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Cytomolecular assay of size nucleolar organizer regions (NORs) polymorphism in Pietrain pigs

Cytomolekularna analiza polimorfizmu wielkości regionów jąderkotwórczych (NORs) u świń rasy pietrain

Summary. The aim of this study was to identify and classify the size variants of nucleolar organizing regions (NORs) as markers useful for genetic characteristics of Pietrain breed pigs. On the basis of cytomolecular analysis carried out by silver staining (Ag-I) and fluorescence *in situ* hybridization (FISH) techniques, four size variants of rDNA-FISH-signals and Ag-NORs silver deposits were classified. Results of cytomolecular assay of size NORs polymorphism can be used in inter-breed or inter-species comparative surveys and studies of evolutionary relationships in *Suidae* family.

Key words: pigs, chromosomes, nucleolar organizing regions (NORs), genetic markers, FISH technique

INTRODUCTION

Investigation of chromosome distribution of repeated sequences like ribosomal DNA is a key for evolutional cytogenetics concerning wild animals or detection of aneuploidy and chromosomal polymorphisms in farm animals. In domestic pigs, rDNA sequences are organized into two distinct gene classes. The 5.8S, 18S and 28S ribosomal RNA genes are localized as tandemly repeated clusters at the secondary constrictions of chro-

mosomes 8. and 10., called nucleolar organizer regions (NORs). The locations of rRNA gene clusters revealed by fluorescence *in situ* hybridization technique (FISH) with human rDNA probe correspond with the positions of silver stained NORs. Both fluorescent signals and silver deposits on NOR-bearing chromosomes demonstrate a clear size diversity resulting from number variation of repeated rDNA sequences and different level of their transcriptional activity. This phenomenon meets the criteria of polymorphism, and polymorphic NORs may be considered as chromosome markers (a special category of genetic markers). Size polymorphism of NORs (examined mainly by silver staining and only sporadically by FISH) has been reported in numerous pig breeds and hybrid lines in the world, including populations bred in Poland (Polish Landrace, Polish Large White, Hampshire, Duroc, Pietrain, Pulawska, Zlotnicka Spotted, 990 and 890 hybrid line) [Miyake *et al*. 1988; Mellink *et al*. 1991, 1994, 1996, Solinas-Toldo *et al*. 1992, Świtoński i Pietrzak 1992, Lomholt *et al*. 1995, Świtoński *et al*. 1997a, b, Słota 1998, Danielak-Czech *et al*. 1999, 2006, 2009].

The aim of this study was double cytomolecular identification and classification of NORs size variants in order to apply them as a chromosome markers for genetic characteristic of Pietrain breed.

MATERIAL AND METHODS

A cytogenetic analysis based on the FISH and Ag-I techniques was performed in population of 16 Pietrain pigs from individual farm. Studies were carried out on metaphase chromosomes 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 6.08 (Poland). 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: 0.101-0.150; III: 0.151-0.200; IV: 0.201-0.250 for pair 8).

FISH experiments were performed using biotynylated human 5.2kb *Bg/* II-*Eco*RI 18S+28S rDNA probe [Pinkel *et al*. 1986; Wachtler *et al*. 1986]. FITC-detected NORs were analyzed in DAPI counterstained chromosomes with fluorescence microscope equipped with the computer-assisted image analysis system LUCIA-FISH (Laboratory Imaging Ltd, Prague, Czech Republic). The rDNA FISH-signal variants were classified, proportionally to the size and intensity, as: 1 – small and weak, and 2, 3, 4 – large and strong.

RESULTS AND DISCUSSION

FISH and silver staining confirmed the location of rRNA genes in 8p11 and 10p11 chromosome regions of Pietrain pigs and revealed size and number polymorphism of NORs in the studied pigs. The detailed results of size variants evaluation are shown in Table 1.

Table 1. The rDNA-FISH signals and Ag-NORs size variants on 10. and 8. chromosome in Pietrain pigs
Tabela 1. Warianty wielkości sygnałów rDNA-FISH i Ag-NORs na chromosomach 10. i 8. u świń rasy pietrain

