Boar taint through the eyes of genetics: A comparison of the Czech indigenous pig breed and commercial breeds in four gene polymorphisms related to skatole and androstenone levels

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Abstract

The problem of boar taint has recently become a frequent topic due to the ban on surgical castration of piglets without anaesthesia. Genetic selection based on markers that affect the molecular synthesis and degradation of the two main compounds of boar taint - androstenone and skatole - appears to be one of the possibilities. Many genes and their expression profiles associated with androstenone and skatole levels are known, and studies usually analyse them in hybrids. Our study focused on pure pig breeds and their comparison in the genotypes of the *CYP2E1*, *TEAD3*, *HSD3B*, and *CYB5A* genes. We examined four common commercial breeds (Large White, Landrace, Pietrain, Duroc) and compared them with the original Czech autochthonous Přeštice Black Pied breed, which is also classified as a gene source, with the breed being highlighted for its meat quality. Our research shows that there are differences between purebred individuals. Due to breeding programs, genotyping of breeding boars and sows is not only possible but also relevant.

Swine, gene resource, genotyping, genetic markers

The Přeštice Black Pied breed (PBP) is a Czech indigenous breed with a contribution of various European breeds to the PBP gene pool (Václavková and Bělková 2019). Since 1992, the PBP pig breed has been declared an Animal Genetic Resource. The breed has been bred in a closed population since 1996. It has been prized since the past to this day for its unique meat characteristics.

Previous studies examined the relationship of gene expression and levels of androstenone and skatole (e.g. Moe et al. 2007; Moe et al. 2008; Drag et al. 2017) and research involved genotyping in the single-nucleotide polymorphisms (SNPs) of these genes (e.g. Zamaratskaia et al. 2008; Mörlein et al. 2012; Robic et al. 2012). The *CYP2E1* gene product is one of the most important enzymes in the synthesis and metabolic pathway of skatole (Diaz and Squires 2000). The *TEAD3* (*TEF-5*) gene and its product appear to be among the most important in androstenone metabolism, the presence of which causes a urine-like pork odour (Robic et al. 2011). Robic et al. (2014) review a list of genes involved in steroid pathways, including *HSD3B* and *CYB5*. The enzyme 3β-hydroxysteroid dehydrogenase (gene *HSD3B*) is part of the first phase of the metabolic process in hepatic degradation of androstenone (Doran et al. 2004). Cytochrome b5 (*CYB5A*) is part of the molecular pathways of 16-androstenone steroid synthesis (Davis and Squires 1999).

Knowledge of the genetic population structure of breeding individuals is important for breeding management and, above all, for the preservation of genetic variability. Selection against boar taint can also affect other important economic, production, and reproductive traits. Duarte et al. (2021) provide an overview of the correlations between androstenone, skatole, indole and traits such as gestation length, litter mortality, total number born,

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Table 1. Primer sequences and amplification fragment size of studied markers.

Marker	Primer sequence	Amplification fragment size (bp)	Reference
CYP2E1	F 5′ CCC TTA ATT TTC TAC AGT AA 3′ R 5′ GCA ACC CCA GTG GTA C 3′	209	Mörlein et al. 2012
TEAD3	F 5′ AGG ACG CCT TCA CCC TAG AC 3′ R 5′ GGC TCC AAC TCC AGA TGT TC 3′	312	Robic et al. 2012
HSD3B	F 5′ TGC AGA GGG AGC AAT GAC TA 3′ R 5′ CTT CCT GAA TCG TGG CTT TC 3′	512	Kim et al. 2013
	CONTROL F 5′ TAT TAC ACC CTG GAA GAG GAT 3′		
	MUTANT F 5′ GCC TGA GGT TCG CCG CT 3′	74	Zamaratskaia et al. 2008
CYB5A	WILDTYPE F 5′ GCC TGA GGT TCG CCG CG 3′		
	EXON R 5′ TAC ACT TTG TGG TGC AGG ATT AG 3′	128	

testes weight, *Glandula bulbourethralis*, and others. Mathur et al. (2013) presented the differences between the two dam lines and the sire lines in the correlation of boar taint compounds and reproduction traits.

Many studies involved genotyping in the SNPs of these genes focused on the final hybrids (e.g. Zamaratskaia et al. 2008; Mörlein et al. 2012). However, it is important to address the frequencies of genotypes and alleles in genes of interest in pure breeds, as they carry the genetic pool. Even more important is the information on the genotyping of breeds that are classified as genetic resources, such as the PBP breed in our country. This research focuses on the description of the genetic structure in four genes of the PBP breed genetic resource and its comparison with the genetic structure of conventional breeds.

