Reducing Boar Taint in Pigs Using SNP Markers

F.S. Schenkel[*] and E.J. Squires[*]

Introduction

Boar taint is an off-flavour that occurs in the fat of a significant portion of adult, uncastrated, male pigs. Pigs that are tainted have very low market value compared to pigs that are free of taint. Boar taint is the result of an accumulation of two compounds called androstenone and skatole in the carcass (Bonneau (1982)). Pigs that produce less of these compounds or that are able to efficiently break down and excrete these compounds are also less susceptible to taint (Zamaratskaia and Squires (2009)). Male pigs are usually castrated to prevent boar taint, but this reduces feed efficiency, lean gain and has a negative impact on animal welfare. Alternatives to surgical castration are immunocastration (Pauly *et al*. (2009)) and selection of pigs that have reduced propensity to produce boar taint (Zamaratskaia and Squires (2009)). The latter approach potentially can yield to a lasting solution for boar taint. In terms of production efficiencies, it is anticipated that the use of entire male pigs will improve profits per pig by more than $5, which is based on analyses that were conducted previously by de Lange and Squires (1995) and adjusted to 2010 economic conditions in Canada.

The heritability of both androstenone and skatole is moderate to high, but previous attempts to select for pigs with low boar taint have resulted in reproductive problems (reviewed in Zamaratskaia and Squires (2009)). The development of specific genetic markers for boar taint would minimize these negative effects on reproduction. It has been posited that genetic markers within the affected metabolic pathways can be used as tools to identify pigs with a greater chance of developing boar taint and removing them from the breeding pool (Squires and Schenkel (2008)). A recent report from Norway (Moe *et al*. (2009)) is the first association study of a large number of single nucleotide polymorphisms (SNPs) with boar taint in pigs. They found significant marker effects for fat androstenone in Duroc, but not in Landrace, and significant marker effects for skatole in both breeds.

The objective of this paper is to report on the development of a panel of genetic markers for boar taint based on the identification of SNPs in candidate genes that encode the enzymes involved in the synthesis and degradation of the boar taint compounds, androstenone and skatole.

Material and methods

Data: The selection of candidate genes was supported by the results from a number of functional studies carried out at University of Guelph over the past 20 years, which characterized the metabolites and enzymes of the metabolic pathways involved in the synthesis and degradation of androstenone and skatole (e.g., Squires (1989); Meadus *et al*. (1993); Diaz *et al*. (2000; 2003)).

[*] Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada

Initially, sequences of different candidate genes amplified by PCR from pools of cDNA obtained from animals from the extremes of the boar taint phenotypes in each of the 8 lines of pigs were compared for SNP discovery. Then a total of 111 SNPs located on 40 candidate genes were genotyped in 1270 pigs from Duroc (n=109), Hampshire (n=119), LW-Duroc (n=219), Landrace (n=185), Large White n=219), Pietran (n=76), Sire-line (n=144) and Yorkshire (n=199) genetic lines. Fat samples were analyzed for 5α-androstenone by Enzyme Linked Immunoassay (ELISA) (Squires and Lundström (1997)) and for skatole by high performance liquid chromatography (HPLC) with fluorescence detection (Lanthier *et al*. (2007)). Animals with low levels of androstenone were only included in the study if they had bulbourethral glands longer than 10 cm or plasma estrone sulfate greater than 20 ng/ml (Sinclair *et al.* (2001)), in order to control for low androstenone levels due to sexual immaturity.

Data editing: SNP genotypes were recoded as Gen= -1 for a homozygote (MM), 0 for a heterozygote (Mm), and 1 for the other homozygote (mm). Redundant SNPs (one from each pair of highly correlated SNPs (r>0.95)) and SNPs with very low allele frequency (f< 0.01) across all 8 lines were removed from the analyses. Because the distribution levels of fat androstenone and skatole are highly skewed, the natural logarithm of both boar taint compounds was analyzed instead.

Statistical analyses: A two step procedure was carried out. The first step was a principal component (PC) regression analysis, where a single SNP was fit in the model along with PC for all other SNPs, which explained 95% of the total variation in the (co)variance matrix of SNP genotypes, followed by a backward model selection applied to the PC terms in the initial model. This procedure was implemented as described in Pant et al. (2010) and aimed to screen the SNPs to enter the second step, minimizing the effect of multicollinearity among SNPs due to the presence of linkage disequilibrium. The full model including the single SNP and all PC terms explaining 95% of the total variation of the remaining SNPs was:

$$y_j = \beta_0 + \beta_k * Gen_k + \sum_{i=1}^{n-1} \beta_i * PC_i + \varepsilon_j$$

where: y_j= phenotype (ln(andr) or ln(skat)) of the jth pig; β_0= intercept; β_k= regression coefficient for the additive effect of the k-th SNP; β_i= multiple linear regression coefficient for the i-th PC (excluding the k-th SNP); n= number of SNPs; and ε_j= random error.

