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CHROMOSOME MARKERS SURVEY OF DUROC PIGS

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The aim of this study was to evaluate the nucleolar organizing regions (NORs) size variants as markers for genetic characteristics of the Duroc breed pigs. Size polymorphism of NORs in 16 Duroc pigs was identified by Ag-I staining method and fluorescent in situ hybridization (FISH). On the basis of microscope and computer image analysis four size variants of rDNA-FISH-signal and Ag-NOR silver deposits were classified. In general, the proportions of rDNA signals and silver deposits in 8 and 10 chromosome pairs were similar. The results obtained suggested that these markers can be used for characteristics of Duroc breed.

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; Mellink et al., 1996). 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. fullfils the criteria of polymorphism, and polymorphic NORs may be considered as chromosome markers. Size polymorphism of NORs has been found in numerous breeds of domestic pig (Mellink et al., 1994; Słota, 1998; Świtoński et al., 1997 a, b). In Polish pigs this phenomenon has been examined by FISH in several breeds (Polish Landrace, Duroc, Piertain, Polish Large White and Hampshire) (Danielak-Czech et al., 1999).

The aim of this study was to characterize Diuroc breed pigs on the basis of chromosome markers including size variants of nucleolar organizing regions.

Materials and methods

A cytogenetic analysis based on the FISH and Ag-I techniques was performed in population of 16 Duroc pigs from individual farm. Studies were carried out on 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 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: 0.101–0.115; III: 0.151–0.200; IV: 0.201–0.250 for pair 8) according to Słota (1998). FISH experiments were performed using biotynylated human 5.2 kb *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). Proportionally to intensity, rDNA FISH-signal variants were classified 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 at the paracentromeric area of chromosomes 8 and 10 in the karyotype of the Duroc pigs investigated. FISH signals were consistently observed in four NOR sites, whereas silver deposits, ranging from 2 to 4, were often visible on three chromosomes only — two in pair 10 and one 8. NORs revealed by double identification were more distinct morphologically and greater on both 10 than on pair 8, what could be well exemplified by size categories characterizing the pig no 6 (Fig. 1, Tab. 1).

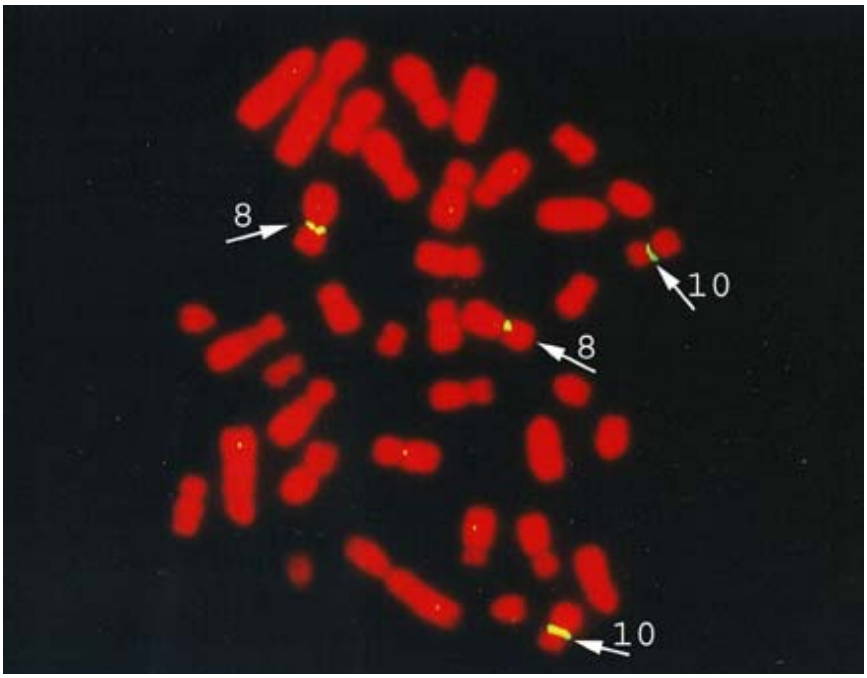

Figure 1. The rDNA fluorescent signals in NORs — metaphase chromosomes of Duroc pigs (animal no 6). Arrows indicate FISH signals on chromosomes of 10 pair (variants 4+ / 2+) and chromosomes of 8 pair (variants 2+ / 3+).