Materials and Methods

Fifty breeding boars of four common commercial breeds Large White (LW), Pietrain (PN), Duroc (D), Landrace (LA) and the Czech autochthonous native breed Přeštice Black Pied (PBP) which has been declared a genetic resource, were selected for the study. A total of 250 breeding boars born in the years 2017–2019 (LW), 2016–2019 (LA), 2015–2019 (D), 2015–2019 (PN), 2012–2015 (PBP) included in breeding programmes in the Czech Republic, were genotyped. Four genetic markers associated with boar taint *CYP2E1* (g.2412C>T), *TEAD3* (g.726 C>T), *HSD3B* (g.165262G>A), *CYB5A* (c.-8G>T) were analysed by PCR-RFLP and allele-specific PCR.

DNA isolation and purification were performed from hair, tissue, and blood samples using a commercial Genomic DNA Mini Kit (Blood/Cultured Cell; Geneaid; New Taipei City; Taiwan) and NucleoSpin®Tissue (Macherey-Nagel GmbH & Co. KG; Duren; Germany) according to the manufacturer's instructions.

The conditions of PCR reactions and RFLP were determined according to the references, including sets of specific primers (Table 1). The reaction mixture consisted of 12.5 µl PPP Master Mix (Top Bio; Vestec; Czech Republic), 9.5 µl PCR water (Top Bio; Vestec; Czech Republic), 1.0 µl of each primer, 1.0 µl DNA.

The polymorphisms were analysed by endonuclease restriction (Table 2). The $CYP2E1^T$ allele remained uncleaved at 209 bp, while the $CYP2E1^C$ allele cleaved into fragments 109 and 100 bp. The allele $TEAD3^C$ was represented by fragments 160, 65, 48, 39 bp, and the allele $TEAD3^T$ by 225, 48, 39 bp fragments. Fragments 442 and 70 bp represented the $HSD3B^A$ allele, fragments 259, 183, 70 bp represented the $HSD3B^G$. For the CYB5A marker, two allele-specific consecutive PCRs were performed according to Zamaratskaia et al. (2008) to distinguish firstly $CYB5A^G/CYB5A^T$, $CYB5A^T/CYB5A^T$ firstly and secondly the $CYB5A^G$/ $CYB5A^G$ genotype.

Data analysis was performed with the software Genepop v. 4.7 (Rousset 2008), MS Excel and software R version 4.1.3. The genotype and allele frequencies, the Hardy-Weinberg equilibrium (HWE), Pearson's χ^2 test were determined. Differences between breeds were compared using genotype frequencies.

Table 2. Conditions of endonuclease restriction.

Marker	Buffer	Restriction enzyme	Fragment size (bp)	Incubation temperature (°C)	Incubation time	Agarose gel (%)
CYP2E1	CutSmart	BtsMutI	109, 100	55	3 h	4
TEAD3	CutSmart	HaeIII	225, 160, 65, 48, 39	37	3 h	4
HSD3B	CutSmart	BsmAI	442, 259, 183	55	3 h	3
CYB5A	-	-	74, 128	-	-	4

Results

Fifty individuals from each of the five studied breeds were successfully genotyped in *CYP2E1*, *TEAD3*, *HSD3B* and *CYB5A*. Table 3 provides an overview of genotype frequencies. In the *CYP2E1* the homozygous *CC* genotype did not occur in PBP and PN, and the frequency of *TT* genotype was the highest in these two breeds. The homozygous *TT* in the *TEAD3* was completely lacked in LW, and its highest frequency was found in PBP. In the *HSD3B* marker, the frequency of the *GG* genotype was zero in the LA and PN breeds, and the highest frequency in D. In the PBP, LW, LA, PN breeds, no individuals with the *TT* genotype in the *CYB5A* marker were found; in the D breed, there was one individual out of 50 tested.

The results of allele frequencies are presented in Table 4. In the *CYP2E1*, the frequency of the *T* allele was the highest in PN. The highest frequency of the *T* allele in the *TEAD3* was found in PBP. In the *HSD3B* marker, the frequency of the *G* allele was highest in D and none in PN. The highest frequency of the *T* allele in *CYB5A* was in D; very low frequencies in other breeds were followed with no *T* allele in LW.

Table 3. The genotype frequencies of five Czech pig breeds in genes associated with boar taint.

	CYP2E1			*TEAD3*			*HSD3B*			*CYB5A*		
	CC	*CT*	*TT*	*CC*	*TC*	*TT*	*AA*	*GA*	*GG*	*GG*	*GT*	*TT*
PBP	0.00	0.58	0.42	0.34	0.50	0.16	0.60	0.38	0.02	0.96	0.04	0.00
LW	0.22	0.50	0.28	0.82	0.18	0.00	0.76	0.18	0.06	1.00	0.00	0.00
LA	0.40	0.48	0.12	0.50	0.38	0.12	0.90	0.10	0.00	0.82	0.18	0.00
PN	0.00	0.14	0.86	0.34	0.54	0.12	1.00	0.00	0.00	0.92	0.08	0.00
D	0.16	0.50	0.34	0.44	0.52	0.04	0.14	0.46	0.40	0.76	0.22	0.02

PBP – Přeštice Black Pied breed; LW – Large White; LA – Landrace; PN – Pietrain; D – Duroc

Table 4. The allele frequencies of five Czech pig breeds in genes associated with boar taint.

