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Interspecies hybridizations *in situ* with bovine heterosome painting probes for identification of sex chromosomes in fallow deer (*Dama dama*)

Międzygatunkowe hybrydyzacje *in situ* z bydlęcymi sondami malującymi heterosomy do identyfikacji chromosomów płci u daniela *(Dama dama)*

Summary. The phenomenon of syntenic genetic conservation of chromosomes enables application of molecular probes obtained from one species of animals to detect homologous DNA segments in another species. The aim of this study was identification of sex chromosomes in fallow deer (*Dama dama)*, using in interspecies hybridizations *in situ* (Zoo-FISH technique) the bovine heterosomes painting probes. The results obtained showed distinct yellow-green signals in big acrocentric chromosomes X and strong red fluorescence signals in small submetacentric heterosomes Y in all fallow deer metaphase plates. The studies confirmed the high degree of genetic conservation of heterosome synteny groups in the species belonging to *Ruminantia*, which makes it possible to use of bovine, heterologous sex chromosomes painting probes in interspecies comparative, phylogenetic and evolutionary analyses.

Key words: Fallow deer (*Dama dama*), syntenic genetic conservatism, sex chromosomes, bovine heterosomes probes, Zoo-FISH technique

INTRODUCTION

The phenomenon of genetic conservatism makes it possible to compare genomes of different species at the level of nucleotide sequences [Rejduch *et al.* 2004; Kozubska-

-Sobocińska *et al.* 2007a, 2009a], chromosome banding patterns [Rubini *et al.* 1990, Bonnet *et al.* 2001; Chi *et al.* 2005; Kozubska-Sobocińska *et al.* 2006, 2007a, 2007b; Oh *et al.* 2011] and groups of linked or syntenic genes that are often in the same relationships even in taxonomically distant species [Rejduch *et al.* 2010a, 2010b; Danielak--Czech *et al.* 2010; Kozubska-Sobocińska *et al.* 2012].

The 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 another species [Chowdhary *et al.* 1996; Goldammer *et al.* 1996; Révay *et al.* 2000, 2002; Kozubska-Sobocińska *et al.* 2003, 2005, 2012; Huang *et al.* 2005; Kozubska-Sobocińska and Rejduch *2*008].

This study was designed to use two commercial bovine molecular probes (ID Labs), specific to the heterosomes to identify sex chromosomes in fallow deer (*Dama dama*) and establish genetic conservation of heterosome synteny groups in *Ruminantia*.

MATERIAL AND METHODS

Metaphase chromosome spreads of fallow deer with normal karyotype 68, XY were obtained from peripheral blood lymphocyte culture (pokeweed mitogen stimulated) according to the standard protocol. Cytogenetic evaluation involved routine kariotype analysis supplemented with CBG and Ag-I techniques.

In this paper we present identification of heterosomes 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. DAPI-banding was applied to precisely identify the chromosome subregions. Hybridization signals were observed under an Axio Imager.D2 (Zeiss) fluorescent microscope using equipped with Axio Vision computer-assisted image analysis system.

RESULTS

Cytogenetic evaluation of fallow deer studied revealed normal 68,XY kariotype consisted of 33 pairs of autosomes (one pair of long metacentric chromosomes and 32 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 acrocentric X heterosomes and strong red fluorescence signal identifying small submetacentric chromosomes Y in all fallow deer metaphase plates. The results confirmed indirectly homology between bovine sex chromosomes in the other ruminant species: sheep – *Ovis aries*, goat – *Capra hircus*, aoudad – *Ammotragus lervia* of *Bovidae*, as well as red deer – *Cervus elaphus* and goral – *Nemorhaedus caudatus* of *Cervidae*.

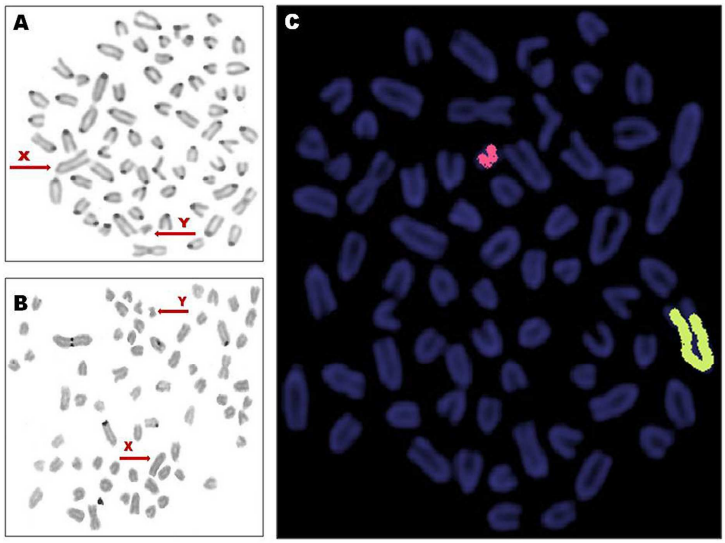

Fig. 1. Metaphase chromosomes of fallow deer: C bands (A), Ag-NOR bands (B), inter-species *in situ* hybridization (Zoo-FISH technique) (C) – yellow-green signal identifies long acrocentric heterosom X, red fluorescence signal labels small submetacentric chromosome Y
Rys. 1. Chromosomy metafazowe daniela. Prążki C (A); prążki Ag-NOR (B); międzygatunkowe hybrydyzacje *in situ* (Zoo-FISH technique) (C) – żółto-zielony sygnał identyfikuje długi akrocentryczny heterosom X, czerwony sygnał fluorescencyjny znakuje mały metacentryczny chromosom Y

DISCUSSION

The comparative studies in the Ruminantia showed chromosome band homology of cattle, sheep, goats, aoudad, water buffaloes (from the Bovdae family) as well as fallow deer, goral and red deer (from the Cervidae family) [Iannuzzi and Di Meo 1995; Słota *et al.* 2001; Kozubska-Sobocińska *et al.* 2007a, 2012; Oh *et al.* 2011]. 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] and 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, 2009 b, 2012; 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 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 confirmed usefullness of hetrosomes-specific bovine molecular probes for identification of sex chromosomes in follow deer (Dama dama).

Cytogenetic 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 of Bovidae and fallow deer of Cervidae. 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 ruminant species, which make it possible to apply of bovine heterosomes probes in cytogenetic diagnostics.

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Streszczenie. Zjawisko syntenicznego konserwatyzmu genetycznego chromosomów umożliwia zastosowanie sond molekularnych otrzymanych dla jednego gatunku zwierząt do detekcji homologicznych fragmentów DNA innych gatunków. Celem badań była identyfikacja chromosomów płci daniela (*Dama dama*) przy wykorzystaniu w międzygatunkowych hybrydyzacjach *in situ* (technika Zoo-FISH) bydlęcych sond malujących heterosomy. Uzyskane wyniki ujawniły wyraźne żółtozielone sygnały w dużych akrocentrycznych chromosomach X i mocne czerwone sygnały fluorescencyjne na małych submetacentrycznych heterosomach Y we wszystkich płytkach chromosomów metafazowych daniela. Badania potwierdziły wysoki stopień konserwatyzmu genetycznego grup syntenicznych heterosomów u gatunków należących do *Ruminantia*, co stwarza możliwość wykorzystania bydlęcych, heterologicznych sond malujących chromosomy płci w międzygatunkowych analizach porównawczych, filogenetycznych i ewolucyjnych.

Słowa kluczowe: daniel (*Dama dama*), syntoniczny konserwatyzm genetyczny, chromosomy płci, bydlęce sondy malujące hererosomy, technika Zoo-FISH