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Genetic variability of the *CRC* and *MYF4* genes in genetic resource, Přeštice Black-Pied pig

Abstract

The study of molecular genetic variability in genetic resources of farm animals is necessary for optimal conduct of their breeding and for understanding of relations between molecular markers and traits. The determination of variability in the CRC and the MYF4 genes and the evaluation of associations between these genetic markers and the total number born, the number born alive and the number weaned of piglets, weaning weight of piglets and farrowing interval were carried out in population of 102 Přeštice Black-Pied sows. The frequency of the allele CRC^n (0.083) was found out to be markedly lower than the frequency of the allele CRC^N and there was observed no sow of homozygous recessive genotype in the investigated sample population. In MYF4 locus, the higher frequency of the MYF^B allele was detected (0.711). A significant influence of the CRC gene polymorphism on number of piglets alive born in the $1^{\rm st}$ and the $1^{\rm st}$ - $6^{\rm th}$ litters was discovered. In the total number of born piglets the significant disparity were ascertained between the $MYF4^A/MYF4^A$ and $MYF4^B/MYF4^B$, $MYF4^A/MYF4^A$ and $MYF4^B/MYF4^B$ sows in the $2^{\rm nd}$ - $6^{\rm th}$ litters and the $1^{\rm st}$ - $6^{\rm th}$ litters. The obtained data are exploitable in genetic structure mapping, management of breeding and improvement of this breed and comparison with other pig genetic resources.

Key Words: genetic resource, pig, Přeštice Black-Pied, litter size, CRC gene, MYF4 gene

Zusammenfassung

Titel der Arbeit: Variabilität der Gene CRC und MYF4 im Genreservoir des Přešticer Schwarzbunten Schweines

Die Analyse der molekular-genetischen Variabilität der genetischen Herkünfte der Haustiere ist für ein optimales Zuchtmanagement sowie das Verständnis der Beziehungen zwischen den Molekularmarkern und Nutzmerkmalen notwendig. Untersucht wurden die Beziehungen zwischen der Variabilität der genetischen Marker *CRC* und *MYF4* und der Anzahl gesamt geborener, lebend geborener und Absatzferkel, den Absatzgewichten am 21. Tag sowie der Zwischentragezeit in einer 102 Muttersauen umfassenden Herde des Přešticer Schwarzbunten Schweines. Es ergaben sich deutlich niedrigere Frequenzen der Allele *CRC*ⁿ (0,083) als der Allele *CRC*^N und es wurden bei keiner Sau der untersuchten Population homozygot rezessive Genotypen gefunden. Am Lokus *MYF4* wurde eine höhere Allelfrequenz bei *MYF4*^B (0,711) beobachtet. Sowohl bei den 1. als auch den 1. bis 6. Würfen wurde ein signifikanter Einfluss des *CRC* Genpolymorphismus auf die Anzahl lebend geborener Ferkel nachgewiesen. Nachweisbare Unterschiede fanden sich bei der Anzahl gesamt geborener Ferkel der Sauen mit den Genotypen *MYF4*^A/*MYF4*^A und *MYF4*^A/*MYF4*^B, *MYF4*^A/*MYF4*^A und *MYF4*^B/*MYF4*^B bei den 2. bis 6. und den 1. bis 6. Würfen. Die gefundenen Ergebnisse sind sowohl zur Beurteilung der genetischen Struktur der Rasse, dem Zuchtmanagement und der Selektion als auch für den Vergleich mit anderen genetischen Schweineherkünften nutzbar.

Schlüsselwörter: Genreservoir, Schwein, Přešticer Schwarzbuntes Schwein, Wurfleistung, CRC Gen, MYF4 Gen

Introduction

One of goals of animal genetics is to locate and identify the loci that are responsible for economical important traits. Genetic resources of farm animals are very interesting branch of research, because these populations are not influenced by selection as much as the commercial hybrid lines. For preservation and conservation is needful to study genetic polymorphisms and their associations with production traits, as in improved breeds of pigs.

In the Czech Republic, the Přeštice Black-Pied pigs (PBP) have been declared as the genetic resource in 1991. At present, there are approximately 500 sows PBP bred in the Czech Republic, 350 of which are included in the population of genetic resources. These pigs are bred mainly in West Bohemia. The following 16 breeds have participated in their genetic improvement: local Bavarian pigs, Large White, Middle White, Swabian – Hall, Berkshire, Large Black, Suffolk, Sussex, Essex, Mirgorod, Livny, Wessex Saddleback, Pietrain, Welsh, Landrase and Hampshire (FIEDLER and SMITAL, 2001). The Přeštice Black-Pied pigs are characterised by high fertility and excellent maternal properties but their disadvantage is higher back-fat thickness, lower % of lean muscle and worse conversion of fodder.

