

**Sociable Swine:**  
**prospects of indirect genetic effects for the improvement of  
productivity, welfare and quality**

**Naomi Duijvesteijn**

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**Sociable Swine:**  
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## **Abstract**

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Social interactions between pigs can originate from heritable traits and are then referred to as indirect genetic effects (IGE), meaning that a pig can influence the trait value of a pen mate genetically. The aim of this thesis was to investigate the contribution of IGE to breeding goal traits in pig breeding programs. This was investigated by incorporating IGE in mixed models containing random effects, or by using genomic information to detect associations between the phenotype and the direct and indirect genetic effects of the focal pig and its group mates.

One of the traits investigated was androstenone, a pheromone released by the boar to attract the sow, but also one of the components causing boar taint: an off-flavour to the meat when cooked or heated. Androstenone showed to be affected by IGE, which significantly contributed to the total genetic variance.

A region on chromosome 6 was significantly associated with the direct effect on androstenone. Several candidate genes were identified which are involved in the synthesis and metabolism of androgens. SNPs on chromosome 9 and 14 were significantly associated with the indirect effect on androstenone, but no clear candidate genes could be identified. Besides associations between phenotype and genotype, also a methodology to model SNPs for indirect genetic effects was presented.

The model which includes IGE showed to significantly fit the data better in several traits such as growth in pigs. To quantify the added value of IGE, a validation study was performed. No significant improvement was observed in ability to predict observed phenotypes between a classical animal model and a model including IGE. The structure and size of the dataset for a large part influenced the outcome of the validation. Therefore we cannot confirm or reject the added value of IGE.

Current policies aim at reducing interventions such as tail biting and castration which would require pigs to fit in a new social environment. Selection for IGE could beneficially improve social behavior between pigs to better fit the new environment.

Application of outcomes from research is of vital significance and therefore communication and understanding among and between stakeholders is essential. A Qualitative Behaviour Assessment (QBA) was performed to investigate the differences or similarities between animal scientists, pig farmers and urban citizens in their opinion on pig behaviour. Results showed that the pig farmers observed the

behaviour of pigs more positively than the urban citizens and the animal scientists. This study confirms the importance of understanding the perception of the different stakeholders which can be used to find shared solutions, especially on sensitive topics such as animal welfare.

***Voor mijn vader***



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# **1**

## **General introduction**



### 1.1 Pig production

Pig production takes place in many different environments and is subjected to physical as well as societal challenges. Obvious physical challenges are high temperatures resulting in heat stress and high pathogen load resulting in diseases. The societal environment (government regulation, involvement of animal welfare NGO's) also has a large influence on pig farming systems and therefore also the environment pigs experience. In the Netherlands, two trends are observed. First is a trend of intensification resulting in large scale pig farms. In the year 1995, 14 % of the pigs slaughtered were from farms with more than 2,000 pigs. This proportion increased to 58% in 2012 (CBS, 2013). In the US a comparable trend is observed as well. In 2005, 82% of the farms had more than 5,000 pigs. This proportion increased to 88% in 2008 (NASS, 2009). Another trend is an increase in the number of farms producing for a higher welfare segment. The turnover for animal proteins produced from welfare friendly farms (including organically produced products) increased from €284 million in 2011 to €421 million in 2012. Out of this, the increase in meat sales from pigs was largest among the different animal species with an increase of 89% in the Netherlands (Bakker, 2012). The increase in market segment was mainly from the emerging middle segment where welfare standards were higher than conventional housing, but not as high as the organic standard, such as one and two stars of the Better Life Hallmark. Better Life Hallmark is supported by the Dutch Society for the Protection of Animals and guarantees a certain level of welfare for animals raised for meat production. The rating ranges from one star to three stars. An increase in turnover for organically produced foods of 8.8% was observed worldwide from 2009 to 2010 (Bakker, 2012).

Political interest and interest by NGO's in the regulation of (pig) farming, with more attention to animal welfare, has resulted in change in environments and experiences for farm animals. In the EU, legislation on housing of sows has changed so that gestating sows should be placed in group-housed systems from January 2013 (EU, 2008). A ban on castration without anaesthesia is already in place in some countries (e.g. Switzerland, The Netherlands, and Norway). In UK and Ireland, pigs are not castrated any more (Fredriksen et al., 2009). Several main players in the pig and pork industry in the EU have signed the Declaration of Brussels to voluntarily end the practice of surgically castrating pigs by 1 January 2018 (EU, 2010). A similar trend on banning tail docking is observed. In the Netherlands a declaration has been signed by producers and stakeholders representing feed industry, university, animal protection and farmer organisations, to work towards a sustainable solution to stop tail docking (declaration of Dalfsen). Attention to animal welfare has resulted in more research on the social environment of the pig.

### 1.2 Pig breeding programs in a changing environment

Commercial pig breeding companies adapt their breeding program continuously to changes in the market and production environment, but also to societal changes. As described previously, the environment of the pig is changing and breeding goals can be designed to adapt pigs to the production environment. The ongoing discussion is whether the animal should be adapted to the environment or whether the environment should be adapted to fit the needs of the animal, although both adaptations seem necessary. Production in an environment with higher welfare standards would be beneficial for the animal, but it often results in reduced productivity and economic profitability. On the other hand, more efficient production often results in more constraints for animals. Animal populations tend to adapt to their environments given the proper selection pressure. Unforeseen trade-offs might occur in commercial breeding programs where selection pressure is greater in the production environment than in nature. A known trade-off is seen in dairy cattle where selection pressure on a higher milk production resulted in a decrease in fertility (Rauw et al., 1998). Unwanted behaviour such as tail biting in pigs or feather pecking in chicken, might also be trade-offs in livestock populations selected for higher economic efficiency.

Direct selection on behaviour can be quite complex to implement in a breeding program. Behavioural traits are often difficult and expensive to phenotype although selection for these traits would be possible due to availability of considerable genetic variation, as reported in a number of studies (e.g. aggression; Turner et al., 2006 and coping style; Velie et al., 2009). In addition, behavioural expressions might have underlying biological mechanisms and have genetic correlations with performance traits that are unknown to us. Therefore, inclusion of behavioural traits in breeding programs could lead to unforeseen trade-offs.

The environment a pig experiences can be physical when it can be described in terms of factors due to space allowance, number of feeders, light intensity. Research on these factors often results in changes in management. For example, research on reducing tail biting suggests changes in environmental enrichment, nutrition and health (EFSA, 2007). Internal factors such as age, gender and genetics also contribute to the occurrence of tail biting. Besides those factors, a pig is also influenced by its pen mates. Social interactions between pigs can have a positive impact on the animal itself (better growth; Bergsma et al., 2013, or social nosing; Camerlink et al., 2013) or a negative impact (aggression; Turner et al., 2006, or tail biting; Schrøder-Petersen and Simonsen, 2001).

### **1.3 Indirect genetic effects**

Social interactions between pigs can be genetically determined and are then referred to as indirect genetic effects (IGE) (Griffing, 1967; Moore et al., 1997; Wolf et al., 1998; Muir, 2005; Bijma et al., 2007). These are also known as associative, social-, or competitive genetic effects. In the last 20 years, indirect genetic effects have been estimated for a variety of traits in several livestock species. Survival in chicken (Ellen et al., 2008; Peeters et al., 2012) and quail (Muir, 2005), bite marks in mink (Alemu et al., 2014b), growth in pigs (Bergsma et al., 2008; Chen et al., 2009; Bergsma et al., 2013) and fin length and fin erosion in Atlantic cod (Nielsen et al., 2014) have all been shown to be influenced by indirect genetic effects. Consideration of indirect genetic effects in breeding programs can lead to additional genetic gains, while ignoring them can even result in an adverse effect (Griffing, 1967; Griffing, 1976; Wolf et al., 1998; Muir, 2005; Bijma, 2011). As classical breeding programs often target only the direct effect an individual has on its own phenotype and neglect the indirect effect the individual has on its pen mates, this method can reduce the overall performance of a group of pigs in a pen. Indirect genetic effects can be incorporated in the classical quantitative genetic framework (Muir and Schinckel, 2002), which makes predictions of breeding values and variance components possible. Especially within animal breeding, this has resulted in more empirical research and more understanding of the effect IGEs have on the response to selection (Bijma, 2013).

Including IGEs in breeding programs makes use of the heritable part of the social interactions. Selection including IGEs does not have to target unwanted behaviours directly. For example, selection on IGEs in average daily gain could indirectly improve social interactions between pigs and directly improve average daily gain of the population. How and which behaviours are affected when selecting on IGEs in pigs, is unknown until now. Possible changes in behaviour due to selection for IGEs include reduced activity, changes in fighting behaviour, faster stabilizing of social ranking after regrouping (Canario et al., 2009, Rodenburg et al., 2010), reduced sexual activity and changes in feeding behaviour (Turner, 2011).

### **1.4 Current developments in pig breeding**

Currently, commercial livestock breeding companies use genomic (DNA) information to increase the reliability of the estimated breeding values for selection candidates. A large number of Single Nucleotide Polymorphisms (SNPs) are being used to detect associations between genetic markers and traits of interest. The effects SNPs have on the phenotype are estimated using sophisticated statistical methods and directly used to estimate genomic estimated breeding

## 1 General introduction

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values (GEBVs) for selection candidates (Meuwissen et al., 2001). Genomic selection can be very beneficial for traits with a low heritability (disease resistance), traits expressed late in life (sow lifetime productivity), traits which are expensive to measure (physiological measurements such as boar taint in pigs), or traits that cannot be directly measured on the selection candidates (meat quality). In the context of IGEs, association studies will possibly gain insight into the underlying genes which contribute to IGEs (e.g. genes involved in stress responses, social dominance, genes regulating activity etc.). In addition to more insight into the biological background of IGEs, an increase in the accuracy of the estimated breeding value could contribute to a higher response to selection.

So far, hardly any pig breeding company has included IGEs into its breeding program. This is due to several reasons. Firstly, the type of behavioural changes associated with selection on IGEs are not known, secondly the magnitude of additional response is unknown and thirdly estimates of IGEs are limited and seem to vary in magnitude depending on the statistical model used. Furthermore, in the current genomics era, little is known about how genomic selection for traits affected by IGEs should be implemented in breeding programs or which genes are involved in processes affecting IGEs.

### 1.5 Aim and outline of this thesis

This thesis is part of two larger research projects: “Genetics of social interactions in livestock: Improving health, welfare, and productivity in laying hens and pigs” and “Seeking sociable swine? Incorporating social genetic effects into pig breeding programs to achieve balanced improvement in productivity and welfare”.

The broader goal of both the projects is to better understand the inheritance of traits affected by social interactions in commercial livestock populations. The ultimate goal is to exploit this knowledge in livestock breeding programs to improve welfare and productivity. More specifically, the second project investigates the opportunities to improve social interactions among pigs by incorporating IGEs in the breeding program and by investigating the implications of selection on IGEs for behaviour and welfare. Future directions for welfare improvement through enhanced social performance will be formulated and discussed in collaboration with stakeholders (e.g. producers, food industry, retail, consumers, and animal welfare organisations). This should enable a balanced selection for sociable, productive pigs that are mentally and physically healthy. In total 4 PhD-projects are included in the second project with focus on genetics (this thesis), genotype by environment interactions including the effect selection for IGEs has on behaviour (Camerlink, 2014), and physiological and emotional state of

animals with different IGEs (Reimert, 2014). The fourth PhD (Benard, 2014) facilitated and studied the incorporation of the societal concern expressed by stakeholders into the project by creating a feedback-loop in which stakeholders and researchers share their disciplinary and experiential knowledge to define preferred directions along the research processes.

The aim of this thesis is to investigate the contribution of IGEs to selection traits in pig breeding programs. This was investigated by incorporating IGEs in quantitative genetic frameworks, or by using genomic information to detect associations between the phenotype and the direct and indirect genetic effects.

The Chapters 2,3 and 4 focus on the trait androstenone. Castration of boars will be prohibited from January 2018 in the EU for welfare reasons. Pork from some entire males could have 'boar taint'; an off-flavour and odour when cooked or heated. One of the components causing boar taint is androstenone. The level of androstenone depends on the stage of puberty and genetics ( $h^2$  ranges between 0.25 and 0.88). One of the approaches to reduce androstenone by breeding is the use of Single Nucleotide Polymorphisms (SNPs). Since the availability of high-density SNP chips, a genome-wide association study (GWAS) was initiated using the SNP array to identify the chromosomal regions and specific SNPs influencing androstenone levels in a commercial breeding population (Chapter 2). In Chapter 3, the social environment is also considered, as androstenone is a pheromone released from the saliva and can be spread through contact between pigs. In the presence of sows, androstenone is released to attract sows and induce a standing response. The contribution of social interactions between group-housed boars to the expression of androstenone is unknown and is studied in Chapter 3 where heritable social effects (IGE) are estimated, but also the non-heritable contribution of the pen and compartment are included in the model to estimate variance components for androstenone. Finally in Chapter 4, the knowledge of Chapter 2 and 3 is used to accurately model androstenone in an association study where direct SNP effects and indirect SNP effects are estimated.

Chapter 5 deals with the validation of IGEs. The first objective of this study was to estimate direct and indirect genetic components for average daily gain in two purebred sire lines. The second objective was to validate the IGE by comparing prediction using a classical model with a model including IGEs. The models were evaluated by correlating predicted phenotypes with observed phenotypes. Furthermore, practical implementation issues of indirect genetic effects in pig breeding programs will be discussed.

In Chapter 6 investigations of possible differences between stakeholders in the perception of animal behavior and welfare have been reported. In this chapter, we

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use Qualitative Behavior Assessment (QBA) to explore whether stakeholder groups (pig farmers, animal scientists and citizens) observe pigs differently. The assessment of behavior primarily relies on human perception: different stakeholders were asked to define an animal's mood by using descriptive terms such as 'active', 'happy' or 'irritated' by viewing video fragments showing different pig behaviors.

In the general discussion (Chapter 7) results of this thesis are discussed from a broader perspective. Three main topics are discussed: raising entire males, indirect genetic effects applied and involvement of stakeholders in research projects related to animal welfare.

# 2

## **A genome-wide association study on androstenone levels in pigs reveals a cluster of candidate genes on chromosome 6**

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## Abstract

In many countries, male piglets are castrated shortly after birth because a proportion of un-castrated male pigs produce meat with an unpleasant flavour and odour. Main compounds of boar taint are androstenone and skatole. The aim of this high-density genome-wide association study was to identify single nucleotide polymorphisms (SNPs) associated with androstenone levels in a commercial sire line of pigs. The identification of major genetic effects causing boar taint would accelerate the reduction of boar taint through breeding to finally eliminate the need for castration.

The Illumina Porcine 60K+SNP Beadchip was genotyped on 987 pigs divergent for androstenone concentration from a commercial Duroc-based sire line. The association analysis with 47,897 SNPs revealed that androstenone levels in fat tissue were significantly affected by 37 SNPs on pig chromosomes SSC1 and SSC6. Among them, the 5 most significant SNPs explained together 13.7% of the genetic variance in androstenone. On SSC6, a larger region of 10 Mb was shown to be associated with androstenone covering several candidate genes potentially involved in the synthesis and metabolism of androgens. Besides known candidate genes, such as cytochrome P450 A19 (*CYP2A19*), sulfotransferases *SULT2A1*, and *SULT2B1*, also new members of the cytochrome P450 *CYP2* gene subfamilies and of the hydroxysteroid-dehydrogenases (*HSD17B14*) were found. In addition, the gene encoding the  $\beta$ -chain of the luteinizing hormone (*LHB*) which induces steroid synthesis in the Leydig cells of the testis at onset of puberty maps to this area on SSC6. Interestingly, the gene encoding the  $\alpha$ -chain of LH is also located in one of the highly significant areas on SSC1.

This study reveals several areas of the genome at high resolution responsible for variation of androstenone levels in intact boars. Major genetic factors on SSC1 and SSC6 showing moderate to large effects on androstenone concentration were identified in this commercial breeding line of pigs. Known and new candidate genes cluster especially on SSC6. For one of the most significant SNP variants, the difference in the proportion of animals surpassing the threshold of consumer acceptance between the two homozygous genotypes was as much as 15.6%.

Key words: genome wide association study, androstenone, boar taint, pigs

## **2.1 Introduction**

In many countries, male piglets are castrated shortly after birth to prevent boar taint, which is an urine-like, unpleasant flavour and odour released at cooking or heating of pork (Bonneau, 1997). However, recent discussions on the pain associated with castration of the piglets early in life have led to a ban on castration without anaesthesia in some countries. In addition, studies have shown that uncastrated males grow faster and have an improved feed efficiency due to reduced fat deposition (Xue et al., 1997; Čandek-Potokar et al., 1998; Metz and Claus, 2003). In future, if uncastrated males will be finished, boar taint needs to be prevented. Two of the major components related to the boar taint are androstenone and skatole (Patterson, 1968; Vold, 1970; Malmfors and Lundström, 1983). Androstenone (5 $\alpha$ -androst-16-en-3-one) is a male sex pheromone produced by the testes and stored in adipose tissue causing a perspiration-like odour (Perry et al., 1980; Bonneau, 1982). Androstenone precursors are also transported to the salivary glands which are capable to produce high levels of androstenone during sexual excitement (Gower, 1972; Claus, 1979). Skatole possesses strong faecal odour and is produced by the bacterial breakdown of the amino-acid tryptophane in the lower gut (Yokoyama and Carlson, 1979). Skatole then diffuses into fat tissue. There is considerable variation for androstenone and skatole between and within lines of pigs. Especially androstenone has high heritability estimates ranging from 0.25 to 0.88 (Sellier et al., 1998; Sellier et al., 2000). Somewhat lower heritabilities have been reported for skatole, between 0.19 and 0.55 (Pedersen, 1998; Tajet and Andresen, 2006). Two linkage studies using microsatellite markers have identified several QTL regions for androstenone and skatole in experimental crosses with 485 and 187 F2 animals, respectively (Quintanilla et al., 2003; Lee et al., 2005) pointing towards several areas in the genome affecting these traits. Also, single candidate genes involved in androstenone synthesis and metabolism of androstenone and skatole have been analyzed at the level of RNA and protein expression and in single SNP association studies (reviewed by Robic et al., 2008). However, no conclusive results showing functional mutations affecting androstenone and skatole levels in fat tissue have been described until now. Recently, large-scale microarray expression studies have reported hundreds of differentially expressed genes which might be involved in synthesis and degradation of androstenone and skatole in testis and liver (Moe et al., 2007; Moe et al., 2008). Subsequent analysis of SNPs in 121 differentially expressed genes identified 10 genes associated with one of the two traits (Moe et al., 2009). Recently, Markljung et al. (2008) reported 2 QTL for androstenone in 139 animals from a cross between Hampshire and Landrace

animals. Although these studies are of limited size and resolution, they indicate that several genetic factors seem to be involved in determining the levels of these boar taint compounds.

Recently, the first high-density 60K porcine SNP array has been developed (Ramos et al., 2009) that offers a much higher resolution. A genome-wide association study (GWAS) was initiated using the SNP array to identify the chromosomal regions and specific SNPs influencing boar taint levels in a commercial breeding population. However, mean skatole levels (75 ng/g fat) in this population were far below the threshold accepted by consumers of 250 ng/g fat (Walstra et al., 1999). To reduce genotyping costs, a selective genotyping strategy for androstenone was applied. In this study, we present the results of a GWAS in pigs by genotyping 987 un-castrated male pigs from a commercial breeding population with large phenotypic variability for androstenone levels in fat, using the 60K (64,232) SNP array. The GWA resulted in an increased resolution compared to previous linkage studies. A large cluster of candidate genes within a 10 Mb region on SSC6 was identified. In addition, three new areas on SSC1 were detected that affect androstenone levels in this breeding line.

## 2.2 Methods

### 2.2.1 Animals and phenotypes

This experiment was conducted strictly in line with the regulations of the Dutch law on the protection of animals. Phenotypic measurements on androstenone were obtained from 1,663 boars slaughtered at a mean hot carcass weight of 95.71 kg. All the boars were purebred animals from a composite Duroc sire-line. Boar taint compounds were measured using fat samples from the neck collected from the left carcass side. The samples were stored under vacuum at -20°C. For androstenone, a fat extraction was done on the fat samples as described by Tuomola et al. (1997). Thereafter, androstenone concentrations in liquid fat were estimated by time-resolved fluoro-immunoassay at the Hormone laboratory, Oslo.

Androstenone was not normally distributed and therefore log-transformed (ln-androstenone). The log transformed androstenone values were analysed using the following statistical model using ASREML (Gilmour et al., 2002):

$$y_{ijklm} = \mu + b_1 * hcw_{ijklm} + b_2 * age_{ijklm} + b_3 * fat_{ijklm} + batch_i + pen_{ij} + litter_{ik} + a_l + e_{ijklm}$$

Where  $y$  = ln-androstenone;  $hcw$  = effect of hot carcass weight as covariate;  $age$  = effect of age at slaughter as covariate;  $fat$  = effect of fat depth at slaughter as covariate;  $batch$  = the random effect of the  $i^{th}$  batch,  $pen$  = the random effect of the  $j^{th}$  pen within the  $i^{th}$  batch;  $litter$  = the random effect of the  $k^{th}$  litter within the  $i^{th}$

batch;  $a$  = additive genetic effect of the  $l^{\text{th}}$  animal;  $e$  = residual effect. Systematic environmental effects were estimated using the full dataset (N=1663). In the genome-wide association study, androstenone levels adjusted for systematic environmental effects were used and these were calculated as:

$$y_{ijklm}^* = y_{ijklm} - \hat{\mu} - \hat{b}_1 * hcw_{ijklm} - \hat{b}_2 * age_{ijklm} - \hat{b}_3 * fat_{ijklm} - batch\hat{h}_i - pen\hat{n}_{ij} - \hat{litter}_{ik}$$

### 2.2.2 Selective genotyping

A simulation study was performed in order to select about 1000 animals from 1663 candidates for genotyping in an optimal way using the existing pedigree (Duijvesteijn and de Koning, 2009). Ten markers and 1 QTL were simulated on 1 chromosome and also 1 chromosome was simulated without a QTL for determining the false-positive rate for a given threshold. Four alternatives for selecting 1000 individuals to be genotyped out of 1663 candidates were compared: 1. random, 2. selecting large half-sib families, 3. selecting high and low phenotypes, 4. selecting high and low phenotypes within full sib families. ANOVA was used to analyze each marker and determine the F-statistic. Selection of high and low phenotypes within full sibs showed the highest power (results not shown). Applying the selection of high and low phenotypes (within full sib families) to our data set consisting of 1663 pigs resulted in 987 pigs selected for genotyping. These pigs originated from 57 sires and 212 dams. Among them, 45 sires and 11 dams were available for genotyping.

### 2.2.3 Genotyping and quality control

Genotyping was performed using the PorcineSNP60 Beadchip of Illumina (San Diego, CA, USA) (Ramos et al., 2009). A total of 1043 samples (including sires and dams) were genotyped for 64,232 SNPs at Service XS (Leiden, The Netherlands) and data quality was evaluated. The average call rate for all samples was  $98.4\% \pm 3.4$ . A total of 63 animals were removed due to pedigree errors (<99% correct genotypes). After quality control, 943 animals were available for the GWAS with 106 singletons and 313 divergent full sib pairs (2 or more full sibs). For the SNPs, a threshold of 30 pedigree errors or more was applied and 190 SNPs were removed. In addition, 10,210 SNPs were removed because of low quality score (GenCall score <0.7). A minor allele frequency of 0.01 was applied removing another 4,925 SNPs of which 980 were monomorphic. In total, 47,897 SNPs remained for the GWAS.

## 2 GWAS on androstenone

**Table 2.1** Descriptive statistics for traits measured<sup>1</sup>.

Trait	N	mean	SD	min	max
<i>Boar taint compounds</i>					
Androstenone (µg/g)	943	1.88	1.67	0.07	10.10
Skatole (ng/g)	942	91.11	97.48	6.00	928.00
Indole (ng/g)	942	54.15	64.79	8.00	678.00
Ln-androstenone	943	0.25	0.91	-2.66	2.31
<i>Finishing traits</i>					
Hot carcass weight	943	95.71	10.95	67.60	136.20
Fat depth at slaughter	943	14.96	2.93	7.60	27.60
Age at slaughter	943	179.80	9.26	152.00	247.00

<sup>1</sup>Mean, standard deviation (SD), minimum (min) and maximum (max) values are presented for all the phenotypes included in the association study (N).

### 2.2.4 Genome-wide association analysis

Corrected log-transformed androstenone was analyzed as a quantitative trait under an additive model using the QFAM module of PLINK (Purcell et al., 2007). The more stringent within-sib-ship test within QFAM was performed which is robust for population stratification compared to the total-sib-ship test. Nominal scores were permuted to obtain an empirical p-value while maintaining familial correlation between genotype and phenotype. The permutation procedure employed by QFAM corrects for relatedness within families and was performed 1,000,000 times. Genomic control was used to correct for score inflation introduced by relatedness between family units (sib ships) (Devlin and Roeder, 1999). False-discovery rate (FDR) was applied to correct for multiple-testing. The R package q-value (Dabney et al., 2010) was used to calculate a FDR-based q value to measure the statistical significance at the genome-wide level for association studies. The cut-off of significant association at the whole genome level was set at q-value  $\leq 0.05$ . The total variance explained by a SNP was calculated using ASReml version 2.0 (Gilmour et al., 2002). For ASReml the full model (as described earlier and including the polygenic effect) was used for the animals genotyped including the SNP as a random effect.

The fraction of the phenotypic variance explained by the SNP =  $\frac{\sigma_{SNP}^2}{\sigma_p^2}$ .

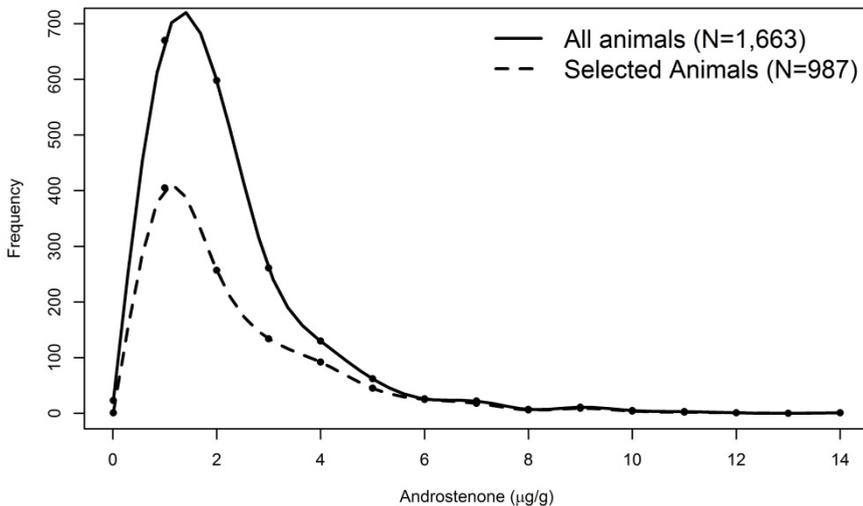
Linkage disequilibrium (LD) between SNPs was quantified as  $r^2$  on all the animals of the GWA study using Haploview (V4.2; Barrett et al., 2005) and the LD block was defined by the criteria of Gabriel et al. (2002).

### 2.2.5 Identification of candidate genes

Porcine transcripts and annotation were downloaded from the porcine Ensembl data base (build9) and aligned with the human RefSeq mRNA sequences using BLAT (Kent, 2002). The human-porcine comparative map was calculated based on the orthologous human-porcine transcripts and for the syntenic regions annotations were downloaded from the NCBI database (build37). Additional candidate genes present in human but not identified in the BLAT search against the human transcriptome were mapped to SSC6 performing a BLAST alignment with the porcine cDNA (*SULT2A1*) or the human homolog (*SULT2B1*, *HSD17B14*) against the porcine genome sequence (build9).

### 2.3 Results

The descriptive statistics of the phenotypic measurements of the boars used for the GWAS are given in Table 2.1. Animals were slaughtered at a mean age of 179.80 d with an average carcass weight of 95.7 kg. The average androstenone level was 1.88  $\mu\text{g/g}$  melted fat, and the average skatole and indole levels were 91.11 ng/g and 54.15 ng/g, respectively. The change of the distribution of androstenone concentrations after selection of divergent sib pairs is shown in Figure 2.1 indicating that not only extreme androstenone levels are represented.

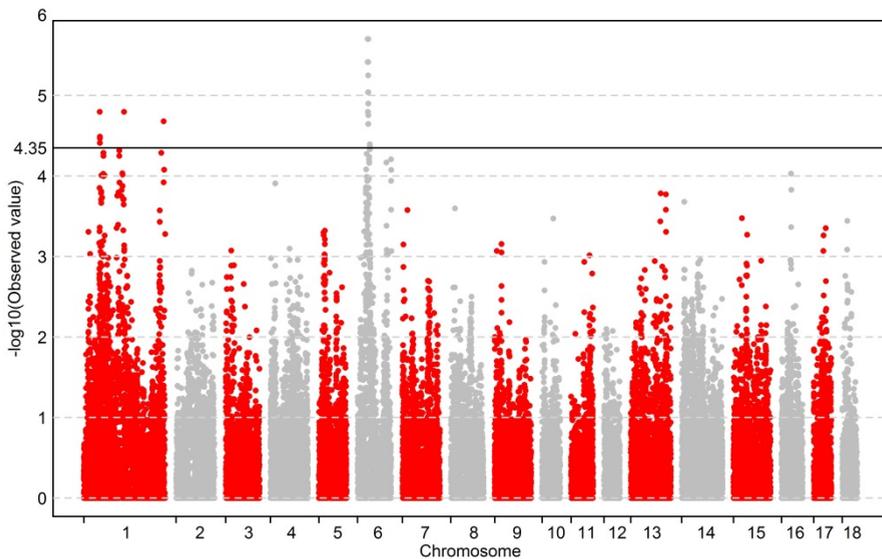


**Figure 2.1** Distribution of androstenone for the full dataset (N=1663) and after selective genotyping was applied (N=987).

