Ann. Anim. Sci., Vol. 12, No. 4 (2012) 495–500, DOI: 10.2478/v10220-012-0041-3

COMPARATIVE SEX CHROMOSOME HYBRIDIZATIONS IN *RUMINANTIA**

Anna Kozubska-Sobocińska[1], Barbara Rejduch[1], Barbara Danielak-Czech[1], Marek Babicz[2], Agnieszka Bąk[1]

[1]Department of Animal Cytogenetics and Molecular Genetics, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
[2]Department of Pig Breeding and Production Technology, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland

Abstract

The syntenic conservation nature of some chromosomes enables the use of several molecular probes obtained from one species of animals to detect homologous DNA segments in other species. The aim of this study was to analyse homology between sex chromosomes in several species belonging to the suborder *Ruminantia* (sheep – *Ovis aries*, fallow deer – *Dama dama*, aoudad – *Ammotragus lervia*, red deer – *Cervus elaphus*) using bovine heterosome painting probes in FISH technique. The results obtained showed strong red fluorescence signals in small metacentric heterosomes Y and distinct yellow-green signals in large acrocentric chromosomes X of all compared species.

Key words: *Ruminantia*, genetic conservatism, sex chromosomes, bovine heterosome probes, FISH

Genetic conservatism makes it possible to compare genomes of different species at the level of nucleotide sequences (Kozubska-Sobocińska et al., 2007, 2009 a; Rejduch et al., 2009), chromosome banding patterns (Di Berardino et al., 2001; Kozubska-Sobocińska et al., 2006, 2007) and groups of linked or syntenic genes that are often in the same relationships even in taxonomically distant species (Hayes, 1995; Danielak-Czech et al., 2010; Rejduch et al., 2010 a, 2010 b).

This syntenic conservation nature of some chromosomes makes it possible to use a number of molecular probes obtained by microdissection or chromosome sorting in one species of animals, for FISH chromosome painting in other species (Chowdhary et al., 1996; Révay et al., 2000; Kozubska-Sobocińska et al., 2003).

This study was designed to use the heterosomes specific bovine molecular probes to identify sex chromosomes in sheep (*Ovis aries*), fallow deer (*Dama dama*), aoudad (*Ammotragus lervia*) and red deer (*Cervus elaphus*) and establish genetic conservation of heterosome synteny groups in *Ruminantia*.

*This study was financed from statutory activity, project no. 04-3.05.1.

Material and methods

Metaphase chromosome spreads of sheep, goat, fallow deer, aoudad and red deer were obtained from peripheral blood lymphocyte culture (pokeweed mitogen stimulated) according to the routine protocol.

In this paper we present identification of heterosomes by FISH technique with two commercial bovine probes (ID Labs): Bovine IDetect™ Chr X Point Probe GREEN – Cat. No. IDBF 1061 and Bovine IDetect™ Chr Y Point Probe RED – Cat. No. IDBR 1059. Fluorescence *in situ* hybridization was performed according the manufacturer's procedure. DAPI-banding was applied to precisely identify the chromosome subregions. Hybridization signals were observed under an OPTON-Axiophot fluorescent microscope using triple attenuation filters DAPI/FITC/Texas Red and the computer image analysis system LUCIA-FISH (Laboratory Imaging Ltd, Prague, Czech Republic).

Results

The results of cross-species hybridizations of sex chromosomes of four species from the suborder Ruminantia with two commercial bovine chromosome painting probes specific for heterosomes (Chr X Point Probe GREEN and Bovine IDetectTM Chr Y Point Probe RED) are displayed in Figure 1 A–E.

As shown, the distinct red fluorescence signals visible in metaphase plates identify small metacentric chromosomes Y in sheep (Fig. 1 B), aoudad (Fig. 1 C), red deer (Fig. 1 D) and fallow deer (Fig. 1 E).

In turn, yellow-green fluorescence signals corresponding to acrocentric X heterosomes in above mentioned species are presented in Figure 1 B–E, respectively.

Discussion

The first comparative study in the *Bovidae* family showed band homology on the chromosomes of cattle, sheep, goats and water buffaloes (Evans et al., 1973).

Comparison of GTG-banded, haploid sets of sheep (2n=54) and aoudad (*Ammotragus lervia*) chromosomes (2n=58) revealed complete chromosome homology in the karyotypes of both species and indicated that centric fusions of autosomes led to evolutionary rearrangements (Słota et al., 2001).