The effect of the *CRC* gene (ryanodine receptor 1) on the manifestation of malignant hyperthermia syndrome (MHS), carcass traits and meat quality in pigs has been studied in detail (FUJII et al., 1991; FISHER and MELLETT, 1997; de SMET et al., 1998). In the *CRC*^N/*CRC*^N genotype higher pH₁ and pH₂₄, backfat thickness and average daily gain and lower carcass lean were discovered (LARZUL et al., 1997; KUHN et al., 1998; KŘENKOVÁ et al., 1999; TOR et al., 2001). The sows of *CRC*ⁿ/*CRC*ⁿ genotype achieve lower total number of born piglets, number of piglets born alive, number of weaned piglets and number of litters per life (WITTMANN et al., 1992; DVOŘÁK, 1994). Likewise, the negative influence of *CRC*ⁿ allele on sperm quality traits (volume, sperm concentration, mobility) in boars has proven (GREGOR and HARDGE, 1995; URBAN and KUCIEL, 2001).

The myogenin gene (*MYF4*), as well as *MYF3*, *MYF5* and *MYF6* genes, is a member of MYOD gene family (WEINTRAUB et al., 1991). The MYOD gene family controls a muscle fibre formation during embryonic development in mammals; the myogenin together with the *MYF3* gene induce the terminal transformation of myoblasts into myofibers (te PAS and VISSCHER, 1994). In the *MYF4* gene three *MspI* polymorphic sites, in the promoter region, the second intron and the 3'side of the gene, have been observed (ERNST et al., 1993; SOUMILLION et al., 1997).

The polymorphism at the 3'side of the MYF4 gene impacts on carcass traits and lean meat content in pigs. For example, CIESLAK et al. (2000) noted, that MYF4^A/MYF4^A homozygous animals obtain significantly higher half carcass meat weight, meat (%), ham meat weight, ham meat ratio, loin meat weight, loin meat ratio and loin eye area. Not much is known about the relationship between the polymorphism at the 3'side and fertility in pigs. It is associated with birth weight and mortality of piglets (te PAS et al., 1999). KANIAK – POLOK et al. (2001) described differences in several reproductive traits (e.g. number of piglets born alive, number of piglets at the 21 day, farrowing interval) between the sows of MYF4^A/MYF4^A and MYF4^B/MYF4^B genotypes. The primary target of this study is to assess the genetic variation within Přeštice Black-Pied pigs by means of molecular genetic markers CRC and MYF4. A secondary aim is to evaluate the associations between polymorphism of these genes and reproduction traits in sows.

Materials and Methods

In the investigated population, there were included 102 Přeštice Black-Pied sows from several pedigree breedings. The genomic DNA was isolated from blood samples by using QIAamp®DNA Blood Mini Kit (QIAGEN GMBH).

The particular parts of the *CRC* and *MYF4* gene sequences were amplified by polymerase chain reactions as described by BRENIG and BREM (1992) and SOUMILLION et al. (1997), respectively. The predicted sizes of PCR products were 134 bp (the *CRC* gene) and 353 bp (the *MYF4* gene).

For the detection of mutant alleles the RFLP method was used. The restriction of the CRC gene PCR product was carried out by endonuclease Hin6I (FUJII et al., 1991). If the restriction site is present (wild allele CRC^N), the PCR product is cut into two fragments (84 and 50 bp). In the presence of the mutant allele (allele CRC^n), the PCR product stays intact. The PCR product of the MYF4 gene is digested by endonuclease MspI into 219 bp and 134 long fragments (allele $MYF4^A$). No restriction site is involved in the allele $MYF4^B$. Therefore, the PCR product is not cleaved (SOUMILLION et al., 1997).

The polymorphism degree of these candidate loci was determined by rate of heterozygosity (NEI, 1978) and polymorphism information content - PIC (BOTSTEIN et al., 1980). A general linear model (SAS, 2000) was used for the analysis of the associations between polymorphisms in the *CRC* and *MYF4* genes and total number of born piglets - TNB, number of piglets born alive - NBA, number of weaned piglets - NW, weaning weight of piglets (at the age of 21 days) - WW and farrowing interval - IF. Because of low significance of the 1^{st} litters, we evaluated the effect of *CRC* and *MYF4* genotypes on reproductive traits in sows for the 1^{st} , the 2^{nd} – 6^{th} and the 1^{st} – 6^{th} litters separately. In the fixed effect were included: genes *MYF4*, *CRC*, herd, year of sow birth and litter parity.