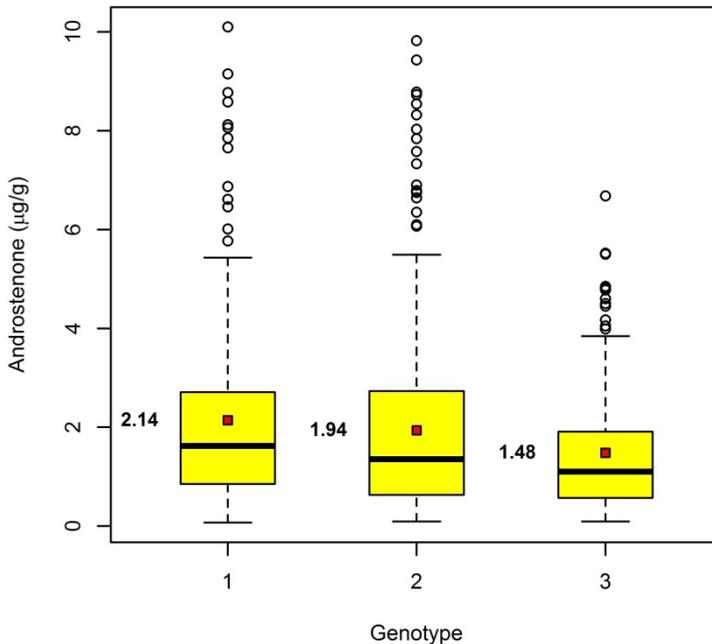
## 2 GWAS on androstenone

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The GWA analysis using the threshold for FDR of  $q \leq 0.05$  showed that 37 SNPs were genome-wide significantly associated with log-androstenone (Figure 2.2 and additional file 1 available online). Among them, thirty-five SNPs are located in regions with multiple significant SNPs. Three regions were identified on SSC1, and one larger region on SSC6. The region between 36.9 Mb and 44.9 Mb on SSC6 encompasses a large cluster of 31 significant SNPs. A single SNP analysis of the most significant SNPs on SSC1 and SSC6 using a mixed model and including a polygenic effect thereby correcting for other genetic factors affecting androstenone (background genome) is shown in additional file 1 (available online). The fraction of the phenotypic variance explained by a single SNP varies between 1.5% and 5.8%.



**Figure 2.2** Association between ln-androstenone and 40,525 mapped SNPs across 18 autosomes using an additive model. Each dot represents one SNP. On the y-axis are  $-\log_{10}$  (p-values), and on the x-axis are the physical positions of the SNPs by chromosome. Cut-off value is 4.35 which equals a FDR  $q$ -value  $\leq 0.05$ .



**Figure 2.3** Box plots of the distribution of the untransformed androstenone concentrations for the SNP MARC0049189 (nr 15). The mean is given in bold.

Figure 2.3 shows the means for the untransformed androstenone levels of the three genotypes of SNP nr 15 on SSC6. There is a difference of 0.66  $\mu\text{g/g}$  between the mean level of the two homozygous genotypes. Correction for systematic environmental effects hardly affects the differences between the genotypes (data not shown). Moreover, among the animals homozygous for the allele associated with high androstenone levels, 39.6% of the animals surpass the threshold of consumer acceptance (2  $\mu\text{g/g}$ ). This proportion is markedly reduced by 15.6% in the homozygous low genotypes (24.0% above 2  $\mu\text{g/g}$ ). A more detailed view of SSC6 is shown in supplemental figure 2.1. The high density of genes presently annotated in Ensembl on SSC6 ( $n=351$ , additional file 3 available online) is even more pronounced in the area of interest with a total of 24 genes between 36,9 Mb and 40 Mb. The homologous region in human on HSA 19q13 between 50 Mb and 52,2 Mb is also very gene-rich with a total of 139 genes and 255 transcripts being annotated until now.



androstenone levels. Only isolated SNPs approach the significance threshold on SSC6 and SSC16 at 122Mb and 105Mb, respectively.

## 2.4 Discussion

### 2.4.1 Filtering of SNP data and statistical analyses

Quality control of the SNPs was based on the GenCall score, MAF and pedigree errors. Hardy-Weinberg equilibrium (HWE) was not considered relevant as a quality control tool as HWE is underpowered to detect genotyping errors (Cox and Kraft, 2006) and only extreme sib pairs have been genotyped. GWA studies are particularly prone to spurious associations because ten thousands of associations are tested inflating the rate of false positives (McCarthy et al., 2008; Pearson and Manolio, 2008). In this study, FDR was used to control for false-positive associations due to multiple testing. The genomic control approach was used to account for spurious association due to population stratification (Devlin and Roeder, 1999; Devlin et al., 2001) and because the breeding line is a composite line derived from three different breeds. Correction for the inflation by division reduces the unadjusted p-value to adjusted levels and accounts for relatedness between the sib ships and possible population stratification. However, in this study the deviation from the chi-square distribution under the null-hypothesis (no association) was very low ( $\lambda_{GC}=1.06$ ).

**Table 2.2** Candidate genes derived from porcine Ensembl build9.

SSC	Start position	End position	Porcine transcript	Gene
SSC1	58190063	58192283	ENSSSCT00000004751	Glycoprotein hormones, $\alpha$ chain
SSC6	33615478	33622941	ENSSSCT00000003325	<i>CYP2A19</i>
SSC6	33821587	33821766	ENSSSCG00000003001	<i>CYP2A6</i>
SSC6	37189463	37189682	ENSSSCT00000003463	Sulfotransferase
SSC6	37567155	37586075	ENSSSCT00000003479	<i>HSD17B14</i>
SSC6	37754569	37755346	ENSSSCT00000003498	<i>LHB</i>

### 2.4.2 QTL areas

Mainly two chromosomes harbour highly significant associations with fat androstenone levels, a rather broad area of 10Mb on SSC6, and three different regions on SSC1. For each region on SSC1 only 1-4 SNPs pass the significance level, whereas on SSC6 a total of 31 highly significant SNPs are detected. This is for the first time that an association with androstenone or related boar taint traits has been reported on SSC1. However, several studies have described QTL effects for

## 2 GWAS on androstenone

traits related to boar taint on SSC6 (Figure 2.5). In an experimental F2 cross with Large White x Meishan, Lee et al. (2005) reported a QTL for androstenone level in fat on SSC6 partially overlapping with the area identified here. In the same study, QTLs from a sensory panel for subjective pork flavour and boar flavour in lean meat were described that are also located nearby the area in our study. Finally, Szyda et al. (2003) identified a QTL for smell intensity in a Duroc X Norwegian Landrace cross covering the area of interest. Considering the low resolution of these QTL studies, it is not possible to conclude whether they might be caused by the same genes segregating as in our study. The remaining boar taint QTL previously identified on SSC6 for smell intensity (Grindflek et al., 2001), subjective pork odour and skatole measurements (Lee et al., 2005; Varona et al., 2005) are located distal or proximal on SSC6. None of the other QTL studies investigating androstenone or sensory panel traits identified effects on SSC6 (Quintanilla et al., 2003; Markljung et al., 2008).

**Table 2.3** Overview of the identified QTL and flanking microsatellites on SSC6 for traits related to boar taint<sup>1</sup>.

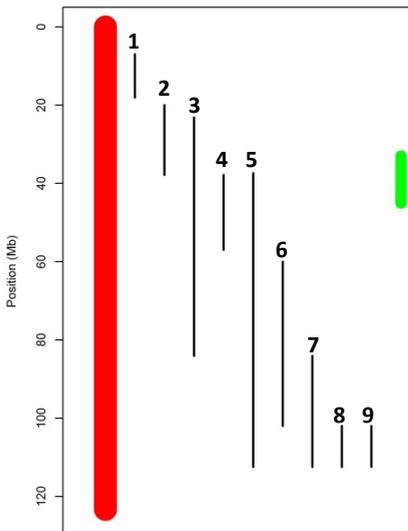
Nr.	Trait	Flanking markers	Reference
1	Subjective pork odor	SW1353 - SW1057	Lee et al., 2005
2	Subjective pork flavor in lean	SWR1130 (SW492) - SW782	Lee et al., 2005
3	Smell intensity	S0087 - S0003	Szyda et al., 2003
4	Androstenone, laboratory	SW782 - SW1823 (SW316)	Lee et al., 2005
5	Subjective boar flavor in lean	SW782 - SW322	Lee et al., 2005
6	Skatole, laboratory	S0059 (SW1473) - S0121 (S0299)	Varona et al., 2005
7	Smell intensity	S0003 - SW322	Grindflek et al., 2001
8	Skatole, sensory panel	S0121 (S0299) - SW322	Lee et al., 2005
9	Skatole, laboratory	S0121 (S0299) - SW322	Lee et al., 2005

<sup>1</sup>When the flanking marker could not be placed on the physical map (*Sus scrofa* build9) then the nearest marker on the MARC map was used to estimate the physical position (marker name in brackets) of the marker.

### 2.4.3 Candidate Genes

For the two major areas of interest, genes potentially affecting steroid synthesis and metabolism of androstenone are listed in Table 2.2. The region on SSC 6, which is an extremely gene-dense area, shows several candidate genes located closely together between 37 – 38Mb. Hydroxysteroid sulfotransferaseA1 (*SULT2A1*) maps to the homologous region in human and has not been annotated in the pig genome sequence. However, a BLAT search with porcine cDNA identifies 100% homology

with two exons and including 3'untranslated sequence (pos.380-508, and 780-999). *SULT2A1* catalyses the sulfoconjugation of 16-androstene-steroids in liver (Sinclair and Squires, 2005) and testis (Sinclair et al., 2005) and has been analysed earlier as a candidate gene for androstenone. Testicular activity of the enzyme was shown to be negatively correlated with fat androstenone levels in Yorkshire boars (Sinclair et al., 2006). However, Moe et al. (2007) found an increased expression of *SULT2A1* mRNA in testis of Duroc and Landrace boars with high androstenone levels. In the same study, *SULT2B1* expression was also increased in Landrace animals with high androstenone levels. *SULT2B1* is located near *SULT2A1* in human, but it has not been annotated in the pig genome sequence and cannot be located by BLAT alignment either. The overexpression of *SULT2B1* does not explain the role of the sulfotransferases as inactivating enzymes (Moe et al., 2007). *SULT2B1* is selective for the sulfation of 3 $\beta$ -hydroxysteroids, and Falany et al. (2006) suggest a role in regulating the responsiveness of cells to adrenal androgens by reducing their conversion to more potent androgens and estrogens. In human, *SULT2B1b* is not expressed in the liver, however the different physiological functions of the two isoforms remain to be analyzed.



**Figure 2.5** Location of the QTL from PigQTLdb for boar taint traits on the physical map of *Sus scrofa* build9 SSC6. The references and traits of the QTLs are given in Table 2.3. Positions in Mb were deduced from a BLAST alignment with the microsatellite markers. The green bar indicates the region found in this GWA study between 33Mb and 45Mb.

Another conjugating enzyme also located in this area is *HSD17B14*. Differential expression of different hydroxysteroid dehydrogenases of the HSD17 $\beta$  family (*HSD17B4*, *HSD17B11*) in the testis has been reported by Moe et al. (2007). *HSD17B4* catalyses the last step of androgen and estrogen synthesis. However, the function of *HSD17B14* has only recently been investigated in human, and Lukacik et al. (2007) suggest a role for the local inactivation of steroids in the nervous system and placenta. Northern blot analysis of human tissues shows that the gene is highly expressed in the liver, but not in testis. Adjacent, another new candidate gene, *LHB* is located, forming the  $\beta$ -chain precursor of the luteinizing hormone (LH). At onset of puberty, LH is secreted by the pituitary gland and induces steroid synthesis in the Leydig cells of the testis. Interestingly, the gene encoding the  $\alpha$ -chain of this glycoprotein hormone (CGA chorionic gonadotropin A) is located on SSC1 in the area of 58 Mb which also shows a significant effect in this study. No known or potentially interesting candidate genes could be pinpointed for the remaining two regions around 146 and 290 Mb on SSC1. Finally, the region on SSC6 extends to a second peak of SNPs nearly reaching the significance threshold around 33 Mb (additional file 2.2). Several cytochrome P450 genes of the *CYP2* family are located there. This *CYP2ABFGST* cluster contains genes from multiple subfamilies (Hu et al., 2008). In human, *CYP2A6*, *CYP2A7*, *CYP2B6*, *CYP2A13*, *CYP2F1* and *CYP2S1* cluster together. From these, aromatase (*CYP2A19*), which is the homolog of *CYP2A6* in pigs, is known to catalyse the synthesis of estrogens from androgens. The pig expresses two isoforms in the testis. Moe et al. (2007) have shown an upregulation of both isoforms in testis and liver of high-androstenone boars. Recently, Moe et al. (2009) reported SNPs within candidate genes associated with androstenone levels in a commercial Duroc line. However, none of the candidate genes reported by Moe et al. (2009) overlap with the major regions identified here.

Taken together, there is overwhelming evidence from previous QTL studies, candidate genes and differential expression that the region on SSC6 contains genetic elements affecting androstenone levels in boars. In order to disentangle the effects of the regions containing the *CYP450* genes and the area around 37 Mb, a mixed-model analysis combining the effects of two SNPs (H3GA0052956 at 33.5 and MARC0049189 at 38.3 Mb) was performed. In this model the fraction of the phenotypic variance explained by both SNPs is 2.1% and 3.6% and together 5.7%. This means that both regions explain a part of the effect of the whole region but due to the high LD between the SNPs they capture the same variation individually (5.76%, additional file 2.1). Therefore, both areas remain relevant for the determination of androstenone levels in this population. This breed is a composite line which could explain this large extent of LD. More data from other unrelated

lines or crossbred animals showing the same effect are needed to further reduce the region of interest.

### **2.4.4 Effect size and application for breeding**

Due to the skewed distribution of androstenone levels, even the use of a single marker would reduce the proportion of animals surpassing the threshold for consumer acceptance of 2 µg/g fat considerably. The difference between the two homozygous genotypes amounts to 15.6% (Figure 2.3). Sorting all offspring by the estimated androstenone effect of marker 50 and comparing the haplotypes of the 10 highest animals shows that all individuals are homozygous for the first haplotype shown in Figure 2.4. Furthermore, this haplotype is completely absent in the group of 10 animals with the lowest effects (data not shown). The 5 major SNPs (SNP nr. 1, 5, 6 on SSC1 and SNP nr. 15, 124 on SSC6) on SSC1 and 6 together explain 8.8 % of the phenotypic variance, and considering a heritability of 64% (Merks et al., 2009) they account for 13.7% of the additive genetic variance.

A sustainable breeding scheme takes also into account the correlated effects on other production and reproduction traits. In general, the genetic correlations with growth, fatness and muscle depth are very low and favourable and therefore no serious negative effects on genetic progress due to selection against androstenone are to be expected (Merks et al., 2009). Also, the positive genetic correlation with skatole would reduce skatole levels indirectly. However, the genetic correlation with fertility traits needs special attention. Male fertility data are not available on the animals in this study because they were slaughtered as commercial fatteners. Female fertility observations are only available on related animals and therefore estimates of genetic parameters have large standard errors (data not shown). A more extended study is underway to monitor the effects of selection against androstenone on male and female fertility. Furthermore, the effects in other lines that form part of the crossbreeding scheme to produce fattening pigs will be investigated.

## **2.5 Conclusion**

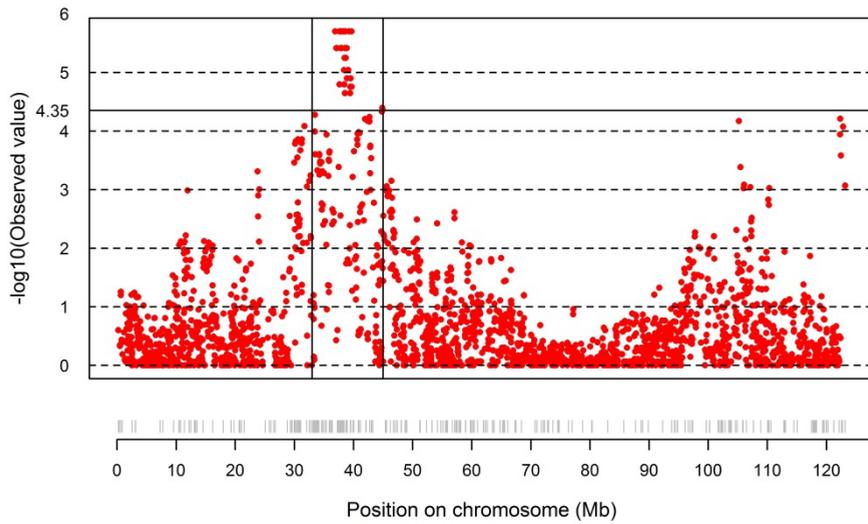
This study clearly shows the large increase in resolution of high-density SNP panels compared to earlier linkage studies using microsatellite markers (Figure 2.5). Several regions in the genome affect androstenone levels in fat in this commercial breeding line of pigs. The genome-wide significant SNPs detected on SSC1 and SSC6 show moderate to large effects explaining a fraction of the phenotypic variance of 2-6%. The candidate genes identified in these areas in the pig genome or via the comparative map in human include genes investigated in earlier reports. In

addition, new genes from the pathways of the synthesis and metabolism of androstenone such as *LHA*, *LHB*, and *HSD17B14* are detected. The rather large LD block seen in this population around 33-45 Mb on SSC6 prevents to disentangle the combined effects of these genes and to pinpoint more specifically the responsible genetic elements. Nevertheless, the most significant SNPs can already be used to accelerate genetic progress in breeding against androstenone in this sire line. However, genetic correlations with production traits and especially possible negative effects on fertility traits will deserve special attention.

### **Acknowledgements**

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**Supplemental figure 2.1** Above a  $-\log$  p-value of 4.35 a SNP is considered significant. The start positions of porcine genes (n=351) from Ensembl are plotted underneath as vertical grey bar based on the sequence of *Sus scrofa* build9. Vertical bars indicate the interval chosen for LD analysis in Figure 2.4.





# 3

## **Direct and associative effects for androstenone and genetic correlations with backfat and growth in entire male pigs**

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## Abstract

In the pig industry, male piglets are surgically castrated early in life to prevent boar taint. Boar taint is mainly caused by androstenone and skatole. Androstenone is a pheromone that can be released from the salivary glands when the boar is sexually aroused. Boars are housed in groups and as a consequence boars can influence each other's phenotype by (non-)heritable social interactions. The influence of these social interactions on androstenone are not well understood. The objective of this study is to investigate whether androstenone levels are affected by (non-) heritable social interactions and estimate its genetic correlation with growth rate and backfat. The dataset contained 6,245 boars, of which 4,455 had androstenone observations (68%). The average pen size was 7 and boars were housed in 899 unique pen-groups (boars within a single pen) and 344 unique compartment-groups (boars within a unique 'room' within a barn during time). Four models including different random effects, were compared for androstenone. Direct genetic, associative (also known as social genetic or indirect genetic effects), group, compartment, common environment and residual effects were included as random effects in the full model (M3). Including random pen and compartment effects (M2) (non-heritable social effects) significantly improved the model compared to including only direct, common environment and residual as random effects (M1,  $p < 0.001$ ), and including associative effects even more (M3,  $p < 0.001$ ). The sum of the direct and associative variance components determines the total genetic variance of the trait. The associative effect explained 11.7% of the total genetic variance. Backfat thickness was analysed using M2 and growth using M3. The genetic correlation between backfat (direct genetic variance) and total genetic variance for androstenone was close to zero. Backfat and the direct and associative effects for androstenone had genetic correlations of  $0.14 \pm 0.08$  and  $-0.25 \pm 0.18$ , respectively. The genetic correlation between total genetic variances for growth rate and androstenone was  $0.33 \pm 0.18$ . The genetic correlation between direct effects was  $0.11 \pm 0.09$  and between associative effects was  $0.42 \pm 0.31$ . The genetic correlations and current selection towards lower backfat and higher growth rate, suggest that no major change in androstenone is expected when breeding goals are not changed.

For selection against boar taint and therefore also against androstenone, results recommend that at least the social environment of the boars should be considered.

Key words: androstenone, associative effects, boar taint, pigs

### 3.1 Introduction

In the pig industry, male piglets are surgically castrated early in life to prevent boar taint, an unpleasant odor of the meat when heated or cooked. Boar taint is mainly caused by skatole (Vold, 1970) and androstenone (5 $\alpha$ -androst-16-en-3-one; (Patterson, 1968b)). Skatole is a product of bacterial degradation of tryptophan in the hind gut. Androstenone is a testicular steroid hormone which causes the urine like odour. The level of androstenone depends on the stage of puberty and genetics ( $h^2$  between 0.25-0.88; Sellier et al., 1998). Storage of androstenone is in the adipose tissue and androstenone can be released from the salivary glands of the boar. When a boar is sexually aroused, saliva containing a mixture of steroid compounds is released to attract females (Pearce and Hughes, 1987) and induce lordosis in receptive sows (Signoret et al., 1975). The levels of exposed pheromones can be influenced by the social environment of the animal (Patterson and Lightfoot, 1984; Zhang et al., 2005). However, the level of androstenone, within groups composed of boars only, is not well understood.

Traditional breeding has selected on the individual performance without considering the social effect that an individual has on its pen mates. This potentially underestimates the heritable variation which could be used for genetic improvement. A genetic model including the direct genetic effect as well as the social genetic effects (referred to as associative effect) of its pen mates was proposed by Griffing (1967). Muir and Schinckel (2002) extended the direct genetic model by incorporating the associative effects into the mixed model equations to predict direct and associative genetic variance components and breeding values.

The aims of our study were to investigate the effect of heritable social interactions on the level of androstenone in boars, estimate genetic correlations between androstenone and growing-finishing traits, and discuss implications for selection against boar taint.

### 3.2 Material and methods

Animal Care and Use Committee approval was not obtained for this study, because the data were obtained from an existing database.

#### 3.2.1 Data records

This work focuses on three traits: androstenone, backfat and growth rate during the growing-finishing period. Measurements of androstenone were obtained from fat in the neck of the boars and were collected in the slaughterhouse. The androstenone level was measured either by time-resolved fluoroimmunoassay by

### 3 Estimation of direct and associative effect for androstenone

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NSVS (Norwegian School of Veterinary Science, Oslo, Norway) described in Tuomola et al. (1997) or by gas chromatography with mass spectrometry by CCL (Co-operative Central Laboratory, Veghel, The Netherlands) described in Verheyden et al. (2007). There is a high correlation of 0.92 between the two methods as reported by Ampuero Kragten et al. (2011). Androstenone is not normally distributed and was therefore log-transformed. The abbreviation AND is used to refer to the androstenone level measured and logAND to the log-transformed androstenone levels used in statistical analysis. All boars were weighed individually at the start and end of the growing-finishing period. Backfat thickness was measured ultrasonically the day before slaughter (using Aloka; Corometrics Medical Systems Inc., Wallingford, CT, USA or Renco; Renco Corp. Minneapolis, MN, USA) or at the slaughter house using Hennessy Grading Probe (HGP; Hennessy and Chong, Auckland, New Zealand. In a comparable dataset (unpublished data), the genetic correlation between ultrasonically measured backfat and HGP backfat was high (0.91) and therefore we considered the two measurements as one trait. Age was calculated as the end date of the growing-finishing period minus date of birth.

#### 3.2.2 Animals

Phenotypic measurements of AND on boars were collected between October 2005 and December 2010 (N=8,294). This study needed accurate information on the pen number and pen mates to be able to identify boars penned together to estimate the (heritable) social effects. Therefore, 38% of the records that did not have pen numbers, had to be discarded. The dataset was completed by adding animals which were penned together with a boar with AND observation (69%). There were boars within the pen with and without observation for AND, because some boars were selected for breeding and could not be tested for AND. These animals are necessary for the estimation of the associative effects. Pens in which less than 2 boars had AND observation (pen size=3) or less than or equal to 25% of the boars (pen size  $\geq 4$ ) had AND observations were discarded (22%), as it is difficult to estimate the effect on pen mates when many pen mates have a missing observation. Minor editing was done by removing pens with gilts or barrows (2%), or pens with less than 3 and more than 11 boars (1%). One genetic line was removed because of low numbers (1%). Also 289 boars (4%) were removed because different genetic lines were penned together. After editing, there were 6,245 boars in the dataset, of which 4,455 boars had an AND observation (68%, Table 3.1).

Purebred boars (farm A, B and C) were either selected and used as an AI (Artificial Insemination) boar or slaughtered. The boars which were selected as AI boar, did

### 3 Estimation of direct and associative effect for androstenone

not have AND observations. Farm D contained crossbred boars and is an experimental farm where different lines are pair wise compared (Institute for Pig Genetics B.V., Beilen, The Netherlands). Four different sire lines were crossed with a F1 sow to produce crossbred pigs comparable to a commercial situation. Three of the four sire lines used to produce the crossbred boars, were similar to the genetic lines of the purebred boars in this study (Table 3.1).

In total, there were 6,245 boars, which originated from 295 sires and 1,146 dams with 2,093 litters. On average there were 3.12 piglets per litter in the dataset, and 1.83 litters per sow. The average pen size was 6.95. In total, 899 groups of pen mates were housed in 209 different physical pens. Those 899 groups are within 344 compartment-groups within a barn. A compartment is defined as a separate 'room' within the barn, and compartment-group as the unique composition of boars within the compartment during time. Due to farm management, animals were not randomly placed in pens, but were more likely to be penned with litter mates. Relatedness was calculated using 5 generations of pedigree. The average relatedness within a pen was 0.22, ranging from 0.02 to 0.57.

**Table 3.1** Number of boars per farm with information on the designs of the different farms.

Farm	Line <sup>1</sup>	No. animals	No. animals with AND observations	No. groups	Average pen size
Purebred					
A	11	728	269	80	9.10
B	22	3,382	2,590	536	6.31
C	33	629	253	88	7.07
Crossbred					
D	14	194	180	27	7.19
	24	557	506	72	7.74
	34	591	499	72	8.21
	54	164	158	23	7.13
	<b>Total</b>	<b>1,506</b>	<b>1,343</b>	<b>194</b>	<b>7.76</b>
<b>Total</b>		<b>6,245</b>	<b>4,455</b>	<b>898</b>	<b>6.95</b>

<sup>1</sup>The first number is corresponding to the sire line and the second number to the dam line.

#### 3.2.3 Univariate analyses

A mixed model in ASReml (Gilmour et al., 2006) was fitted to determine which fixed effects should be included in the final models ( $p < 0.20$ ). The effects of line ( $p < 0.001$ ), lab method ( $p < 0.001$ ), the interaction between weight at the end of the growing-finishing period and line ( $p < 0.001$ ) and number of pen mates ( $p = 0.04$ ) were significant in all models. Farm was fully confounded with line, and therefore not included in the model. Age was not significant, and although backfat thickness was significant, it was not included as a fixed effect. Correcting for the ability to store (more or less) AND due to the amount of adipose tissue in the pig, is part of the biological mechanism behind the excretion of AND. In this study, it is important to have AND not corrected for backfat as we are analyzing the complete process (storage and excretion) of AND. A permanent sow effect was found not to be significant.

Residual maximum likelihood (ReML) as implemented in ASReml (Gilmour et al., 2006), was used for estimating the genetic parameters using an animal model (Henderson, 1975; Lynch and Walsh, 1998). Records for logAND were analyzed with four univariate mixed models. A classical animal model (model 1), a social model including non-heritable social effects of the pen mates (random group effect, model 2), a social model with non-heritable social effects of the group (random group effect) and non-heritable effects of the compartment (random compartment effect, model 3), and a model including both heritable social effects and non-heritable social effects of the group and a non-heritable social effect of the compartment (model 4).

$$\text{Model 1: } \mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wl} + \mathbf{e},$$

$$\text{Model 2: } \mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wl} + \mathbf{Vg} + \mathbf{e},$$

$$\text{Model 3: } \mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wl} + \mathbf{Vg} + \mathbf{Uc} + \mathbf{e},$$

$$\text{Model 4: } \mathbf{y} = \mathbf{Xb} + \mathbf{Z}_D \mathbf{a}_D + \mathbf{Z}_S \mathbf{a}_S + \mathbf{Wl} + \mathbf{Vg} + \mathbf{Uc} + \mathbf{e}$$

where  $\mathbf{y}$  is the vector of phenotypes;  $\mathbf{b}$  is a vector of fixed effects with incidence matrix  $\mathbf{X}$ ;  $\mathbf{a}$  ( $\mathbf{a}_D$ ) is a vector of direct additive genetic effects with incidence matrix  $\mathbf{Z}$  ( $\mathbf{Z}_D$ );  $\mathbf{a}_S$  is a vector of associative additive genetic effects with the incidence matrix  $\mathbf{Z}_S$ ;  $\mathbf{l}$  is the vector for the non-genetic effects from individuals born in the same litter with incidence matrix  $\mathbf{W}$  and  $\mathbf{l} \sim N(0, I\sigma_l^2)$ ;  $\mathbf{g}$  is the vector of non-genetic effects due to the group in which the boars are penned during the growing-finishing period with incidence matrix  $\mathbf{V}$  and  $\mathbf{g} \sim N(0, I\sigma_g^2)$ ; and  $\mathbf{c}$  is the vector of non-genetic effects due to the same compartment in a barn where groups were housed during

the growing-finishing period with incidence matrix  $\mathbf{U}$  and  $\mathbf{c} \sim N(0, I\sigma_c^2)$ , and  $\mathbf{e}$  is the vector of residuals with  $\mathbf{e} \sim N(0, I\sigma_e^2)$ . The vectors  $\mathbf{a}_D$  and  $\mathbf{a}_S$  have a multivariate normal distribution ( $MVN \sim (0, \mathbf{C} \otimes \mathbf{A})$ ) where

$$\mathbf{C} = \begin{bmatrix} \sigma_{A_D}^2 & \sigma_{A_{DS}} \\ \sigma_{A_{DS}} & \sigma_{A_S}^2 \end{bmatrix} \text{ and } \mathbf{A} \text{ is the numerator relationship matrix calculated using}$$

5 generations and  $\otimes$  is the Kronecker product.

When the model does not include associative effects, the  $\mathbf{Z}_D\mathbf{a}_D$  and  $\mathbf{Z}_S\mathbf{a}_S$  matrix reduces to  $\mathbf{Za}$  and model 4 becomes equal to model 3. The  $\mathbf{Za}$  and  $\mathbf{Z}_D\mathbf{a}_D$  are identical, but named differently to emphasize the difference between  $\mathbf{Z}_D\mathbf{a}_D$  from  $\mathbf{Z}_S\mathbf{a}_S$  in model 4. The  $\mathbf{Z}_S$  matrix has a 1 on the off-diagonal for each pen mate, and zeros elsewhere (Muir, 2005).

