the centric fusion in the evolution (Buckland and Evans, 1978b; Iannuzzi, 1991). However, in goral, the present study revealed no evidence of Robertsonian translocation.

The karyogram of the male goral RBG-banded metaphase was compared with the GTG-banded karyotype of a male goral to complete the karyotype of goral (Fig. 3). A G-banded ideogram of a male goral is presented in Fig. 4. The male goral karyotype was shown to harbor 56 chromosomes, with 27 pairs of acrocentric autosomes, a submetacentric X chromosome and an acrocentric Y chromosome. The sub-bands are numbered according to the International System for Human Cytogenetic Nomenclature recommendation for describing the human karyotype (ISCN, 1985).

A previous study on the Korean goral revealed the lowest level of heterozygosity among the Caprinae subfamilies (Kim et al., 2004). In this paper, we present the chromosomal characteristics of male and female gorals in Korea. While goral is a member of the Bovidae family, it does not share any of the common chromosomal characteristics of this group. Groves and Grubb (1985) suggested that goral and serow should be assigned to one genus on the basis of morphological analysis, but the present study offers no cytogenetic evidence to support their case.

Historically, taxonomy has been based on morphological criteria. To date, this appears have been reasonably accurate for Bovidae species. Fortunately, there is little difference in the band patterns of various wild and domestic bovid in spite of their unique appearance, but this is insufficient to clarify the phylogenic relationships between them. According to a recent research, the karyotypes of red goral and Sumatran serow have different diploid chromosome as well as different types of chromosomal rearrangements (Huang et al., 2005). The available data for goral support the idea that goral did not originate from a common bovid ancestor, because the chromosomal differences are far more significant than those associated with other Bovidae species. Studies using microsatellite, mitochondrial DNA or Zoo-FISH will be useful to investigate the evolutionary relationships with other species of artiodactyla. Above all, the evolutionary relationship between the gorals in other isolated areas and other goral species should be thoroughly investigated.

REFERENCES

An, J., Choi, S.K., Sommer, J., Louis, E., Brenneman, R., Zemanova, B., Hajokova, P., Park, G., Min, M.S., Kim, K.S., et al. (2010). A core set of microsatellite markers for conservation genetics studies of Korean goral (*Naemorhedus caudatus*) and its cross-species amplification in Caprinae species. J. Vet. Sci. *11*, 351-353.

Buckland, R.A., and Evans, H.J. (1978a). Cytogenetic aspects of phylogeny in the Bovidae. I. C-banding. Cytogenet. Cell Genet. *21*, 42-63.

Buckland, R.A., and Evans, H.J. (1978b). Cytogenetic aspects of phylogeny in the Bovidae. II. C-banding. Cytogenet. Cell Genet. *21*, 64-71.

Chowdhary, S.P., Harbitz, I., Davies, W., and Gustavsson, I. (1991).

Chromosomal localization of the glucose phosphate isomerase (GPI) gene in cattle, sheep and goat by *in situ* hybridization chromosomal banding homology versus molecular conservation in Bovidae. Hereditas *114*, 161-170.

Claro, F., Hayes, H., and Cribiu, E.P. (1994). The C-, G-, and R-banded karyotype of the scimitar-horned oryx (*Oryx dammah*). Hereditas *120*, 1-6.

Claro, F., Hayes, H., and Cribiu, E.P. (1995). Identification of p and q arms of the blesbok (*Damaliscus dorcas phillipsi, Alcelaphinae*) RBG-banded chromosomes with comparison to other wild and domestic bovids. Cytogenet. Cell Genet. *70*, 268-272.

Claro, F., Hayes, H., and Cribiu, E.P. (1996). The karyotype of the addax and its comparison with karyotypes of other species of Hippotraginae antelopes. Hereditas *124*, 223-227.

Cribiu, E.P., Asmonde, J.F., Durand, V., Greth, A., and Anagariyah, S. (1990). Robertsonian chromosome polymorphism in the Arabian oryx (*Oryx leucoryx*). Cytogenet. Cell Genet. *54*, 161-163.

Di Berardino, D., Rønne, M., Burguete, I., Lioi, M.B., Taibi, L., and Matassino, D. (1987). R-banding pattern of the prometaphase chromosomes of the goat. J. Hered. *78*, 225-230.

Di Berardina, D., Hayes, H., Fries, R., and Long, S. (1990). International system for cytogenetic nomenclature of domestic animals. The Second International Conference on Standardization of Domestic Animal Karyotypes, INRA, Jouy-en Josas, France, 22nd-26th May, 1989. Cytogenet. Cell Genet. *53*, 65-79.