Karyotype studies of different species of *Cervidae* family (elk, roe deer, red deer, sika deer and fallow deer) living in the wild, conducted by Gustavsson and Sundt (1968), concerned routinely stained metaphase chromosomes, which were classified according to size and morphology. For the *Dama dama* species, the 68,XY or 68,XX karyotype as well as the number of arms of autosomal chromosomes (68) were determined. Concerning sex chromosomes, X was identified as the acrocentric chromosome and Y as a small submetacentric.

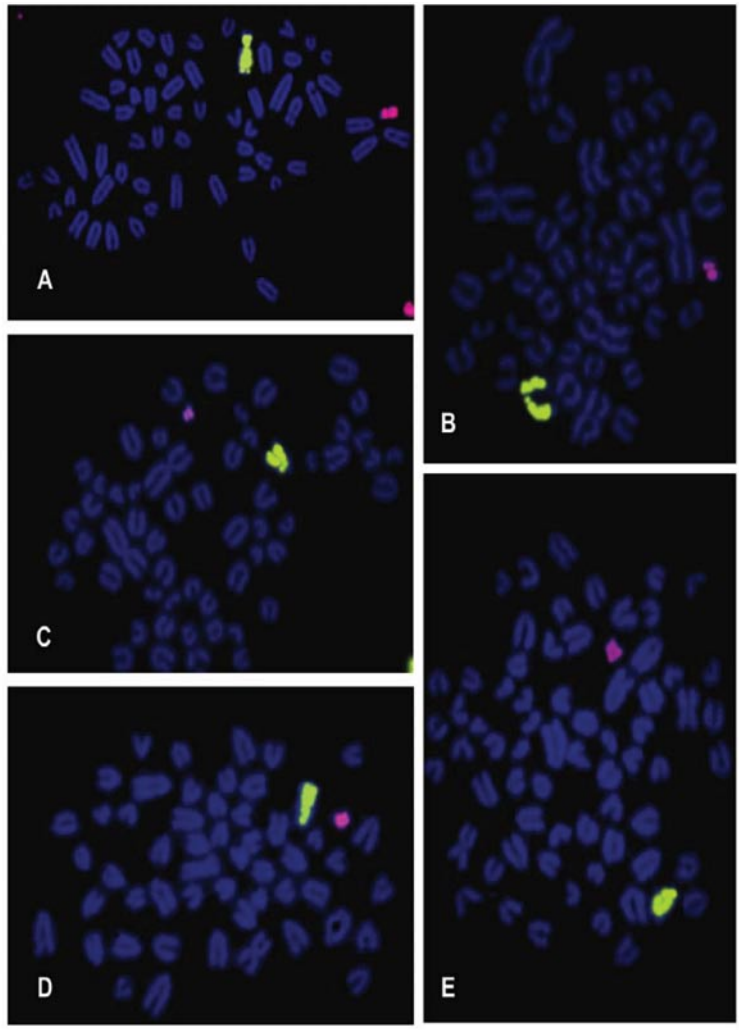

Figure 1. The cross-species hybridizations with bovine probes specific to the heterosomes:
(A) metaphase chromosomes of bull – 60,XY, (B) sheep metaphase plate – 54,XY, (C) aoudad metaphase plate – 58,XY, (D) red deer metaphase plate – 68,XY, (E) fallow deer metaphase plate – 68,XY.
Red fluorescence signals identify small metacentric chromosomes Y in cattle, sheep, aoudad, red deer and fallow deer.
Yellow-green fluorescence signals correspond to acrocentric X heterosomes in sheep, aoudad, red deer and fallow deer and submetacentric X in cattle.

In the next step of determination the karyotype of fallow deer, the following differential staining techniques were used: GTG, with 350–450 G-bands obtained on metaphase chromosomes (Rubini et al., 1990; Kozubska-Sobocińska et al., 2006, 2007) and RBA, with 527 bands obtained on prometaphase chromosomes (Lioi et al., 1994).

A remarkable homology of most autosomes of the fallow deer and the roe deer (*Capreolus capreolus*) were revealed by comparison between the G-banded karyotypes (Rubini et al., 1990). According to these authors, the metacentric pair in the fallow deer retains the same band patterns as the two acrocentric pairs in the roe deer, while the X chromosomes of the roe deer differ as a result of pericentric inversion.