The phenotypic variance for each model was (Bouwman et al., 2010):

$$\text{Model 1: } \sigma_P^2 = \sigma_{A_D}^2 + \sigma_I^2 + \sigma_e^2$$

$$\text{Model 2: } \sigma_P^2 = \sigma_{A_D}^2 + \sigma_I^2 + \sigma_g^2 + \sigma_e^2$$

$$\text{Model 3: } \sigma_P^2 = \sigma_{A_D}^2 + \sigma_I^2 + \sigma_g^2 + \sigma_c^2 + \sigma_e^2$$

$$\text{Model 4: } \sigma_P^2 = \sigma_{A_D}^2 + (n-1)\bar{r} \left[ 2\sigma_{A_{DS}} + (n-2)\sigma_{A_S}^2 \right] + (n-1)\sigma_{A_S}^2 + \sigma_I^2 + \sigma_g^2 + \sigma_c^2 + \sigma_e^2$$

where  $n$  is the number of boars within a group and  $\bar{r}$  is the average additive genetic relatedness between the pen mates of the same group.

The ratio of the total explained additive genetic variance over phenotypic variance is called heritability,  $h^2 = \frac{\sigma_A^2}{\sigma_P^2}$ . Including associative effects changes the additive

genetic variance ( $\sigma_A^2$ ) into total heritable variance ( $\sigma_{TBV}^2$ ). Genetic variance of the Total Breeding Value (TBV) is calculated as:

$$\sigma_{TBV}^2 = \sigma_{A_D}^2 + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma_{A_S}^2,$$

where  $\sigma_{A_D}^2$  is comparable to  $\sigma_A^2$ , whereas  $2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma_{A_S}^2$  originates from the additional genetic variance due to associative effects (Bijma et al., 2007).

The  $\sigma_{A_D}^2$  is the variance of the Direct Breeding Value (DBV),  $\sigma_{A_S}^2$  is the variance of the Social Breeding Value (SBV). The TBV is the sum of the individual's DBV and  $(n-1)$  times its SBV:

$$TBV_i = A_{D,i} + (n-1)A_{S,i}.$$

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The  $\sigma_{TBV}^2$  represents the total heritable variation that can be used for response to selection (Ellen et al., 2007):

$$\Delta G = i\rho\sigma_{TBV},$$

where  $i$  is the selection intensity,  $\rho$  the accuracy and  $\sigma_{TBV}$  is the square root of  $\sigma_{TBV}^2$ .

The equation for the ratio between the total heritable variance over phenotypic variance then becomes  $T^2 = \frac{\sigma_{TBV}^2}{\sigma_P^2}$ .

$T^2$  and  $h^2$  are both expressed on the same scale, which gives a possibility to judge the contribution of associative effects to the genetic variance that can be used for genetic improvement by breeding organizations. Even when  $\sigma_{A_s}^2$  is small, the contribution to the genetic variance can be substantial, especially when group sizes are large, as shown by the factor  $(n-1)^2$ .

#### 3.2.4 Dependency of associative effects on group size

The number of pen mates might affect the magnitude of the associative effects. In larger groups, the time to interact with each pen mate may be smaller (Ellen et al., 2007). Depending on the trait of interest, the magnitude of the associative effect may therefore be reduced in larger groups. The time spend fighting with a particular group mate, for example, is probably smaller in larger groups. For other traits, such as the spread of an infectious disease, the number of pen mates may not influence the magnitude of associative effects. This phenomenon is referred to as dilution (Bijma, 2010b). Not considering dilution might overestimate the  $\sigma_{TBV}^2$  and the potential of a population to respond to selection with large groups. The dilution of the associative effect is modelled by including a regression coefficient in front of the associative effect in the mixed model (Canario et al., 2010):

$$A_{S,j}(n) = \left(\frac{\bar{n}-1}{n-1}\right)^d A_{s,j},$$

where  $d$  is the dilution coefficient varying between 0 and 1 and  $n$  is the group size. Hence, non-zero elements of  $\mathbf{Z}_s$  are no longer equal to 1, but equal  $[(\bar{n}-1)/(n-1)]^d$ .

When  $d$  is 0, the associative effect is independent of group size. When  $d$  is 1 there is complete dilution, meaning that the associative effect is proportional to  $\frac{1}{n-1}$ ,

and an individual's total associative effect summed over all group mates is constant. Further information on dilution is in Bijma (2010b). The dilution coefficient was estimated for the associative effect of logAND, by finding the maximum likelihood value for  $d$  in the range from 0 to 1.

### 3.2.5 Bivariate analyses

Bivariate analyses were conducted to estimate the genetic and phenotypic correlations between logAND and growing-finishing traits using ASReml (Gilmour et al., 2006). The model for logAND was the same as model 4. Growing-finishing traits investigated were backfat thickness and growth rate (growth during the growing-finishing period). Fixed effects for the growing-finishing traits differed. For backfat, fixed effects were: line, line\*weight at end of the growing-finishing period (kg), feeding system (*ad libitum* or restricted), backfat measurement method and loin depth (mm). For growth, only line and feeding system were fixed effects with a p-value <0.2. The random effect in the models used for the growing-finishing traits were the same as in model 3 (only non-heritable social effects included) and model 4 ((non-)heritable social effects included) described in the univariate analyses section. The best fitting model was chosen based on a likelihood-ratio test. Genetic correlations between DBV, SBV and TBV were calculated between the growing-finishing traits and logAND.

The genetic correlation between two traits for the TBV is defined as (K. Peeters, Animal Breeding and Genomics Centre, Wageningen University and Research Centre, Wageningen, The Netherlands, personal communication):

$$r_{12\_TBV} = \frac{\sigma_{A_{12\_D}} + (n-1)\sigma_{A_{1\_D\_2\_S}} + (n-1)\sigma_{A_{2\_D\_1\_S}} + (n-1)^2\sigma_{A_{12\_S}}}{\sqrt{(\sigma_{A_{1\_D}}^2 + 2(n-1)\sigma_{A_{1\_DS}} + (n-1)^2\sigma_{A_{1\_S}}^2)(\sigma_{A_{2\_D}}^2 + 2(n-1)\sigma_{A_{2\_DS}} + (n-1)^2\sigma_{A_{2\_S}}^2)}}$$

### 3.3 Results and discussion

The descriptive statistics of the data are shown in Table 3.2. Boars were penned with an average weight around 28 kg and weighed on average 118 kg at the end of the growing-finishing period. The average growth rate was 899 g/d during this period.

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**Table 3.2** Number of observations and mean, minimum (min) and maximum (max) and standard deviation (SD) for growing-finishing traits.

	N	mean	min	max	SD
logAND <sup>1</sup>					
NSVS (µg/g)	1,611	0.22	-3.0	2.3	0.80
CCL (µg/g)	2,844	-0.20	-3.0	2.1	0.88
Weight start (kg)	6,200	27.5	13.0	70.0	4.85
Weight end (kg) <sup>1</sup>	6,244	117.9	70.0	195.0	12.40
Growth rate (g/d) <sup>1</sup>	6,200	898.9	415.0	1,546.0	118.06
Age (days)	6,244	174.8	146.0	213.0	9.20
Backfat thickness (mm) <sup>1</sup>	6,243	10.86	5.00	25.20	2.50

<sup>1</sup>logAND = log transformed androstenone level; NSVS= Norwegian School of Veterinary Science, Oslo, Norway; CCL= Co-operative Central Laboratory, Veghel, The Netherlands

<sup>2</sup>at the end of the growing-finishing period.

#### 3.3.1 Univariate analyses

Results from the univariate analyses for logAND are given in Table 3.3. The heritability for logAND was 0.63 in the classical animal model (model 1). By adding a random group effect, the heritability decreased from 0.63 to 0.61. The variance explained by the group decreased from 0.022 to 0.016 when a compartment effect was added to the model (model 2 vs. model 3). The model including associative effects (model 4) had a small associative genetic variance (0.002), but contributed considerably to the  $\sigma_{TBV}^2$  (11.7% respectively) due to the factor  $(n-1)^2$ . The estimates for group (and compartment) reduces when including associative effects, which was also found in other studies (Arango et al., 2005; Bouwman et al., 2010; Hsu et al., 2010; Bergsma et al., 2013). This could indicate a confounding between group and compartment effect and the associative effect (Cantet and Cappa, 2008). The effect of group was fully absorbed by including associative effects in the model, where compartment still explained a small variance (0.004±0.01) though not significant different from zero. Inclusion of (non-)heritable social effects reduced the estimated litter variance (from 0.039 to 0.032 respectively) and similar results were reported by Bergsma et al. (2008) and Bouwman et al. (2010). In some cases, litter is for a large part confounded with group as families are more likely to be placed together in a pen. Therefore the litter variance will be partly absorbed when (non-) heritable social effects are included in the model.

### 3 Estimation of direct and associative effect for androstenone

**Table 3.3** Results from the univariate analyses. Log-likelihoods (LogL) and estimates of parameters<sup>1</sup> for each model.

	LogL	$\sigma_{A_D}^2$	$\sigma_{A_{DS}}$	$\sigma_{A_S}^2$	$\sigma_g^2$	$\sigma_c^2$	$\sigma_l^2$	$\sigma_e^2$	$\sigma_p^2$	$\sigma_{TBV}^2$	$h^2 / T^2$
Model 1	-784.59	0.40±0.04					0.039±0.01	0.20±0.02	0.64±0.02		0.63±0.05
Model 2	-777.53	0.39±0.04			0.022±0.01		0.032±0.01	0.19±0.02	0.64±0.02		0.61±0.05
Model 3	-775.96	0.38±0.04			0.016±0.01	0.009±0.01	0.031±0.01	0.20±0.02	0.64±0.02		0.60±0.05
Model 4	-771.22	0.35±0.04	0.006±0.004	0.002±0.001	0.000±0.00	0.004±0.01	0.032±0.01	0.22±0.02	0.64±0.02	0.49±0.05	0.75±0.06

<sup>1</sup>  $\sigma_{A_D}^2$ ,  $\sigma_{A_{DS}}$  and  $\sigma_{A_S}^2$  = estimates of direct genetic variance, direct-associative genetic covariance and associative genetic variance.  $\sigma_g^2$ ,  $\sigma_c^2$ ,  $\sigma_l^2$  and  $\sigma_e^2$  = estimates of group, compartment, litter and residual variance.  $\sigma_p^2$  = phenotypic variance.  $\sigma_{TBV}^2$  = variance of the total breeding value (TBV).  $T^2 = \sigma_{TBV}^2 / \sigma_p^2$  and  $h^2$  = heritability.

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The comparison between the models is shown in Table 3.4, where a likelihood-ratio test was performed between all pairs of the four models. The classical animal model (model 1) performs worse than the other three models ( $P < 0.001$ ) where heritable or non-heritable social effects or both, were added. The difference between model 2 and model 3, where a group effect was present and a compartment effect was added (model 3), had only a minor effect and was borderline significant ( $P = 0.076$ ). Model 4, including a heritable social effect, performed significantly better than including only non-heritable social effects ( $P = 0.006$  compared to model 2 and  $P = 0.009$  compared to model 3).

The correlation between the direct and social breeding values for logAND was positive (0.24), however not significantly different from zero. The positive correlation could indicate a stimulating effect of the secretion of AND by the pen mates. When the genetic correlation between the direct and associative effect is positive, boars with a high DBV will have a high SBV. The SBV is passed on to its pen mates.

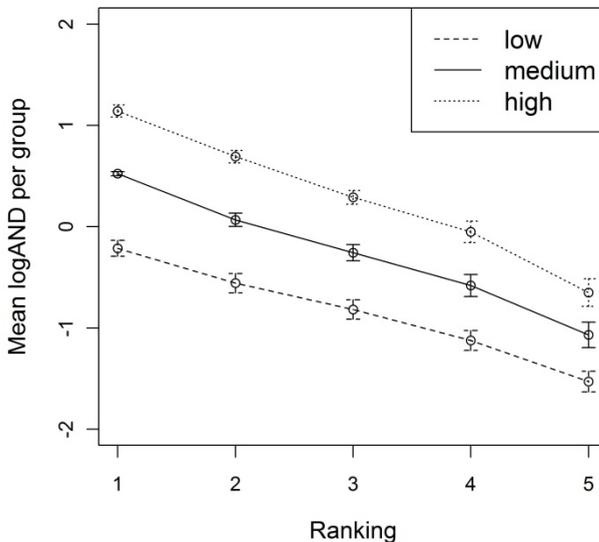
Giersing et al. (2000) suggested a stimulating effect of AND for boars on the other boars within the pen. In that study, pens were classified as high, medium and low based on the boar within the pen with the highest AND level. This study reported that a high maximum level of AND within a pen resulted also in a higher level of the second highest AND boar within that pen. Moreover, a higher mean AND level was reported between the pens classified as high compared to the classes medium and low. From these observations, Giersing et al. (2000) concluded a stimulation effect of AND between boars within the same pen. To investigate whether this claim is correct, the same approach was repeated in this study and results are shown in Figure 3.1. Five-hundred boars from 100 different group were ranked based on the highest logAND level within the group and 34% of the groups was classified as high, 33% as medium and 33% as low.

**Table 3.4** Likelihood-ratio test for the four models.

Models compared	LRT <sup>1</sup>	P-value	Df <sup>2</sup>
2 vs. 1	14.12	<0.001	1
3 vs. 1	17.26	<0.001	2
4 vs. 1	26.74	<0.001	4
3 vs. 2	3.14	0.076	1
4 vs. 2	12.62	0.006	3
4 vs. 3	9.48	0.009	2

<sup>1</sup>LRT= chi-square test statistic for the likelihood-ratio test ( $-2(\text{LogL}_{\text{reduced model}} - \text{LogL}_{\text{full model}})$ ).

<sup>2</sup>Degrees of freedom for the chi-square test statistic defined as the difference in number of (co)variances fitted for the two models.



**Figure 3.1** The effect of ranking on average logAND levels per class. A group was classified as either high (N=34), medium (N=33) or low (N=33) according to the maximum logAND level within a group (only laboratory method from CCL; Co-operative Central Laboratory, Veghel, The Netherlands). One hundred groups were equally divided into the three classes. Only hundred groups were selected based on logAND level not missing and equal group sizes (N=5). On the x-axis is the ranking of the boars within the group in order from highest (rank=1, high logAND) to lowest (rank=5, low logAND). The y-axis gives the mean logAND level. Means are given with standard error bars per class and ranking.

The results from Figure 3.1 are comparable to the results shown by Giersing et al. (2000; Figure 3.2). In both figures, the effect of the maximum logAND level within a pen seems to have a major effect on the level of logAND by the pen mates. Nevertheless, results from the models in this study suggest only a small effect of the social environment of the boars. In model 3, where only non-heritable social effects were included, the variance explained by the group and compartment was only 3.9%, which is only minor compared to the direct genetic variance which was 60%. Therefore, suggesting a stimulating effect based on the level of logAND between pen mates using raw data without correcting for any (non-)heritable effects, is not correct and results in an overestimation of the effect of the social environment. The significant improvement of the model when associative effects were taken into account, does suggest that social interactions between the boars

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do affect the secretion of AND in groups although the effect seems to be minor. The biological mechanism behind these results is still unknown.

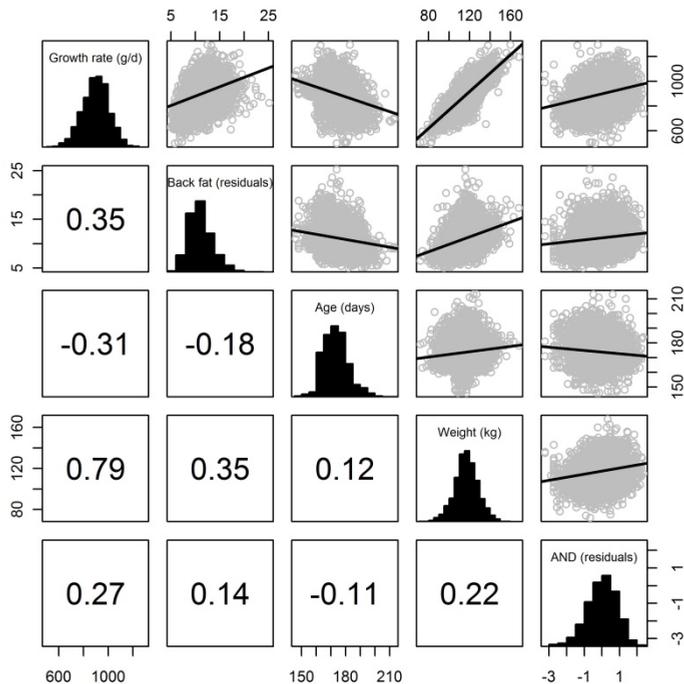
Pauly et al. (2009) found no significant difference in AND levels between individual and group housed boars, which is not supporting the hypotheses that AND has a stimulating effect on pen mates (boars only). Also boars are less sensitive to AND than gilts. Dorries et al. (1995) reported that gilts were better in identifying lower AND concentrations than boars in a food reward experiment. The presence of gilts or sows in the neighbourhood of a boar has a stimulating effect on AND levels of the boars (Narendran et al., 1982; Patterson and Lightfoot, 1984). In this study, there were gilts kept in the same compartment as boars, but the effect of sex ratio within a compartment was not significant on AND for farm D (no information available on the other farms). However, in this study there was a lack of information on the number of females that achieved sexual maturity or the number of females that had been in oestrus. On average, gilts have their first oestrus at 190 days (Rekwot et al., 2001; Kuehn et al., 2009) but when exposed to a boar, earlier oestrus can be shown (Thompson and Savage, 1978). On average, the boars and gilts attained an age of 175 days in this study, and probably many gilts didn't attained puberty yet. This could explain why the number of gilts in the compartment did not have a large influence on the level of AND in the boars. Also no physical contact was possible between the members of adjacent pens to influence the secretion of AND in the boars.

#### 3.3.2 Dilution of associative effects

Different dilution ( $d$ ) factors were used between 0 and 1 with step size of 0.25. The difference between complete dilution ( $d=1$ ) and no dilution ( $d=0$ ) was borderline significant ( $p=0.04$ ), where no dilution ( $d=0$ ) fitted the model best. This is suggesting that magnitude of the associative effect is not influenced by pen size. This study contained group sizes varying between 3 and 11 boars, and extrapolation towards larger groups ( $>15$ ) is not possible. Though AND is a pheromone and can be spread by air or contact, eventually distance to reach the other pen mate if group size becomes very large, will be limited and  $d$  will become larger than 0. In the current situation with large groups up to 11 boars, will be affected more by associative effects for AND than smaller groups.

#### 3.3.3 Bivariate analyses

Figure 3.2 shows the raw relations between logAND and the growing-finishing traits growth rate, backfat (corrected for the different measurements), age and weight (kg) at the end of the growing-finishing period and the distribution of the traits.



**Figure 3.2** Overview of the raw phenotypic correlations between growth during growing-finishing (g/d), backfat, age (days), weight (kg), logAND and the distribution of these traits. Upper-diagonal: the raw data plotted with a regression line. On the diagonal is the distribution of the traits in a histogram. The lower-diagonal gives the correlation between the two traits. Backfat was corrected for method of measurement and residuals were used. LogAND was corrected for lab methods and residuals were used.

Age and weight at slaughter were included to investigate the effect on logAND. In this study, the correlation between age (175 days on average) and logAND is -0.11, which is only minor. In several studies the effect of age at slaughter on AND has been studied and was related to sexual maturity. Sexual maturity of the boar has a large influence level on AND (Babol et al., 1995) Sexual maturity is difficult to measure as it is a continuous process, but on average boars attain puberty around 6-7 months of age (Lagerlöf and Carlquist, 1961; Andersson et al., 1999) and can be influenced by different factors such as genetics (Schinckel et al., 1984), nutrition, season and lighting conditions (Andersson et al., 1999). Due to low variation in the trait age, a clear relation between age and logAND cannot be made in this study. The correlation between logAND and weight (at end of the growing-finishing period) is 0.22 (Figure 3.2). Over the last 20 years, slaughter weight of pigs has

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increased from 84.3 kg to 92.4 kg in The Netherlands (PVE, 2010). The increase of slaughter weight results in more boars that attain maturation, and consequently increases the risk for boar taint.

Correlations of logAND with growth rate and backfat were positive (0.27 and 0.14 respectively). Growth rate and backfat had a positive correlation of 0.35. All traits were approximately normally distributed.

**Table 3.5** Estimates for parameters<sup>1</sup> with SE for the bivariate analyses between log-transformed androstenone (logAND) and backfat in entire male pigs.

	logAND	Backfat (mm)
$\sigma_{A_D}^2$	0.355±0.04	1.170±0.124
$\sigma_{A_{DS}}$	0.007±0.004	
$\sigma_{A_S}^2$	0.002±0.001	
$\sigma_g^2$	0.000±0.00	0.112±0.025
$\sigma_c^2$	0.005±0.005	0.139±0.027
$\sigma_l^2$	0.031±0.01	0.002±0.03
$\sigma_e^2$	0.220±0.02	1.148±0.07
$\sigma_p^2$	0.65±0.02	2.57±0.07
$\sigma_{TBV}^2$	0.50±0.06	
$h^2 / T^2$	0.78±0.08	0.46±0.04
$r_{g(TBV(\log AND), DBV(backfat))}$		0.03±0.08
$r_{g(DBV(\log AND), DBV(backfat))}$		0.14±0.08
$r_{g(SBV(\log AND), DBV(backfat))}$		-0.25±0.18

<sup>1</sup>  $\sigma_{A_D}^2$ ,  $\sigma_{A_{DS}}$  and  $\sigma_{A_S}^2$  = estimates of direct genetic variance, direct-associative genetic covariance and associative genetic variance.  $\sigma_g^2$ ,  $\sigma_c^2$ ,  $\sigma_l^2$  and  $\sigma_e^2$  = estimates of group, compartment, litter and residual variance.  $\sigma_p^2$  = phenotypic variance (calculated according to model 4 for logAND and model 3 for backfat).  $\sigma_{TBV}^2$  = variance of the total breeding value (TBV).  $T^2 = \sigma_{TBV}^2 / \sigma_p^2$ ,  $h^2$  = heritability.  $r_{g(TBV(\log AND), DBV(backfat))}$  = genetic correlation between the TBV (logAND) and the direct breeding value (DBV) (backfat).  $r_{g(DBV(\log AND), DBV(backfat))}$  = genetic correlation between the DBVs of both traits.  $r_{g(SBV(\log AND), DBV(backfat))}$  = genetic correlation between the social breeding value (SBV logAND) and DBV (backfat).

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**Table 3.6** Estimates for parameters<sup>1</sup> with SE for the bivariate analyses between log-transformed androstenone (logAND) and growth in the growing-finishing period (g/d) in entire male pigs.

	logAND	Growth (days)
$\sigma_{A_D}^2$	0.355±0.04	2523±397
$\sigma_{A_{DS}}$	0.008±0.004	-46.8±57.7
$\sigma_{A_S}^2$	0.002±0.001	34.9±15.4
$\sigma_g^2$	0.000±0.00	414.5±155
$\sigma_c^2$	0.005±0.005	1038±175
$\sigma_l^2$	0.03±0.01	1065±170
$\sigma_e^2$	0.22±0.02	6154±277
$\sigma_p^2$	0.65±0.02	11506±275
$\sigma_{TBV}^2$	0.51±0.06	3202±760
$T^2$	0.79±0.08	0.28±0.06
$r_{g(DS)}$	0.29±0.19	-0.16±0.18
$r_g(TBV(\log AND),TBV(growth))$		0.33±0.18
$r_g(DBV(\log AND),DBV(growth))$		0.11±0.09
$r_g(SBV(\log AND),SBV(growth))$		0.42±0.31

<sup>1</sup>  $\sigma_{A_D}^2$ ,  $\sigma_{A_{DS}}$  and  $\sigma_{A_S}^2$  = estimates of direct genetic variance, direct-associative genetic covariance and associative genetic variance.  $\sigma_g^2$ ,  $\sigma_c^2$ ,  $\sigma_l^2$  and  $\sigma_e^2$  = estimates of group, compartment, litter and residual variance.  $\sigma_p^2$  = phenotypic variance (calculated according to model 4).  $\sigma_{TBV}^2$  = variance of the total breeding value (TBV).  $T^2 = \sigma_{TBV}^2 / \sigma_p^2$ .  $r_{g(DS)}$  = genetic correlation between the direct breeding value (DBV) and the social breeding value (SBV).  $r_g(TBV(\log AND),TBV(growth))$  = genetic correlation between the TBVs of both traits.  $r_g(DBV(\log AND),DBV(growth))$  = genetic correlation between the DBVs of both traits.  $r_g(SBV(\log AND),SBV(growth))$  = genetic correlation between the SBVs of both traits.

Results from the bivariate analyses between logAND and backfat are shown in Table 3.5. A model with heritable social effect for backfat was not significantly different from a model where these effects were excluded (results not shown), which was also found in earlier studies (Bergsma et al., 2008; Hsu et al., 2010). Therefore, the model for backfat included only non-heritable social effects (group

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and compartment) comparable to model 3. Model 4 was used for logAND as it was the best fitting model in the univariate analyses. Estimates for logAND in the bivariate analyses were similar to the results from the univariate analyses. Backfat had a heritability of 0.46 and the variance for the non-heritable social components (group and compartment) explained ~10% of the phenotypic variance. The genetic correlation between the TBV of logAND and backfat (DBV) was close to zero ( $0.03 \pm 0.08$ ). The correlation between the DBV of AND with the DBV of backfat was positive ( $0.14 \pm 0.08$ ) and between the SBV of AND and the DBV of backfat was negative ( $-0.25 \pm 0.18$ ). All genetic correlations were not significantly different from zero. A positive correlation between AND and backfat is to be expected, as AND is stored in adipose tissue.

Growth rate during the growing-finishing period was analysed following model 4, where a social heritable effect was added because it was significantly different from model 3 where only non-heritable social effects were included (results not shown). Both group and compartment contributed considerably to the phenotypic variance (~13%). The  $T^2$  of growth rate was 0.28 and the genetic variance explained by  $\sigma_{A_d}^2$  was 0.23 (Table 3.6). Also other studies found (non-)heritable social effects for growth rate in pigs (Arango et al., 2005; Bergsma et al., 2008; Chen et al., 2008; Hsu et al., 2010; Bergsma et al., 2013). The testing program and populations used in the study by Bergsma et al. (unpublished data) were comparable with this study and estimates for the heritable variance explained by growth rate ( $T^2$ ) was 0.34 and a genetic correlation between the direct and associative effect of 0.01. The negative covariance found in this study between direct and associative effect (-47) also contributed to a lower total heritable variance ( $\sigma_{TBV}^2$ ) resulting in a lower  $T^2$  compared to the results of Bergsma et al. (unpublished data). The difference between the two studies is the sex ratio within the datasets. Estimates by Bergsma et al. (unpublished data) were based on pens consisting of barrows, gilts and boars, whereas only boars were used in this study. Underlying the negative covariance could be behaviours that are more or less expressed in boars compared to gilts and barrows. Boars showed significantly more aggressive behaviour and attempts to mount compared to barrows (Cronin et al., 2003). Boars also displayed more aggressive behaviour than gilts when grouped in a mixed pens (Rydmer et al., 2006) or in single-sex pens (Salmon and Edwards, 2006).

The genetic correlation between the DBVs for growth and logAND were  $0.11 \pm 0.09$ , but not significantly different from zero. Sellier et al. (2000) found a genetic

correlation of -0.16 at an average live weight of 118 kg. At 99 kg of live weight the genetic correlation was 0.04, though none of the genetic correlations were significant statistically. In a comparable dataset, the genetic correlation between growth and logAND was 0.19 (Merks et al., 2010). The genetic correlation between the SBVs was also positive ( $0.42 \pm 0.31$ ) as well as the genetic correlation between the TBVs ( $0.33 \pm 0.18$ ).

#### **3.3.4 Consequences for selection**

Breeding programs for sire lines are directed towards increased growth, feed efficiency and low backfat thickness. Andresen (1976) selected boars on fatness and growth rate. In the 7<sup>th</sup> and 8<sup>th</sup> generation, significantly higher levels of AND were found in boars selected for increased fatness and a low growth rate compared with boars selected on leanness and a high growth rate. A simulation study by Merks et al. (2009) reported a small reduction in logAND when selection was only on production traits. The underlying genetic correlation were -0.11 between growth and logAND and +0.07 between backfat and logAND, which resulted in a reduction of 0.05 µg/g AND per generation. The genetic correlation between logAND and growth is not consistent across studies. Studies with positive correlations (Merks et al., 2010) and negative correlations (Sellier et al., 2000; Merks et al., 2009) were found and genetic correlations were not significantly different from zero. Genetic correlations between logAND and backfat thickness are close to zero (this study) or positive (Sellier et al., 2000; Merks et al., 2009). The effect of selection against logAND, might have consequences for male and female fertility. Genetic correlations between logAND and other male sex steroids were high (0.80-0.95) (Grindflek et al., 2011a), however correlations between semen quality and quantity traits and logAND seem to be non-significant (Merks et al., 2010). Correlations with female fertility, point towards delayed maturation of gilts (Willeke et al., 1987) and correlations with other female fertility traits are low negative or non-significant (Merks et al., 2010) when selection is on lower logAND levels.

#### **3.4 Conclusion**

The results show that (non-)heritable social effect significantly affect logAND, however the estimates are relatively small. The explained variance for the non-heritable social effects was 4%. The associative effect explained almost 12% of the total genetic variance, but was small (0.002) compared to the direct genetic variance which was 0.35. For breeding purposes, at least the social environment has to be considered when selection is on AND.