Ford, C.E., Pollock, D.L., and Gustavsson, I. (1980). Proceedings of the First International Conference for the Standardisation of Banded Karyotypes of Domestic Animals. University of Reading, England. 2nd-6th August 1976. Hereditas *92*, 145-162.

Gallagher, D.S. Jr., and Womack, J.E. (1992). Chromosome conservation in the Bovidae. J. Hered. *83*, 287-298.

Groves, C.P., and Grubb, P. (1985), The biology and management of mountain ungulates: Reclassification of the serows and gorals (*Nemorhaedus*: Bovidae). (London: Croom Helm). 45-50.

Grubb, P., Wilson, D.E., and Reeder, D.M. (2005), Mammal Species of the World. 3rd ed. (Johns Hopkins University Press).

Hayes, H., Petit, E., and Dutrillaux, B. (1991). Comparison of RBG-banded karyotypes of cattle, sheep, and goats. Cytogenet. Cell Genet. *57*, 51-55.

Hayes, H., Petit, E., Bouniol, C., and Popescu, C.P. (1993). Localization of the α-S2-casein gene(CASAS2) to the homologous cattle, sheep, and goat chromosomes 4 by *in situ* hybridization. Cytogenet. Cell Genet. *64*, 281-285.

Huang, L., Nie, W.H., Wang, J.H., Su, W.T., and Yang, F.T. (2005). Phylogenomic study of the subfamily Caprinae by cross-species chromosome painting with Chinese mantjac paints. Chromosome Res. *13*, 389-399

Iannuzzi, L. (1994). Standard karyotype of the river buffalo (*Bubalus bubalis* L., 2n = 50). Cytogenet. Cell Genet. *67*, 102-113.

Iannuzzi, L., Di Meo, G.P., Perucatti, A., Ferrara, L., and Gustavsson, I. (1991). Sister chromatid exchange in chromosomes of cattle from three different breeds reared under similar conditions. Hereditas *114*, 201-205.

Iannuzzi, L., Rangel-Figueido, T., Di Meo, G.P., and Ferrara, L. (1993). A new centric fusion translocation in cattle, rob(16;18). Hereditas *119*, 239-243.

Iannuzzi, L., Di Meo, G.P., Perucatti, A., and Ferrara, L. (1995). G- and R-banding comparison of sheep (*Ovis aries* L.) chromosomes. Cytogenet. Cell Genet. *68*, 85-90.

ISCN (1985). An international system for human cytogenetic nomenclature. (1985). Report of the Standing Committee on Human Cytogenetic Nomenclature. Birth Defects Orig. Artic. Ser. *21*, 1-117.

Jim, I.M. (1985). Nemorhaedus goral. Mammalian Species *335*, 1-5.

Kaftanovskaya, H.M., and Serov, O.L. (1994). High-resolution GTG-banded chromosomes of cattle, sheep, and goat: a comparative study. J. Hered. *85*, 395-400.

Kim, K.S., Min, M.S., An, J.H., and Lee, H. (2004). Cross-species amplication of bovidae microsatellites and low diversity of the endangered Korean goral. J. Hered. *95*, 521-525.

Min, M.S., Okumura, H., Jo, D.J., An, J.H., Kim, K.S., Kim, C.B., Shin, N.S., Lee, M.H., Han, C.H., Voloshina, I.B., et al. (2004). Molecular phylogenetics status of the Korean goral and Japanese serow based on partial sequence of the mitochondrial cytochrome *b* gene. Mol. Cells *17*, 365-372

Robinson, T.J., Wilson, V., Gallager, D.S., Taylor, Jr. J.F., Davis, S.K., Harrison, W.R., and Elder, F.F.B. (1996). Chromosomal evolution in duiker antelope (Cephalophinae:Bovidae) karyotype comparisons, fluorescence *in situ* hybridization, and rampant X chromosome variation. Cytogenet. Cell Genet. *73*, 116-122.

Seabright, M. (1971). A rapid banding technique for human chromosomes. Lancet *2*, 971-972.

Soma, H., Kada, H., Matayoshi, K., Kiyokawa, T., Ito, T., Miyashita, M., and Nagase, K. (1980). Some chromosomal aspects of *Naemorhedus goral* (Goral) and *Procapra gutturosa* (Mongolian Gazelle). Proc. Japan Acad. *56*, Ser. B

Sumner, A.T. (1972). A simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. *75*, 304-306.

Wurster, D.H. (1972). Sex-chromosome translocations and karyotypes in bovid tribes. Cytogenetics *11*, 197-207.