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[1]Departament of Animal Cytogenetics and Molecular Genetics
National Institute of Animal Production, Krakowska 1, 32-083 Balice/Kraków
e-mail: barbara.czech@izoo.krakow.pl
[2]Department of Pig Breeding and Production Technology
University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin
e-mail: marek.babicz@up.lublin.pl

BARBARA DANIELAK-CZECH[1], MAREK BABICZ[2],
ANNA KOZUBSKA-SOBOCIŃSKA[1], BARBARA REJDUCH[1]

Size polymorphism survey of nucleolar organizer regions (NORs) in Hampshire boars

Ocena polimorfizmu wielkości regionów jąderkotwórczych (NOR) u knurów rasy hampshire

Summary. Previous studies revealed that precisely quantified and normalized NORs size variants may be considered as chromosome markers helpful in pig breeds characterization and gene mapping. The aim of the present study was to identify and classify the NORs size variants as markers useful for genetic characteristics of Hampshire pigs. On the basis of cytogenetic and molecular analysis (carried out by Ag-I and FISH techniques) four size variants of FISH-signals and Ag-NORs deposits were classified. Nucleolar organizer regions were more morphologically distinct and greater on both chromosomes of pair 10 than on pair 8, which suggests a dominant role of chromosome 10 in the global production of ribosomal RNA. Results of cytomolecular survey of NORs size polymorphism can be used for inter-breed or inter-species comparative assays and phylogenetic studies in *Suidae* family.

Key words: Hampshire boars, nucleolar organizing regions (NOR), size variants, chromosome markers, FISH technique, Ag-I technique

INTRODUCTION

In the domestic pig genome, the ribosomal DNA is organized into two classes of tandemly repeated clusters. The major class, encoding 18S, 5.8S and 28S rRNA is present at the secondary constrictions of chromosomes 8, 10 and 16, whereas the minor class encoding 5S RNA exists only on chromosome 14 [Miyake *et al*. 1988; Bosma *et al*. 1991; Mellink *et al*. 1991, 1994, 1996; Lomholt *et al*. 1995]. The locations of rDNA gene clusters, identified by fluorescent *in situ* hybridization with the specific DNA probe (FISH), have been proven to correspond with the positions of silver-stainable nucleolar

organizer regions (Ag-NORs) [Solinas-Toldo *et al*. 1992]. Both fluorescent rDNA signals and silver deposits on NOR-bearing chromosomes demonstrate clear size diversity.

Variation of FISH-signals with regard to size and intensity is suggested to be a result of different number of repeated rDNA sequences, whereas the size variation of Ag-NORs reflects the level of their transcriptional activity. This phenomenon, especially as referred to chromosome pair 10, fulfills the criteria of polymorphism. Polymorphic NORs may be considered as chromosome markers, which are the special category of genetic markers. Size polymorphism of NORs has been found in numerous breeds of the domestic pig [Mellink *et al*. 1994; Świtoński *et al*. 1997 a, b; Słota 1998]. In Polish pigs this phenomenon has been examined recently by FISH in several breeds (Polish Landrace, Polish Large White, Pulawska, Duroc, Piertain) [Danielak-Czech *et al*. 1999, 2006, 2009, 2011].

The aim of this study was to survey size polymorphic variants of nucleolar organizing regions (NORs) in Hampshire pigs in order to use them for genetic characteristic of this breed.

MATERIAL AND METHODS

A cytogenetic analysis based on the FISH and Ag-I techniques was performed in population of (imported for some years up to former decade from Western Europe countries) 16 unrelated, 2–5 years old Hampshire boars from the Animal Breeding and Insemination Station in Bydgoszcz.

Studies were carried out on (collected in several years) metaphase chromosomes preparations obtained after routine lymphocyte cultures *in vitro*.

Evaluation of silver deposits obtained by Ag-I technique was accomplished by the computer image analysis system (MultiScan). The Ag-NORs polymorphism was expressed in the relative value of silver deposits calculated from a ratio of the silver deposit area to the whole chromosome-bearing NOR area. Values of the Ag-NORs relative area were classified into four categories (I: 0.101-0.200; II: 0.201-0.300; III: 0.301-0.400; IV: 0.401-0.500 for chromosome pair 10 and I: 0.051-0.100; II: 0.101-0.115; III: 0.151-0.200; IV: 0.201-0.250 for pair 8) according to our previous papers [Danielak-Czech *et al*. 1999, 2006, 2009, 2011].

FISH experiments were performed using biotynylated human *5.2kb Bg/ II-EcoRI* 18S+28S rDNA probe [Pinkel *et al*. 1986, Wachtler *et al*. 1986]. FITC-detected NORs were analyzed in DAPI counterstained chromosomes in Axio Imager.D2 (Zeiss) fluorescence microscope equipped with Axio Vision computer-assisted image analysis system. Proportionally to intensity, rDNA FISH-signal variants were classified as 1 – small and weak, and 2, 3, 4 – large and strong.

