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Polymorphism of Nucleolar Organizer Regions on the 8th Pair of Pig Chromosomes

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Polymorphism of nucleolar organizer regions on the 8th pair of pig chromosomes was analysed. The relative size of silver stained NORs was measured in metaphasal plates of 30 animals. The relative size of silver stained NORs ranged from 0.102 to 0.188 m^2 in males and from 0.119 to 0.208 m^2 in females. The most frequently observed nucleolar organizer regions represented the third and the fourth size category (26.67% and 23.33% individuals, respectively).

Key words: Pig, nucleolar organizer regions.

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Nucleolar organizer regions, NORs, are fragments of chromosomes in which genes coding for ribosomal RNA (rRNA), making up ribosomal subunits, occur (CHARON & ŚWITOŃSKI 2000; PIEŃKOWSKA & ŚWITOŃSKI 1993). NORs are responsible for the structure, organization and formation of nucleoli in the process of cell protein synthesis. In pigs nucleolar organizer regions occur on two chromosome pairs, 8 and 10. Nucleolar organizer regions, due to considerable polymorphism, can be used as cytogenetic markers in the development of genetic distance of different breeds. The research also shows the applicability of linkage and mapping of genes conditioning functional characters of animals to analysis (SŁOTA 1998; ŚWITOŃSKI *et al.* 1997; ŚWITOŃSKI *et al.* 1994).

The aim of the present research is to analyse the frequency of occurrence and size of nucleolar organizer regions on chromosome pair 8 in pigs.

Material and Methods

The present research involved pigs representing three breeds, from herds of the Kujawy and Pomorze Province. Peripheral blood samples of 30 individuals (15 males and 15 females) were taken in test-tubes containing heparin as an anticoagulant. The blood sample was used to obtain the tissue culture, using Parker's, Eagle's medium or RPMI 1640 as medium (OLSZEWSKA *et al.* 1981). For karyotype analysis, the preparations were stained following standard procedures, with the use of Giemsa stain. Nucleolar organizer regions were identified with silver staining method Ag-I. The preparation image was computer analysed with the MultiScan-Karyotype software (KOZUBSKA-SOBOCIŃSKA *et al.* 1999) in order to measure the surface area of silver deposit and chromosome 8 as a whole. The size of Ag-NOR (μm^2) was compared with the surface area of the whole chromosome, defining its relative size. For each individual, measurements were taken in a few metaphasal plates, then the average value was calculated and classified into the adequate size category. Five size categories were assumed, depending on the NOR size obtained in chromosome pair 8 following the classification of SŁOTA (1998):

- First category – 0.000-0.050 μm^2
- Second category – 0.051-0.100 μm^2
- Third category – 0.101-0.150 μm^2

The studies were carried out in accordance with the permission of the Ethical Committee in Bydgoszcz (No.16/2001).

– Fourth category – 0.151-0.200 μm^2

– Fifth category – 0.201-0.250 μm^2

The measurements were grouped according to animal gender and the presence of silver deposits observed in both chromosomes or in a single chromosome of pair 8. Mean values for boars and sows were calculated. The frequency of Ag-NOR occurrence and percentage share of respective size categories of these regions in males and females were determined.

Results and Discussion

The results are presented in two tables. In two males NORs occurred on two chromosomes of pair 8. Six boars showed the occurrence of Ag-NORs on one homologue only, while in seven no Ag-NORs were noted (Table 1). Out of all the females examined, only one had nucleolar organizer regions on both homologues of the 8th chromosome pair. Eight sows showed the presence of Ag-NORs on a single chromosome, while six did not have silver deposits in the metaphasal plates in any chromosome of pair 8 (Table 1). In the examined

Table 1

Measurements of the relative size of nucleolar organizer regions on the 8th chromosome pair in pigs

Gender	L.p.	Ag-NORs on both homologues			L.p.	Ag-NORs on one homologue		
		size range (μm^2)	mean size (μm^2)	size category		size range (μm^2)	mean size (μm^2)	size category
boars	1.	0.095-0.152	0.120	III	1.	0.091-0.112	0.102	III
	2.	0.152-0.173	0.164	IV	2.	0.149-0.241	0.188	IV
					3.	0.109-0.115	0.112	III
					4.	0.119-0.155	0.137	III
					5.	0.141-0.156	0.149	III
					6.	0.168-0.171	0.170	IV
	total		0.138				0.146	
sows	1.	0.2010.217	0.208	V	1.	0.231-0.241	0.236	V
					2.	0.139-0.175	0.160	IV
					3.	0.182-0.210	0.196	IV
					4.	0.114-0.128	0.121	III
					5.	0.115-0.122	0.119	III
					6.	0.159-0.168	0.164	IV
					7.	0.118-0.185	0.152	IV
					8.	0.138-0.150	0.144	III
	total		0.208			0.114-0.241	0.165	
total		0.158				0.157		