#### **Acknowledgements**

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# 4

## **Boar taint in entire male pigs: a GWAS for direct and indirect genetic effects on androstenone**

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## **Abstract**

Androstenone is one of the compounds causing boar taint of pork, and is highly heritable ( $\sim 0.6$ ). Recently, indirect genetic effects (IGEs, also known as associative effects or social genetic effects) were found for androstenone, meaning that pen mates (boars) affect each other's androstenone level genetically. Similar to estimating variance components with a direct-indirect animal model, direct and indirect genetic SNP effects can be estimated for androstenone. This study aims to detect SNPs with significant direct and indirect genetic effects on androstenone. The dataset consisted of 1,282 non-castrated boars (993 boars genotyped), from 184 groups of pen members. After quality control, 46,421 SNPs were included in the analysis. One model for single-SNP regression was fitted, where both the direct SNP-effect of the individual itself and the indirect SNP-effects of its pen mates were included. None of the SNPs (direct or indirect) were found genome-wide significant. One QTL on SSC6 was chromosome-wide significant for the direct effect. A single SNP on SSC9 and two regions and a single SNP on SSC14 were found for the indirect effect. A backwards elimination method and haplotype analysis were used to quantify the variance explained by the SNPs. The backwards elimination method identified four independent regions affecting androstenone. The QTL on SSC6 explained 2.1% and 2.6% of the phenotypic variance using the backwards elimination method or the haplotype analysis. The QTLs on SSC14 explained 3.4% and 2.7% of the phenotypic variance using the backwards eliminations method or the haplotype analysis. The single association on SSC9 explained 2.2% of the phenotypic variance. All significant QTL together explained 7-8% of phenotypic variance, and 40-44% of the total genetic variance available for response to selection. Besides the newly discovered QTL and the confirmation of known QTL, this study also presents a methodology to model SNPs for indirect genetic effects.

Key words: androstenone, indirect genetic effect, genome-wide association study, QTL, pigs, boar taint

### 4.1 Introduction

Castration of young male piglets, is a welfare concern in many European countries and a ban is expected in the coming years (EU, 2010). Castration is applied to circumvent boar taint; an unpleasant odor to meat when cooked or heated. Main compounds causing boar taint are androstenone, skatole and indole. Both skatole and indole are synthesized in the large intestine from tryptophan (Jensen et al., 1995), while androstenone is a testicular steroid (Patterson, 1968) excreted via saliva as a pheromone to attract the opposite sex (Pearce and Hughes, 1987) and induces lordosis in receptive sows (Signoret et al., 1975).

All components causing boar taint are heritable with a varying heritability estimate from moderate (0.2) to high (0.9) (Robic et al., 2008). Androstenone levels can be influenced by the social environment (Patterson and Lightfoot, 1984; Giersing et al., 2000) which can contain both heritable and non-heritable components. Recently, indirect genetic effects (IGE's, also referred to as associative effects) were found for androstenone, meaning that other pen mates (only boars) influence the level of androstenone of a given pen mate genetically (Duijvesteijn et al., 2012). Both the DGE (direct genetic effect) and IGE's contribute to the total genetic variance, that determines a population's potential to respond to selection (Griffing, 1967; Muir, 2005; Bijma, 2011). The IGE's contributed 12% to the total genetic variance, the DGE contributed 71% and their covariance contributed 17% (Duijvesteijn et al., 2012).

Underlying genes for the DGEs on androstenone have been discussed in several linkage (Quintanilla et al., 2003; Lee et al., 2005) and genome-wide association studies (Duijvesteijn et al., 2010; Grindflek et al., 2011b). Similar to estimating variance components for a direct-indirect animal model, SNP effects for the DGE and IGEs can be estimated for androstenone. Using this new approach, this study aims to detect SNP associations for androstenone.

### 4.2 Material and Methods

Animal Care and Use Committee approval was not obtained for this study, because the data were obtained from an existing database.

#### 4.2.1 Animals

The original dataset of Duijvesteijn et al. (2012) was used, and contained 6,245 boars of which 68% had a phenotypic measurement for androstenone. In total, 1,634 boars were genotyped using the Porcine 60K Beadchip (Illumina, San Diego, CA, USA). A minimum callrate of 95% per individual for the genotypes was applied

#### 4 GWAS for direct and indirect effect on androstenone

and 19 boars didn't pass this quality control. Besides the callrate, genotypes of the pen members need to be available to perform an association study for indirect genetic effect. Therefore, only groups of pen members where at least 40% of the boars was genotyped were selected, which reduced the dataset to 1,282 boars (993 boars genotyped, 1,151 boars with androstenone levels) from 184 groups of pen members. The average pen size was 7 and varied between 3 and 11, where pens with 3 or 4 pigs had at least 50% of the boars genotyped. Due to farm management, boars were not randomly placed in groups. The average relatedness between the boars was 0.21 within a group, ranging between 0.02 to 0.53 using 5 generations of pedigree. The boars were housed in 112 compartments-groups, where a compartment is defined as a separate 'room' within the barn, and compartment-group as the unique composition of boars within the compartment during time.

The boars originated from 3 different farms (A,B and C) of which farm A and B only kept purebred boars, while farm C was an experimental farm (Institute for Pig Genetics B.V., Beilen, The Netherlands) with only crossbred animals. The crossbred animals were bred from boars originating from three different sire lines (1,2, and 3) crossed with an F1 sow. Two of the three sire lines (1 and 2) were from the same sire line as the purebred boars in this study (Table 4.1).

**Table 4.1** The number of animals per farm with information on the designs of the different farms.

Farm	Line <sup>1</sup>	No. animals	No. animals genotyped	No. groups	Average group size
Purebred					
A	11	598	405	94	6.4
B	22	14	9	3	4.7
Crossbred					
C	14	274	247	36	7.6
	24	319	263	39	8.2
	34	77	69	11	7.0
Total		670	579	86	7.8
<b>Total</b>		<b>1,282</b>	<b>993</b>	<b>183</b>	<b>7.0</b>

<sup>1</sup>The first number corresponds to the sire line and the second to the dam line.

### 4.2.2 SNP quality

Samples were genotyped using the Porcine 60K Beadchip (Illumina, San Diego, CA, USA) at Service XS (Leiden, The Netherlands). A total of 64,232 SNPs were genotyped, of which 58,822 were mapped using Sus Scrofa Build10.2 and were included before applying quality control. SNPs were coded as missing when the GenCall Score (GC score) was <0.7. Then a minimum callrate of 0.95 was applied on the SNPs and a minor allele frequency (MAF) above 0.01 was required for each SNP. In total 46,421 SNPs remained in the final analyses.

### 4.2.3 Genome-wide association

The direct genetic effects of the individual's own SNPs and the indirect genetic effects of the SNPs of each of its pen mates on androstenone were estimated. Fixed effects in the genome-wide association (GWA) model included were: line, method of laboratory for the chemical analyses of androstenone (Duijvesteijn et al., 2012), the interaction between weight at the end of the growing-finishing period, line, and number of pen members. Also the first three eigenvectors of a principal component analysis (PCA) of the genomic relationship matrix were added as fixed effects to account for population stratification (see below). No fixed effect for herd-year-month was fitted, it was partly confounded with line and was not found to be significant in the model. The data was analysed in 46,421 consecutive runs using ASReml (Gilmour et al., 2009). The SNP effects were fitted one at a time, both as the direct effect of the individual and the summed indirect effects of its pen mates:

$$y_{ijklmnop} = \mu + \text{line}_k + \text{lab}_l + \text{line}_k * \text{weight} + \text{npn}_m + \text{pc1} + \text{pc2} + \text{pc3} + \beta * \text{SNP}_i + \beta * \sum_j \text{SNP} + A_{D,i} + \sum_j A_{I,j} + \text{group}_n + \text{comp}_o + \text{litter}_p + e_{ijklmnop}$$

Where  $y_{ijklmnop}$  is log transformed androstenone for animal  $i$  from line  $k$  analysed in lab  $l$  and grouped in pensize  $m$ , group  $n$ , compartment  $o$  and born in litter  $p$ , PCA1, PCA2 and PCA3 are the first three principle components of the kinship matrix, and  $\beta$  is the effect of the SNP genotype of animal  $i$  or pen mates  $j$ , coded as the allele count (0, 1 or 2) minus  $2p$  ( $p$  is the allele frequency of the counted allele). To allow pens containing animals with missing genotypes to be included in the analysis, missing genotypes were replaced with the population mean, which was zero, because  $2p$  was subtracted from the allele count. Thus animals with missing genotypes were included in the estimation of the SNP effects, both for the direct and the indirect SNP effects.

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The  $A_{D,i}$  is the direct polygenetic effect, and  $\sum A_{I,j}$  the sum of the indirect polygenetic effects for the pen members of animal  $i$ . The direct and indirect genetic effect, group, compartment, litter are assumed random. Variance component estimation was not feasible (due to the limited size) and therefore the estimated variance components from the larger dataset presented in Duijvesteijn et al., (2012) were used (Table 3.3). The dataset used for this study was a subset of that larger dataset.

A genomic kinship matrix of the genotyped animals was created to derive axes of genetic variation (principal components) using R package GenABEL (Aulchenko et al., 2007). The first three eigenvectors from the PCA were used to account for population stratification. Population structure in this dataset is likely to cause population stratification as different populations are included in the same study sample. The first principle components of the 993 x 993 kinship matrix represent broad differences across individuals within the study sample and capture the population structure (explain largest part of the genetic variation). In this study, the first three principal components explained 30% of the genetic variation. Including more eigenvectors as fixed effects (5, 10 or 20) showed a decrease of the inflation factor (up to 1.2), but also reduced the p-values of the well-known QTL on SSC6 to such a level that the QTL was no longer identified. Therefore, we decided to include the first three principal components.

To investigate deviation of the p-values from their expected distribution under the null hypotheses of no genetic association, a quantile-quantile (Q-Q) plot was constructed where the observed p-values were plotted against their theoretical distribution. P-values were adjusted using the genomic control (GC) approach when inflation or deflation was detected (Devlin and Roeder, 1999). Inflation or deflation was expressed as  $\lambda$ . When  $\lambda > 1.1$  (WTCCC, 2007), the F-values were divided by  $\lambda$  and p-values were re-calculated. Deflation is rarely observed, though when present, p-values were not be adjusted

Given the large number of tests (46,421), highly significant findings are expected by chance. To control the number of false discoveries, the false discovery rate (FDR) was calculated, using the qvalue package (Storey and Tibshirani, 2003) in R. This package calculates a  $q$ -value based on the distribution of the p-values as a measure of significance in terms of the FDR. The  $q$ -value is therefore dependent on the set of p-values, and results can be different when for example subsets of the larger dataset are analysed. The  $q$ -value for declaring a significant association was set to 0.1, meaning that 10% of the significant associations are on average allowed to be false positives. The significance threshold was used for genome- and chromosome-

wide associations. Chromosome-wide associations can be informative, as chromosome  $q$ -values might indicate SNPs to be suggestive associated, even when none were found significant in the genome-wide analysis.

### 4.2.4 Estimation of variance explained by Quantitative Trait Loci (QTL)

To quantify the variance explained by significant SNPs ( $q$ -value  $\leq 0.10$ ), two different approaches were applied. Both approaches aim at getting closer to the causative mutation by determining the most significant SNPs, and to calculate the variance explained by the significant SNP. The latter, is important for quantifying the impact on breeding programs when SNPs are implemented using marker assisted selection (MAS). To get an accurate estimate for the variance explained, it is important to account for Linkage Disequilibrium (LD) between significant SNPs. Because we performed a single SNP analyses, the significant SNPs might be in high LD with each other, and without accounting for it, will result in an overestimation of the variance explained.

The first approach was a backwards elimination method. All SNPs were fitted simultaneously to determine the least significant SNP. The least significant SNP was removed and the procedure was repeated until one or more SNP(s) ended up significant ( $p$ -value  $\leq 0.05$ ). The genetic variance explained by the significant direct SNP was calculated from the allele substitution effect and the allele frequency as:  $V = 2pq\alpha^2$  (Falconer et al., 1996), where  $p$  and  $q$  are the major and minor allele of the SNP and  $\alpha$  is the estimated allele substitution effect. LD between significant SNPs was not taken into account when the genetic variance explained by the SNP was calculated.

The second approach to estimate the genetic variance explained by the SNPs used haplotypes and was carried out only when multiple SNPs on a chromosome were found to be significant from the GWA study. All significant SNPs per chromosome were used and were forced into one haplotype. Haplotypes were inferred (computed using expectation-maximization algorithm) per animal using PLINK (Purcell et al., 2007). Each animal has two haplotypes; either 0, 1 or 2 copies of each of the available ones. Haplotypes per animal with a probability  $\leq 0.5$  were set to missing and were added to a 'bin' haplotype. Haplotypes with a frequency  $<1\%$  were also added to the 'bin' haplotype. When significant SNPs were found for indirect genetic effects, the haplotypes of the pen mates were modelled. The variance explained by the haplotypes was calculated as:  $V = E[(X - \mu)^2]$ , where  $\mu$

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is  $\sum p\alpha$ ,  $p$  is the frequency of the haplotype in the population and  $\alpha$  is the estimated haplotype effect. Because each boar has 2 haplotypes,  $V$  becomes  $2\sum[(\alpha - \mu)^2 p]$ .

The variance explained ( $V$ ) for both the backwards elimination method and the haplotypes was expressed relative to the phenotypic variance ( $\sigma_P^2$ ) and to the total genetic variance ( $\sigma_{A_T}^2$ ). The phenotypic variance was calculated assuming unrelated groups members (Bergsma et al., 2008):

$$\sigma_P^2 = \sigma_{A_D}^2 + (n-1)\sigma_{A_I}^2 + \sigma_I^2 + \sigma_g^2 + \sigma_c^2 + \sigma_e^2, \quad [4.1]$$

where  $n$  is the number of pen members,  $\sigma_{A_D}^2$  is the direct additive effect,  $\sigma_{A_I}^2$  is the indirect genetic effect,  $\sigma_I^2$  is the litter variance,  $\sigma_g^2$  is the group variance,  $\sigma_c^2$  is the compartment variance and  $\sigma_e^2$  is the residual variance. The total genetic variation available for response to selection is the variance of the total breeding values of individuals:

$$A_{T,i} = A_{D,i} + (n-1)A_{I,i}, \quad [4.2]$$

which is the sum of the direct genetic effect of animal  $i$  on its own androstenone level plus its total indirect genetic effect on the androstenone level of each of its pen mates. The total genetic variation available for response to selection (Bijma et al., 2007) is given by:

$$\sigma_{A_T}^2 = \sigma_{A_D}^2 + 2(n-1)\sigma_{A_{DI}} + (n-1)^2\sigma_{A_I}^2 \quad [4.3]$$

where  $\sigma_{A_{DI}}$  is the direct-indirect genetic covariance.

The total breeding value of an animal reflects the genetic impact of that animal on trait values in the population, and therefore depends only on the genes of the animal itself (Equation 4.2). The phenotype of an individual, in contrast, is determined by the direct effects originating from the individual itself (including both genetic and non-genetic components) and the sum of the indirect effects originating from each of its pen mates. Consequently, as illustrated by Equations 4.1 and 4.3, the total heritable variance is not part of the phenotypic variance. Therefore, as described below, the variance due to the SNP-effects is expressed in two ways. First, variance due to the SNP-effects was expressed relative to the phenotypic variance, to illustrate the proportion of the observed variance explained by the SNPs. Second, variance due to the SNP-effects was expressed relative to the total heritable variance, to illustrate the proportion of the genetic variance for response to selection explained by the SNPs.

Both the phenotypic and genetic variance explained by the SNP were calculated for SNPs with a direct genetic effect as  $\frac{V}{\sigma_P^2}$  and  $\frac{V}{\sigma_{A_r}^2}$ . The variance explained by indirect SNPs was calculated differently, because indirect effects are expressed once on the phenotype of each of the  $(n-1)$  group mates of an individual. Therefore the proportional contribution of indirect SNPs to phenotypic variance becomes  $\frac{(n-1)*V}{\sigma_P^2}$ . The proportion of total genetic variance explained by the indirect SNPs or haplotypes which can be used for the response to selection is  $\frac{(n-1)^2 * V}{\sigma_{A_r}^2}$ . The square of  $(n-1)$  is taken as is it the variance of  $(n-1)$  which is the contribution to  $\sigma_{A_r}^2$ .

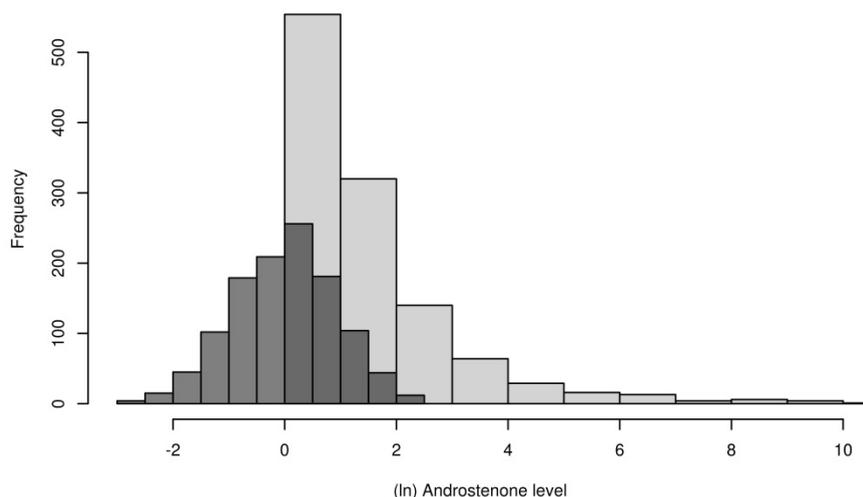
#### ***QTL comparison and identification of candidate genes***

All earlier reported QTL on androstenone and related traits such as perception of pork by consumer panels were available at the Pig QTLdb (<http://www.animalgenome.org/cgi-bin/QTLdb/SS/index>). The left and right flanking marker of those QTL were searched at the reference genome (build10.2) to identify the physical position of microsatellites or identify the position of SNPs that has been mapped on a different reference genome. When the physical position of a microsatellite was not identified, the closest neighbouring marker according to the linkage map from MARC USDA (<http://www.marc.usda.gov/genome/>) was used. The location of the QTL was compared to significant regions found in this study. The significant associated regions from the GWAS were used to identify new candidate genes. The position of the left flanking region minus 1 Mb and the position of the right flanking SNP plus 1 Mb were used to search for candidate genes. Gene annotation for the significant associated regions was performed using BIOMART software in the Ensembl Sscrofa 10.2 (<http://www.ensembl.org/biomart>). Gene names were used to match with previously identified candidate genes.

### 4.3 Results and discussion

#### 4.3.1 GWAS quality and results

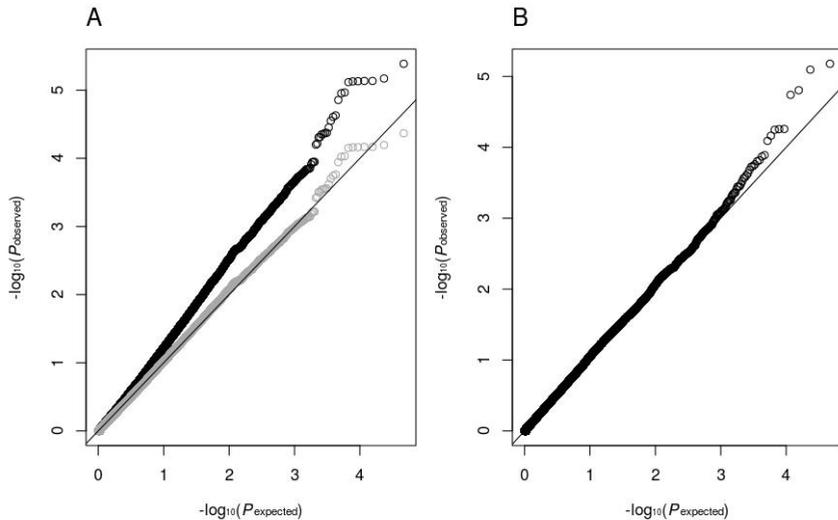
Androstenone was not normally distributed and therefore log-transformed (Figure 4.1). The mean non-transformed androstenone value was 1.50, with a maximum of 10.38 (N=1,151).



**Figure 4.1** Histogram of androstenone (light grey) and log-transformed androstenone (dark grey) of 1,151 boars.

The inflation factor  $\lambda$  was calculated for both the direct and the indirect SNP effects. The  $\lambda$  was 1.33 for the direct effect and 1.07 for the indirect effect. Genomic control was applied only on the direct SNP effect, after which the corrected  $\lambda$  was 1.05 (Figure 4.2A). As the  $\lambda$  for the indirect SNP effect was low ( $<1.1$ ), genomic control was not applied (Figure 4.2B).

FDR rate was applied genome-wide and both the direct and indirect SNPs had a  $q$ value  $> 0.1$ , resulting in that no SNPs were indicated genome-wide significant. After applying FDR per chromosome, suggestive significant associations were found. For the direct SNPs, 10 SNPs were chromosome-wide significant on one chromosome. The significant SNPs were located in a region from 48.6 through 50.9 Mb on SSC6. For the indirect SNPs, 9 SNPs were chromosome-wide significant on two chromosomes (SSC9 and SSC14) after applying FDR. In total 19 SNPs were chromosome-wide significant for the direct or indirect effect and information on those SNPs is available in Supplemental Table 1 (online).



**Figure 4.2** A quantile-quantile (Q-Q) plot of observed p-values versus expectation under null hypothesis. The black line is the expected line under the null distribution. The black dots indicate uncorrected p-values and grey dots indicate adjusted p-values after genomic control. **(A)** Q-Q plot of the p-values for direct effect of all SNPs. **(B)** Q-Q plot of the p-values for indirect effect of all SNPs.

### 4.3.2 SSC6

The 48.6 through 50.9 Mb region on SSC6 was chromosome-wide significant associated with a direct effect on androsteneone. The backwards elimination method started with 10 SNPs and ended with one significant SNP (ASGA0089838) that explained 2.1% of the phenotypic variance and 2.7% of the total genetic variance (Table 4.2). LD between the significant single SNPs was high as shown in Figure 4.3A. The haplotype method resulted in three haplotypes from 10 SNPs to estimate the variance explained by this region (Table 4.3). The most frequent haplotype accounted for 55% of the observations, and together the 3 haplotypes represented 98% of the haplotypes within the population. The haplotypes together explained 2.6% of the phenotypic variance and 3.2% of the total genetic variance, which was in line with what was found using the backwards elimination method. The haplotypes 1 and 2 are opposite in the allele coding and haplotype 3 shows a recombination in the middle of the haplotype, where the first 5 SNPs are similar to haplotype 1 and the last 5 are similar to haplotype 2. The fourth SNP was

#### **4 GWAS for direct and indirect effect on androstenone**

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ASGA0089838, which was also selected as significant SNP from the backwards elimination method. Also the evolutionary distance between haplotype 1 and 2 is the largest where haplotype 3 is in the middle as shown in Figure 4.4. Altogether these results show that the divergent haplotypes (determined mainly by SNP ASGA0089838) have a different evolutionary background (Figure 4.4). It is possible that because of introgression of Asian pig genomes, these different haplotypes exist in European commercial breeds.

The identified region on SSC6 in this study overlaps with the QTL region for direct effect reported by Duijvesteijn et al., 2010, who performed an association study only on line 11 (referred to as a Duroc-based sire line in (Duijvesteijn et al., 2010)). The boars from line 11 used in the present study were all included in the association study by Duijvesteijn et al. (2010). However, due to restrictions on the number of pen members genotyped only 598 animals were included in the present study compared to 987 in Duijvesteijn et al. (2010). Lee et al. (2005) found 3 QTL covering a similar but larger region for androstenone in fat, subjective boar flavor in lean pork and subjective pork flavor in lean pork (Pig QTLdb Id: 4219, 4215, 4200). Szyda et al. (2003) found one QTL on smell intensity covering a similar but larger region (Pig QTLdb Id: 668). Due to a low resolution of QTL studies using microsatellites (Szyda et al., 2003; Lee et al., 2005) it is difficult to say whether these QTL have similar underlying genes as the QTL found here. A more recent study using a high-density SNP panel by Grindflek et al. (2011b) detected a chromosome-wide significant region between 46.3 through 56.5 Mb (build10.2) for androstenone in subcutaneous fat and androstenone in plasma in a Norwegian Duroc line. That region is very similar to the identified region in this study. Candidate genes in this extremely gene-dense region have been discussed extensively in previous studies (Sinclair et al., 2006; Moe et al., 2007; Leung et al., 2010) and are summarized in Duijvesteijn et al. (2010). Mentioned and most likely candidate genes are the sulfotransferases SULT2A1, and SULT2B1, the hydroxysteroid-dehydrogenases (HSD17B14) and cytochrome P450 A19 (CYP2A19). A recent study by Hildago et al. (2014) who conducted an RNA-sequencing analysis on the region on SSC6, could not identify the causative mutation in SULT2A1.

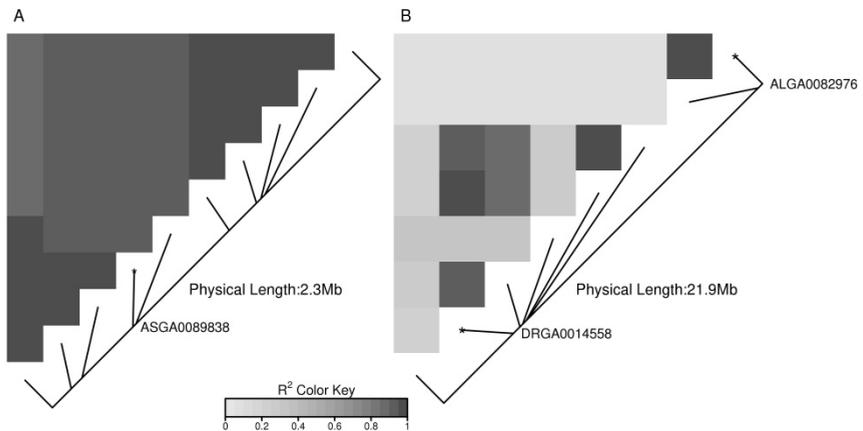
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**Table 4.3** Haplotypes from SNPs between 48.5 and 50.9 Mb at SSC6 with the estimated effect ( $\alpha$ ) and standard error (SE  $\alpha$ ) of the effect, the haplotype frequency (p), and the variance explained by the haplotype (V). The light and dark grey shading indicates the part of the haplotype which defines the divergence of the phylogenetic tree for the haplotypes (Figure 4.4).

Haplotype number	$\alpha^1$	SE $\alpha$	p	V	Haplotype <sup>2</sup>
haplo1	0.31	0.15	0.42	4.4E-03	G G G <b>A</b> A G A G G A
haplo2	0.14	0.15	0.55	2.8E-03	A A A <b>G</b> G A G A A G
haplo3	0.22	0.22	0.01	5.0E-07	G G G <b>A</b> A A G A A G
bin	0.000	-	0.02	7.2E-04	

<sup>1</sup>Estimated effect for the haplotypes were estimated against the bin haplotype which was set to zero.

<sup>2</sup>In bold are the significant SNPs found using the backwards elimination method (Table 4.2)



**Figure 4.3** LD plots with  $r^2$  as the LD measure for the significant SNPs per chromosome (Shin et al., 2006). The significant SNPs from the backwards elimination method are indicated by name and with \*. (A) SSC6. (B) SSC14.

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**Table 4.2** SNPs significantly associated with androstenone after the backwards elimination, for both direct and indirect effects ( $P < 0.05$ ). With the SNP effects ( $\alpha$ ) with the standard error (SE  $\alpha$ ) and the minor allele frequency (MAF), the variance explained by the SNP ( $V$ ), the proportion the SNP explained of the phenotypic variance (%  $\sigma_p^2$  expl.) and of the total genetic variance (%  $\sigma_{A_T}^2$  expl.).

Effect	SNP	SSC <sup>1</sup>	Position (bp.)	Allele coding	Counted allele	MAF	$\alpha$	SE $\alpha$	V	% $\sigma_p^2$ expl. <sup>2</sup>	% $\sigma_{A_T}^2$ expl. <sup>3</sup>
direct	ASGA0089838	6	49146524	AG	A	0.45	-0.16	0.04	0.0131	2.13	2.66
indirect	MARC0008206	9	115090777	AC	C	0.07	0.13	0.03	0.0023	2.23	16.72
indirect	DRGA0014558	14	130394571	AG	A	0.07	-0.13	0.04	0.0020	1.96	14.68
indirect	ALGA0082976	14	147512113	AG	A	0.11	-0.09	0.03	0.0014	1.40	10.48

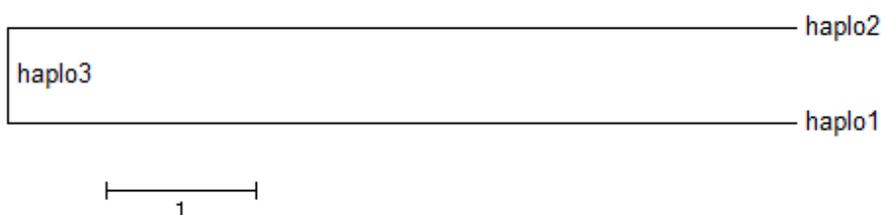
<sup>1</sup>SSC= *sus scrofa* chromosome

<sup>2</sup>For SNPs with a direct effect:  $\frac{V}{\sigma_p^2}$ ; for SNPs with an indirect effect:  $\frac{(n-1)V}{\sigma_p^2}$ ,  $\sigma_p^2$  is 0.62

<sup>3</sup>For SNPs with a direct effect:  $\frac{V}{\sigma_{A_T}^2}$ ; for SNPs with an indirect effect:  $\frac{(n-1)^2V}{\sigma_{A_T}^2}$ ,  $\sigma_{A_T}^2$  is 0.49

### 4.3.3 SSC9

The single SNP (MARC0008206) for the indirect genetic effect was chromosome-wide significant on SSC9 and explained 2.2% of the phenotypic variance and 17% of the total genetic variance. Although the effect is relatively large, we suspect a spurious association due to population stratification. The population studied here is strongly stratified: sire line 1 is a synthetic and dam line 4 is a two-way cross. Although a fixed effect for line and the three principal components of the kinship matrix were included in the model, it is still possible that this stratification caused a spurious association.



