

## Determination of Breed Differences in Ag-NORs Occurrence in Pigs

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The investigations involved pigs of three breeds: Pietrain, Zlotnicka Spotted and Polish Landrace. The polymorphism in the number and size of nucleolar organizer regions was analyzed in animals of different breeds. It was observed that the average size of Ag-NORs in pigs was higher in chromosome pair 10 than in pair 8. The largest nucleolar organizer regions were observed in Polish Landrace pigs. The silver stained NORs were most frequently identified in the Pietrain breed.

Key words: Pigs, nucleolar organizer regions.

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The nucleolar organizer regions identified as secondary constrictions in mitotic chromosomes, referred to as NORs (Nucleolar Organizer Regions), are responsible for the structure and organization of nucleoli during the cell cycle, which are then a base for the establishment of ribosomal subunits, which facilitate the synthesis of protein (CHARON & ŚWITOŃSKI 2000). The number of chromosomes with nucleolar organizer regions and the location of secondary constrictions is specific for each organism, allowing identification of the species, defining the respective taxon, as well as evaluating the adequacy of its karyotype.

As for *Sus scrofa domestica*, the nucleolar organizer regions occur in the eighth and ninth pair of chromosomes, on short arms, close to the centromere (SŁOTA 1998). The size and variation of the number of NORs defines the polymorphism of chromosomes equipped with these active regions.

The present paper aims at defining the polymorphism of NORs as far as their size and number in different pig breeds are concerned.

### Material and Methods

The research involved pigs of three breeds: Pietrain, Zlotnicka Spotted, and Polish Landrace (10

animals of each breed). A blood lymphocytes culture on Parker's, Eagle's and RPMI 1640 media were performed in order to obtain preparations of metaphasal chromosomes (OLSZEWSKA *et al.* 1981). The preparations were stained, following the standard procedure, with Giemsa stain. The nucleolar organizer regions were identified with silver staining method Ag – I. Measurements were taken of the surface area of silver deposits and chromosomes as a whole, in which they occurred, applying MultiScan Karyotype software (KOZUBSKA-SOBOCIŃSKA *et al.* 1999). A few metaphasal plates were evaluated for each individual, identifying the presence of (+) or absence (-) of deposits on the chromosomes of a given pair. The frequency of occurrence of Ag-NORs in animals of different breeds was defined, and the mean number per individual was calculated, depending on the breed, in reference to chromosome pairs 8 and 10. The share of silver deposits was calculated against their theoretically possible number. One individual can have four NORs, and so for 10 individuals of a given breed 100 % accounted for 40 Ag-NORs. Relative Ag-NORs values were determined in  $\mu\text{m}^2$ , referring their surface area to the surface area of the chromosome as a whole. The size range was given for the breed. Mean values were calculated for

each breed, size categories followed the classification by SŁOTA (1998).

## Results and Discussion

The results are presented in three tables.

Table 1 gives the number of individuals with 0, 1 or 2 Ag-NORs observed in respective chromosome pairs.

Chromosome pair 8 in the studied animals was most frequently observed with a single silver deposit. Only in three cases did two nucleolar organizer regions occur on this pair of homologues. Ag-NORs on chromosome pair 8 were not observed in half of the examined individuals of the Zlotnicka Spotted and Polish Landrace. Out of 10 Pietrain individuals, none had two nucleolar organizer regions (Table 1). SŁOTA 1998 reported that the most frequent occurrence of two silver deposits are in chromosome pair 8. Out of 175 individuals researched, 106 showed this number of Ag-NOR, 46 – a single deposit, and only 23 individuals had no nucleolar organizer region.

Table 1

Number of individuals demonstrating the presence of Ag-NORs on chromosomes of different pig breeds

Breed	Chromosome pair 8			Chromosome pair 10		
	variant:			variant:		
	+/+	+/-	-/-	+/+	+/-	-/-
Pietrain	–	7	3	10	–	–
Zlotnicka Spotted	2	2	5	6	4	–
Polish Landrace	1	4	5	6	4	–
Total	3	14	13	22	8	–

On chromosome pair 10 in each breed two silver deposits were most frequent. All the individuals of the Pietrain breed had Ag-NORs on both homologues. No metaphasal plate was found without nucleolar organizer regions on chromosome pair 10 (Table 1). SŁOTA (1998) reported that all the individuals investigated showed the occurrence of Ag-NORs on both homologues. ŚWITOŃSKI *et al.* (1997) observed the occurrence of two Ag-NORs on both homologues of pair 10 in all the Zlotnicka Spotted individuals examined.

On chromosome pair 8 the mean number of Ag-NOR was low, similar in the individuals of all breeds (Table 2). The percentage share of deposits on this pair of homologues accounted for 15 % only in Polish Landrace and 17.5 % both in Pietrain and Zlotnicka Spotted. The values presented in Table 2 demonstrate a small share of silver deposits on chromosome pair 8, which suggests a diversified activity of NORs or no activity at all. Only 20 Ag-NORs were observed in 30 pigs, which accounted for 0.67 regions per individual.