	CYP2E1[a]		*TEAD3*[b]		*HSD3B*[c]		*CYB5A*[d]	
	C	*T*	*C*	*T*	*A*	*G*	*G*	*T*
PBP	0.29	0.71	0.59	0.41	0.79	0.21	0.98	0.02
LW	0.47	0.53	0.91	0.09	0.85	0.15	1.00	0.00
LA	0.64	0.36	0.69	0.31	0.95	0.05	0.91	0.09
PN	0.07	0.93	0.61	0.39	1.00	0.00	0.96	0.04
D	0.41	0.59	0.70	0.30	0.37	0.63	0.87	0.13

PBP – Přeštice Black Pied breed; LW – Large White; LA – Landrace; PN – Pietrain; D – Duroc
[a] The allele *T* is favourable, the allele C is associated with higher skatole concentration in *CYP2E1* g.2412C>T
[b] The allele *T* is associated with lower androstenone concentration in *TEAD3* g.726 C>T
[c] The allele *G* is associated with lower androstenone concentration in *HSD3B* g.165262G>A
[d] The allele *T* is associated with lower androstenone concentration in *CYB5A* (c.-8G>T)

Table 5. Significant differences between five Czech pig breeds in genes associated with boar taint.

	LW	LA	PN	D
PBP	*CYP2E1 P* = 0.0017	*CYP2E1 P* < 0.0000	*CYP2E1 P* < 0.0000	*CYP2E1 P* = 0.1280
	TEAD3 P < 0.0000			
		HSD3B P = 0.0028	*HSD3B P* < 0.0000	*HSD3B P* < 0.0000
			CYB5A P = 0.0251	*CYB5A P* = 0.0150
LW			*CYP2E1 P* < 0.0000	
		TEAD3 P = 0.0012	*TEAD3 P* < 0.0000	*TEAD3 P* = 0.0003
			HSD3B P = 0.0011	*HSD3B P* < 0.0000
		CYB5A P = 0.0071		*CYB5A P* = 0.0011
LA			*CYP2E1 P* < 0.0000	*CYP2E1 P* = 0.0055
				HSD3B P < 0.0000
PN				*CYP2E1 P* < 0.0000
				HSD3B P < 0.0000
				CYB5A P = 0.0809

PBP – Přeštice Black Pied breed; LW – Large White; LA – Landrace; PN – Pietrain; D – Duroc

The tests revealed a significant difference ($P < 0.05$) between the frequency of genotypes among the five studied breeds (Table 5). The PBP breed differed from all others in the *CYP2E1* marker. All breeds in individual markers were tested for HWE, only PBP in *CYP2E1* and LW in *HSD3B* were not in HWE.

Discussion

CYP2E1 is associated with skatole degradation and is inhibited by androstenone (Doran et al. 2002). Skatole levels in adipose tissue are negatively correlated with the expression of the *CYP2E1* gene (Lin et al. 2006). Substitution of g.2412 C>T affects skatole levels, as described by Mörlein et al. (2012) in two commercial pig populations, and thus the *CC* genotype is associated with a higher skatole content. Significantly, higher skatole levels were associated with the *CC* genotype compared to the *CT* and *TT* genotypes. Aluwé et al. (2011) reported higher levels of skatole in back fat in LW and LA compared to PN boars. Muñoz et al. (2018) noted differences in the frequencies of alleles *C* and *T*; some breeds had higher frequencies *T* and other frequencies *C*. For example, Basque had the frequency of alleles *C* 1.00, Mangalitsa 0.90 and Swabian-Hall 0.80. Mörlein et al. (2012) found that the frequency of *CC*, *CT,* and *TT* genotypes in Duroc-sired crossbred was 0.25, 0.52 and 0.23 respectively. In our study, none of the individuals tested in the PBP and PN breeds had a *CC* genotype. The desired *TT* genotype had the highest frequency in PN and PBP. However, the less preferred *C* allele occurred in the *CT* genotype in both breeds. The frequency of *C* allele was higher in PBP (0.29) in comparison with frequency in PN (0.07).

In the *TEAD3* gene, Robic et al. (2012) described the polymorphism g.726 C>T and its correlation with androstenone concentrations in the Large White (LW) and Large White × Meishan cross. The *T* allele was associated with lower androstenone levels compared to androstenone in the *CC* genotype in LW. On the other hand, they found higher androstenone concentrations associated with the *T* allele in commercial French hybrids. Further, Robic et al. (2012) did not find a significant difference in genotype frequencies between the two breeds of Norwegian Landrace and Duroc. The frequency of the *T* allele was 0.37 and 0.30 (two sets of N. LA) and 0.52 and 0.45 (two sets of D). We found the frequency of the *T* allele 0.31 in LA and 0.30 in D. The preferred *TT* genotype was not

observed in LW at all; in PBP, incidence of *TT* was the highest, although it was only 8 out of 50.