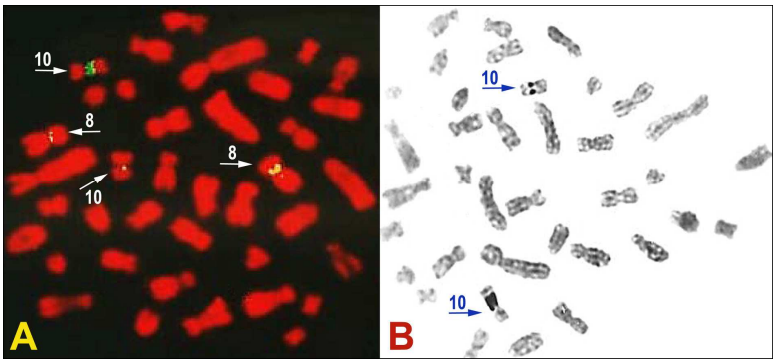
RESULTS

Cytomolecular double identification of NORs, carried out by silver staining and FISH techniques, confirmed the location of rRNA genes at the SSC8p11 and SSC10p11 chromosome regions in the karyotype of the Hampshire pigs investigated. FISH signals were consistently observed in four NOR sites, whereas silver deposits (ranging from 2 to 4) were the most often visible on three chromosomes only – two in pair 10 and one 8. NORs revealed by double identification were more morphologically distinct and greater on both 10

than on pair 8, what could be well exemplified by size categories characterizing the pig no. 15 (Tab. 1). These observations suggest a dominant role of chromosome 10 in the production of ribosomal RNA, which is in agreement with Mellink *et al.* [1994]. The detailed results of size variant survey in all Hampshire boars examined are shown in Table 1.

Table 1. The FISH signals and Ag-NORs size variants on 10. and 8. chromosome in Hampshire boars
Tabela 1. Warianty wielkości sygnałów FISH i Ag-NOR na chromosomach 10. i 8. u knurów rasy hampshire

Boar Knur	FISH signal size variants Warianty wielkości sygnałów FISH				Ag-NOR size variants Warianty wielkości Ag-NOR			
	10	10	8	8	10	10	8	8
1	3	1	2	2	0.366/III	0.123/I	0.112/II	0.105/II
2	3	1	4	2	0.395/III	0.145/I	0.210/IV	0.227/II-
3	4	2	2	1	0.457/IV	0.219/II	0.132/II	-
4	2	1	3	3	0.202/II	0.135/I	0.166/III	-
5	3	1	2	1	0.380/III	0.130/I	0.131/II	0.070/I
6	3	3	1	1	0.350 /III	0.381/III	0.085/I	0.093 /I
7	4	1	2	2	0.468 /IV	0.185/I	0.145/II	-
8	3	1	2	1	0.320/III	0.173/I	0.140/II	0.058/I
9	3	1	2	1	0.392/III	0.114/I	-	-
10	4	3	3	2	0.478/IV	0.330/III	0.152/III	0.146/II-
11	2	2	3	1	0.250/II	0.238 /II	0.170/III	-
12	4	3	1	1	0.474/IV	0.342 /III	0.080/I	-
13	3	2	2	1	0.344/III	0.227/II	0.122/II	-
14	4	4	1	1	0.415/IV	0.419/IV	0.095/I	-
15	3	2	2	1	0.301/III	0.249/II	0.114/II	0.060/II
16	3	1	2	1	0.330/III	0.125/I	-	-



A: arrows indicate FISH-signal size variants on chromosome pair 10 (3+ / 1+) and pair 8 (2+ / 1+).
B: arrows indicate Ag-NOR size variants on chromosome pair 10 (3+ / 1+).
A: strzałki wskazują warianty wielkości sygnałów FISH na chromosomach pary 10. (3+ / 1+) i pary 8. (2+ / 1+).
B: strzałki wskazują warianty wielkości Ag-NOR na chromosomach pary 10. (3+ / 1+).

Fig. 1. The FISH signals in nucleolar organizer regions on metaphase chromosomes of Hampshire boar (animal no. 16 in Tab. 1)
Rys. 1. Sygnały FISH w regionach jąderkotwórczych na chromosomach metafazowych knura rasy Hampshire (osobnik nr 16 w tab. 1)

The experiment described revealed size and number polymorphism of NORs in the studied Hampshire pigs, which is especially visible in case of boar no. 9 and 16 (Fig. 1, Tab. 1).