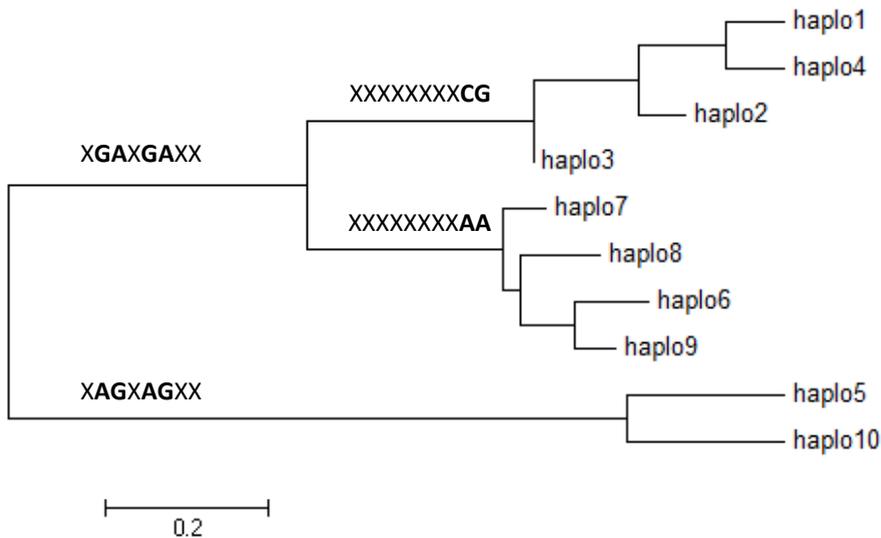
**Figure 4.4** Phylogenetic tree indicating evolutionary distances between the 3 haplotypes of 10 SNPs on SSC6. The analysis used the Tamura-Nei evolutionary distance method (Tamura et al., 2004) and the neighbor-joining algorithm of Saitou and Nei (1987) and the tree was reconstructed using MEGA software version 5 (Tamura et al., 2011). The length of the bar equals 1 nucleotide substitutions per site.

### 4.3.4 SSC14

On SSC14, two chromosome-wide significant associated regions, with an indirect effect, were found (Table 4.2). The first region was from 130.4 through 131.4 Mb. The second region was from 147.2 through 147.5. Also a single SNP at 125.6 Mb was associated. The backwards elimination method resulted in two significant SNPs (DRGA0014558 and ALGA0082976) that explained 3.4% of the phenotypic variance and 25.2% of the total genetic variance. LD between the two regions and the single SNP was low as shown in Figure 4.3B. Using haplotypes to estimate the variance explained, resulted in 10 haplotypes from 8 SNPs. The most frequent haplotype accounted for 70% of the observations, together the 10 haplotypes represented 98% of the haplotypes within the population. The haplotypes together explain 2.7% of the phenotypic variance and 19.9% of the total genetic variance (Table 4.4). The differences between the haplotypes is caused by two SNPs that are also the significant SNPs from the backwards elimination. The evolutionary tree shows two main branches, of which the first is separated by SNP DRGA0014558 (haplotype 5

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and 10 are separated from the rest) and the second by SNP ALGA0082976 (haplotype 1,2,3 and 4 are separated from haplotype 6,7,8, and 9; Figure 4.5). No previous studies have investigated QTL for the indirect genetic effect on androstenone. In total 8 genes were identified in the first region and 4 genes in the second region. Identification of candidate genes is difficult, also because this is the first study investigating the effect of genes from pen mates on a specific individual for androstenone. Candidate genes should influence pen mates in their expression of androstenone and should not influence the expression of their own androstenone level. This is because the significant region for the indirect effect on SSC14 does not contain SNPs significant for the direct effect. Genes influencing social behaviour or dominance are examples of possible candidate genes. None of the identified genes are involved in any of these mechanisms. Currently the knowledge on these complex mechanisms is still limited (Jensen et al., 2008) and the identified genes often have a unknown function, because gene annotation of the pig genome is not completed yet.



**Figure 4.5** Phylogenetic tree indicating evolutionary distances between the 10 haplotypes of 8 SNPs on SSC14. The analysis used the Tamura-Nei evolutionary distance method (Tamura et al., 2004) and the neighbor-joining algorithm of Saitou and Nei (1987) and the tree was reconstructed using MEGA software version 5 (Tamura et al., 2011). The length of the bar equals 0.2 nucleotide substitutions per site.

**Table 4.4** Haplotypes from SNPs at 125.6, 130.4-131.4 and 147 Mb at SSC14 with the estimated effect ( $\alpha$ ) and standard error (SE  $\alpha$ ) of the effect, the haplotype frequency (p), and the variance explained by the haplotype (V). The light and dark grey shading indicates the part of the haplotype which defines the divergence of the phylogenetic tree for the haplotypes (Figure 4.5).

Haplotype number	$\alpha^1$	SE $\alpha$	p	V	Haplotype <sup>2</sup>							
haplo1	0.00	0.06	0.701	1.9E-04	A	<b>G</b>	A	G	G	A	C	<b>G</b>
haplo2	-0.03	0.07	0.053	1.3E-05	G	<b>G</b>	A	G	G	A	C	<b>G</b>
haplo3	-0.02	0.07	0.049	1.0E-07	G	<b>G</b>	A	A	G	A	C	<b>G</b>
haplo4	0.00	0.07	0.042	8.5E-06	A	<b>G</b>	A	A	G	A	C	<b>G</b>
haplo5	-0.09	0.11	0.035	1.7E-04	G	<b>A</b>	G	A	A	G	C	<b>G</b>
haplo6	-0.09	0.08	0.038	2.2E-04	A	<b>G</b>	A	G	G	A	A	<b>A</b>
haplo7	-0.20	0.08	0.011	3.5E-04	G	<b>G</b>	A	A	G	A	A	<b>A</b>
haplo8	-0.06	0.09	0.014	2.8E-05	G	<b>G</b>	A	G	G	A	A	<b>A</b>
haplo9	-0.09	0.11	0.013	6.4E-05	A	<b>G</b>	A	A	G	A	A	<b>A</b>
haplo10	-0.13	0.11	0.026	3.1E-04	G	<b>A</b>	G	A	A	G	A	<b>A</b>
bin	0.00	-	0.018	6.3E-06								

<sup>1</sup>Estimated effect for the haplotypes were estimated against the bin haplotype which was set to zero.

<sup>2</sup>In bold are the significant SNPs found using the backwards elimination method (Table 4.2)

#### 4.3.5 Variance explained by QTL

Only a few SNP associations were found in this study, indicating that most QTL involved in the genetic control of androstenone were either rare (direct and indirect), or not large enough (direct) to be detected due to a lack of power of the study, suggesting that the direct effect on androstenone is controlled by many small or rare QTL. The MAF of the significantly associated indirect SNPs was low (0.07 and 0.11). Given the effect sizes, the contribution of indirect SNP effects to phenotypic and total genetic variance are larger than for the direct SNP effect, because of the multiplication by the number of pen mates (Table 4.3). This may explain that SNPs with a lower MAF can be found significant for indirect effects rather than for direct effects.

The four significant SNPs for the direct and indirect effect together explained 7.7% of the phenotypic variance and 44.5% of the total genetic variance using the backwards elimination method (Table 4.3). The haplotypes and the single associated SNP on SSC9, together explained 7.4% of the phenotypic variance and

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39.8% of the total genetic variance. Both methods seem to be comparable in estimation the variance explained. Important for the haplotype estimation is a good representation of the population (together the haplotypes should reflect >90% of the haplotypes in the population) to have an adequate estimation of the haplotype effects.

The effect sizes of the indirect SNPs seem small, its contribution to the total genetic variance is substantial, which is also reflected in the variance explained by the indirect SNPs. The indirect SNPs explain around 40% of the total genetic variance, whereas the direct SNPs at SSC6 explain around 3.2% of the total genetic variance. These percentages are probably overestimated, due to the Beavis effect (Beavis, 1998), which is a tendency for significant effects to be overestimated when many effects are tested for significance. Especially the indirect SNP effects seem to be overestimated because the sum of the variances explained by the SNPs is greater than the variance component ( $\sigma_{A_{Ti}}^2$  0.0057 vs. 0.002).

##### 4.3.6 Methodology

This study shows a methodology to model SNPs for indirect genetic effects. Only one earlier study (Biscarini et al., 2010) reported an association study for direct and indirect genetic effects in livestock species, which was performed on feather score data in laying hens. The difference in method between the study by Biscarini et al. (2010) and this study is the genetic model. In this study, the variance components were set to fixed values and included both  $\sigma_{A_D}^2$  and  $\sigma_{A_I}^2$  and the other variance components described by Duijvesteijn et al. (2012). Biscarini et al. (2010) used a two-step method, where in the first model no genetic effect (except for the SNP) were included, while in the second model the significant SNPs were included using an animal model without an indirect variance component. Because the study of Biscarini et al. (2010) involved a limited dataset (N=662), it was not feasible to run a direct-indirect animal model.

The  $\lambda$  for the direct effect was rather large which could be an indication for population stratification or a misfit of the model. Due to different lines in this study, population stratification is present, but a fixed effect for line and the first three principle components were fitted to correct for stratification. A more elegant way to account for population stratification might have been to fit a pedigree-genomic relationship matrix H (Legarra et al., 2009), as a substitute for the A matrix based on pedigree information. The H matrix would join genotyped and ungenotyped animals and improve the estimation of true relationships between animals. However, this solution is not so straightforward when different

populations and crossbreds of those populations are included. The different allele frequencies between populations should be taken into account when calculating the H matrix. Until now, a solid solution has not been suggested and therefore the use of the H matrix is beyond the scope of this study. Another cause of the inflated  $\lambda$  could be a misfit of the model, as the variance components were fixed *a priori* rather than estimated from the data in this study. Although the variances were estimated from a larger dataset where the animals used in this study were also included, different subsets of the larger dataset could result in different estimated variances. Only the p-values for direct SNP effects showed inflation, suggesting that the estimated direct genetic variance may have been too low, causing SNPs to absorb direct genetic variance, resulting in inflation of the p-values. To investigate this potential cause, we doubled the direct genetic variance while keeping the other variances at their original level. The  $\lambda$  for the direct SNP effects decreased to 1.3, but the reduction was not substantial, suggesting that the direct genetic variance used was not the major cause of the inflated  $\lambda$ .

FDR was applied to account for multiple testing. Because we used many markers, significance tests at different markers will not be independent as a result of LD. To investigate whether our significance threshold was sensitive to linkage disequilibrium between markers, we calculated q-values using only every tenth marker. The resulting q-value was very similar to the value obtained using all markers, suggesting that statistical dependence of significance tests is not a major issue when using FDR.

No SNPs were found to be genome-wide significant. When QTL would be spread randomly across the chromosomes, also no significant chromosome-wide results would have been expected. However, the distribution of p-values varied among chromosomes. Some chromosomes showed more SNPs with low p-values than expected by chance, indicating presence of QTL. Therefore, applying FDR per chromosome resulted in different results than when applied for the whole genome (Supplemental Figure 4.1).

### **CONCLUSIONS**

No genome-wide associated SNPs were found for direct and indirect effects on androstenone, but only chromosome-wide significant SNPs. In total, four independent regions were found to influence the androstenone level in boars. One region was associated with the direct genetic effect and 3 regions were associated with the indirect genetic effect. All independent significantly associated SNPs together explained around 7.9% of the phenotypic variance using the backwards

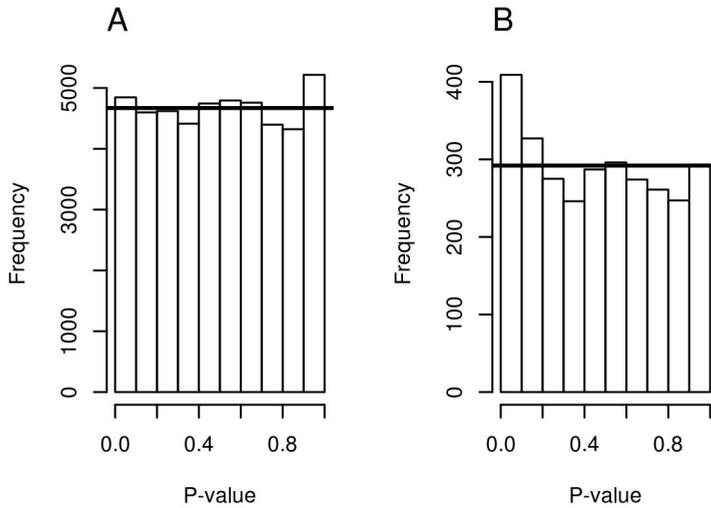
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elimination method. Haplotype analyses resulted in 7.44% of the phenotypic variance explained. Using the backwards eliminations method or the haplotype analysis, 44.5% or 39.8% of the total genetic variance was available for response to selection. Altogether this study shows a new way of modelling SNPs for traits which have an indirect genetic effect and could provide a method to select SNPs which affect the phenotype of their pen mates. These SNPs together with SNPs with a direct effect could be used in breeding programs to better predict the breeding value of animals using marker assisted selection (MAS).

#### **Acknowledgements**

We would like to acknowledge D.J. de Koning and the anonymous reviewers for valuable comments and suggestions. This research is part of the project 'Seeking Sociable Swine? Incorporating social genetic effects into pig breeding programs to achieve balanced improvement in productivity and welfare' and is funded by the program 'The value of Animal Welfare' of the Netherlands Organization for Scientific Research (NWO), and part of it was coordinated by the Dutch Technology Foundation (STW).



**Supplemental Figure 4.1** Histogram of p-values of direct SNPs where the horizontal black line indicates the expected frequency per bin. On the y-axis the frequency per bin is shown. The x-axis represents the p-values per bins of 0.2. **(A)** All p-values across 18 autosomes (N=46,421) with 4,642 expected SNPs per bin. **(B)** P-values for SSC6 (N=2,915) with 292 expected SNPs per bin.



# 5

## **Validation of indirect genetic effects for average daily gain in pigs**

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## **Abstract**

Social interactions between group-housed animals can have favourable or unfavourable effect on performance of individuals. Ignoring these social interactions in genetic selection can adversely affect response to selection. Therefore, appropriate models for genetic evaluation and selection are required. One of the approaches is to extend the classical animal model by including the genetic effect an individual has on its pen mates (IGE). This approach was validated using data from two purebred sire lines. The first objective of this study was to estimate direct effects and IGEs for average daily gain (ADG). The second objective was to validate the IGEs by comparing the predictive ability of a classical animal model to a model including IGEs based on correlations between predicted and observed phenotypes. A total of 41,144 records were used. There were 16,522 records from sire line 1 and 24,622 from sire line 2. Models including IGEs fitted the data significantly better than a classical animal model. However, no significant improvement was observed in ability to predict observed phenotypes between a classical animal model and a model including IGEs. Results differed among sire lines, validation years and farms, and were non-conclusive. Further research with more data including more sires and more groups and with closely monitored pen recording is suggested to investigate the additional benefits of genetic evaluation models including IGEs in pig breeding programs.

Key words: average daily gain, indirect genetic effects, pigs, validation

### 5.1 Introduction

Social interactions between group-housed animals can have favourable or adverse effects on performance of individuals. Ignoring these social interactions can reduce response to selection. As an example, average daily gain (ADG) is one of the most commonly recorded and selected traits in finishing pigs. However, response to selection for this trait has been short of expectations in many environments (Gunsett, 2005; Knap and Wang, 2012). One of the reasons could be that the genetic evaluations of individuals did not consider the genetic effect that an animal has on its pen mates (referred to as indirect genetic effects; IGE). Animals have a direct effect on their own trait value, but could also have an indirect effect on the trait value of their pen mates (Griffing, 1967).

In previous studies (Arango et al., 2005; Bergsma et al., 2008; Chen et al., 2009; Hsu et al., 2010; Bergsma et al., 2013), variance components for models including IGEs have been estimated for finishing traits in pigs. Results between studies are variable depending upon the components of the model. However, these studies did not include a validation step by prediction of future phenotypes using models including IGEs. Another method to validate is to estimate response to selection in a selection experiment. Gunsett (2005) conducted a multiple-generation group selection experiment for 6 years, where half sib families were selected. Phenotypic trend in this experiment was in the desired direction. However, another one generation selection experiment by (Camerlink et al., 2014) with high and low IGEs for ADG did not show an improvement of ADG due to IGEs. This could be due to the fact that pigs, compared to laying hens for example, are housed in more variable circumstances. Pig breeding companies house selection candidates throughout the world, resulting in different social environments for pigs by variable group sizes, grouping strategies, space allowance and seasonal influences by light or temperature. Therefore, it might be more challenging to predict the added value of including IGEs in pig breeding programs compared to other livestock species such as laying hens.

The first objective of this study was to estimate direct and IGEs for ADG in two purebred sire lines. The second objective was to validate the usefulness of IGEs by comparing predictions of phenotypes using a classical animal model to a model including IGEs.

### 5.2 Material and Methods

Animal Care and Use Committee approval was not obtained for this study, because the data were obtained from an existing database.

### 5.2.1 Animals

The dataset used for this study consisted of 41,144 progeny from 454 sires and 2,851 dams. Average daily gain (ADG) of these pigs was recorded from 2006 through 2013 (Table 5.1). Fifty-four percent of the tested animals were boars, the other 46% were gilts, and no castrates were tested. Animals were housed in groups on two nucleus farms and originated from two purebred sire lines. Both farms housed both sire lines. Farm 1 was larger with 22,368 records, compared to farm 2 which had 18,776 records. Farm 1 had an average pen size of 11.0 (range 6-16) and farm 2 had an average pen size of 11.4 (range 6-16). Group composition was defined according to the pigs penned together at the beginning of the finishing period. The pigs were not regrouped throughout the finishing period although group composition could change by early removal of some of the pen mates (for slaughter, selection, or health condition). Pigs were not randomly placed in pens. Full sibs were more likely to be grouped together. At farm 1, the pigs were from an average of 3.11 different sires per pen and 2.7 different sires at farm 2. The average relatedness within a pen mates was 0.27 in both farms. It ranged from 0.03 to 0.57 based on 5 generations of pedigree information.

Sire line 1 (S1) was smaller and consisted of 16,522 records while sire line 2 (S2) consisted of 24,622 records.

Sire line 1 was a Yorkshire based synthetic and sire line 2 was a Duroc based synthetic. Number of animals for each farm and line, their means (standard deviation) for ADG as well as start and end weights are given in Table 5.1.

**Table 5.1** Number of animals per farm and line and means (standard deviation) for average daily gain (ADG), start weight and end weight.

Farm	Sire line	N observations	ADG (g/d)	Start weight (kg)	End weight (kg)
1	S1	12,595	1,022 (130)	32.9 (7.3)	115.8 (13.5)
	S2	9,773	955 (110)	32.7 (7.0)	116.7 (14.2)
2	S1	3,927	1,050 (124)	37.8 (6.8)	122.0 (11.6)
	S2	14,849	986 (114)	33.9 (7.7)	118.5 (11.7)

### 5.2.2 Estimation of genetic parameters

Residual maximum likelihood (ReML) approach as in ASReml (Gilmour et al., 2009), was used to estimate the genetic parameters using a classical animal model (model 1) (Henderson, 1975; Lynch and Walsh, 1998) and a model including indirect genetic effects (IGE, model 2) (Muir and Schinckel, 2002). Fixed effects for ADG were: line, sex, farm\*department, number of pen mates and age on test. Genetic

parameters were estimated for sire lines 1 and 2 separately (S1 and S2) and also pooled together in a combined dataset (COM). Following models were used:

Model 1: Classical animal model

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wl} + \mathbf{Vg} + \mathbf{Uc} + \mathbf{Tf} + \mathbf{e}$$

Model 2: Animal model including IGEs

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_D a_D} + \mathbf{Z_{I(d)} a_{I,n}} + \mathbf{Wl} + \mathbf{Vg} + \mathbf{Uc} + \mathbf{Tf} + \mathbf{e}$$

where  $\mathbf{y}$  is the vector of ADG;  $\mathbf{b}$  is a vector of fixed effects;  $\mathbf{a}$  ( $\mathbf{a_D}$ ) is a vector of direct additive genetic effects;  $\mathbf{l}$  is the vector for the non-genetic effects from individuals born in the same litter;  $\mathbf{g}$  is the vector of non-genetic effects due to the group in which the pigs are penned during the finishing period;  $\mathbf{c}$  is the vector of non-genetic effects due to the same compartment in a barn where groups were housed during the finishing period;  $\mathbf{f}$  is the vector of non-genetic effects due to littermates within the same group during the finishing period,  $\mathbf{e}$  is the vector of residuals, and  $\mathbf{X}$ ,  $\mathbf{Z_D}$ ,  $\mathbf{Z_I}$ ,  $\mathbf{W}$ ,  $\mathbf{V}$ ,  $\mathbf{U}$ , and  $\mathbf{T}$  are incidence matrices.

The  $\mathbf{a}_{I,n}$  is a vector of indirect additive genetic effects referring to the average pen size with the incidence matrix  $\mathbf{Z_{I(d)}}$  which depends on the relationship of IGEs and group size, referred to as dilution ( $d$ ) (Bijma, 2010b):

$$\mathbf{Z_{I(d)}}(i, j) = \left( \frac{\bar{n} - 1}{n - 1} \right)^d \text{ when } j \text{ is a pen mate of } i, \text{ otherwise } \mathbf{Z_{I(d)}}(i, j) = 0,$$

where  $\bar{n}$  denotes the average group size.

Dilution indicates the proportional decrease of the IGE when group size increases. When  $d=0$ , the IGE is independent of group size. When  $d=1$ , there is complete dilution since IGE is proportional to  $\frac{1}{n-1}$ . An individual's total IGE summed over all pen mates is constant. Dilution was set to 0.5. as a deterministic search for the best fitting  $d$  did not indicate a significant improvement of the model for values between 0 and 1. A  $d$  with the value of 0.5 makes  $\sigma_p^2$  independent of group size with unrelated animals (Bijma, 2010b). We assumed unrelated groups to exclude influences of relatedness. Considering IGEs, the phenotypic variance depends on relatedness between pen mates. Therefore, it was assumed that pen mates were unrelated for more effective comparisons of genetic parameters with other studies. Parameters estimated from model 1 and 2 were used to determine the total heritable variance ( $\sigma_{TBV}^2$ ) and the phenotypic variance ( $\sigma_p^2$ ) (Bijma, 2010b). The total heritable variance determines the potential of a population to respond to selection, and was calculated as follows according to (Bijma et al., 2007):

## 5 Validation of indirect genetic effects

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$$\sigma_{TBV}^2 = \sigma_{A_D}^2 + 2(\bar{n}-1)\sigma_{A_{DI}} + (\bar{n}-1)^2\sigma_{A_I}^2, \quad [5.1]$$

where  $\sigma_{A_D}^2$  is the direct genetic variance,  $\sigma_{A_I}^2$  is the indirect genetic variance and  $\sigma_{A_{DI}}$  is the covariance between  $\sigma_{A_D}^2$  and  $\sigma_{A_I}^2$ . The  $\sigma_{A_D}^2$  is the variance of the direct breeding value which corresponds to the classical breeding value from the classical model,  $\sigma_{A_I}^2$  is the variance of the indirect breeding value.

The phenotypic variance for the classical animal model is:

$$\sigma_p^2 = \sigma_A^2 + \sigma_I^2 + \sigma_g^2 + \sigma_c^2 + \sigma_f^2 + \sigma_e^2. \quad [5.2]$$

Where  $\sigma_A^2$  is the additive genetic variance which is the variance of the classical breeding values. The phenotypic variance including IGEs and assuming unrelated pen mates was defined as:

$$\sigma_p^2 = \sigma_{A_D}^2 + (\bar{n}-1)\sigma_{A_I}^2 + \sigma_I^2 + \sigma_g^2 + \sigma_c^2 + \sigma_f^2 + \sigma_e^2 \quad [5.3]$$

Improvement due to a model including IGEs over a classical animal model was tested using a likelihood-ratio test. Comparison of models was also done by expressing the total heritable variance per model over the phenotypic variance. In the classical animal model also referred to as heritability ( $h^2$ ) and in the model including IGEs is  $T^2 = \frac{\sigma_{TBV}^2}{\sigma_p^2}$  (Bergsma et al., 2008). The comparison between  $h^2$  and  $T^2$  shows the proportional contribution of the IGEs to the heritable variance.

### 5.2.3 Validation by predicting phenotypes

The classical animal model and a model including IGE were compared with respect to their ability to predict observed phenotypes. Known phenotypes were set to missing for calculation of predicated phenotypes. The predicated phenotypes were then compared to the known phenotype. The correlation between the predicted and known phenotype was used as a measure of the predictive ability of the model. First, we describe the estimation of the different breeding values and phenotypes used to compare the two models. Secondly, we describe the procedure for selection of animals used for prediction and finally the different correlation coefficients.

Following phenotypes were predicted:

$$\hat{P}_{C,i} = \hat{A}_i$$

$$\hat{P}_{D,i} = \hat{A}_{D,i}$$

$$\hat{P}_{I,i} = \sum_{n-1} \hat{A}_{I,j}$$

$$\hat{P}_{DI,i} = \hat{A}_{D,i} + \sum_{n-1} \hat{A}_{I,j}$$

The predicted phenotype ( $\hat{P}_{C,i}$ ) of individual  $i$  is the direct genetic effect based on the classical model. The predicted phenotype ( $\hat{P}_{D,i}$ ) of individual  $i$  is the direct genetic effect based on the model including IGEs. The predicted phenotype ( $\hat{P}_{I,i}$ ) of individual  $i$  is the sum of indirect genetic effects of  $j$  pen mates based on the model including IGEs. The predicted phenotype ( $\hat{P}_{DI,i}$ ) of individual  $i$  based on the model including IGEs therefore becomes (Ellen et al., 2010):  $\hat{P}_{DI,i} = \hat{A}_{D,i} + \sum_{n-1} \hat{A}_{I,j}$ .

Correlations between the  $\hat{P}_C$ ,  $\hat{P}_D$ ,  $\hat{P}_I$  and  $\hat{P}_{DI}$  with the ADG corrected for fixed effects (ADGc) were calculated.

To determine the predictive abilities of the classical animal model vs. a model including IGEs, the data was split into a training dataset and a validation dataset. To simulate an animal breeding program, the year following the training dataset was set to missing (Table 5.2), so we predicted future records. The variance components were fixed to the values from Table 5.3 using parameters from the appropriate dataset. It was necessary to fix parameters as training datasets of different sizes were used that would have otherwise lead to different parameters.

Although all animals in the validation dataset were predicted, only a subset of those animals was used to determine the predictive abilities of the two models. Some sires were only present in the validation dataset with no offspring information in the training dataset, making prediction almost random. Therefore, validation dataset was restricted to those sires that had offspring in the training as well as validation dataset and accuracy of their estimated breeding value was above 0.7 (based on the classical model). Accuracy was calculated as:

$$r = \sqrt{\frac{1 - PEV}{(1 + f)\sigma_A^2}}$$

where PEV is the Predicted Error Variance and  $f$  is the inbreeding

coefficient of the individual (Gilmour et al., 2009). The breeding values of offspring of the selected sires were used for the validation.

To estimate correlation between the predicted phenotypes ( $\hat{P}_C$ ,  $\hat{P}_D$ ,  $\hat{P}_I$  and  $\hat{P}_{DI}$ ) and ADGc, a bivariate model was used with a fixed effect for birth year of the validation dataset (Gilmour et al., 2009). Birth year was included as a fixed effect to

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account for effect of genetic selection as the records were from a population under selection. Ignoring the effect of selection could have resulted in differences between the levels of predicted phenotypes over the years due to selection.

Correlations were calculated for each birth year of the validation dataset and for each farm using the COM dataset. Validation was also performed per sire line and correlations were calculated for each birth year of the validation dataset within each sire line. Differences between farms were not investigated each sire line due to size limitation of the dataset.

**Table 5.2** Number of animals and groups in the training and validation datasets.

Training dataset		Validation dataset		
Birth year	N Sires <sup>1</sup>	Birth year	N Predicted phenotypes	N Groups
2006-2009	14	2010	831	151
2006-2010	21	2011	888	166
2006-2011	17	2012	930	176
2006-2012	16	2013	968	147

<sup>1</sup>Sires were selected when accuracy of their estimated breeding value for ADG (using a classical animal model) was above 0.7.

### 5.3 Results

#### 5.3.1 Genetic parameters

The estimated genetic parameters for the classical model and model including IGEs for the combined dataset (COM) and per sire line (S1 and S2) are given in Table 5.3. The non-genetic terms (the sum of variance components for litter, pen, compartment and interaction of group by full-sib family) in the model explained from 26% to 42% of the phenotypic variance. Models including IGEs showed a better fit, as the difference with a classical animal model in log-likelihood was significant (e.g. COM dataset  $\chi^2_2 = 12.52$ ,  $p = 0.008$ ). Heritability using the classical model ranged from 0.19 to 0.23 among the three datasets. In the models with IGEs,  $T^2$  ranged from 0.33 to 0.42. The direct and indirect genetic variances of all models were significantly different from zero. The impact of IGEs in line S2 was smaller compared to line S1, because of the lower (30%) variance contributed by the IGEs. This is partly due to a negative but non-significant covariance between the direct and indirect genetic effects ( $\sigma_{A_{DI}} = -12$ ) as compared to positive and significant variance in line S1 ( $\sigma_{A_{DI}} = 78$ ). The estimates for variance components such as  $\sigma_{A_D}^2$

and  $\sigma_{A_i}^2$  of the combined dataset (COM) were within the range of those obtained using datasets S1 and S2, separately.

### 5.3.2 Validation

#### *COM dataset*

Phenotypes of 3,617 animals were predicted from 640 groups in the COM dataset. The average accuracy of the EBVs of the sires used for the prediction was 0.83 (range 0.70-0.92) for the classical estimated breeding value (CEBV), similar for direct estimated breeding value (DEBV) and 0.52 for indirect estimated breeding value (IEBV) (range 0.18-0.72). In total 68 sires were used for the prediction. Phenotypes for years 2010 through 2013 were predicted using 831 to 968 animals per year (Table 5.2).

The overall analysis with dataset COM showed no significant differences in the correlation between observed and predicted phenotypes using a classical animal model compared a model including IGEs (Table 5.4). The predicted phenotype from the classical model ( $\hat{P}_C$ ) and predicted phenotype for the direct genetic effect from the model including IGEs ( $\hat{P}_D$ ) were highly correlated (0.91), which is expected as they both explain the direct genetic effect of the animal itself. The correlations varied among years (Figure 5.1A). There was a higher correlation using a model including IGEs for 2013. For that year, the correlation between ADGc and predicted phenotype for the model including IGEs ( $\hat{P}_{DI}$ ) was 0.34 compared to 0.24 for the  $\hat{P}_C$ , and was highest for predicted phenotype for the indirect genetic effects ( $\hat{P}_I$ ) (0.43). The previous years (2010 through 2012), did not show an improvement when using a model including IGEs. There was even a decrease in the correlation during 2012. The correlation for the  $\hat{P}_C$ ,  $\hat{P}_D$  and  $\hat{P}_{DI}$  were 0.19, 0.10 and 0.08 respectively. During the years 2010 and 2012 the correlation between the  $\hat{P}_I$  and ADGc was even negative. The correlations of  $\hat{P}_C$ ,  $\hat{P}_D$  and  $\hat{P}_{DI}$  with ADGc were higher in farm 2 compared to farm 1 (Table 5.4). There were no clear differences between correlations estimated for the two farms for the  $\hat{P}_I$ .