Table 2

Frequency of occurrence and size category of nucleolar organizer regions on the 8th chromosome pair in pigs

	Boars (%)	Sows (%)	Total (%)
1. Occurrence:			
Ag-NORs on both homologues	13.39	6.67	10.00
Ag-NORs on one homologue	40.00	53.33	46.67
no Ag-NORs	46.67	40.00	43.33
2. Percentage share of category			
first category	–	–	–
second category	–	–	–
third category	33.33	20.00	26.67
fourth category	20.00	26.67	23.33
fifth category	–	13.33	6.67

individuals, a total of 10% showed the occurrence of nucleolar organizer regions on both chromosomes of pair 8 (Table 2). The occurrence of Ag-NORs on a single chromosome was only noted for 46.67% of individuals. In 43.33% of pigs no silver deposits were recorded (Table 2). In research reported by ŚWITOŃSKI *et al.* (1997) silver deposits were noted in all of 40 pigs. Most individuals (22 animals) showed the presence of Ag-NORs on both chromosomes, while 18 animals on one chromosome. Other studies have indicated variation in the number of active NORs in respective individuals, and even no evidence of genetic conditioning of the number of active regions on the 8th chromosome pair in pigs (SŁOTA 1998; SŁOTA *et al.* 2000).

The size of nucleolar organizer regions in the pigs examined ranged from 0.091 to 0.241 μm^2 (Table 1). High individual variation was identified, just as reported by other authors (SŁOTA *et al.* 2000; ŚWITOŃSKI *et al.* 1997). The mean size of the silver deposits from a few measurements in respective individuals ranged from 0.102 to 0.208 μm^2 (Table 1). Large differences were recorded in the size of nucleolar organizer regions which occurred on both chromosomes in individuals of different gender (Table 1). The results, however, concern only two boars and one sow, and so they cannot be generalized. The analysis of the size of nucleolar organizer regions in each individual on a single chromosome only showed that there was greater size variation in boars, from 0.091 to 0.241 μm^2. The calculated mean values of relative Ag-NOR sizes ranged from 0.102 to 0.188 μm^2. Sows showed a slightly smaller size range (0.114-0.241 μm^2). Their nucleolar organizer regions were larger. The mean size for respective individuals ranged from 0.119 to 0.236 μm^2 (Table 1). The mean size of the silver deposits measured on the 8th pair of chromosomes in pigs was greater in females: 0.165 μm^2 than in males: 0.146 μm^2. Taking into consideration the measurements in individuals of both sexes in total, the average relative size was 0.157 μm^2 (Table 1). Słota reports the size of silver deposits on the 8th chromosome pair in pigs as ranging from 0.060 to 0.244 μm^2; the most frequently observed value ranged from 0.101 to 0.150 μm^2 (SŁOTA 1998). The present results are thus similar.

The percentage share of the size category shows that in 33.33% of males NORs represented the third size category, and in 20.00% of boars – the fourth category (Table 2). None of the males examined showed Ag-NORs in the first, second and fifth size category, while 46.67% of individuals of this gender did not have any Ag-NOR at all. Out of all the females, 26.67% revealed nucleolar organizer regions in the fourth size category, 20.00% of sows showed NORs of the third size category. No female possessed Ag-NORs representing the first and second size categories.

In males the size of nucleolar organizer regions most frequently represented the third size category, and in females the fourth size category. However, if all the animals examined are taken into account, the greatest number of individuals (26.67%) had Ag-NORs of the third size category (Table 2). Similarly, SŁOTA (1998) suggested that the most frequent size category of NORs on the 8th chromosome pair was the third size category; he did not observe regions of the first category.

According to SŁOTA *et al.* (2000) and ŚWITOŃSKI *et al.* (1994) the size variants of nucleolar organizer regions can be used for gene mapping or polymorphic DNA sequences based on family research and linkage analysis.

Conclusions

Mean values of the relative size of silver deposits on the 8th chromosome pair in boars ranged from 0.102 to 0.188 μm^2.

The nucleolar organizer regions observed in females showed average values from 0.119 to 0.236 μm^2.

Out of all the animals researched, nucleolar organizer regions representing the third (26.67% individuals) and the fourth (23.33%) size category were most frequent.

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