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 evaluation in all Duroc pigs examined are shown in Table 1. Maximum differences in relative quantities of rDNA in all NOR-bearing chromosomes were estimated at about x 4 (range 1 to 4) within animals. Correspondingly, Ag-NORs measured variants were classified into size categories (I–IV) ranging from 0.109 (I) to 0.477 (IV) for chromosome pair 10, and 0.075 (I) to 0.245 (IV) for pair 8. 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, Słotę, 1998 and Danielak-Czech et al., 1999. The experiment described revealed size and number polymorphism of NORs in the studied Duroc pigs. NORs variation assays has been reported in different populations and breeds in the world (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). 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. In Duroc pigs investigated, 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 Złotnicka Spotted breeds (.Mellink et al., 1994; Świtonski i in., 1997b, Słota, 1998; Danielak-Czech i in., 1999). 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).

Table 1

The rDNA FISH-signal and Ag-NOR size variants on 10 and 8 chromosome pairs of Duroc pigs

Pig No	rDNA FISH-signal variants				Ag-NOR size variants			
	10	10	8	8	10	10	8	8
1	4	1	3	1	0,465/IV	0,323/III	0,108/II	0,085/I
2	3	1	4	4	0,370/III	0,124/I	**0,245/I**	—
3	4	1	2	1	0,460/IV	**0,109/I**	**V**	—
4	2	1	3	1	0,202/II	0,130/I	0,102/II	—
5	3	1	3	2	0,375/III	0,126/I	0,170/III	0,101/II
6	**4**	**2**	**3**	**2**	**0,413/I**	**0,270/II**	0,166/III	**0,135/II**
7	3	1	2	2	**V**	0,177/I	**0,198/III**	0,114/II
8	3	1	2	1	0,322/III	0,145/I	**I**	**0,075/I**
9	3	1	2	2	0,319/III	0,126/I	0,06/II	—
10	4	3	3	3	0,303/III	0,319/III	—	—
11	2	1	3	2	**0,477/I**	0,177/I	0,42/II	—
12	4	2	2	1	**V**	0,212/II	—	—
13	3	2	3	1	0,212/II	0,214/II	0,70/III	—
14	3	3	2	1	0,474/IV	0,350/III	0,05/II	—
15	3	2	2	2	0,335/III	0,281/II	0,64/III	0,110/II
16	3	2	3	2	0,379/III	0,219/II	0,20/II	0,148/II
					0,360/III		0,14/II	
					0,320/III		0,60/III	

Chromosome markers of NORs — the rDNA signal and silver deposit size variants classified in the presented study supplement genetic characteristics of Duroc breed pigs, which had been earlier drawn up on the basis of centromeric heterochromatin markers (Christensen and Pedersen, 1991; Słota, 1998).

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S t r e s z c z e n i e

Celem badań była ocena wariantów wielkości regionów jąderkotwórczych (NORs) jako markerów genetycznych przydatnych do charakterystyki świń rasy Duroc. Metodą barwienia Ag-I i fluorescencyjnej hybrydyzacji in situ (FISH) zidentyfikowano polimorfizm wielkości NORs u 16 świń rasy Duroc. Na podstawie mikroskopowej i komputerowej analizy obrazu sklasyfikowano cztery warianty wielkości sygnałów rDNA-FISH oraz depozytów srebrowych Ag-NOR. Generalnie, proporcje sygnałów rDNA i depozytów srebrowych w chromosomach 8 i 10 pary były podobne. Uzyskane rezultaty sugerują, że markery te mogą być wykorzystane do charakterystyki rasy Duroc.

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ИССЛЕДОВАНИЕ ХРОМОСОМНЫХ МАРКЕРОВ СВИНЕЙ ПОРОДЫ ДЮРОК

А н н о т а ц и я

Целью этого исследования была оценка вариантов размера участков, которые формируют ядра (NORs) как маркеров генетических особенностей свиней породы Duroc. Полиморфизм размера NOR в 16 свиней породы Duroc был идентифицирован методом Ag-расцветки и методом флуоресцентной гибридизации in situ (FISH). Микроскопический и компьютерный анализ изображения четырех вариантов размера рДНК- FISH сигнала и серебряных компонентов в 8 и 10 парах хромосом, показал, что они были аналогичными. Полученные результаты свидетельствуют о том, что эти маркеры можно использовать для характеристики свиней породы Duroc.

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ДОСЛІДЖЕННЯ ХРОМОСОМНИХ МАРКЕРІВ СВИНЕЙ ПОРОДИ ДЮРОК

Р е з ю м е

Метою цього дослідження була оцінка варіантів розміру ділянок, які формують ядра (NORs) як маркерів генетичних особливостей свиней породи Duroc. Поліморфізм розміру NOR у 16 свиней породи Duroc був ідентифікований методом Ag-забарвлення і методом флуоресцентної гібридизації in situ (FISH). Мікроскопічний та комп'ютерний аналіз зображення чотирьох варіантів розміру рДНК-FISH сигналу та срібних компонентів у 8 та 10 парах хромосом, показав, що вони були аналогічними. Отримані результати свідчать про те, що ці маркери можна використовувати для характеристики свиней породи Duroc.

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