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**Table 5.3** Genetic parameters ( $\sigma_{A_D}^2$ ,  $\sigma_{A_{DI}}$ ,  $\sigma_{A_I}^2$ ,  $\sigma_{TBV}^2$ ,  $\sigma_p^2$ ), pen effects ( $\sigma_g^2$ ), compartment effects ( $\sigma_c^2$ ), litter effects ( $\sigma_l^2$ ) and littermates within a group effects ( $\sigma_f^2$ ) and heritability for average daily gain using a classical animal model and a model including IGE (with standard errors in parentheses) for all three datasets, along with the difference in log-likelihood between the models<sup>1</sup>.

Dataset <sup>2</sup>	Model <sup>3</sup>	$\sigma_{A_D}^2$	$\sigma_{A_{DI}}$	$\sigma_{A_I}^2$	$\sigma_g^2$	$\sigma_c^2$	$\sigma_l^2$	$\sigma_f^2$	$\sigma_{TBV}^2$	$\sigma_p^2$	$r_g$ <sup>4</sup>	$h^2 / T^2$ <sup>5</sup>	LogL
COM	C	2,893 (189)			1,270(58)	2,249(182)	603(53)	661(71)		13,643(214)		0.21(0.01)	0
	IGE	2,762(187)	27(19)	13(3)	1,121(67)	2,013(172)	606(53)	660(71)	4,641(541)	13,339(206)	0.14(0.10)	0.35(0.04)	5
S1	C	2,772(306)			1,753(118)	2,928(306)	860(95)	604(117)		14,954(353)		0.19(0.02)	0
	IGE	2,552(295)	78(34)	20(7)	1,481(137)	2,553(287)	877(95)	595(117)	6,122(1,119)	14,466(337)	0.34(0.16)	0.42(0.08)	7
S2	C	2,872(238)			973(64)	1,412(146)	457(62)	591(89)		12,268(204)		0.23(0.02)	0
	IGE	2,857(240)	-12(24)	13(4)	883(75)	1,203(136)	452(62)	587(89)	3,985(635)	12,063(198)	-0.06(0.12)	0.33(0.05)	4

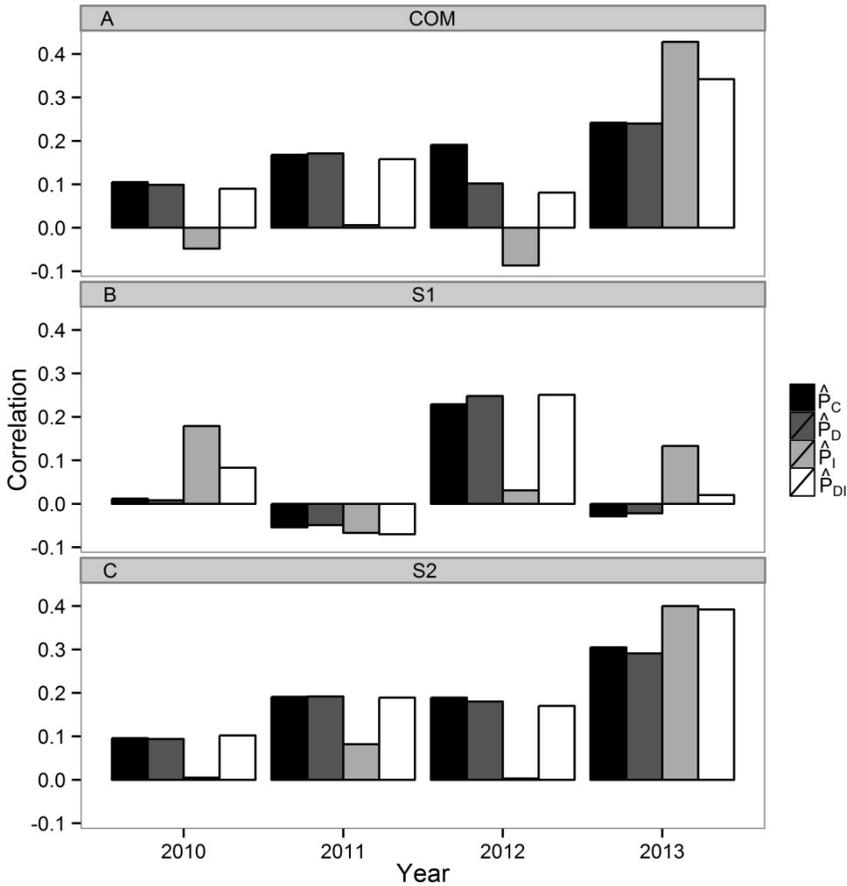
<sup>1</sup>  $\sigma_{TBV}^2$  and  $\sigma_p^2$  are derived using Equation 1 and 2.

<sup>2</sup> COM = combined dataset, S1 = Sire line 1 dataset, S2 = Sire line 2 dataset.

<sup>3</sup> C = classical animal model, IGE = model including indirect genetic effects.

<sup>4</sup>  $r_g$  = correlation between direct and indirect genetic effects.

<sup>5</sup>  $h^2$  for classical animal model,  $T^2$  for models including IGE.



**Figure 5.1** Correlations between the different predicted phenotypes and average daily gain corrected for fixed effects per year.  $\hat{P}_C = \hat{A}$ ,  $\hat{P}_D = \hat{A}_D$ ,  $\hat{P}_I = \sum_{n-1} \hat{A}_I$ ,  $\hat{P}_{DI} = \hat{A}_D + \sum_{n-1} \hat{A}_I$

**A.** Combined dataset (COM). **B.** Sire line 1 (S1). **C.** Sire line 2 (S2).

*S1 dataset*

The dataset from S1 was smaller and contained 16,522 records only. The number of animals in the validation was also small. A total of 945 animals from 25 sires and 220 groups were used. The genetic parameters indicated a stronger effect of the IGEs compared to S2. Nevertheless, the validation by predicting the phenotypes for S1 did not result in a significant improvement using a model with IGEs compared to the classical animal model (Table 5.5). In general, the correlations were low, independent of the model. They were 0.07 for  $\hat{P}_C$  and 0.06 for  $\hat{P}_{DI}$ . Results varied

## 5 Validation of indirect genetic effects

between years. During 2012 the correlations were highest for  $\hat{P}_C$  (0.23) and  $\hat{P}_{DI}$  (0.25) (Figure 5.1B). During 2011, all correlations ( $\hat{P}_C$ ,  $\hat{P}_D$ ,  $\hat{P}_I$  and  $\hat{P}_{DI}$ ) were negative. In 2013, the  $\hat{P}_I$  correlated best with ADGc (0.13).

### *S2 dataset*

The dataset from S2 was larger and contained 24,622 records. A total of 2,482 animals from 40 sires and 405 groups were used. Validation by prediction of phenotypes for S2 did not result in a significant improvement using a model with IGEs compared to the classical animal model (Table 5.5). Although the correlation between  $\hat{P}_I$  and ADGc (0.25) was higher than  $\hat{P}_C$  (0.21) or  $\hat{P}_{DI}$  (0.22). Validation across years showed a trend of increase in correlation ranging from 0.10 in 2010 to 0.31 in 2013 for  $\hat{P}_C$  and from 0.10 in 2010 to 0.39 in 2013 for  $\hat{P}_{DI}$  (Figure 5.1C). In 2013, the  $\hat{P}_I$  correlated best with ADGc (0.40).

**Table 5.4** Correlations between the predicted phenotypes and average daily gain corrected for fixed effects for the combined dataset (COM).

	All	Farm 1	Farm 2
$\hat{P}_C$	0.18 (0.02)	0.15 (0.02)	0.23 (0.02)
$\hat{P}_D$	0.16 (0.02)	0.13 (0.02)	0.20 (0.02)
$\hat{P}_I$	0.14 (0.02)	0.09 (0.02)	0.08 (0.02)
$\hat{P}_{DI}$	0.19 (0.02)	0.15 (0.02)	0.21 (0.02)

$$\hat{P}_C = \hat{A}, \hat{P}_D = \hat{A}_D, \hat{P}_I = \sum_{n-1} \hat{A}_I, \hat{P}_{DI} = \hat{A}_D + \sum_{n-1} \hat{A}_I$$

**Table 5.5** Correlations between the predicted phenotypes and average daily gain corrected for fixed effects for sire line 1 (S1) and sire lines 2 (S2).

	S1	S2
$\hat{P}_C$	0.07 (0.03)	0.21 (0.02)
$\hat{P}_D$	0.08 (0.03)	0.20 (0.02)
$\hat{P}_I$	0.09 (0.03)	0.25 (0.02)
$\hat{P}_{DI}$	0.06 (0.03)	0.22 (0.02)

$$\hat{P}_C = \hat{A}, \hat{P}_D = \hat{A}_D, \hat{P}_I = \sum_{n-1} \hat{A}_I, \hat{P}_{DI} = \hat{A}_D + \sum_{n-1} \hat{A}_I$$

## 5.4 Discussion

### 5.4.1 Genetic parameters

The models including IGEs provided significantly better predictions than the classical model. The estimated genetic parameters from the combined dataset (COM) were similar to previous estimates from Bergsma et al. (2013). Some animals of this study were also included in the study of Bergsma et al. (2013). The  $T^2$  in Bergsma et al. (2013) was 0.34 compared to 0.35 using COM in this study. For this study, a relatively small dataset from a larger dataset was selected based on knowledge on accurate pen registration at the farms, which is important when estimating and validating IGEs. The total heritable variance was about 60% greater using IGEs than using a classical animal model. The model used by Bergsma et al. (2013) was extended by including an additional random effect for the interaction between group and full-sib family. This interaction was highly significant and explained around 5% of the phenotypic variance (see Chapter 7 section 'Indirect Genetic Effects Applied' for more details).

The estimates of IGEs in the two sire lines were different. There was a positive genetic correlation between  $\sigma_{A_D}^2$  and  $\sigma_{A_I}^2$  in S1 it was negative (not significant) in S2 (Table 5.3). However, the 95% confidence intervals of the genetic correlations of S1 and S2 overlap (S1: 0.03-0.65 and S2: -0.30-0.18) and therefore are not significantly different from each other.

### 5.4.2 Validation

The empirical results could not show a significant improvement of predicted phenotypes using a model including IGEs compared to the classical model. The results between years and between genetic lines seem to be variable. There are two possibilities why we didn't find a significant difference between the two models. First, the model including IGEs does not result in an improvement or second, given the present dataset we could not prove that the model including IGEs is better, due to limitations in the dataset.

Retrospectively, we performed a power calculation on the present dataset to test whether we could expect to find a significant difference. We tested the difference in accuracy of the predicted phenotypes between a classical model and model including IGEs over the difference in standard error (SE) on the accuracies between the two models (see Appendix for derivation and calculation). Results indicate that given the size of the present dataset, we could not expect a significant difference between the two models ( $P > 0.05$ ).

## 5 Validation of indirect genetic effects

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Thorough validation of models including IGEs require several conditions to be fulfilled. First, observed phenotypes of complete set of pens mates need to be predicted. Therefore setting the observations in a subset of the dataset at random for the purpose of prediction is not possible. Prediction of phenotypes needs to be performed in batches. In this study batches according to birth year were considered. The current dataset is from two purebred sire lines used for selecting the next generation. Each year, 40 sires will be selected from 2,500 tested boars. Therefore, each validation year will only have around 40 sires per line. In addition these sires need to have offspring both in the training dataset as well as in the validation dataset with an accuracy of the estimated breeding value of 0.7 (based on the classical model). In the current dataset this condition reduced the number of sires used in the validation to 68 in the COM dataset, and to 25 for S1 and 40 for S2. All the full sibs in the validation set of these sires, independent of whether they were penned together, will have a similar predicted phenotype using the classical model. For a model including IGEs, full sibs in the validation set will only have a similar predicted phenotype when penned together. The above discussion illustrates that the number of sires in a study for a large part determines the accuracy of prediction, and a sufficient number of sires is required for meaningful conclusions. Larger datasets are necessary to have sufficient number of sires and to increase the accuracy of the correlation coefficients.

Second, the accuracy of the indirect estimated breeding value (IEBV) of the sires was lower than the classical (CEBV) or direct estimated breeding value (DEBV). The accuracy of the sires for the IEBV ranged from 0.18 and 0.72 compared to the CEBV of the sires which was between 0.70 and 0.92. The accuracy of the IEBV depends both on the number of groups in which the sire has offspring, and on the number of individuals. A lower accuracy of the sires for the IEBV results into lower accuracies of the predicted phenotypes of the offspring of these sires for the IEBV. An increase in the accuracy can be achieved by increasing the number of pens by reducing the number of pigs per pen and keeping family groups together. Keeping half- or full-sib group together should lead to the highest accuracies and response to selection for traits affected by IGEs (Bijma, 2010a).

There is a conflict between the ideal group composition for estimating variance components and for achieving the highest response to selection for traits affected by IGEs. Variance components are best estimated in groups not composed of complete family groups, while accuracies would be highest for family groups. A solution is to keep two distinct families within each group, where the number of groups rather than the number of animals is determining the accuracy of the variance components. This strategy will probably also yield good accuracies of the

breeding values, although more research is needed to confirm this (Ellen et al., submitted).

To achieve two distinct families and smaller group sizes in pig breeding to be able to both estimate variance components and achieve a high response to selection, the penning strategy of pigs needs to be adjusted. This will be time consuming as changes are often dependent on the construction of new farms. An alternative is to collect a lot more data still using in the current penning strategy.

Third, accurate pen recording is essential for estimation of the IEBV of an individual. When an animal is recorded in the wrong pen, the model will estimate its effect on an individual which was never a pen mate. In the current dataset, there was no monitoring of the accuracy of pen recording. Therefore, influence of inaccuracies in pen recording cannot be estimated. Furthermore, in pig production social groups change over the lifespan due to cross-fostering, mixing of unacquainted pigs after weaning and at the beginning of the finishing period. Influence of instable social group could affect the accuracy of prediction in two ways (Ellen et al., submitted). First, the assumption that every pig equally influences other pen mates is violated when pigs are removed from the pen or switched to another pen. In this study, animals were grouped in the same pen from start till the end of the finishing period, although earlier removal of pigs (for slaughter, selection or health condition) still occurred. Secondly, pigs are often not randomly removed from the pen, but based on individual performance which is partly genetic. Both factors can bias the estimation of the IEBV of a pig.

The variation in predictive abilities for the IEBV is reflected when data is split in the different birth years. Correlations between the predicted phenotypes and the ADGc varied for different breeds and birth years between -0.09 and 0.43. Correlations between years were even more variable and unpredictable as fewer sires were used per year. Prediction of phenotypes using a model with IGEs was best for the birth year 2013. Surprisingly, the  $\hat{P}_i$  in 2013 outperformed all the other predicted phenotypes. Possibly, the accuracy of the pen recording improved, because monitoring of pen recording started in 2013 (personal communication: Arjan Neerhof, TOPIGS). The effect of monitoring pen recording on the predictive ability of models should be estimated as part of the future validation studies.

### 5.5 Conclusion

Models including IGEs fitted the data significantly better than a classical animal model. Validation on the other hand could not confirm this improvement. No significant improvement was observed by comparing the correlations of predicted

phenotypes with observed phenotypes from a classical animal model to those from a model including IGEs. The number of sires used to predict the performance of the offspring was the limiting factor in the current study. Inaccuracies in pen recording could have also affected the predictive ability of the model including IGEs. Further research with more data including more sires and more groups with closely monitored pen recording is necessary to investigate the additional advantage of including IGEs in genetic evaluation models used in pig breeding programs.

### 5.6 Acknowledgements

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### 5.7 Appendix

#### 5.7.1 Calculating power of validation study

To evaluate whether a significant difference between the two models was expected given the present dataset, an analyses of the power of the study was conducted. Expected difference in accuracy between the two models is determined and the standard error (SE) SE on the difference of the accuracies between the two models.

Hypotheses tested:

$$H_0 = \rho_C = \rho_{DI}$$

$$H_{alt} = \rho_C < \rho_{DI}$$

where subscript C indicates the classical model and DI the model including IGEs.

Given the present dataset, we wanted to investigate whether the model including IGEs is significantly better than the classical model and therefore a 1-tailed test is performed with the .05 probability level. The critical value of z is 1.65.

First, the variance of the phenotypes needs to be calculated where the predicted phenotype is:

$$\hat{P} = \hat{A}_{D,i} + (n-1)\hat{A}_{I,j}$$

where  $\hat{A}_{D,i} = \frac{1}{2}\hat{A}_{D,sire} + \frac{1}{2}A_{D,dam}$  and  $\hat{A}_{I,j} = \frac{1}{2}\hat{A}_{I,sire} + \frac{1}{2}A_{I,dam}$

The variance of the predicted phenotypes is:

$$\text{var}(\hat{P}) = \text{var}(\hat{A}_D) + (n-1)\text{var}(\hat{A}_I)$$

$$\text{var}(\hat{A}_D) = \frac{1}{4}(\rho_{D,sire}^2 + \rho_{D,dam}^2)\sigma_{A_D}^2$$

$$\text{var}(\hat{A}_I) = \frac{1}{4}(\rho_{I,sire}^2 + \rho_{I,dam}^2)\sigma_{A_I}^2$$

Accuracy of the classical, direct and indirect EBV's from the sires and dams of the validated animals were calculated as described in the Material and Methods section.

For the classical model, terms with indirect effects can be omitted. The reliability of the predicted phenotypes is calculated as:

$$\rho_{P,\hat{P}}^2 = \frac{\text{var}(\hat{P})}{\sigma_P^2}$$

The difference in the accuracy between the two models equals:

$$\Delta = \rho_{P,\hat{P}_{DI}} - \rho_{P,\hat{P}_C}$$

The standard error on the accuracy of the predicted phenotypes was calculated as:

$$SE_{(P,\hat{P})} = \frac{1 - \rho_{P,\hat{P}}^2}{\sqrt{N-1}}, \text{ where } N \text{ is the number of observations.}$$

The SE on the difference of the accuracies between the two models was therefore:

$$SE(\Delta) = \frac{\sqrt{(1 - \rho_{P,\hat{P}_{DI}}^2)^2 + (1 - \rho_{P,\hat{P}_C}^2)^2}}{\sqrt{N-1}}$$

To test whether the model including IGEs will result in a significant improvement:

$$z = \frac{\Delta}{SE(\Delta)} \geq 1.65.$$

Using the input parameters from Table 5.6, results in a z of 0.67 which is < 1.65 (Table 5.7) and we can reject  $H_{alt} = \rho_C < \rho_{DI}$ , where  $\rho_C$  is the accuracy of the

classical model and  $\rho_{DI}$  is the accuracy of the model including IGEs. Estimated breeding values from full-sibs in the validation dataset based on the classical model, will have similar breeding values. Similar to full-sibs which were penned together will have similar EBVs using the model including IGEs. The results from the power analyses ignore that animals can have similar breeding values.

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**Table 5.6** Input parameters to calculate the power of the validation<sup>1</sup>.

	Classic	IGE model
$\rho_{D,sire}^2$	0.69	0.58
$\rho_{D,dam}^2$	0.45	0.43
$\rho_{I,sire}^2$		0.27
$\rho_{I,dam}^2$		0.17
N	3,617	

<sup>1</sup>Variance components are given in Table 5.3 from dataset COM.

**Table 5.7** Output parameters from the power analyses

	Classic	IGE model
$\rho_{P,\hat{p}}^2$	0.06	0.05
$SE_{(P,\hat{p})}$	0.016	0.016
$SE(\Delta)$	0.022	
$\Delta$	0.015	
<b>z</b>	0.670	

# 6

## **Same pig, different conclusions: stakeholders differ in Qualitative Behaviour Assessment**

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## **Abstract**

Animal welfare in pig production is frequently a topic of debate and is sensitive in nature. This debate is partly due to differences in values, forms, convictions, interests and knowledge among the stakeholders that constitute differences among their frames of reference with respect to pigs and their welfare. Differences in frames of reference by stakeholder groups are studied widely, but not specifically with respect to animal behaviour or welfare. We explored this phenomenon using a Qualitative Behaviour Assessment (QBA). Participating stakeholders were classified into two expert groups consisting of pig farmers (N=11) and animal scientists (N=18) and a lay-group consisting of urban citizens (N=15). The stakeholders were asked to observe the behaviour of a specific pig in each of the nine videos and to assign a score for each video using 21 predefined terms describing the mood, such as 'happy' or 'irritated'. They were asked to complete two additional questionnaires to obtain information on their frames of reference. Results from the QBA showed that the pig farmers observed the behaviour of pigs more positively than the urban citizens and the animal scientists. This was evident from the consistently higher scores on the positive terms to assess pig behaviour. The questionnaires revealed that the farmers had a different frames of reference regarding pigs and different understanding of welfare, which might explain the differences in assessment. In a follow-up stakeholder workshop, which focussed on differences in observation, QBA showed to be an effective tool to stimulate mutual learning among stakeholders, which is necessary to find shared solutions.

Key words: Animal welfare, Perspectives, Dialogue, Qualitative behaviour assessment, Pigs

## 6.1 Introduction

In the Netherlands, animal welfare in animal production has surpassed the stage of hype and has acquired a permanent place on the political, scientific and private agenda (Hopster, 2010). Despite of agreement on the need of welfare improvement, the issue is still frequently a topic of debate (Eijsackers and Scholten, 2010). This is partly the result of different visions between stakeholder groups on how to treat animals (Te Velde et al., 2002; Lassen et al., 2006; Miele et al., 2011). These different visions between stakeholder groups may make it difficult to reach agreements on approaches for improvement of animal welfare.

Different stakeholder groups tend to signal different problems in animal welfare and suggest different solutions. Their visions around animal welfare are constructed according to so-called *frames of reference* (Te Velde et al., 2002). This is a frame that helps to make sense of complex realities: it provides a perspective to structure knowledge, position experiences and to judge and respond to issues (Schön and Rein, 1994). A frame of reference is based on the entire set of a person's norms, values, knowledge, convictions and interests (see Table 6.1) (Te Velde et al., 2002). These variables are usually in coherence with each other (Vinken and Soeters, 2004), as people prefer to have a harmony between them (Festinger et al., 1956). Values are the most stable variable (Rokeach, 1973), and conflicting visions between persons or groups are frequently the result of implicit value conflicts. Value conflicts often do not arise on the question whether single values are wrong or right, but on the order in which a set of values is prioritized (Schwartz and Bilsky, 1990).

**Table 6.1** Variables that form the frame of reference (Te Velde et al., 2002)

Variable	Meaning
Values	The things that matter to people
Norms	The translation of values into behavioural rules
Convictions	Taken for granted assumptions
Interests	The issues people are concerned with
Knowledge	Constructs from experiences, facts, stories and associations

In this study, we explored to what extent stakeholder groups observe pig behaviour differently and whether that is related to differences in frames of reference. It is known that observations are not only shaped by the given scene or object, but also by peoples framings. This implies that people with different framings, who are observing the same scene or object, might have different observations (Raftopoulos and Machamer, 2012). Different observations of the same situation might result in different convictions and beliefs and thereby strengthen and even validates one's own frame of reference.

Differences in observation might therefore have an important role in the animal welfare debate. In an earlier study, we noticed that pig farmers were very critical to scientific insights on pigs and their welfare when presented by animal scientists during an interactive symposium. This was partly due to differences in observation (Benard et al., 2013). The pig farmers and the animal scientists appeared to have a different approach for observing: the pig farmers were used to observe pigs by scanning their surroundings and noticing abnormalities, whereas animal scientists were used to observe pigs from a rather modelled with an emphasis on the individual animal. Consequently, they did not recognize some of each other's observations ('blind spots'), which contributed to disagreement on the importance of the natural foraging behaviour for good pig welfare.

In this study, we applied the Qualitative Behaviour Assessment (QBA) to assess differences in observation between stakeholder groups. QBA was originally developed as a tool for quantitative assessment of animal welfare (Wemelsfelder et al., 2001; Rousing and Wemelsfelder, 2006; Wemelsfelder, 2007; Temple et al., 2011). It primarily relies on human perception: different observers are asked to define an animal's mood by using descriptive terms such as 'active', 'happy' or 'irritated' (Wemelsfelder, 2007). The method was first described to evaluate the animal's overall welfare state (Wemelsfelder et al., 2000; Wemelsfelder et al., 2001) and studies have been performed on a wide range of animal species. This assessment is unique in the sense that it goes beyond identifying pain and distress, and also addresses positive aspects of animal welfare by observing the animal as a whole. Differences in QBA assessment between stakeholder groups was studied by Wemelsfelder et al (2012), whereby agreement on terms was found between pig farmers, veterinarians and animal activists.

The aim of this study is to investigate potential differences between stakeholder groups by using QBA similar to Wemelsfelder et al. (2012), but with an alternative approach by using QBA to specifically address differences in observations. In the discussion, we illustrate how the insights of this study can be used in multi-stakeholder learning processes. Multi-stakeholder learning processes are

acknowledged as the most constructive approach to find shared solutions among stakeholders on complex issues such as animal welfare improvement in pig production (Hisschemöller and Hoppe, 1995). These processes aim to develop congruency among visions by a process of mutual learning. An important element in this is frame reflection, which is defined by Schön and Rein (1994) as: “*to put themselves in the shoes of other actors in the environment (...) and to overcome the blindness induced by their own ways of framing the policy situation*”. By exploring a shared perspective on the problem, “*horizons become fused*” (Gadamer, 1965), and thereby the chance of success in creating solutions that match the diversity of societal perspectives increases.

### **6.2 Material and methods**

The use of the QBA method for assessing differences and similarities between stakeholder groups was first tested in a pilot study, which served to amend the protocol for the main study. We first describe the use of the pilot study to the main study, after which the materials and methods of the main study are described.

#### **6.2.1 Pilot study**

In November 2011, a pilot QBA was carried out with 15 participants. Twelve participants were animal scientists from different disciplines within Wageningen University (Animal Breeding and Genomics; Adaptation Physiology Group), and three participants were representatives from either an animal welfare organization, a farm branch organization, or the meat industry. They were shown 16 videos of 2 min each. Based on this pilot study, the number of videos for the main study was reduced to 10. In addition, videos were more focussed on the behaviour of a single animal rather than the whole group, resulting in videos of variable length. Furthermore, the word ‘curious’ was added to the scoring list on pig moods (described in the section ‘scoring form and questionnaires’) as suggested by participants of the pilot study.

#### **6.2.2 Main study**

##### *QBA participants*

Based on framing differences described in literature (Te Velde et al., 2002; Vanhonacker et al., 2008; Miele et al., 2011; Benard and Cock Buning, 2013; Benard et al., 2013) three stakeholder groups were selected, which were expected to have different frames of reference. The first two groups consisted of pig farmers (N=11) and animal scientists (N=18). They were defined as ‘experts’, meaning that they had frequent contacts and prior knowledge of pigs. The third stakeholder group

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were urban citizens (N=15). They were defined as 'lay-people', meaning that they had no or limited contact with farm animals in general. All participants were recruited through personal invitation by a person who was known to the participant (colleague or farm advisor). Participants were naïve in the sense that they were not familiar with negotiating or stakeholder learning processes, such as dialogues, at a professional level.

Pig farmers were selected from a rural area in the Netherlands (province Noord-Brabant) where there is a dense pig population compared to the average in the Netherlands (>6,000 pigs/km<sup>2</sup> compared to 376 pigs/km<sup>2</sup> (CBS, 2013). They had conventional intensive pig farms (N=9) or complied with slightly higher animal welfare standards (one 'star' on a three point 'star'-scaling system for farming systems with higher animal welfare standards ("Beter Leven kenmerk") (N=2). A majority of stakeholder in this group consisted of man (10 out of 11), with an average age of 35 (range 20–49). Five pig farmers had an education level of BSc. or higher. All pig farmers daily ate meat from a standard welfare segment, of which pork was the most favourable meat.

The animal scientists were all part of the Centre for Animal Welfare and Adaptation (CAWA), which is a collaboration between two of the largest research groups on welfare of production animals in the Netherlands: the chair group Adaptation Physiology of Wageningen University and the department of Animal Welfare of Wageningen UR Livestock Research in Lelystad. Scientists had on average 11 years of experience of working with pigs. The scientists consisted of 7 males and 11 females, with an average age of 39 (range 24-52). They all had an education level of BSc. or higher. Six scientists ate meat daily, nine scientists between 3 to 5 times a week, one less than 3 to 5 times a week, one less than 1 time a week, and one was a vegetarian. Half of the scientists ate meat from a standard welfare segment and the other half from a higher welfare segment. They had no specific preference for a particular type of meat.

The urban citizens, had no agricultural background or direct link with agriculture and were living in the Randstad of the Netherlands (a Dutch metropole). Nine out of 15 had never visited a pig farm and the others once. The group of urban citizens consisted of 3 males and 12 females, with an average age of 27 (range 20-34). They all had an education level of BSc. or higher and worked at a non-agricultural department at the VU University Amsterdam. Two urban citizens daily ate meat, two urban citizens were vegetarians and nine purchased meat from a higher animal welfare segment than standard. Chicken and beef were the most favourable meat.