DISCUSSION

The NORs variation assays has been described in different domestic populations and breeds in the world, among them in Polish Landrace, Polish Large White, Zlotnicka Spotted, Pulawska, Duroc and Piertain bred in Poland [Świtoński and Pietrzak 1992; Mellink *et al*. 1994; Liu *et al*. 1995; Świtoński *et al*. 1997 a, b; Słota 1998; Danielak-Czech *et al*. 1999; 2006, 2009, 2011]. Following this, the surveys were applied to differentiation of pig breeds as well as estimation of genetic distance or evolutionary relationships in domestic pigs or between domestic and wild pigs. Reported in this paper, similar NORs variability evaluation in Hampshire pigs (despite marginal importance of this breed in pig production due to acid meat condition), substantially supplement overall knowledge concerning these chromosome markers which may be the basis of subsequent comparative studies.

In Hampshire pigs studied, like in the other Polish pig breeds, the FISH signals and Ag-deposits on chromosome 10 were regularly classified as higher size variant values than on chromosome 8. However, the cases of unusually large NORs on chromosome 8 were reported in Pietrain and Yorkshire pigs as well as in the primitive Meishan and Zlotnicka Spotted breeds [Mellink *et al*. 1994; Świtonski *et al.* 1997b; Słota 1998, Danielak-Czech *et al.* 1999]. Generally, our results are in agreement with the hypothesis that size polymorphism of rDNA signals and active Ag-NORs corresponds to the length variation of the tandemly repeated DNA sequences generated by unequal crossing-over due to an incorrect meiotic pairing of homologous chromosomes [Harding *et al.* 1992].

Chromosome markers of NORs – the rDNA signal and silver deposit size variants classified in the presented study supplement genetic characteristics of Hampshire breed pigs, which had been earlier drawn up on the basis of centromeric hetrochromatin markers.

Results obtained in the present study suggest that the polymorphic co-dominant and easily scored rDNA signals and Ag-NORs, demonstrating Mendelian segregation, can be assigned into genetic markers, useful in gene mapping. For this purpose, the size polymorphism of silver deposits, quantified earlier only on the basis of arbitrary microscope evaluation, started to be classified into categories on the basis of their measurements [Świtoński and Pietrzak 1992; Świtoński *et al*. 1997a; Komisarek *et al*. 1998b; Słota 1998]. The two measured size variants of Ag-NORs on chromosome 10 were used in the Polish "Pig Genome Mapping Project" (accomplished in 1997) for the identification of quantitative trait loci (QTLs) of genes controlling fattening, and carcass and meat quality traits in pigs [Żurkowski *et al.* 1995; Komisarek *et al*. 1998a]. However, Ag-NORs variants on chromosome 8, considered at the beginning of this project, were finally not classified as genetic markers due to their unstable activity and non-hereditary character [Komisarek *et al*. 1998b]. These results strongly suggest that future gene mapping experiments should rather apply rDNA FISH-signal variants as markers of chromosome 8, since FISH-signal variants are stable and consistently observed, even in the case of a small gene copy number or when transcriptional activity of some gene clusters fails to occur.

The two types of genetic markers – four fluorescent rDNA *in situ* hybridization signals and Ag-NORs size variants, classified in the present report, can be useful for characterization of pig breeds and QTLs mapping.

CONCLUSIONS

1. Chromosome markers of NORs – the rDNA signal and silver deposit size variants supplement genetic characteristics of Hampshire boars, which had been earlier drawn up on the basis of centromeric hetrochromatin markers.

2. The NORs size polymorphism survey can be applied for studies on differentiation of pig breeds as well as estimation of genetic distance or evolutionary relationships in domestic pigs or between domestic and wild pigs.

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Streszczenie. Wcześniejsze badania wykazały, że precyzyjnie określone i znormalizowane warianty wielkości NOR można uznać za markery chromosomowe, pomocne w charakterystyce rasowej świń i mapowaniu genów. Celem prezentowanych badań była identyfikacja i klasyfikacja wariantów wielkości regionów jąderkotwórczych (NOR) jako markerów genetycznych przydatnych do charakterystyki świń rasy hampshire. Na podstawie cytogenetycznej i molekularnej analizy (przeprowadzonej technikami Ag-I i FISH) sklasyfikowano cztery warianty wielkości sygnałów FISH oraz depozytów Ag-NOR. Regiony jąderkotwórcze były morfologicznie wyraźniejsze i większe na obu chromosomach 10 pary niż w parze 8, co sugeruje dominującą rolę chromosomu 10 w globalnej produkcji rybosomalnego RNA. Wyniki cytomolekularnej oceny polimorfizmu wielkości NOR mogą zostać wykorzystane w międzyrasowych lub międzygatunkowych analizach porównawczych oraz badaniach filogenetycznych w rodzinie *Suidae*.

Słowa kluczowe: knury rasy hampshire, regiony jąderkotwórcze (NOR), warianty wielkości, markery chromosomowe, technika FISH, metoda Ag-I