Animals Zwierzęta	Size variants – FISH Warianty wielkości – FISH 10 : 10 : 8 : 8				Size variants – Ag-NORs Warianty wielkości – Ag-NORs 10 : 10 : 8 : 8			
1.	2	1	3	2	0.212/II	0.177/1	0.170III	-
2.	**3**	**2**	**3**	**2**	**0.320/III**	**0.219/II**	**0.160/III**	**0.150/II**
3.	3	1	3	2	0.375/III	0.126/I	0.166/III	0.102/II
4.	2	1	3	1	0.202/II	0.130/I	0.170/III	-
5.	**3**	**1**	**4**	**4**	**0.360/III**	**0.125/I**	**0.246/IV**	**0.237/IV**
6.	2	2	3	3	0.250/II	0.220/II	0.190/III	0.185/III
7.	4	1	2	1	0.460/IV	0.110/I	0.0.102/II	-
8.	4	1	3	1	0.465/IV	0.181/I	0.190/III	-
9.	3	1	3	3	0.340/III	0.136/I	0.197/III	0.179/III
10.	3	1	4	2	0.319/III	0.145/I	0.220/IV	0.133/II
11.	3	1	4	3	0.303/III	0.126/I	0.215/IV	0.191/III
12.	3	2	4	2	0.325/III	0.260/II	0.222/IV	0.098/I
13.	3	2	4	3	0.328/III	0.214/II	0.205/IV	0.189/III
14.	3	2	4	4	0.360/III	0.281/II	0.216/IV	0.180/III
15.	3	3	4	1	0.379/III	0.350/III	0.210/IV	-
16.	2	1	3	3	0.255/II	0.182/I	0.140/III	0.129/II

FISH signals were consistently observed in four NOR sites, whereas silver deposits were often visible on three chromosomes only – two in pair 10. and one 8. Maximum differences in relative quantities of rDNA in all NOR-bearing chromosomes were estimated at about × 4 (range 1 to 4) within animals. Correspondingly, Ag-NORs measured variants were classified into size categories (I– IV) ranging from 0.110 (I) to 0.465 (IV) for chromosome pair 10, and 0.098 (I) to 0.246 (IV) for pair 8 (Tab. 1). The size diversity of rDNA signals and Ag-NORs was distinct enough to classify adequately into four size variants, comparable to the scale presented by Mellink *et al*. [1994], Słota [1998] and Danielak-Czech *et al*. [1999]. In one case only NORs in both chromosome pairs were ascertained to be expressed almost evenly and classified by the same size categories (the animal no 2) (Tab. 1, Fig. 1a, b). In remaining animals under study, the FISH signals and Ag-deposits on chromosome 8. were regularly classified as higher size variant values than on chromosome 10., what could be well exemplified by the most morphologically distinct and great NORs areas on both chromosome 8 in the pig no 5 (Tab. 1, Fig. 1c). As known from published data, some cases of unusually large NORs on chromosome 8 were found in previous studies in Pietrain and Yorkshire pigs as well as in the primitive Asiatic Meishan and Polish indigenous Złotnicka Spotted breeds. On the other hand, the results of NORs variation assays for the majority of Landrace populations currently bred in Europe pointed out the tendency to occurrence of prominent nucleolar organizing regions on chromosome 10., suggesting a dominant role of this chromosome in the production of ribosomal RNA [Mellink *et al*. 1994, Świtoński and Pietrzak 1992, Świtoński i in. 1997a, b, Słota 1998, Danielak-Czech *et al.* 1999, 2006, 2009].

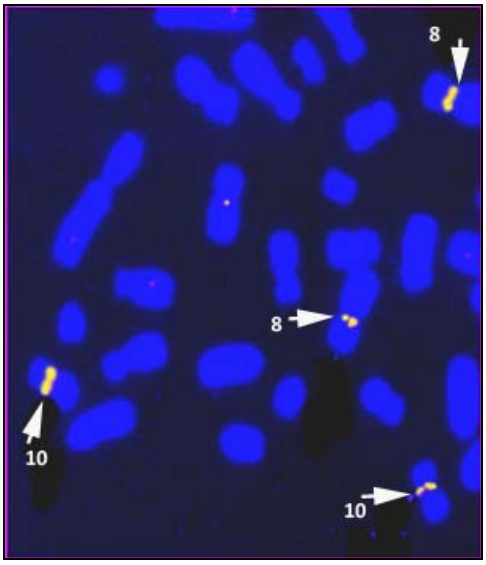

Fig. 1. Size polymorphism of NORs in metaphase chromosomes of Pietrain pigs: NORs after fluorescent *in situ* hybridization with the 18S + 28S rDNA probe in the pig no 2. Arrows show fluorescent signals (rDNA-FISH) on chromosome of 10. and 8. pair, classified as size variants: 3^+ and 2^+
Ryc. 1. Polimorfizm wielkości NORs w chromosomach metafazowych świń rasy pietrain: NORs po fluorescencyjnej hybrydyzacji *in situ* z sondą 18S + 28S rDNA u świni nr 2. Strzałki wskazują sygnały fluorescencyjne (rDNA-FISH) na chromosomach 10. i 8. pary, sklasyfikowane jako warianty wielkości 3^+ i 2^+