**Table 6.2** Description of the nine videos which were shown to the three stakeholder groups

Video	Performed behaviour
1	Pig is trying to lie down on a part of the pen where another pig is lying, and touching this pig's body
2	Pig is sniffing and touching a pen mate
3	Pig is drinking water from the drinker nipple which results in water dripping on the head of a pig lying under the drinker nipple. This pig shakes his head in return
4	Pig is chewing the tail of a pen mate
5	Pig is sniffing, touching and biting on a ball hanging on a chain attached to the wall
6	Pig is biting and sniffing a jute sack which is attached to the pen wall
7	Pig is chewing on straw and sniffing in the straw while lying with eyes open
8	Pig is lying in the straw with eyes closed
9	Pig is sniffing in the straw while lying with eyes open

#### *Video recordings and selection of videos*

Video recordings of the pigs were collected at the animal facilities of Wageningen University (Wageningen, the Netherlands) in 2012. Pigs were housed in groups of six with a space allowance of 1 m<sup>2</sup> per pig (0.2 m<sup>2</sup> above minimum requirements of the Dutch legislation). Half of the pens had a conventional half slatted and half solid concrete floor, whereas the other pens had a deep litter bedding of sawdust and straw. All pens contained a metal chain with ball (in line with Dutch legislation) and a jute sack as a distraction material. Video footage was collected when a pig in the pen had specific behaviours such as drinking, sleeping, playing or oral manipulation of pen mate(s).

Nine video fragments were selected to represent a wide-range of behaviours and expressions which were either active or passive (Table 6.2). Video fragments were selected based on the behaviour shown (variation between the video fragments), the clarity in which the behaviour was expressed, and representation of both housing conditions (barren and straw-enriched). The length of the videos varied between 34 s and 1.55 min depending on the length of the expressed behaviour. At the beginning of the video, an arrow indicated on which pig the scoring should be performed. The nine videos were played in row, whereby the second video was repeated as the tenth video to be able to calculate the intra-observer reliability.

#### *Scoring form for videos and questionnaires*

In order to assess a pig on the videos, the method described in the Welfare Quality® Assessment protocol for sows and piglets (2009) was used (for Welfare Quality® see e.g. Blokhuis, 2008). All 20 terms from the protocol were used. The word 'curious' was added based on the pilot study. This led to the following list of

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terms: 1: active, 2: relaxed, 3: fearful, 4: agitated, 5: calm, 6: content, 7: tense, 8: enjoying, 9: frustrated, 10: sociable, 11: bored, 12: playful, 13: positively occupied, 14: listless, 15: lively, 16: indifferent, 17: irritable, 18: aimless, 19: happy, 20: distressed, and 21: curious. These descriptive terms with an expressive connotation were used to reflect the mood of an animal in a certain situation (Wemelsfelder, 2007). All terms were translated into Dutch for common interpretation by the participants. The participants were given time to read and understand the terms, although the meaning of the terms was not openly discussed before scoring started. The score-sheet included all 21 terms. Each term was followed by a horizontal line (125 mm in length) from minimum (not fitting the mood of the pig) to maximum (completely fitting the mood of the pig). Participants ticked the line at an appropriate point based on their assessment of the video.

In addition there were two questionnaires (available upon request). Questionnaire 1 was to gain insight in the background of the participants and included general information such as age and education, questions on contact with animals and questions on meat consumption. Questionnaire 1 was used to define the stakeholder groups as described earlier. Questionnaire 2 was comparable to the questionnaire published in Wemelsfelder et al. (2012), and served to gain a better understanding of the participants' framings of pigs. This questionnaire consisted of three parts: part 1 'how do you view pigs' (continuous scale from disagree to agree), part 2 'situations involving pigs' (continuous scale from 'not at all' to 'very much'), and part 3 'what do you think pigs can do' (5-point Likert scale; 'yes, very sure' to 'no, very sure' including a neutral midpoint 'not sure'). When the questions were following a continuous scale, the participants ticked the scale at an for them appropriate point. All questions were written in Dutch for a clear interpretation by the participants. The results of the completed questionnaires of all participants are available upon request.

### *Assessment procedure*

In November 2012, the three stakeholder groups were subjected to the QBA on separate locations, each in a location which was familiar to them. The stakeholder groups received identical instructions prior to the start of the video assessment. They were told not to communicate and to remain silent until the end of the session. First, the participants were asked to fill in the two questionnaires. Then, videos were displayed using an overhead projector with sound. A test video was shown to get acquainted with the scoring method. Thereafter, the 10 main videos were shown. The number of each video was clearly indicated at the beginning. Directly after each video, participants were given one minute to score the video

using the lines besides the 21 terms listed on a score-sheet. At the end of the session, the score-sheets were collected.

### *Statistical analysis of the QBA*

For the analysis of the QBA, the distance in millimetres between the left end of the scale ('minimum') and the tick of the participant was measured. These distances were collected in one matrix in an Excel spread sheet. These distances were subsequently analysed using a principal component analysis (PCA, no rotation) on stakeholder group level using R package FactoMineR (Markljung et al., 2008). PCA was used to reduce the dimensionality of the dataset (both the number of terms and the number of participants are large). A PCA summarizes the variables into a smaller number of terms, and may clarify coherence between groups (e.g. participants) for the different terms. Two main dimensions were generated to describe the variance between pig behaviour and each video fragment on each of these dimensions. Since all participants scored the same video fragments, each video received as many scores on each dimension of a PCA as there were participants included in this study. Results were then grouped per stakeholder group to identify similarities or differences between the stakeholder groups.

The units of the variables were scaled to unit variance and presented in a correlation circle where the first two principle dimensions were presented. Every term (N=21) was placed in the correlation circle indicated with an arrow. The more an arrow had an absolute magnitude close to 1 on the first dimension (x-axis) or second (y-axis) dimension, the more weight it had as a descriptor for that dimension. The angle between two arrows represented the strength of the correlation between two terms. An angle of 90 degrees indicated no correlation between the terms. To investigate differences between stakeholder groups, the average PCA score of each stakeholder group was graphically presented in a factor map. This factor map corresponds to the correlation circle in the sense that the interpretation of the x-axis and y-axis is the same. Significant differences between stakeholder groups were indicated by constructing a confidence ellipse (95% confidence interval) around the group mean (which is determined by the variability of the individuals within the stakeholder group). The construction of the confidence ellipses followed the parametric bootstrap method as described by Delholm et al. (2012). To determine whether the ranking of videos was different between stakeholder groups, the correlation between the stakeholder groups for the first and second dimension over the nine different videos was calculated, i.e. this would indicate whether all stakeholder groups would find video x the most positive or negative compared to the other videos. Insight in the ranking of videos is especially

relevant to the comparison between this study and studies which performed a QBA using Free Choice Profiling whereby observers generate their own terms for the observed behaviours. The alternative approach to QBA did not enable comparison between studies based on level, but did enable comparison based on how video fragments were ranked between different stakeholder groups.

Intra-observer reliability was determined to indicate whether the responses of the participants were stable over time by comparing the results from video 2 and video 10, which was the same video fragment repeated. A correlation coefficient between the responses for the two videos was calculated per participant. The correlation coefficients were averaged per stakeholder group to see whether video 2 was interpreted differently from video 10, within the three stakeholder groups. A PCA was performed to determine whether video 2 was interpreted differently from video 10 when all participants were analysed together. A confidence ellipse was drawn to determine whether the differences between video 2 and 10 were significant.

### *Statistical analysis of the questionnaires*

Means with standard errors were calculated by stakeholder group for part 1 and part 2 of questionnaire 2. Comparisons between stakeholder groups were analysed by ANOVA and the Tukey test. Pairwise  $p$ -values were used to indicate significant differences between the groups. Part 3 of questionnaire 2 followed a 5-point Likert scale and a Kruskal-Wallis test was used to test for significant differences between stakeholder groups. For questions where a significant difference between groups was observed, a post-hoc analysis for pairwise comparison using a Mann-Whitney U test was performed between the stakeholder groups to investigate differences between the three groups.

## **6.3 Results**

The three stakeholder groups, namely pig farmers, animal scientists, and urban citizens showed remarkable differences and similarities in how they observed the behaviour of the pigs in the videos. These results are described in the first paragraph. The stakeholder groups also had different visions on pigs and their welfare in general, as became apparent from the questionnaires, which is described in the second paragraph.

**Table 6.3** The three most positive and most negative significant correlations ( $p < 0.05$ ) between the first dimension from the PCA and the terms from the scoring list, and between the second dimension and the terms from the scoring list, for each stakeholder group

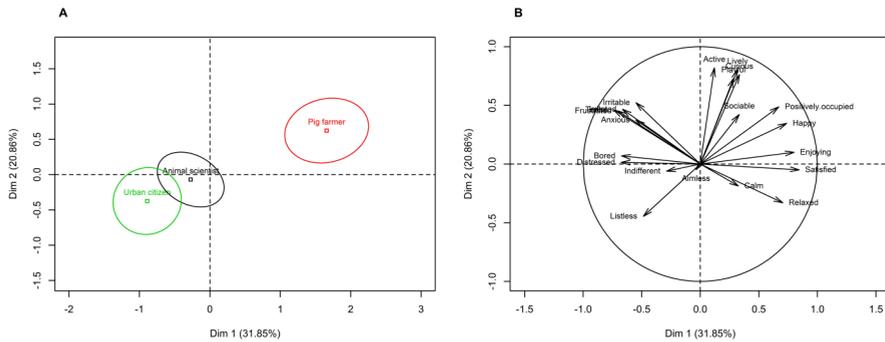
	Dimension 1				Dimension 2			
	Positively correlated term	Positive correlation	Negatively correlated term	Negative correlation	Positively correlated term	Positive correlation	Negatively correlated term	Negative correlation
Urban citizens	Positively occupied	0.81	Distressed	-0.61	Tense	0.84	Listless	-0.25
	Happy	0.77	Indifferent	-0.65	Frustrated	0.83	Satisfied	-0.43
	Satisfied	0.71	Listless	-0.68	Irritable	0.78	Relaxed	-0.73
Pig farmers	Satisfied	0.86	Frustrated	-0.69	Active	0.88	Calm	-0.32
	Enjoying	0.83	Irritated	-0.72	Lively	0.87	Listless	-0.52
	Happy	0.76	Tense	-0.78	Playful	0.80		
Animal scientists	Calm	0.60	Tense	-0.84	Lively	0.81	Listless	-0.44
	Enjoying	0.68	Frustrated	-0.81	Curious	0.79		
	Satisfied	0.78	Irritated	-0.80	Active	0.75		

### 6.3.1 Qualitative Behaviour Assessment (QBA)

The first two dimensions of the principle component analysis (PCA) explained the most variance between the pig behaviours and the video fragments. The first dimension showed strong correlations with the terms 'happy', 'satisfied' and 'enjoying' for all three stakeholder groups (Table 6.3). On the opposite site of the axis of this first dimension, the terms 'frustrated', 'irritated' and 'tense' correlated with the pig farmers and the animal scientists, whereas the terms 'distressed', 'indifferent', and 'listless' correlated with the urban citizens (Table 6.3). This dimension explained 28.6% of the variation among the videos for the urban citizens, 35.5% for the pig farmers, and 34.7% for the animal scientists. The terms explaining most variation for the first dimension, showed consensus between the stakeholder groups.

The second dimension showed a strong correlation with the terms 'active' and 'lively' for both the pig farmers and the animal scientists, whereas the terms 'tense', 'frustrated', and 'irritable' showed a strong correlation for the urban citizens. On the opposite site of the axis of the second dimension, all three stakeholder groups correlated with the term 'listless' (Table 6.3). The second dimension explained 26.1% of the variation among the videos for the urban citizens, 19.7% for the pig farmers, and 19.0% for the animal scientists.

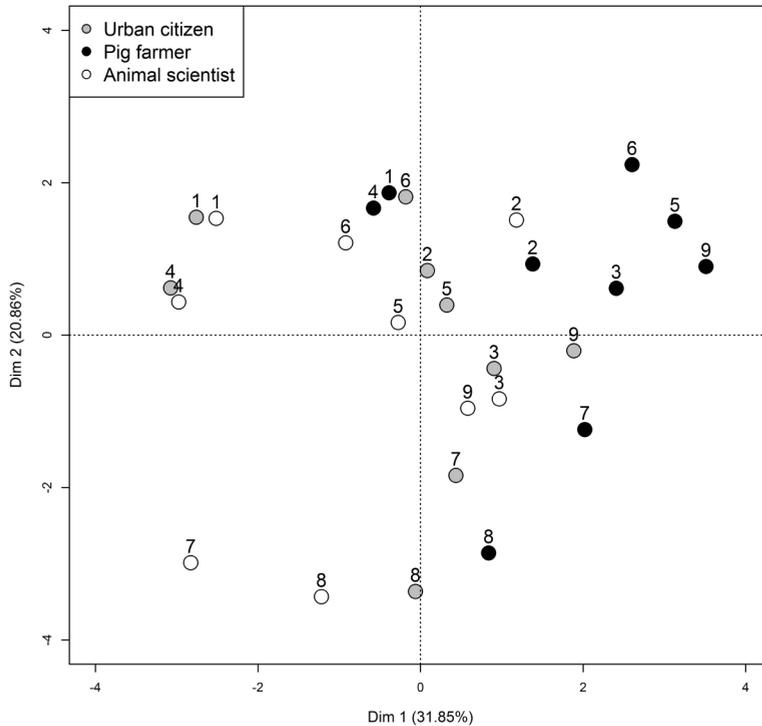
The pig farmers scored the videos significantly different compared to the animal scientists and the urban citizens (Figure 6.1a). This difference was well reflected in a combined analysis, where the stakeholder group was included as a categorical variable. In this combined analysis the first dimension reflected 'satisfied', 'enjoying' and 'happy' on one side of the axis and 'distressed', 'frustrated' and 'tense' on the other side of the same axis. The second dimension was reflected by 'active', 'lively' and 'curious' on one side of the axis and by 'calm', 'relaxed' and 'listless' on the other side of the same axis (Figure 6.1b). When terms are closely located to each other in Figure 6.1b, the correlation between the two terms is also high, for example 'enjoying' and 'satisfied' have a correlation of 0.79. The first dimension explained 31.9% of the variation between the stakeholder groups, and the second dimension explained 20.9% of the variation. The stakeholder groups significantly differed on the first dimension ( $r^2 = 0.15$ ;  $p < 0.001$ ), whereby the pig farmers had a higher coordinate (1.49) than the urban citizens (-1.06) and the animal scientists (-0.45) (Figure 1a). In addition, the stakeholder groups also differed on the second dimension ( $r^2 = 0.03$ ;  $p = 0.001$ ), whereby the pig farmers had a higher coordinate (0.56) on the axis than the urban citizens (-0.43) (Figure 6.1a).



**Figure 6.1** PCA analysis of the combined analyses. The x-axis indicates the first dimension and the y-axis indicates the second dimension. **(A)** Observer plot with the three stakeholder groups. The ellipse represents a 95% confidence interval around the stakeholder group mean. **(B)** Correlation circle with an arrow per variable. Length of the arrow indicates the correlation with the dimension. The angle between two arrows represents the strength of the correlation between two terms. An angle of 90 degrees indicates no correlation between the terms. Figure 1b corresponds to the observer plot in the sense that the interpretation of the x-axis and y-axis is the same. For example, the terms ‘distressed’ and ‘satisfied’ are strongly correlated with the first dimension.

The stakeholders ranked the videos similar regarding their judgement of the most positive or negative video (Figure 6.2). For example, video 4 was scored as most negative for all three stakeholder groups, even though its placement on the dimensions, indicating the scoring of subjective terms on moods, was quite different between the three stakeholder groups (Figure 6.2). Correlations were relatively high on both dimensions. Correlations for the first dimension were 0.63 between pig farmers-urban citizens, 0.71 between urban citizens-animal scientists and 0.90 between pig farmers-animal scientists. Correlations for the second dimension were 0.91 between pig farmers-urban citizens, 0.96 between urban citizens-animal scientists and 0.97 between pig farmers-animal scientists. Intra-observer correlation was relatively high with 0.61 for pig farmers, 0.68 for animal scientists, and 0.66 for urban citizens. No significant difference was found between the two identical videos, using a 95% confidence ellipse in the PCA analysis. This indicates a high repeatability within participants and consensus on the interpretation of the terms over different videos.

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**Figure 6.2** PCA analysis of the videos per stakeholder group. This figure corresponds to Figure 6.1 in the sense that the interpretation of the x-axis and y-axis is the same. The numbers written next to the symbols indicate the nine different videos (Table 6.2).

### 6.3.2 Questionnaires

The stakeholder groups significantly differed in their perceptions on eight of the ten questions of the questionnaire ‘How do you view pigs’ (Questionnaire 2 – part 1 as described in the Materials and methods) (Table 6.4). The pig farmers consistently scored higher on questions on the appearance of pigs (i.e. the questions ‘I like pigs’, ‘Pigs are fascinating’ and ‘Pigs are handsome’) compared to the urban citizens. In addition, the pig farmers showed less fear for pigs compared to urban citizens and were significantly less bothered by the smell or dirtiness of a pig than the urban citizens and animal scientists. In contrast, pig farmers were less likely to stroke or pat a pig than both the urban citizens and animal scientists, and they were less inclined to talk to a pig compared to animal scientists. The animal scientists were more inclined to talk to pigs than the pig farmers or urban citizens.

**Table 6.4** Means and standard errors (SE) for each stakeholder group on the questionnaire 'How do you view pigs' (Questionnaire 2 – part 1). The higher the score on a scale from 0 –75, the more the stakeholder would agree with the statement

	Urban citizens (N=15)		Pig farmers (N=11)		Animal scientists (N=18)		$p^*$
	Mean	SE	Mean	SE	Mean	SE	
I like pigs	48.5 <sup>a</sup>	3.7	68.8 <sup>b</sup>	1.8	63.2 <sup>b</sup>	1.7	<0.001
I think pigs are fascinating animals	37.7 <sup>a</sup>	4.5	69.2 <sup>b</sup>	1.8	62.7 <sup>b</sup>	1.8	<0.001
I think pigs are handsome animals	24.0 <sup>a</sup>	4.2	57.1 <sup>b</sup>	4.2	36.4 <sup>a</sup>	3.7	<0.001
I think pigs are cute	58.2	3.9	61.5	3.8	57.9	2.5	0.74
Would feel frightened	27.2 <sup>a</sup>	5.4	2.5 <sup>b</sup>	0.7	7.9 <sup>b</sup>	2.4	<0.001
Would you be bothered by their smell or dirtiness	38.1 <sup>a</sup>	5.5	4.3 <sup>b</sup>	1.7	27.1 <sup>b</sup>	3.6	<0.001
Would you feel like going up to them and stroking or patting them	41.3 <sup>a</sup>	5.5	21.9 <sup>b</sup>	6.9	43.6 <sup>a</sup>	3.8	0.02
Would you feel like talking to them	33.7	6.9	25.3 <sup>a</sup>	7.9	48.7 <sup>b</sup>	4.7	0.04
Would you feel that you could communicate with them in some way	34.8 <sup>a</sup>	5.5	45.2	7.5	51.7 <sup>b</sup>	2.7	0.05
Would you feel that they could communicate with you in some way	35.3	4.3	42.6	8.0	50.1	3.0	0.08

\* $p = p$  value to indicate whether the stakeholder groups were significantly different

<sup>a, b</sup> Averages within a row with different superscript letters differ significantly by  $p < 0.05$

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**Table 6.5** Means and standard errors (SE) for each stakeholder group on the questionnaire ‘Situations involving pigs’ (Questionnaire 2 – part 2). The higher the score on a scale from 0 –75, the more happy the stakeholder felt about the situation. ‘Who’ indicates whether the stakeholder felt happy himself in the situation (You) or that he or she thought the pig felt happy about the situation (Pig)

	Who	Urban citizens (N=15)		Pig farmers (N=11)		Animal scientists (N=18)		<i>p</i> *
		Mean	SE	Mean	SE	Mean	SE	
You see a pig lying in the corner, its skin badly scratched after losing a fight with another unfamiliar pig	Pig	14.9	2.7	11.5	4.3	12.8	2.2	0.72
	You	18.7	4.1	20.8	7.3	18.3	2.5	0.92
You see a pig rolling about in a wallow of mud on a hot sunny day	Pig	64.0	3.3	54.2	7.0	60.5	1.7	0.24
	You	58.9 <sup>a</sup>	3.5	35.2 <sup>b</sup>	8.0	51.9 <sup>a</sup>	3.9	0.01
You see a young pig unable to feed because other pigs are blocking its way to a feeder	Pig	17.4	3.2	10.5 <sup>a</sup>	4.2	21.9 <sup>b</sup>	2.5	0.06
	You	21.9	3.3	15.5 <sup>a</sup>	6.6	29.5 <sup>b</sup>	2.7	0.06
You see a group of piglets scampering about together in fresh straw	Pig	59.3	3.5	52.2	8.3	56.4	1.9	0.57
	You	53.3	3.3	42.3	8.6	51.0	2.3	0.25
You see a piglet squealing as it is castrated	Pig	7.1	2.9	13.5	4.8	10.6	3.5	0.51
	You	10.4	2.9	17.3	6.2	13.6	3.3	0.52

\**p* = *p* value to indicate whether the stakeholder groups were significantly different

<sup>a, b</sup> Averages within a row with different superscript letters differ significantly by *p* < 0.05

**Table 6.6** Means and standard errors (SE) for each stakeholder group on the questionnaire 'What do you think pigs can do' (Questionnaire 2 – part 3). The score went from 1: Yes (very sure) to 5: No (very sure)

	Urban citizens (N=15)		Pig farmers (N=11)		Animal scientists (N=18)		$p^*$
	Mean	SE	Mean	SE	Mean	SE	
Remember something that happened yesterday	2.2 <sup>a</sup>	0.1	1.9	0.3	1.3 <sup>b</sup>	0.1	0.003
Actively think about something that happened yesterday	2.0	0.2	2.3	0.3	1.9	0.1	0.50
Anticipate something that might happen tomorrow	3.0 <sup>a</sup>	0.2	4.2 <sup>b</sup>	0.3	3.3	0.3	0.04
Actively think about something that might happen tomorrow	3.4 <sup>a</sup>	0.2	4.3 <sup>b</sup>	0.3	3.6	0.3	0.03
Recognize a particular stockperson	1.5	0.1	1.5	0.2	1.2	0.1	0.06
Prefer to be handled by a particular stockperson out of a group of familiar stockpersons	1.7	0.1	2.7	0.4	1.7	0.2	0.24
Recognize an object they saw two or three months ago	2.4	0.2	3.1 <sup>a</sup>	0.3	2.1 <sup>b</sup>	0.2	0.02
Favour particular individual pigs but dislike others	1.7	0.2	2.5	0.3	1.6	0.1	0.75
Deceive another pig	2.3	0.2	3.1	0.4	2.8	0.2	0.31
Go to the aid of another unrelated adult pig	2.0	0.1	2.9	0.4	2.3	0.2	0.29
Form a picture in its mind of where things are in the area in which it lives	1.9	0.1	2.5	0.4	1.9	0.2	0.01

\* $p$  =  $p$  value to indicate whether the stakeholder groups were significantly different

<sup>a, b</sup> Averages within a row with different superscript letters differ significantly by  $p < 0.05$

The stakeholder groups significantly differed in two out of five questions on how they would feel, or how they thought that the pig would feel, in the different situations (Questionnaire 2 – part 2) (Table 6.5). Both the animal scientists and urban citizens felt happier than the pig farmers when imagining a pig rolling in the mud. How the pig would feel when rolling in the mud was judged the same by the stakeholder groups. The pig farmers felt worse as compared to the animal scientists when picturing a pig being blocked at the feeder. The pig farmers also, more than the animal scientists, thought that the pig would feel worse when it was blocked at the feeder. On this question, the urban citizens did not significantly differ from the other two stakeholder groups, with a score in between both groups (Table 6.5).

The 11 questions on ‘What you think pigs can do’ (Questionnaire 2 – part 3) resulted in five significant differences between the stakeholder groups (Table 6.6). The animal scientists were more sure that pigs could ‘remember something that happened yesterday’ as compared to the urban citizens. The pig farmers were less sure than the urban citizens that pigs could ‘anticipate something that might happen tomorrow’ or that pigs could ‘actively think about something that might happen tomorrow’. Also, the pig farmers were less sure than the animal scientists that pigs could ‘recognize an object they saw two or three months ago’.

### 6.4 Discussion

In this study, Qualitative Behaviour Assessment was used as a tool to unveil whether three stakeholder groups, i.e. pig farmers, animal scientists and urban citizens, observed pig behaviour differently. The application of QBA to assess differences in observation is an alternative approach as it was originally developed as qualitative tool to assess animal welfare (Wemelsfelder et al., 2001; Rousing and Wemelsfelder, 2006; Wemelsfelder, 2007; Temple et al., 2011). The pig farmers observed pig behaviours more positively than the animal scientists and the urban citizens. This can be concluded based on higher scores on the positive terms used to describe the pig behaviours. Intra-observer analysis indicated that the differences between the stakeholder groups were likely not due to interpretation differences of the terms.

#### 6.4.1 Reflecting on the QBA approach

Originally, QBA has been designed to assess animal welfare, and has as such been applied to a wide range of species (pigs:(Wemelsfelder et al., 2000; Wemelsfelder et al., 2001; Rutherford et al., 2012); dairy and beef cattle:(Rousing and Wemelsfelder, 2006; Stockman et al., 2011); sheep:(Phythian et al., 2013);

horses:(Napolitano et al., 2008); and dogs:(Walker et al., 2010). QBA terms showed significant correlations with animal based welfare parameters, such as physiological parameters (Stockman et al., 2011) and quantitative ethograms (Rutherford et al. 2012; Rousing and Wemelsfelder 2006). QBA is thereby increasingly applied to assess animal welfare (e.g. Temple et al., 2011). However, if different stakeholders give consistently different scores in QBA studies, the conclusions on animal welfare may depend upon the participants. Wemelsfelder et al. (2012) assessed differences between three stakeholder groups using QBA. They showed agreement and consistency between pig farmers, veterinarians and animal activists. Wemelsfelder et al. (2012), however, made use of free-choice profiling whereby observers generate their own terms for the observed behaviours, and they could therefore not compare levels between groups but could only compare ranking of videos. Also in this study the three stakeholder groups did not differ on how they ranked the videos, as rank correlations between the groups were high. However, differences in level between stakeholder groups should also be considered when discussing animal welfare aspects. For example animal scientists emphasize on more play material within pens while pig farmers have the opinion that the current play material offers sufficient distraction to pigs. Therefore, to obtain a balanced assessment on animal welfare from a QBA study, it would be important to compose a group of participants from various backgrounds and with a varying degree of familiarity with the animal species under study.

### **6.4.2 Frames of reference**

Differences between the stakeholder groups were found in the level of how given terms were judged, whereby the pig farmers judged the terms systematically more positively than the animal scientists and urban citizens. For example, they were more inclined to give a higher score to the terms 'satisfied' and 'enjoying'. We realize that due to the small sample size, we did not cover the diversity of perspectives within the stakeholder groups. However, we did not aim to capture the complete diversity, but aimed to gain insight in the mechanism of observing by real life groups. In the current study, differences in observation seemed to be related to the differences in frames of reference, as the outcomes of the questionnaires also showed differences between the pig farmers on the one hand, and the urban citizens and animal scientists on the other hand. All three stakeholder groups (except for the vegetarians) seemed to perceive pigs as production animals, or as a source of food, which became apparent from their consumption behaviour. In the direct human-animal relationship, however, the responses indicated that the pig farmers had a different perception of pigs than the

animal scientists and the urban citizens. The pig farmers view pigs as production animals, but the urban citizens and the animal scientists tended to view pigs more respectfully. For example, although the pig farmers liked pigs and considered them fascinating and handsome animals they kept a more emotional distance from the pigs by not feeling tempted to stroke or pat them or to talk to them, contrary to the animal scientists and the urban citizens.

### **6.4.3 Animal welfare approaches**

The different framings might be the cause of different animal welfare approaches. Animal welfare is generally described in three approaches: the biological functioning, feeling and natural living approach (Fraser, 1997). The pig farmers showed a *biological functioning approach*, which emphasizes the health, fertility and productivity of animals (e.g. the pig farmers felt unhappy when a pig was denied access to a feeder, questionnaire 2). Both the animal scientists and the urban citizens expressed a natural feeling pigs as with a natural living being, which emphasize the need of a good mental wellbeing and the need to behave naturally (e.g. they were happy when a pig could be rolling in the mud, questionnaire 2). Also in other studies, farmers evaluated animal welfare more positively than other stakeholder groups, and valued health as most important, whereas urban-citizens or animal scientists valued natural behaviour as most important (Te Velde et al., 2002; Boogaard et al., 2006; Lassen et al., 2006; Marie, 2006; Vanhonacker et al., 2008; Hubbard and Scott, 2011). From the video fragments it could be clearly observed that pigs were healthy, but some of the natural behaviours could not be fulfilled (e.g. no possibility to root or wallow). This might explain why the pig farmers were more positive than the animal scientists and urban citizens in observing the videos.

### **6.4.4 Application of QBA to stimulate multi-stakeholder learning processes**

Multi-stakeholder learning processes have been addressed as strategy to handle conflicts which involve conflicting framings and polarization (Dunn, 1988; Schön and Rein, 1994; Hisschemöller and Hoppe, 1995; Termeer et al., 2010). The outcome of multi-stakeholder learning processes may highly depend on the presence of different observations. In an earlier study reporting from a symposium organised by animal scientists for pig farmers, we showed how these differences can potentially give rise to misunderstandings or even create a deadlock due to distrust. The fact that participating farmers had different experiences and

observations on which they based their beliefs, was for a few farmers the reasons to question the reliability of scientific findings (Benard et al., 2013).

Based on the outcomes of the here presented study, we organized a workshop in which pig farmers, animal scientists, and farm advisors were brought together to observe and assess a new series of videos and images of pigs (Benard and Cock Buning, under review). All groups jointly watched the behaviour of (groups of) pigs (e.g. calm or restless), the facial expression of pigs, the positioning of the tail, and they judged pigs' vocalizations. Again, the participants differed in their observations and interpretations of the images and sounds. To stimulate dialogue and mutual learning, questions were asked what influenced the scoring, what was understood by the moods or QBA terms, and whether and why they considered certain terms as relevant for pig welfare. Contrary to the symposium reported in Benard et al. (2013), a constructive in-depth discussion was established. This way of organizing a workshop was generally positively valued by the participants. By confronting stakeholder groups with their differences in observation, the differences became explicit and inescapable, and by careful facilitating the dialogue, this may result in "calibration" of the way of observation. Using observation differences in multi-stakeholder learning processes might prevent misunderstandings, raise insights in each other's observations and underlying framing, and may therefore be an effective strategy in creating shared solutions that match the diversity of societal perspectives.