A total of 52 Ag-NORs were noted in pigs on chromosome pair 10, an average of 1.73 regions per individual (Table 2). On chromosome pair 10 the highest mean number, two silver deposits, was recorded in Pietrain (Table 2), which means that 50% of all the deposits which occur in the individuals of this breed was present just on this pair of homologues. Polish Landrace and Zlotnicka Spotted showed a lower number of nucleolar organizer regions, at a level of 1.6, which accounts for 40% of the total possible number of deposits (Table 2). The total for both chromosomes, the greatest number of silver deposits (27) was observed in Pietrain pigs, which accounts for 67.5% of the total possible number of deposits; on average 2.7 Ag-NORs per individual. Zlotnicka Spotted and Polish Landrace showed a lower number of nucleolar organizer regions than Pietrain, respectively: 23 and 22 regions, which accounts for on average 2.3 silver deposits per Zlotnicka Spotted individual, and

Table 2

Frequency of occurrence of Ag-NORs in different pig breeds

Breed	Chromosome pair 8			Chromosome pair 10			Chromosome pair 8 and 10 in total		
	Number of Ag-NORs observed	Mean NORs number per individual	Share of deposits (%)	Number of Ag-NORs observed	Mean NORs number per individual	Share of deposits (%)	Number of Ag-NORs observed	Mean NORs number per individual	Share of deposits (%)
Pietrain	7	0.7	17.5	20	2.0	50.0	27	2.7	67.5
Zlotnicka Spotted	7	0.7	17.5	16	1.6	40.0	23	2.3	57.5
Polish Landrace	6	0.6	15.0	16	1.6	40.0	22	2.2	55.0
Total	20	0.67	16.67	52	1.73	43.3	72	2.4	60.0

Table 3

Size of nucleolar organizer regions on chromosomes of different pig breeds

Breed	Chromosome pair 8			Chromosome pair 10		
	size range ( $\mu\text{m}^2$ )	mean size ( $\mu\text{m}^2$ )	size category	size range ( $\mu\text{m}^2$ )	mean size ( $\mu\text{m}^2$ )	size category
Pietrain	0.109-0.241	0.142	III	0.037-0.228	0.139	II
Zlotnicka Spotted	0.095-0.185	0.147	III	0.069-0.366	0.135	II
Polish Landrace	0.091-0.241	0.185	IV	0.084-0.341	0.196	II
Total	0.091-0.241	0.154	IV	0.037-0.366	0.187	II

2.2 per Polish Landrace individual. SŁOTA (1998) shows that out of all the breeds investigated, the highest number of Ag-NORs was also recorded in Pietrain (3.1) and the lowest – in Polish Landrace (2.4). Polymorphism of the number of silver deposits depending on the pig breed was also noted by MELLINK *et al.* (1994). A higher number of Ag-NORs was observed in wild pigs as compared to graded breeds.

Table 3 presents a size range, mean size and the mean Ag-NOR size category defined for chromosome pair 8 and 10 in animals of a given breed. Polymorphism of the size of nucleolar organizer regions was observed at the interbreed level in pigs.

On chromosome pair 8 the greatest Ag-NOR variation (0.091–0.241  $\mu\text{m}^2$ ) was recorded for Polish Landrace. The size range for Pietrain did not differ considerably (Table 3). The lowest size polymorphism on chromosome pair 8 was noted in Zlotnicka Spotted (0.095–0.185  $\mu\text{m}^2$ ).

Polish Landrace showed the greatest average size of deposits – 0.185  $\mu\text{m}^2$  on homologue pair 8. Average Ag-NOR sizes of Pietrain and Zlotnicka Spotted were similar; Pietrain showed an average size of deposits of 0.142  $\mu\text{m}^2$ , while the average for Zlotnicka Spotted was 0.147  $\mu\text{m}^2$  (Table 3).

Silver deposits of chromosome pair 8 in Polish Landrace represented on average the fourth size category, and in the other breeds – the third category.

As for chromosome pair 10, the greatest size range (0.069–0.366  $\mu\text{m}^2$ ) was recorded for Zlotnicka Spotted, while the lowest Ag-NOR size variation (0.037–0.228  $\mu\text{m}^2$ ) was observed in Pietrain.

On chromosome pair 10, just like on pair 8, nucleolar organizer regions in Polish Landrace showed the greatest average size – 0.196  $\mu\text{m}^2$ . As for Pietrain, the average Ag-NOR size was 0.139  $\mu\text{m}^2$ , and for Zlotnicka Spotted – 0.135  $\mu\text{m}^2$  (Table 3).

Ag-NORs which occur on homologues of pair 10 represented, on average, the second size category in all the breeds examined. While comparing the sizes of nucleolar organizer regions on chromosome pairs 8 and 10, for pigs of three breeds clearly larger nucleolar organizer regions were

noted on chromosomes of pair 10 and 8, respectively, 0.187  $\mu\text{m}^2$  and 0.154  $\mu\text{m}^2$  (Table 3).

SŁOTA *et al.* (1999, 2000) and MELLINK *et al.* (1994) indicated that the silver deposits on pig chromosomes of pair 10 were greater than on those of pair 8 in the individuals analyzed. In the present research Polish Landrace was the only one which showed a greater average Ag-NOR size on homologues of pair 10. In the other breeds the silver deposits were larger, although inconsiderably, on pair 8 (Table 3).

The greatest differences were observed for the average size of silver deposits between Polish Landrace and the other breeds. Ag-NORs of Polish Landrace were considerably larger on both chromosome pairs (Table 3), while Pietrain showed the greatest average number of deposits, their percentage share was greater than in the other breeds (Table 2). The results confirmed differences across breeds in the occurrence, number and size of nucleolar organizer regions on chromosomes in pigs.

## Conclusions

The largest nucleolar organizer regions, as compared with Pietrain and Zlotnicka Spotted breeds, were observed in Polish Landrace pigs: 0.185  $\mu\text{m}^2$  – chromosome pair 8 and 0.196  $\mu\text{m}^2$  – chromosome pair 10.

The greatest average number of Ag-NORs (2.7) per individual and the most frequent occurrence of silver deposits were recorded for Pietrain.

Calculations for the three breeds in total revealed a higher average Ag-NOR size on chromosome pair 10 than on pair 8 in pigs.

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