The models were as follows:

for the 1st litters

$$Y_{ijkl} = \mu + MYF4_i + CRC_j + H*Y_k + e_{ijkl}$$

and for the $2^{nd} - 6^{th}$ and the $1^{st} - 6^{th}$ litters

$$Y_{ijklm}\!=\!\mu + MYF4_{i}\!+\!CRC_{j} + H^{*}Y_{k} + L_{l} + e_{ijklm}$$

where: $Y_{ijkl\ (m)}$ – trait value, μ – general mean, MYF4 $_i$ – the effect of *MYF4* genotype (i = 1, 2, 3), CRC $_j$ – the effect of *CRC* genotype (j =1, 2), H*Y $_k$ – the effect of the interaction between the herd and the year of sow birth (k = 1, 2, 3, 4, 5, 6, 7, 8, 9), L_l – the effect of the litter parity (l = 1, 2, 3, 4, 5, 6), $e_{ijkl(m)}$ – random residual

Table 1 Frequencies of genotypes and alleles in the *CRC* and *MYF4* genes in Přeštice Black-Pied sows (n = 102) (Genotypen- und Allelfrequenz der Gene *CRC* und *MYF4* bei Sauen der Rasse Přešticer Schwarzbuntes Schwein (n - 102))

Locus	Genotype	Frequency of genotypes	Allele	Frequency of alleles
CRC	$CRC^{N}/CRC^{N} \ CRC^{N}/CRC^{n} \ CRC^{n}/CRC^{n}$	0.833 0.167 0	CRC^{N} CRC^{n}	$0.917 \pm 0.019 \\ 0.083 \pm 0.019$
MYF4	MYF4 ^A /MYF4 ^A MYF4 ^A /MYF4 ^B MYF4 ^B /MYF4 ^B	0.078 0.422 0.500	MYF4 ^A MYF4 ^B	0.289 ± 0.032 0.711 ± 0.032

Results

Genetic variability in the CRC and MYF4 gene

In our study a lower frequency of the allele CRC^n (0.083) than the frequency of the allele CRC^N in PBP sows was observed. There was no sow with genotype CRC^n/CRC^n in the investigated population. The frequency of dominant homozygous genotypes CRC^N/CRC^N was very high compared to genotypes CRC^N/CRC^N (Table 1).

The frequency of the allele $MYF4^B$ was found to be higher than the frequency of allele $MYF4^A$. There were more sows with $MYF4^B/MYF4^B$ genotypes than $MYF4^A/MYF4^B$ heterozygotes in the population of PBP. The frequency of genotypes $MYF4^A/MYF4^A$ was clearly the lowest (Table 1).

CRC locus showed lower heterozygosity and polymorphism information content than MYF4 (Table 2), because of missing recessive homozygotes CRC^n/CRC^n .

Table 2 Summary of the polymorphism level for both loci in Přeštice Black-Pied pigs: heterozygosity (Het.) - NEI (1978); polymorphism information content (PIC) - BOTSTEIN et al. (1980) (Polymorphismus-Niveau beider Loci der untersuchten Sauen)

Locus	Het.	PIC
CRC	0.1538	0.1400
MYF4	0.4151	0.3270
Average Het.	0.2844	

Associations of the polymorphism in the CRC gene with reproduction traits

In the 1^{st} litters the polymorphism in the CRC gene influenced significantly NBA (P \leq 0.05). The dominant homozygous sows showed also higher TNB, NW and WW than heterozygous sows (Table 3). No significant differences were found out between CRC^N/CRC^N and CRC^N/CRC^n genotypes in the $2^{nd}-6^{th}$ litters, although homozygous sows were better in TNB, NBA, NW and WW as well as in the 1^{st} litters. However, the sows of CRC^N/CRC^n genotypes had shorter farrowing interval.