This procedure was repeated for each SNP, so that p-values were obtained for all SNPs. In the second step, all SNPs found significantly associated to skatole or androstenone (P<0.05) in one or more breeds in the first step were, then, simultaneously fit in a multiple regression model:

$$y_j = \beta_0 + \sum_{i=1}^{n} \beta_i * Gen_i + e_j$$

where: y_j= phenotype (ln(andr) or ln(skat)) of the jth pig; β_0= intercept; β_i= multiple linear regression coefficient for the additive effect of the i-th SNP; and e_j= random error. The best fitting model for each breed and phenotype was selected using the SAS Proc Reg, via backward model selection (P<0.05).

Results and discussion

Eighty SNPs were associated with fat skatole and androstenone and the strength of the associations varied among the 8 lines of pigs. The number of significant SNPs across lines varied from 5-17 and from 3-16 for skatole and androstenone, respectively (Table 1). Thus different sets of SNPs had an effect on different pig lines and taint compounds. Nevertheless, a large proportion (65%) of the effective SNPs were associated with both skatole and androstenone across lines, which corroborates with the moderate positive genetic correlation between these two boar taint compounds (e.g., Tajet *et al.* (2006)). A substantial amount of the skatole variance was accounted for the SNPs (Table 1). In Duroc line, for instance, about 60% of the skatole variance was explained by 13 SNPs, while in LW-Duroc line, 5 SNP accounted for about 10% of the skatole variance. On average, across all lines, the SNPs explained about 33% of the skatole variance. Similar results were observed for androstenone (Table 1). On average, across all lines, the SNPs explained about 28% of the androstenone variance. Moe *et al.* (2009) also reported significant marker effects for fat androstenone in Duroc, but not in Landrace, and also significant marker effects for skatole in both breeds. Individual markers reported explained from 2.5-16.3% of the total variation in the traits.

Table 1: Summary of marker effects for fat skatole and androstenone

	Line	Number of effective markers	R^2	Favourable allele frequency	Current geometric mean (ng/g fat)	Mean with favourable alleles	% Change
Skatole	Duroc	13	0.59	.05 - .95	40.85	24.02	-41.2
	Hampshire	17	0.42	.28 -.85	97.51	54.41	-44.2
	LW-Duroc	5	0.09	.06 - .87	96.54	77.62	-19.6
	Landrace	13	0.42	.02 - .76	59.15	34.96	-40.9
	LargeWhite	10	0.18	.15 -.74	72.24	48.18	-33.3
	Pietrain	12	0.45	.10 - .88	63.43	29.62	-53.3
	Sire Line	9	0.33	.03 - .92	42.10	22.23	-47.2
	Yorkshire	10	0.19	.30 - .91	24.78	15.93	-35.7
Androstenone	Duroc	11	0.41	.03 - .96	1.38	0.67	-51.6
	Hampshire	3	0.13	.21 - .81	0.79	0.31	-60.9
	LW-Duroc	16	0.35	.06 - .78	2.14	1.23	-42.7
	Landrace	14	0.32	.05 - .79	0.52	0.29	-44.1
	LargeWhite	5	0.08	.07 - .96	0.55	0.38	-31.3
	Pietrain	12	0.51	.10 - .94	0.35	0.18	-47.3
	Sire Line	10	0.27	.09 - .74	0.88	0.54	-38.6
	Yorkshire	7	0.16	.17 - .92	0.55	0.41	-26.0

Simulating the application of the markers to produce pigs that were homozygous for the favourable SNP alleles showed that the average (geometric mean) fat skatole levels would decrease by 20-53% (Table 1) and fat androstenone would decrease by 26-61% (Table 1), depending on the line. It was also determined that none of these markers were associated with negative effects on production traits (Back fat, loin depth, front leg score, rear leg score, subjective live muscle/conformation score, daily gain; data not shown). Because the SNPs

found associated with boar taint in this study need to be validated in other independent samples, the next step in the project includes the validation of the SNP in 5 different lines of pigs from a commercial company. The ultimate goal is to identify effective SNPs in the handful of most important genes, and then use these markers in breeding programs to develop lines of pigs that are free of boar taint but, otherwise, grow as normal boars.

Conclusion

Castration to prevent boar taint limits productivity and increases animal welfare concerns of commercial pork production, so alternative strategies for controlling taint are needed. The development of low boar taint lines of pigs by using genetic markers would provide a lasting solution to the problem. In this study 80 significant SNPs located in 28 candidate genes were associated with levels of fat androstenone and skatole across the 8 lines of pigs, which explained a substantial proportion of the variance in these boar taint compounds and had a large expected impact on the average of the two compounds in all lines.

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