The comparison of R-banded chromosomes of Vietnamese sika deer (*Cervus nippon pseudaxis*, 2n=66) with bovine R-banded chromosomes was described by Bonnet et al. (2001). Next, the probes for twenty-nine Texas nomenclature type I markers for each cattle autosome, sixteen other type I and fourteen microsatellite markers on sika deer chromosomes were used in FISH technique on sika deer chromosomes. A complete correspondence between sika deer and cattle chromosomes was established; however, autosome pair 7 of sika deer presented the most complex rearrangement as compared with cattle chromosomes.

A complete set of Chinese muntjac chromosome-specific painting probes was used in hybridization *in situ* to G-banded chromosomes of Chinese muntjac (*Muntiacus reevesi*), forest musk deer (*Moschus berezovskii*) and gayal (*Bos frontalis*) to investigate the karyotype relationships between these three species (Chi et al., 2005). In total, the 22 autosomal painting probes of Chinese muntjac delineated 33 and 34 conserved chromosomal segments in the genomes of forest musk deer and gayal, respectively. The combined analysis of comparative chromosome painting and interspecies G-band comparison revealed a high degree of G-banding patterns conservation of most homologous segments. Interestingly, the musk deer has retained a highly conserved karyotype that closely resembles the proposed ancestral pecoran karyotype but shares none of the rearrangements characteristic of the *Cervidae* and *Bovidae*.

In studies on heterosomes conservation in *Ruminantia* most interspecies hybridizations were based on bovine probes generally (Kozubska-Sobocińska et al., 2003, 2005, 2009 b; Kozubska-Sobocińska and Rejduch, 2008). The only example of using a probe from *Bos indicus* (obtained from microdissected Yp12 fragment) is identification of a complementary sequence in the X-Y bivalent at metaphase I in *Bos taurus* and performing comparative hybridization (using the Yq12.1-12.6 probe obtained from *Bos indicus*) of the appropriate segment on the q arm of the Y heterosome in *Bos taurus* (Goldammer et al., 1996). A probe specific for the Yp12 fragment was also used to identify the Y chromosome in metaphase plates and spermatozoa (Révay et al., 2000). The high conservation of sex chromosomes in *Ruminantia* is evidenced by hybridization signals obtained by Révay et al. (2002) for bull spermatozoa, following the application of probes (using FISH) obtained by heterosome sorting of the yak (*Bos grunniens*).

The study presented in this paper, consisted of using the heterosomes specific bovine molecular probes to identify sex chromosomes in sheep (*Ovis aries*), fallow deer (*Dama dama*), aoudad (*Ammotragus lervia*) and red deer (*Cervus elaphus*). The experiments carried out revealed genetic conservation of heterosome synteny groups in *Ruminantia*, which make it possible to apply bovine heterosome probes in cytogenetic diagnostics concerning identification of sex chromosomes in somatic and generative cells.

It is worth noting that identification of subtle chromosome mutations and intrachromosomal evolutional rearrangements as well as comparative gene mapping need region- or loci-specific probes to be applied in FISH technique (Rejduch et al., 2009, 2010 a).

The results obtained after comparison of *Bovidae* and *Cervidae* families belonging to the suborder *Ruminantia* suggest that genetic conservatism involving gene syntheny of chromosome X and chromosome Y is the phenomenon frequently observed also between larger systematic units than family.

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Accepted for printing 5 IX 2012

ANNA KOZUBSKA-SOBOCIŃSKA, BARBARA REJDUCH, BARBARA DANIELAK-CZECH, MAREK BABICZ, AGNIESZKA BĄK

Hybrydyzacje porównawcze chromosomów płci u *Ruminantia*

STRESZCZENIE

Synteniczno-konserwatywny charakter wielu chromosomów umożliwia wykorzystanie licznych sond molekularnych, otrzymanych dla jednego gatunku zwierząt, do detekcji homologicznych fragmentów DNA u innych gatunków. Celem tych badań była analiza homologii między chromosomami płci u kilku gatunków należących do podrzędu *Ruminantia* (owcy – *Ovis aries*, daniela – *Dama dama*, owcy grzywiastej – *Ammotragus lervia*, jelenia szlachetnego – *Cervus elaphus*) przy zastosowaniu techniki FISH i bydlęcych sond malujących heterosomy. Uzyskane wyniki ujawniły mocne czerwone sygnały fluorescencyjne na małych metacentrycznych heterosomach Y i wyraźne żółto-zielone sygnały w dużych akrocentrycznych chromosomach X u wszystkich porównywanych gatunków.