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Comparative hybridizations *in situ* for identification heterosomes in aoudad (*Ammotragus lervia*)

Porównawcze hybrydyzacje *in situ* do identyfikacji heterosomów owcy grzywiastej *(Ammotragus lervia*)

Summary. Genetic conservation on the level of genes from syntenic groups enables the use of molecular probes obtained from one species of animals to detect homologous DNA segments in different species. The aim of this study was to identify heterosomes in aoudad (*Ammotragus lervia*) by comparative hybridizations *in situ* technique (Zoo-FISH) with commercial bovine heterosomes painting probes. The results obtained showed distinct yellow-green signals in big acrocentric chromosomes X and strong red fluorescence signals in small metacentric chromosomes Y in all aoudad metaphase plates. The experiments confirmed the high degree of genetic conservation of heterosome synteny groups in the species (cattle and aoudad) belonging to *Bovidae* family, which makes it possible to use bovine, heterologous sex chromosomes painting probes in interspecies comparative, phylogenetic and evolutionary studies.

Key words: aoudad (*Ammotragus lervia*), syntenic genetic conservatism, sex chromosomes, bovine heterosomes probes, Zoo-FISH technique

INTRODUCTION

Interspecies comparative analyses of the genomes are based on the phenomenon of the genetic conservatism. This concerns groups of linked or syntenic genes that are often in the same relationships even in taxonomically distant species [Rejduch *et al.* 2010b, Danielak-Czech *et al.* 2010, Kozubska-Sobocińska and Rejduch *2*008, Kozubska-Sobocińska *et al*. 2012, 2013], microsatellites and nucleotide sequences of genes coding the same products in different species [Rejduch *et al.* 2004, 2010a, Kozubska-

Sobocińska *et al.* 2007a, 2009a] and chromosome banding patterns [Iannuzzi *et al.* 1995, Bonnet *et al.* 2001, Słota *et al.* 2001, Chi *et al.* 2005, Kozubska-Sobocińska *et al.* 2006, 2007b, Oh *et al.* 2011].

Conservation nature of some chromosomes makes it possible to application a number of molecular probes, obtained by microdissection of whole chromosomes or their fragments as well as chromosome sorting in one species of animals, for FISH chromosome painting in another species [Chowdhary *et al.* 1996, Goldammer *et al.* 1996, Huang *et al.* 2005, Kozubska-Sobocińska *et al.* 2005, 2012, 2013].

This study was designed to use two commercial bovine molecular probes (ID Labs) specific to the heterosomes to identify sex chromosomes in aoudad (*Ammotragus lervia*) and establish genetic conservation of heterosome synteny groups in species of *Bovidae* family.

MATERIAL AND METHODS

Metaphase chromosome spreads (150 cells) of three aoudads (two females and one male originating from Krakow's ZOO) were obtained from peripheral blood lymphocyte culture (pokeweed mitogen stimulated), according to the standard protocol and routine kariotype analysis.

In this paper we present identification of sex chromosomes in aoudad by Zoo-FISH technique with two commercial bovine probes (ID Labs): Bovine IDetect^{TM} Chr X Point Probe GREEN and Bovine IDetect^{TM} Chr Y Point Probe RED (Cambio Ltd., Cambridge, UK). Interspecies *in situ* hybridizations were performed according the manufacture's procedure. Hybridization signals were observed under an Axio Imager.D2 (Zeiss) fluorescent microscope equipped with Axio Vision computer-assisted image analysis system.

RESULTS

Cytogenetic evaluation of aoudad studied revealed normal 58,XY kariotype consisted of 28 pairs of autosomes (one pair of long metacentric chromosomes and 27 pairs of acrocentrics) and the pair of heterosomes (long acrocentric X chromosom and small submetacentric Y chromosome) (Fig. 1A, 1B).

The cross-species hybridizations (Zoo-FISH technique), with the use of bovine microdissected whole chromosome painting probes (WCPP), presented in Figure 1C show distinct yellow-green fluorescence signal corresponded to long acrocentric X heterosomes and strong red fluorescence signal identifying small submetacentric chromosomes Y in all aoudad metaphase plates.

The results confirmed indirectly homology between bovine sex chromosomes in the different ruminant species of *Bovidae* (sheep – *Ovis aries*, goat – *Capra hircus*, aoudad – *Ammotragus lervia*), and *Cervidae* family (fallow deer – *Dama dama* and goral – *Nemorhaedus caudatus*) from the suborder *Ruminantia*.