Table 3 Associations between reproduction traits and genotypes of the CRC gene and the MYF4 gene in Přeštice Black-Pied sows (least-square means LSM \pm standard error S_E) (Beziehungen zwischen den Wurfleistungsmerkmalen und Genotypen der Gene CRC und MYF4 der untersuchten Sauen (LSM und S_E))

und Genotypen der Gene CRC und $MTF4$ der untersuchten Sauen (LSM und S_E))									
Genotypes	NL	TNB	NBA	NW	WW	IF			
the 1 st litters									
CRC^N/CRC^N	85	11.52 ± 0.32	$10.57^* \pm 0.32$	9.81 ± 0.29	54.40 ± 1.88	-			
CRC^{N}/CRC^{n}	17	10.68 ± 0.65	$9.17^* \pm 0.64$	9.06 ± 0.59	49.32 ± 3.58	-			
$MYF4^A/MYF4^A$	8	11.03 ± 0.91	9.34 ± 0.90	9.19 ± 0.83	50.52 ± 5.21	-			
$MYF4^A/MYF4^B$	43	11.19 ± 0.43	10.11 ± 0.42	9.61 ± 0.39	52.70 ± 2.34	-			
$MYF4^{B}/MYF4^{B}$	51	11.08 ± 0.36	10.16 ± 0.36	9.51 ± 0.33	52.36 ± 2.03	=			
the 2 nd - 6 th litters									
CRC^N/CRC^N	368	13.11 ± 0.21	11.86 ± 0.19	10.33 ± 0.17	58.17 ± 1.24	169.76 ± 3.08			
CRC^{N}/CRC^{n}	83	12.57 ± 0.40	11.28 ± 0.36	10.23 ± 0.32	56.95 ± 2.19	165.62 ± 5.81			
$MYF4^A/MYF4^A$	39	$14.06^{**,***} \pm 0.55$	11.92 ± 0.50	10.12 ± 0.45	56.34 ± 3.05	165.58 ± 8.10			
$MYF4^A/MYF4^B$	186	$12.04^{***} \pm 0.27$	11.28 ± 0.25	10.27 ± 0.22	57.65 ± 1.51	169.44 ± 3.99			
$MYF4^B/MYF4^B$	226	$12.44^{**} \pm 0.24$	11.52 ± 0.21	10.45 ± 0.19	58.68 ± 1.31	168.05 ± 3.46			
the 1 st - 6 th litters									
CRC^N/CRC^N	453	12.85 ± 0.18	$11.65^* \pm 0.16$	10.27 ± 0.15	57.74 ± 1.03	=			
CRC^{N}/CRC^{n}	100	12.25 ± 0.35	$10.93^* \pm 0.32$	10.04 ± 0.28	55.98 ± 1.87	-			
$MYF4^A/MYF4^A$	47	$13.52^{**,**} \pm 0.48$	11.47 ± 0.44	9.96 ± 0.40	55.66 ± 2.65	-			
$MYF4^A/MYF4^B$	229	$11.92^{**} \pm 0.24$	11.10 ± 0.22	10.19 ± 0.19	57.07 ± 1.27	-			
$MYF4^{B}/MYF4^{B}$	277	$12.22^{**} \pm 0.20$	11.30 ± 0.18	10.32 ± 0.17	57.85 ± 1.09	-			

Note: NL-number of investigated litters, TNB- total number of born piglets, NBA- number of piglets born alive, NW-number of weaned piglets, WW - weaning weight of piglets (at the age of 21 days), IF - farrowing interval;

Values with superscripts in columns show significant differences: $*P \le 0.05$, $**P \le 0.01$, $***P \le 0.001$

The significant associations (P \leq 0.05) between polymorphism in the *CRC* gene and NBA were also observed in a joint analysis of the $1^{st} - 6^{th}$ litters.

Associations of the polymorphism in the MYF4 gene with reproduction traits

The highest TNB, NW and WW in the 1st litters were recorded in heterozygous sows. The $MYF4^B/MYF4^B$ sows produced most born alive piglets but no differences were significant (Table 3). In the $2^{nd}-6^{th}$ litters, we determined significant differences between sows of $MYF4^A/MYF4^A$ and $MYF4^B/MYF4^B$ genotypes (P \leq 0.01) and between sows of $MYF4^A/MYF4^A$ and $MYF4^A/MYF4^B$ genotypes (P \leq 0.001) in TNB. Homozygous $MYF4^A/MYF4^A$ sows excelled in number of born alive piglets. NW and WW from $MYF4^B/MYF4^B$ sows were higher than those from other genotypes. The longest farrowing interval was found out in heterozygous sows.