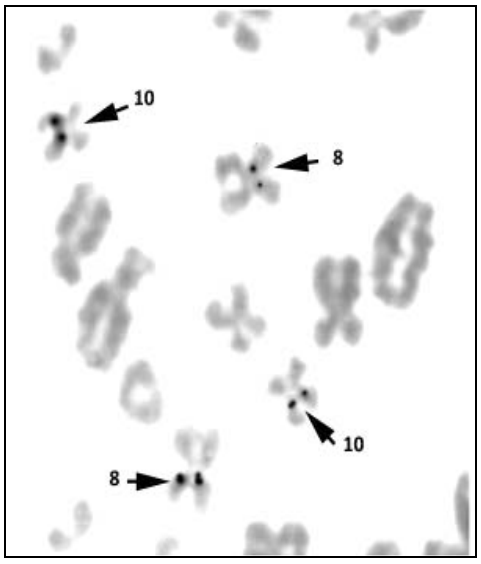

Fig. 2. Size polymorphism of NORs in metaphase chromosomes of Pietrain pigs: NORs after silver staining in the pig no 2. Arrows show silver deposits (Ag-NORs) on chromosomes 10. and 8. pair, classified as size variants: III and II
Ryc. 2. Polimorfizm wielkości NORs w chromosomach metafazowych świń rasy pietrain: NORs po barwieniu srebrowym u świni no 2. Strzałki wskazują depozyty srebrowe (Ag-NORs) na chromosomach 10. i 8. pary, sklasyfikowane jako warianty wielkości: III i II

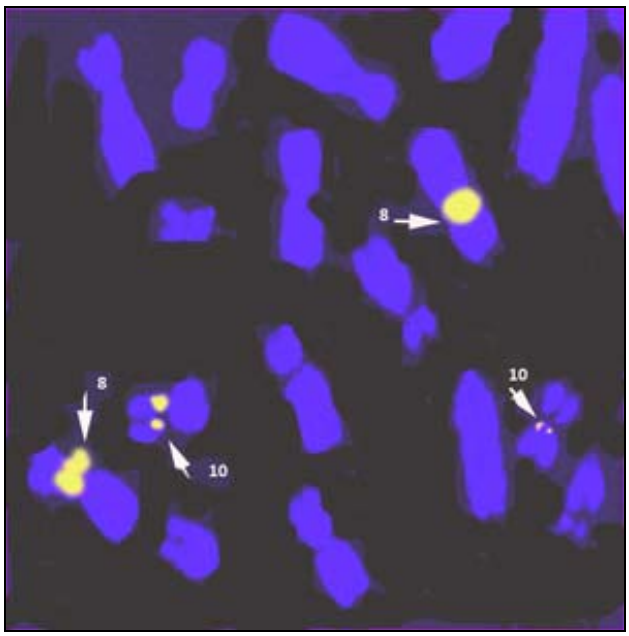

Fig. 3. Size polymorphism of NORs in metaphase chromosomes of Pietrain pigs: NORs after fluorescent *in situ* hybridization with the 18S+ 28S rDNA probe in the pig no 5. Arrows show fluorescence signals (rDNA-FISH) on chromosome 10. pair, classified as size variants: (3+/1+) and 8. pair valued as variants: (4+/4+)

Ryc. 3. Polimorfizm wielkości NORs w chromosomach metafazowych świń rasy pietrain: NORs po fluorescencyjnej hybrydyzacji *in situ* z sondą 18S + 28S rDNA u świni no 5 Strzałki wskazują sygnały fluorescencyjne (rDNA-FISH) na chromosomach 10. pary, sklasyfikowane jako warianty wielkości: (3+/1+) i 8. pary, określone jako warianty: (4+/4+)

On the whole, our findings 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].

CONCLUSIONS

1. Chromosome markers in the form of nucleolar organizing regions – the rDNA--FISH signals and Ag-NORs size variants classified in the presented study supplement genetic characteristics of Pietrain breed pigs, which had been earlier drawn up on the basis of centromeric hetrochromatin markers.

2. The results of cytomolecular assay of NORs polymorphism 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. Celem badań była identyfikacja i klasyfikacja wariantów wielkości regionów jąderkotwórczych (NORs) jako markerów genetycznych przydatnych do charakterystyki świń rasy pietrain. Na podstawie cytomolekularnej analizy przeprowadzonej technikami barwienia srebrowego (Ag-I) i fluorescencyjnej hybrydyzacji *in situ* (FISH) sklasyfikowano cztery warianty wielkości sygnałów rDNA-FISH oraz depozytów srebrowych Ag-NORs. Wyniki cytomolekularnej oceny polimorfizmu wielkości NORs mogą zostać wykorzystane w międzyrasowych lub międzygatunkowych analizach porównawczych oraz badaniach związków ewolucyjnych w rodzinie *Suidae*.

Słowa kluczowe: świnie**,** chromosomy, regiony jąderkotwórcze (NORs), markery genetyczne, technika FISH