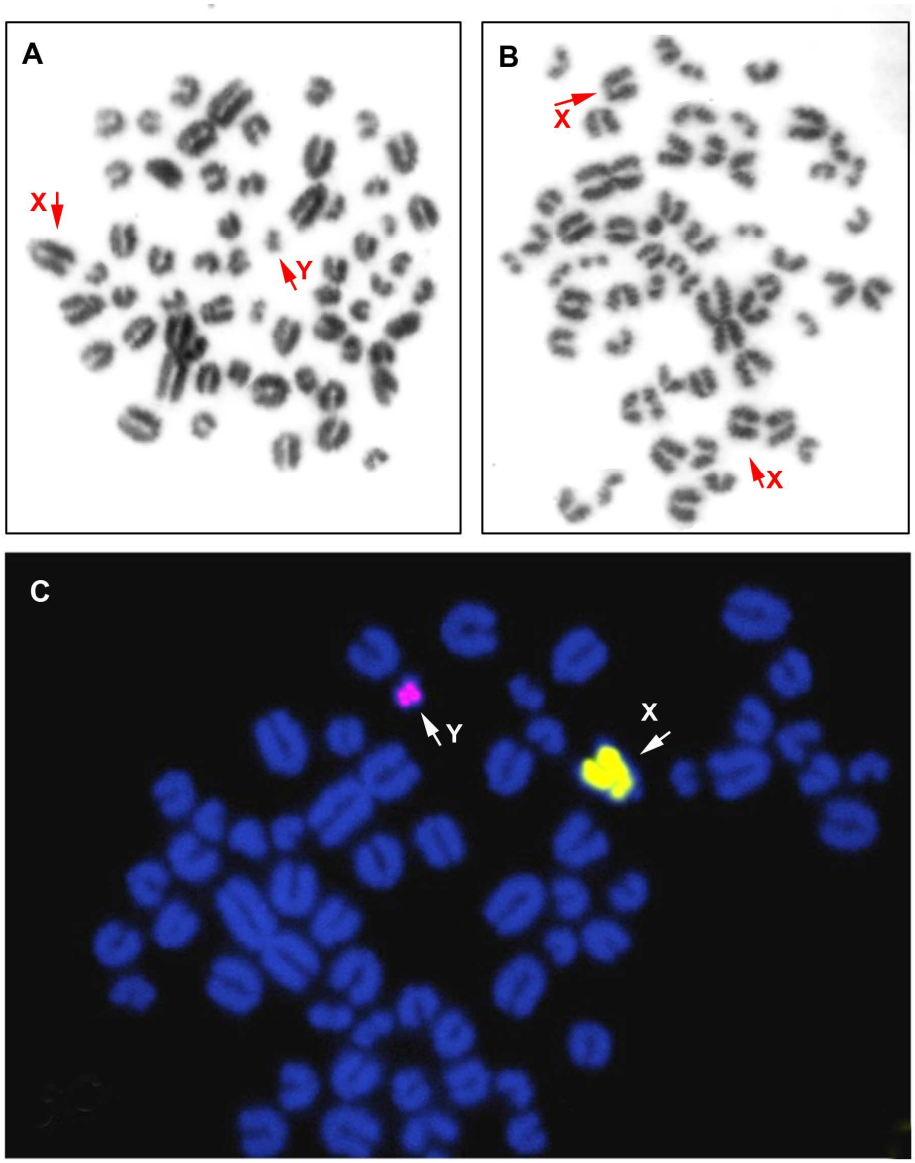

Fig 1. Giemsa stained metaphase chromosomes of aoudad: 58,XY (A), 58,XX (B). Inter-species in situ hybridization (Zoo-FISH technique) (C) – yellow-green signal identifies long acrocentric heterosom X, red fluorescence signal labels small submetacentric chromosome Y

Rys. 1. Chromosomy metafazowe barwione barwnikiem Giemsy: 58,XY (A), 58,XX (B). Międzygatunkowe hybrydyzacje in situ (technika zoo-FISH) (C) – żółto-zielony sygnał identyfikuje długi akrocentryczny heterosom X, czerwony sygnał fluorescencyjny znakuje mały metacentryczny chromosom Y

DISCUSSION

The aoudad (*Ammotragus lervia*) is characterised by a mixture of sheep and goat features, probably is the living representative of a sheep and goat ancestor [Geist 1971]. The cytomolecular comparative studies in the *Ruminantia* showed chromosome band homology of cattle, sheep, goats, aoudad, water buffaloes (from the *Bovdae*) as well as fallow deer, goral and red deer (from the *Cervidae*) [Iannuzzi and Di Meo 1995, Słota *et al.* 2001, Kozubska-Sobocińska *et al.* 2006, 2007a, Oh *et al.* 2011].