Kim et al. (2013) considered *3β-HSD* (*HSD3B*) as a candidate gene for boar taint and investigated its polymorphisms in Duroc. The g.165262G>A SNP polymorphism with the *GG* genotype was associated with lower concentrations of androstenone, and the *G* allele was associated with lower androstenone content. In Duroc, the frequencies of allele *G* and allele *A* were 0.48 and 0.52, respectively. The genotype frequencies of *GG*, *GA,* and *AA* were 0.27, 0.41, and 0.32, respectively, in the same study. In our research, the *GG* genotype did not occur at all in LA and PN. There was not even an individual of the *GA* genotype in PN, the only genotype found in PN was *AA*. The frequency of *AA* genotypes also dominates in other breeds. The preferred *GG* genotype in PBP occurred in only one test individual.

The expression of the *CYB5A* gene correlates with androstenone concentrations and its mutations can reduce androstenone levels (Davis and Squires 1999). Polymorphism G>T in the *CYB5A* gene affects the concentration of porcine androstenone and skatole (Lin et al. 2005). The *T* allele led to lower concentrations of androstenone and skatole in fat and plasma. Lin et al. (2005) found a correlation between *CYB5A* expression levels and androstenone levels in adipose tissue. They also described a G-T polymorphism (in the 5′ UTR) in the *CYB5A* gene associated with androstenone, the mutation caused a decrease in androstenone concentrations. Allele *T* was significantly associated with lower androstenone concentrations and allele *G* with higher fat androstenone levels. They described the highest level of fatty androstenone for the wild-type *GG* genotype, the lowest level for the homozygous mutant *TT*, and *GT* genotype was found to be associated with the mean androstenone level. Lin et al. (2005) investigated whether the *TT* genotype was related to a lower activity of *CYB5A*. In 229 male pigs of different breeds (Duroc, Yorkshire, Landrace, Pietrain, and crosses LW×D and LW×PN), they observed the frequencies of 0.84, 0.13, 0.03 for genotypes *GG*, *GT*, and *TT*, respectively. The allelic frequencies were 0.904 in the *G* allele and much lower at 0.096 in the *T* allele. Peacock et al. (2008) described no *TT* genotype in the Large White, Pietrain, Sire Line and Duroc; the highest frequency was found in Yorkshire boars (0.45). Zamaratskaia et al. (2008) recorded *T* allele frequencies of 0.65 for Yorkshire and 0.13 for Landrace. Muñoz et al. (2018) reported a higher frequency of the *G* allele in *CYB5* in all breeds studied. In the Basque and Nero Siciliano breeds, the frequency of the *G* allele was the same in both, 0.91. Furthermore, in the Turopolje and Schwabish-Hallisches Schwein breeds, the frequency of the *G* allele was 1.00 and the *T* allele did not occur. Similarly, in the breeds we examined, the *TT* genotype did not occur in PBP, LW, LA, PN, and the only individual with this genotype occurred in the Duroc breed. The *GT* genotype did not occur in any of the LW boars, and therefore, the group of animals of this breed included only *GG* individuals. This genotype dominated in all breeds, and the frequency of the preferred *T* allele was low in these populations. The frequency of the *G* allele dominated from 1.00 in LW, 0.98 in PBP, 0.96 in PN, 0.91 in LA to 0.87 in D.

Considering the boar taint issue, the *T* allele in the *CYP2E1* marker is preferred; the results reveal that it had the highest frequency in PN, followed by PBP, and the lowest in LA. The *T* allele is also favoured in *TEAD3* with the highest frequency in PBP and a very low frequency in LW. For the *HSD3B* marker, the preferred allele is *G* with the highest frequency in D and zero occurrence in PN. It was also observed that the preferred *T* allele in *CYB5A* did not occur in LW; its frequency was very low in all breeds, the highest in D.

This research focused on the genotyping of purebred boars of four common commercial breeds of pigs (Large White, Landrace, Pietrain, Duroc) and their comparison with the gene source of the Přeštice Black Pied breed. We found significant differences

between the studied breeds in all four markers. In the *CYB5A* marker, we recorded an overwhelming predominance of one genotype. According to allele frequencies this marker is not polymorphic in the PBP, LW, PN breeds. Our results highlight the possibilities of testing the genotypes of markers associated with boar taint in breeding boars and sows and the differences in allele frequencies in pure breeds. We also point out the importance of maintaining genetic variability in functional genes of the breed which has been declared a gene source.

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