The similar results we detected in the $1^{\rm st}-6^{\rm th}$ litters, where significant differences (P \leq 0.01) in TNB between sows of $MYF4^A/MYF4^A$ and $MYF4^B/MYF4^B$ genotypes as well as between sows of $MYF4^A/MYF4^A$ and $MYF4^A/MYF4^B$ genotypes were evaluated.

Discussion

The absence of CRC^n/CRC^n genotypes and low frequency of the recessive allele in CRC loci were surprising. On the basis of references, we supposed the incidence of the CRC^n allele in PBP sows is more frequent. For instance, ČEPICA et al. (1982) described that an occurrence of this allele is 0.348 (more recent data about PBP are not available). The substantial reduction of the recessive allele numerousness must have been caused by efforts of exclusion of CRC^n/CRC^n and CRC^n/CRC^n genotypes from reproduction in pedigree breedings that were performed despite declaration of Přeštice Black-Pied pigs as the gene reserve. Thereby, the immigration of CRC^n alleles from Landrase breed that was used for the genetic improvement of PBP in 1986 (KLUSÁČEK et al., 1991), could not increase the frequency of the recessive allele in PBP sows either.

If we compared our data with references about the *CRC* gene variability in Landrase (L) and Large White (LW) breed in the Czech Republic, we found slight differences only. BEČKOVÁ et al. (2002) and MATOUŠEK et al. (2003) noted the frequency of the recessive allele 0.01 in Landrase sows and 0.025 – 0.045 in Large White sows. However, the incidence of the *CRC*ⁿ allele in L and LW breed was far higher a few years ago – 0.22 and 0.06, respectively (KAHÁNKOVÁ et al., 1996). Well, just as the frequency of the recessive allele has dropped in L and LW breed, so it has in Přeštice Black-Pied sows.

There are no original data about the MspI variability of the MYF4 gene in Přeštice Black-Pied pigs accessible, so we could not assess if changes of genotypes and alleles frequencies have happened within evolution of this breed. It is remarkable that similar results ($MYF4^B = 0.68$) were obtained in autochthonous breed – Zlotnicka Spotted in Poland (CIESĹAK et al., 2000). In contrast to the PBP breed, there is more frequent occurrence of the $MYF4^A$ allele than the $MYF4^B$ allele in L (0.545 – 0.680) and LW (0.750) breed (KOLAŘÍKOVÁ et al., 2002; PUTNOVÁ et al., 2001). These dissimilarities may be explained by more stringent selection to carcass characteristics and meat quality in L and LW breed, because the $MYF4^A$ allele conditions a superior level of these traits (te PAS et al., 1999).

It is obvious from Table 2, that *CRC* and *MYF4* loci demonstrate a different degree of polymorphism measured by heterozygosity and by polymorphism information content in the PBP population. This finding can be elucidated by marker assisted selection implemented in the *CRC* gene as opposed to the *MYF4* gene.

The evaluation of associations between the variability in the CRC gene and reproductive traits in PBP sows proved the outcomes in other maternal breeds (DVOŘÁK, 1994; KMIEĆ et al., 2002), because the sows of CRC^N/CRC^N genotypes showed the higher TNB, NBA and NW than the heterozygous ones.

Piglets of $MYF4^A/MYF4^A$ genotypes have greater birth weight, which decreases their mortality (te PAS et al., 1999). Therefore, we expected the sows of $MYF4^A/MYF4^A$ genotypes would achieve more weaned piglets compared to $MYF4^B/MYF4^A$ or $MYF4^A/MYF4^B$ genotypes. These assumptions were not shown to be correct in this study. The homozygous $MYF4^A/MYF4^A$ sows surpassed the sows of $MYF4^A/MYF4^B$ and $MYF4^B/MYF4^B$ genotypes in TNB and NBA in the $2^{nd} - 6^{th}$ and the $1^{st} - 6^{th}$ litters. Nevertheless, in NW as well as in WW were the sows of $MYF4^B/MYF4^B$ genotypes the best. Some researches point out potential associations of the polymorphism at the 3'side of the MYF4 gene on fertility (KANIAK – POLOK et al., 2001), but this hypothesis neither has been confirmed nor has been disproved so far.

That said data could assist in genetic structure assessing, the proposal of measures concerning the breeding and improvement of this breed as well as for comparison with other genetic resources of pigs. The loci described here are utilized for this purpose well. The increment of loci number and inclusion of all chromosomes in the evaluation are essential (WITTMANN and DOHY, 1999) for proper genetic appraisal of Přeštice Black-Pied pigs.

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