Comparison of GTG-banded, haploid sets of sheep (2n = 54) and aoudad 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]. The pair of metacentric chromosomes of the aoudad is fully homologous with the first pair of metacentric sheep chromosomes. According to Słota *at al*. [2001], there is a significant homology of all aoudad chromosomes with sheep chromosomes or sheep chromosome p and q arms was observed. Furthermore, the X chromosome of the aoudad, also homologous with acrocentric X chromosome of sheep, is the third chromosome in aoudad karyotype, based on the length.

Next comparison between G-banding patterns on cattle and fallow deer chromosomes, [Kozubska-Sobocińska *et al.* 2007b] and earlier described comparisons of fallow deer and sheep [Kozubska-Sobocińska *et al.* 2006] as well as fallow deer and goat [Kozubska-Sobocińska *et al.* 2007a], confirmed chromosome homology in the *Bovidae* family described by Iannuzzi and Di Meo [1995].

In studies on heterosomes conservation in *Ruminantia* most interspecies hybridizations were based on bovine probes generally [Kozubska-Sobocińska *et al.* 2003, 2005, 2009b, 2012, 2013, Kozubska-Sobocińska and Rejduch 2008]. The example of using a probe from *Bos indicus* (obtained from microdissected of 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]. Moreover, bovine painting probes applied in FISH technique made it possible to determine the frequency of early-dissociation of sex bivalent in rams [Kozubska-Sobocińska *et al.* 2009 b].

The high conservation of sex chromosomes in *Bovidae* 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 confirmed usefullness of hetrosomes-specific bovine molecular probes for identification of sex chromosomes in aoudad (*Ammotragus lervia*).

Cytomolecular comparative studies enable chromosome markers to be identified even in species representing different families, as exemplified by the pairs of homologous chromosomes identified in cattle, sheep, goats, aoudad of *Bovida*e and fallow deer, goral of *Cervidae* [Słota *at al*. 2001,.Kozubska-Sobocińska *et al.* 2007 a, 2012, 2013]. These analogies could be used in evolutionary studies as well as for diagnosing chromosomal changes in wild-living species whose karyotypes are less known than the karyotypes of farm animals.

CONCLUSIONS

The experiments carried out revealed genetic conservation of heterosome synteny groups in species of *Bovidae* family, which make it possible to apply of bovine heterosomes probes in cytogenetic diagnostics.

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Streszczenie. Konserwatyzm genetyczny na poziomie genów z grup syntenicznych umożliwia wykorzystanie sond molekularnych otrzymanych dla jednego gatunku zwierząt do identyfikacji homologicznych fragmentów DNA innych gatunków. Celem badań była identyfikacja heterosomów arui (*Ammotragus lervia*) przy wykorzystaniu techniki porównawczych hybrydyzacji *in situ* (zoo-FISH) z komercyjnymi bydlęcymi sondami malującymi heterosomy. Uzyskane wyniki ujawniły wyraźne żółto-zielone sygnały w dużych akrocentrycznych chromosomach X i mocne czerwone sygnały fluorescencyjne na małych metacentrycznych heterosomach Y we wszystkich płytkach chromosomów metafazowych arui. Eksperymenty potwierdziły wysoki stopień konserwatyzmu genetycznego grup syntenicznych heterosomów u gatunków należących do rodziny *Bovidae* (bydło i arui), co stwarza możliwość wykorzystania bydlęcych, heterologicznych sond malujących chromosomy płci w międzygatunkowych badaniach porównawczych, filogenetycznych i ewolucyjnych.

Słowa kluczowe: arui (*Ammotragus lervia*), synteniczny konserwatyzm genetyczny, chromosomy płci, bydlęce sondy malujące heterosomy, technika zoo-FISH