### **6.5 Conclusions**

In this study, Qualitative Behaviour Assessment was used as a tool to unveil to what extent pig farmers, animal scientists and urban citizens observe pig behaviour differently. Pig farmers observed pig behaviours more positively than animal scientists or urban citizens did, which seemed to be related to different underlying framing about pigs. Differences in observation can trouble multi-stakeholder learning processes as it might lead to different convictions and beliefs on animal welfare. This study shows the need of addressing potential observation differences in multi-stakeholder learning processes and illustrates how this can be achieved.

### **Acknowledgements**

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# 7

## **General discussion**



### 7.1 Introduction

The livestock production is a world-wide industry. In developing countries, this industry sector is moving from backyard farming towards more intensive farming to meet higher demands for animal proteins due to population growth, urbanization, and growth in income. In developed countries, the market share of animal proteins is stagnating and animal production systems are focusing more on sustainable production (Thornton, 2010).

One of the reasons for change in focus for livestock production is genetic progress through breeding. The demand for higher efficiency has resulted in 1% to 3% genetic progress per generation in commercial breeding programs due to single or multi-trait models (Smith, 1984; Merks, 2000). Breeding organisations are continuously adapting their breeding goals to support the needs of farmers and society. There is a world-wide demand for better feed efficiency due to the competition for land between food and feed production, but there is also more attention on some of the undesirable correlated responses such as increase in disease susceptibility and overall stress-sensitivity.

Another driving force for change in focus of livestock production systems is ethical concern which is mostly expressed in developed countries. Animal welfare guidelines are often established by a combination of legislative minimum standards and market-led initiatives (Lawrence and Stott, 2009). Until now, legislation did not ask breeding organisations to include traits related to health or behaviour in the breeding goal, although some breeding organisations decided to do so, on their own.

Pig production including higher welfare standards should not be always associated with loss of productivity and higher costs. Actually a welfare friendly production can still be economically profitable by a balanced improvement in productivity as well as in functional traits such as health and fertility.

An 'early life' example of simultaneous improvements in welfare and productivity is reduction in neonatal mortality. In common public opinion, neonatal mortality is an indication of suffering of young piglets and it causes economic loss for the farmer as well (Mellor and Stafford, 2004). Some pig breeding organisations have included neonatal survival in their breeding programs which has resulted in lower mortality rate while maintaining the rate of increase in the total number of piglets born (Knol et al., 2002). Hence, improvement in neonatal survival has led to better animal welfare as well as higher profitability for the farmer.

A potential very promising improvement for both animal welfare and economic output can be obtained by using indirect genetic effects (IGEs) in breeding

programs. An indirect genetic effect (IGE) is a heritable effects of one individual on the trait value of another individual (Griffing, 1967; Griffing, 1976; Wolf et al., 1998; Bijma et al., 2007). The breeding approach using IGEs incorporates both the direct genetic effect due to the focal individual, and the genetic effect an animal has on its pen mates into the trait value of the focal individual (Griffing, 1967). While traditional methods focused on individual performance only, this strategy could improve growth in pigs, as well as the behaviour of pigs which are housed in groups (Camerlink et al., 2013; Reimert et al., 2013; Camerlink et al., submitted). Since group housing is standard practice in finishing pigs, the pen with 10-20 finishers is the production unit and the pen is of great importance for the production, welfare and health of the pig.

In order to enhance pig welfare within the EU, legislative measures to reduce the number of interventions on pigs such as tail docking and castration are being discussed. A ban on those interventions will results in more or different social interactions between pigs. For example when castration is banned, entire males will be raised which are known to be more aggressive towards each other compared to castrates. The use of IGEs can offer a genetic solution to this problem. The potential of including IGE in pig breeding programs is investigated within the project 'Seeking sociable swine? Incorporating social genetic effects into pig breeding programs to achieve balanced improvement in productivity and welfare'. This thesis is part of that project. Both the impact on productivity and effect on behaviour were studied within the project. Specifically in this thesis, I focused on entire male production where androstenone is the subject under study (Chapters 2, 3 and 4). Chapter 5 describes the practical implications of implementing IGEs for growth in a pig breeding program. In Chapter 6, the differences in observation of pig behaviour between stakeholder groups (e.g. citizens, pig farmers and animal scientists) using Qualitative Behaviour Assessment (QBA) are described. To place the results of this thesis in a broader perspective, this general discussion will focus on three main topics related to the current status and developments to be expected in pig production with emphasis on pig breeding. These three topics are: a) raising entire males, b) indirect genetic effects applied c) involvement of stakeholders.

## 7.2 Raising entire males

Animal welfare concerns are increasing the pressure on pig farmers to stop castration. Usually, castration of entire male piglets is performed surgically by farmers with or without anaesthesia. It causes pain and suffering to the piglets especially when performed without anaesthesia. To possibly improve animal welfare of entire males, a declaration to ban castration by 1 January 2018 was signed by the pig and pork industry within the EU (EU, 2010). Therefore, in the near future all male piglets born will no longer be castrated within the EU. The consequences of stopping castration will be discussed in this section. First, I will briefly discuss the welfare implications of raising entire males and consumer acceptance of entire males as potential risks followed by the opportunity to increase feed efficiency. Thereafter, contribution of breeding to reduce boar taint and possibly improve behaviour will be discussed. Finally the trends in raising entire males will be described.

### 7.2.1 Welfare implications

In early life, welfare of entire males is improved compared to castrated animals, by not having to experience the pain and discomfort of castration. As they grow, entire males become more aggressive and perform more mounting, which could impair their welfare later in life (EFSA, 2004). Several studies have described this phenomenon comparing entire males to females (Rydhmer et al., 2006; Salmon and Edwards, 2006; Boyle and Bjorklund, 2007) and castrates (Tuyttens et al., 2008). More skin lesions are scored at the farm and abattoir for entire males compared to castrates. In addition to these physical indications of reduced welfare, pigs could also experience more fear, stress and pain as a consequence of the aggressive behaviour in the pen.

Sexual behaviour such as mounting is observed more frequently in entire males compared to castrates (Cronin et al., 2003; Rydhmer et al., 2006; Rydhmer et al., 2010). Entire males which mount a lot tend to have a reduced growth (Rydhmer et al., 2006) and an increased risk for leg problems and more skin lesions in single sex pens (Rydhmer et al., 2006).

Entire males have more aggressive behavior than castrates and gilts. Mixing of sexes further elevates the problem. Welfare of gilts is best guaranteed by single sex pens

### 7.2.2 Consumer acceptance of meat from entire males

One of the main issues with raising entire males is that pork from some entire males can emit boar taint. Boar taint is an urine-like, unpleasant flavour and odour released at cooking or heating of pork (Bonneau, 1997). Consumers that experience boar taint may reject the meal or completely stop purchasing pork. However, consumer acceptance of tainted pork is highly variable depending on preparation of the sample, consumer profile (e.g. gender, country), the piece of meat evaluated, location of the test, and breed used in the test (Font-i-Furnols, 2012). Boar taint is mainly caused by two compounds: androstenone (5 $\alpha$ -androst-16-en-3-one; Patterson, 1968) and skatole (Vold, 1970). Skatole levels are more important than the androstenone levels in this respect (Mathur et al., 2012). The percentage of tainted carcasses is highly variable depending on thresholds considered with respect to boar taint compounds. Reported percentages vary between 4 - 15% based on thresholds of androstenone and skatole (Xue et al., 1996; Aluwé et al., 2011). A study based on a Human Nose Score (HNS) where panellists scored a heated sample by a hot-iron showed that 3-7.5% of the carcasses were tainted (score 3 and 4; Mathur et al., 2012; van Wagenberg et al., 2013). Variability is high between farms with a prevalence between 0 and 7.5% (van Wagenberg et al., 2013) which is indicating that farm and management characteristics have a large influence on boar taint.

The potential risk for raising entire males is the risk of boar taint that may change purchasing behavior of consumers leading to reduction in sale of pork products

### 7.2.3 Breeding to reduce boar taint

Although levels of androstenone and skatole are variable, they are also for a large degree genetically determined. The heritability of androstenone and skatole are moderate to high (androstenone: 0.25-0.88; Robic et al., 2008, skatole: 0.23-0.55; Tajet and Andresen, 2006; Windig et al., 2012). Boar taint evaluated using human nose scores (HNS) has a relatively lower heritability of 0.12 (Windig et al., 2012). Genetic correlation between HNS and androstenone was between 0.22 and 0.52 and between HNS and skatole was 0.31 and 0.89 (Mathur et al., 2012). Therefore it seems possible to achieve a reduction in the number of tainted carcasses through breeding. Considering constant selection intensity, boar taint can be eliminated in about four generations of genetic selection using traditional quantitative genetics (Merks et al., 2009) or through genomic selection (Haberland et al., 2014). There

are also differences between lines. Dam lines often have higher concentrations of boar taint compounds compared to sire lines (Windig et al., 2012) due to correlated responses with reproduction traits (Mathur et al., 2013). Therefore, reduction of boar taint needs to be included in breeding goals for sire as well as dam lines.

Breeding to reduce boar taint requires additional data collection relating to androstenone, skatole and human nose scores. Collection of data on boar taint compounds (androstenone and skatole) has a few disadvantages: (1) costs of the phenotyping, (2) need to measure on carcasses (3) cannot be measured on selection candidates and (4) does not directly target the breeding goal.

Over the past years several alternatives have been investigated to reduce the costs of phenotyping (analysing androstenone and skatole is ~ €55 per sample) and to directly target the breeding goal: reducing the number of tainted carcasses. Introduction of the HNS fulfils both criteria, sampling costs are around €1 per sample (Mathur et al., 2012), and targets directly the breeding goal instead of underlying correlated traits. Although HNS has a lower heritability and a lower repeatability, family information on 4 full sibs and 76 half sibs will achieve a comparable accuracy compared to boar taint compounds with family information on 1 full sibs and 19 half sibs (Windig et al., 2012). The disadvantage remains that it cannot be measured directly on the selection candidate and therefore the selection decision has to be delayed until information on sibs (or offspring) becomes available.

Biopsy-based performance testing on selection candidates is a possibility. A small biopsy sample from the neck region can be taken (Baes et al., 2012) to determine androstenone and skatole levels. A high correlation between post-mortem and biopsies were observed for androstenone as well as skatole levels, indicating the usefulness of this approach. A drawback besides high costs of the phenotyping, are welfare concerns. Besides possible inflicted pain and bleeding of the wound (Baes et al., 2012), the appearance of the machine and the method does not seem animal friendly.

Another method to reduce boar taint is using genomic selection as a tool to reduce androstenone, and skatole or HNS. When the association between genetic marker and phenotype is established using at least 1,000 animals with phenotypes and genotypes (reference population), breeding values can be predicted of candidates from a DNA sample (genomic selection: Meuwissen et al., 2001). These candidates only need genotypes to get an accurate breeding value for boar taint phenotypes because the connection between phenotype and genotype has been established previously. This method reduces phenotyping costs, and the selection decision does not need to be delayed until sib or progeny information becomes available.

In recent years, several association studies have been published to find genomic regions associated with androstenone and skatole (Chapter 2 and 4 of this thesis; (Grindflek et al., 2011b; Ramos et al., 2011; Gregersen et al., 2012; Rowe et al., 2012). Overlap of associated regions between the studies is small confirming breed differences, and the assumption that boar taint compounds are under control of many genes. Fine mapping of associated genomic regions to functional genes has proven to be difficult. Hidalgo et al. (2014) has reduced the region on chromosome 6 detected in Chapter 2 and 4 from 3.75 Mb to 1.94 Mb using haplotype analyses. These results were replicated in independent populations confirming the genomic region. The region explains around 3-8% of the additive genetic variance. Fine mapping to find the causal mutation did not succeed, in spite of the effort to perform RNA-seq analysis and allele-specific expression analysis. Even though selection would be more beneficial when the causal mutation was found, known SNPs with an effect on boar taint compounds can be used in pig breeding programs to increase the accuracy of breeding values for boar taint compounds (marker assisted selection: MAS).

Currently, commercial livestock breeding is going through a transition phase from using MAS towards genomic selection (GS). For complex traits such as boar taint traits, which mostly are affected by many hundreds or thousands of polymorphisms each with small effects, MAS will capture less genetic variance than GS. One of the disadvantages which has delayed practical implementation of GS in pig breeding is the costs of the genotyping relative to additional genetic gain. However, genotyping costs have reduced and other alternatives are available such as cheaper low-density SNP chips to (partly) overcome this cost issue.

The breeding strategy used by major breeding organisations to reduce boar taint will be GS. Haberland et al. (2014) indicated that costs using GS + biopsy-based method compared to biopsy-based method only per selection candidate were higher (€380 vs. €330), while annual genetic gain improved marginally. However, reported genotyping costs were high (€150 per selection candidate) and costs could partly be refunded by additional profit in the production traits by increased accuracy of breeding values for these traits. Haberland et al. (2014) also reported that GS was most effective breeding strategy to reduce HNS, which ultimately is the breeding goal.

Genomic selection (GS) is recommended as new breeding strategy to reduce boar taint especially for the trait human nose score as GS is most beneficial on traits with low heritability and traits which are not measured on selection candidates

### **7.2.4 Breeding to improve behaviour**

Behaviour related traits are usually ignored by breeding organisations, mainly because they do not have an obvious economic return and are very labour intensive to measure. Therefore, also little is known about the genetic variation of many behaviour traits in pigs. However, behaviour of an animal can be used as a welfare indicator assuming that the external response of a pig reflects its internal state (Canario et al., 2013). With this in mind, behaviour does offer a lot of opportunities for breeding organisations to select animals which perform well given their environment and therefore select on the internal state of animals (e.g. coping with stress).

In current breeding programs, selection is on individual performance and does not take pen performance into account. Social interactions between animals within a pen, is of great importance for welfare, health and productivity. This means that breeding programs can be improved by incorporating these factors which reflect how pigs cope with external factors.

One method is to include an individual's heritable social effect which influences the performance of pen mates into breeding programs. This heritable social effect is referred to as indirect genetic effect (IGE: see section 7.2.1 'Theory of indirect genetic effects' for more explanation). Inclusion of the individual performance and IGEs in breeding programs, should guarantee equal or better group performance on the trait which is influenced by IGEs. For traits in pig breeding programs, significant contribution of IGEs have been estimated for growth (Chen et al., 2008; Canario et al., 2010; Hsu et al., 2010; Bergsma et al., 2013 and Chapter 5), feed intake (Bergsma et al., 2013) and androstenone (Chapter 3). Transmission of the heritable social effect to pen mates can be done through behaviour related traits. Either via positive interactions (social nosing; Camerlink et al., 2013) or negative interactions (aggression; Turner et al., 2006 or tail biting; Schrøder-Petersen and Simonsen, 2001). Harmful social behaviours such as tail biting often result in reduced economic performance of pen mates by reduced growth or feed intake (Wallgren and Lindahl, 1995; Sinisalo et al., 2012), but including IGEs in breeding programs could potentially reduce those unwanted behaviours.

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In layer hens, it was shown that selection on IGEs for high survival resulted in less feather pecking, less fear-related behaviours, and reduced stress response compared to selection on individual performance only (Bolhuis et al., 2009; Rodenburg et al., 2009). Also differences in functional activity of the serotonergic system and dopaminergic system were found. Results indicate a change in the internal state of animals (stress response), rather than changing one specific behaviour only.

In two studies on divergent IGEs for growth in pigs, it was shown that pigs with a positive effect on their pen mates growth had fewer skin lesions under stable social conditions, suggesting a more rapid rank order establishment (Rodenburg et al., 2010; Canario et al., 2012). A one generation selection experiment where pigs (gilts and castrates) were grouped based on a high or low IGE for growth was conducted to investigate underlying behavioural differences and confirm previous results (N=480). Aggression measured by skin lesions and fighting during regrouping did not differ between high and low IGE pigs. However, pigs with a high IGE showed less aggression after reunion with familiar pigs and also had less non-reciprocal biting in the week after regrouping (Camerlink et al., 2013). During the finishing phase, high IGE pigs showed systematically less biting behaviour; 40% less aggressive biting and 27% less oral manipulation of pen mates. High IGE pigs were also chewing 40% less on distraction material and consumed 30% less of the jute sacks provided. These differences were also expressed in the tail damage, where high IGE pigs had a better tail score (less damage) compared to low IGE pigs (Camerlink et al., submitted). In responses to novel situations, pigs with a high IGE were less fearful than low IGE pigs, as revealed by a shorter latency to touch and less locomotion in a novel arena (Reimert et al., 2013). Reimert et al. (submitted) found lower leukocyte, lymphocyte and haptoglobin levels for high IGE pigs, which supports the hypothesis of a lower stress response by high IGE pigs. These results altogether indicate that selection for high IGEs for growth rate is not only associated with social interactions but also with a change in internal state (Camerlink et al., submitted). Implementation of a breeding strategy for selecting high IGE for growth would therefore not only change one observed behaviour, but also the underlying mechanism which helps in better adaptation to the external environment.

Two remarks on this one generation selection experiment should be made. Firstly, the contrast on growth by selecting sires and dams with high and low IGEs, did not result in significant differences in growth in their offspring (Camerlink et al., 2014). The authors argue that control measures to limit harmful behaviour might have reduced the expression on IGEs on growth and that research under commercial

circumstances needs to be conducted. Further discussion on validation of IGEs can be found in section 7.4.4 'Validation of IGEs'. Secondly, differences observed in behaviour are not on entire males but castrates. Harmful unwanted behaviour is more observed in entire males as previously discussed, and therefore I argue that selection on high and low IGEs for growth could result in more pronounced differences in behaviour. Especially results on a more rapid rank order establishment in high IGE pigs is important in entire males, because they are more sensitive to changes in the social group, resulting in increased aggression as discussed in section 7.2.6. Selecting for growth including IGE in pig breeding programs is the most promising method at the moment to gain beneficial improvement in social behaviours, while not having to compromise on growth performance.

Transition towards entire male production could be supported by selection using IGEs for growth. That will improve growth as well as social interactions between entire males favorably.

### **7.2.5 Increased feed efficiency**

In addition to enhancing animal welfare, raising entire males can be actually beneficial to farmers. Entire males are more efficient in converting feed into gain, which could result in higher economic benefits to pork producers when castration is stopped. This improvement in feed efficiency is determined mainly by two factors: growth rate and body composition. Entire males are more efficient due to more energy use for protein deposition and lower lipid deposition, resulting in higher lean growth (for review see Millet et al., 2011). Entire males have, on average, 14% lower feed conversion ratio (feed/gain) and 7% higher lean meat percentage, which is comparable to about 6 years of selection. Farmers that stop castration can gain between €5 to €8 (Bikker et al., 2010; Backus et al., 2013) per slaughtered pig in the Netherlands, mainly due to reduced feed costs.

Entire males have a higher feed efficiency, which could result in economic benefits to producers.

### 7.2.6 Trends in pork production using entire males

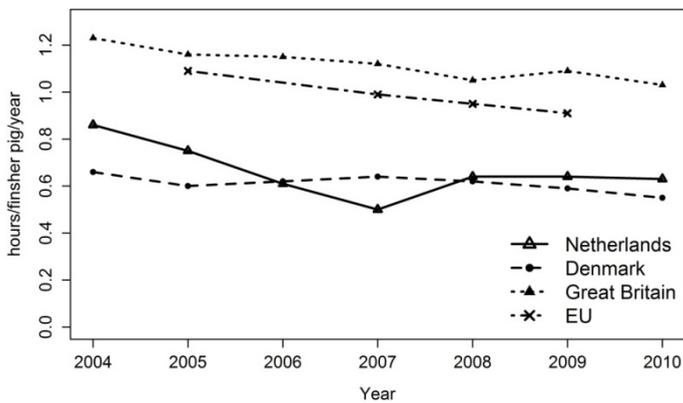
The suggested enhancement in animal welfare by stopping castration is already gaining momentum in Europe. A total of 148 million pigs were slaughtered in the Europe Union during 2012. Germany, Spain and France are the main producers responsible for 45% of the total production (FAOSTAT, 2012). The increased feed efficiency of entire males with increasing feed prices together with an upcoming ban on castration, has already resulted in an increased number of slaughtered entire males. In 2006, almost 100% of the slaughtered male pigs were castrated, but this decreased to almost 50% in 2012 (Newsletter Boars heading for 2018) in the Netherlands. Other European countries show a decline in % slaughtered castrates from 2006 to 2012, though not as much as the Netherlands (Newsletter Boars heading for 2018).

Slaughter weights between countries are highly variable, depending on the market. The slaughter weight has increased considerably, from 84 kg in 1990 to 93 kg in 2012 in the Netherlands (PVE, 2012). Several studies have shown that increase in slaughter weight of entire males should lead to increase in risk of boar taint (Babol et al., 2002; Aluwé et al., 2011). In the Netherlands and in many other European countries routine castration has been performed for many years allowing for higher slaughter weights without the risk of boar taint. Countries such as the UK and Ireland have stopped castration for past several years already and to circumvent the risk for boar taint, entire males are slaughtered at a younger age. However, even in the UK and Ireland, an increase in slaughter weight was observed from 65 kg in 1990 to 79 kg in 2013 (Meatstats, 2014), increasing the risk of boar taint. The increase of slaughter weights is mainly driven by efficiencies associated with heavier pigs by slaughter plants and the trend is expected to continue. Therefore, very low slaughter weights as the solution to reduce boar taint in entire males, is neither adequate nor practical. There is a strong need to find other solutions to reduce boar taint.

The proportion of entire males raised compared to castrates is increasing in Europe. At the same time slaughter weight is also increasing. This further increases the risk of boar taint.

Another trend in pig production which could affect raising entire males is the decrease in human labour and time available per pig. Analysis of the trend suggests that the time spent per piglet has been halved between 2000 and 2009 from 35 minutes to 17 minutes in the Netherlands (Landelijk Biggenprijzenschema 2000 to

2009). A similar trend is observed for hour/spent per year on a finisher pig (Figure 7.1) by the farmer. Countries such as Denmark and the Netherlands have reached a minimum number of minutes to spend on their pigs and have stabilized the last years at around 0.60 hour/pig/year. The EU average and Great Britain (GB) are still reducing their time spent per pig.



**Figure 7.1** Time trend on hours spent per finishing pigs for the EU and EU countries (BPEX, 2004-2010).

This reduction in time spent per pig is only possible when pigs are more self-reliant, which includes viable piglets (Merks et al., 2011), pigs that are more robust towards diseases and can be kept in groups without interventions. In pig production, the composition of social groups is determined by the farmer and can change throughout the life of the pig. Disturbance of the social group (e.g. cross-fostering, mixing at start of the finishing period, or split marketing for slaughter) does result in pigs experiencing stressful situations (Rault, 2012). Especially for entire males, interventions in the social group can have a large influence on the level of aggression. Fredriksen et al. (2008) showed that entire males kept in full sib groups are less aggressive and have fewer skin lesions than entire males kept in mixed groups. A recent study by Rydhmer et al. (2013) showed similar results. The group of entire males which was kept together from weaning onwards was compared to a group which was mixed at the start of the finishing period. The unchanged group had much lower frequency of aggressive interactions when entering the growing–finishing unit compared to the group which was mixed at the start of the finishing period. The number and severity of the skin lesions was also less in the unchanged groups, suggesting that socialized pigs learn social skills that

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enable them to form a stable social rank more rapidly (D'Eath, 2005). However, Fabrega et al. (2013) suggest that these results are short term effects of mixing (less skin lesions after 48 h) and rank order establishment as none of the indicators (behaviour, cortisol or skin lesions) was found to indicate a lower welfare status of mixed pens compared to intact pens when the new social rank for the mixed pens had been established.

Furthermore, several studies found that split marketing (removal of heaviest animals from the pen for slaughter) resulted in more aggression in pens with entire males compared to pens with gilts (Boyle and Bjorklund, 2007; Fredriksen and Hexeberg, 2009). When entire males were kept in unchanged groups and split marketing was applied, they tended to establish hierarchy quicker resulting in less skin lesions (Fàbrega et al., 2013) compared to mixed pens with entire males. Altogether these results indicate that stable social groups are even more important in entire male production compared to castrates.

Entire males could be more aggressive than gilts and castrates and therefore require more attention. However, there is a trend of decrease in availability and labour and time per pigs. Mixing or removal of pigs from a group further elevates the problem. Proper attention to social interactions and development of appropriate grouping strategies is much more important in this respect.

### 7.2.7 Conclusions

To conclude, switching towards entire males will affect the pig breeding goal. Boar taint needs to be incorporated in breeding goals of the sire lines and dam lines to reduce the risk of tainted carcasses reaching the consumer. Genomic selection using HNS or other boar taint related phenotypes need to be implemented to achieve the desired direction of change. Farmers need to pay extra attention to the penning strategy to reduce aggressive behaviours in entire males (single-sex pens, stable pen composition throughout life). From a breeding perspective, unwanted social behaviour between pigs and especially entire males can be beneficially improved by selecting on high IGEs for growth, resulting in a good economic performance and better welfare.

### 7.3 Indirect genetic effects applied

Indirect genetic effects were included in the statistical models described in chapter 3, 4 and 5. In addition to empirical estimates of indirect genetic effects (IGEs) for traits related to entire male production (androstenedione and average daily gain), the results can be extended for wider application. In this section I will discuss four additional aspects of IGEs for more accurate estimation, validation and effective use in breeding programs. First models for estimation of IGEs, second the use of DNA markers for IGE affected traits, third the effect of full sibs within a group and finally the validation of estimated IGEs will be discussed. Initially a general summary of the theory on IGEs is given to lay the foundation for the section 'indirect genetic effects applied'.

#### 7.3.1 Theory of indirect genetic effects

This section briefly summarizes the quantitative genetic theory of indirect genetic effects (IGEs). It could be skipped when familiar with this theory.

An indirect genetic effect (IGE) is a heritable effect of one individual on the trait value of another individual (Griffing, 1967; Griffing, 1976; Wolf et al., 1998; Bijma et al., 2007), which is also referred to as associative effect, social genetic effect or competition effect. The phenotype of an individual considering IGEs is composed of two components; a direct effect originating from the individual itself and the sum of indirect effects originating from  $n - 1$  group mates (Griffing, 1967):

$$P_i = A_{D,i} + E_{D,i} + \sum_{j=1}^{n-1} A_{I,j} + \sum_{j=1}^{n-1} E_{I,j} \quad 7.1$$

Where  $i$  denotes the focal individual,  $j$  denotes a group mate,  $A_{D,i}$  the direct genetic effect (DGE),  $E_{D,i}$  the non-heritable direct effect,  $A_{I,j}$  is the IGE of group mate  $j$  and  $E_{I,j}$  is the non-heritable effect of group mate  $j$ . The phenotypic variance assuming unrelated groups members is (Bijma et al., 2007):

$$\sigma_P^2 = \sigma_{A_D}^2 + \sigma_{E_D}^2 + (n-1)(\sigma_{A_I}^2 + \sigma_{E_I}^2) \quad 7.2$$

where  $\sigma_{A_D}^2$  is the genetic variance due to direct effect, and  $\sigma_{A_I}^2$  is the genetic variance due to indirect effect.

The total breeding value ( $A_T$ ) of an individual is the heritable impact an individual has on the population mean and is expressed as (Bijma et al., 2007):

$$A_{T,i} = A_{D,i} + (n-1)A_{I,i} \quad 7.3$$

It is important to note that the total breeding value of an individual is entirely dependent on the focal individual  $i$  while the phenotype of an individual is determined by direct effects from the focal individual and the sum of the IGEs from each of its group mates.

The potential of a population to respond to selection is proportional to the variance of total breeding values of individuals (Bijma et al., 2007):

$$\sigma_{A_T}^2 = \sigma_{A_D}^2 + 2(n-1)\sigma_{A_{DI}} + (n-1)^2\sigma_{A_I}^2 \quad 7.4$$

where  $\sigma_{A_{DI}}$  is the genetic covariance between direct and indirect effects.

Compared to the classical additive genetic variance the part,  $2(n-1)\sigma_{A_{DI}} + (n-1)^2\sigma_{A_I}^2$  originates from IGEs. Even when  $\sigma_{A_I}^2$  is small, its contribution to the total genetic variance can be substantial, especially when group sizes are large due to multiplication by  $(n-1)^2$ .

The total heritable variance expressed relative to the phenotypic variance equals (Bergsma et al., 2008)

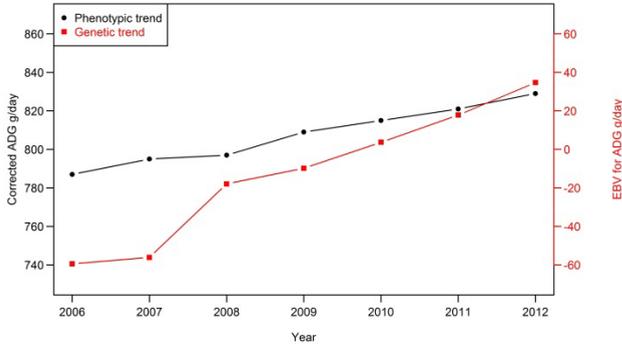
$$T^2 = \frac{\sigma_{A_T}^2}{\sigma_p^2} \quad 7.5$$

$T^2$  can be interpreted as a generalization of the conventional  $h^2$  to account for IGEs.

### 7.3.2 Models for estimation of IGEs

Average daily gain (ADG) is the most commonly recorded finishing trait in pig breeding to increase efficiency of pork production. Differences between genetic and phenotypic trends in this trait are of interest as they are related to modelling for IGEs. The genetic trend for the nucleus level is clearly increasing in time in the desirable direction (Figure 7.2). The phenotypic trend in the commercial crosses is also in the desired direction but more variable (Figure 7.2). However, the rate of improvement in the end product is not completely aligned to the expected response based on the genetic progress at the nucleus level. In six years, the genetic trend increased by 94 g/day, while the phenotypic trend increased by 42 g/day only. As the genetic trend is at the nucleus (purebred) level mainly based on selection in sire lines, 50% of the response could be expected at the end user level, although ADG is also selected for in dam lines. Even then the expectations fall short by 5 g/day. The difference is not very large in this example but the phenomenon is well known. Possible reasons for this shortfall are genotype-environment

interactions (Knap and Wang, 2012) and lack of appropriate genetic evaluation models.



**Figure 7.2** Genetic and phenotypic trends for average daily gain (ADG). Genetic trends were calculated for 2 purebred sire lines described in Chapter 5. The phenotypic trends were based on records from finishing pigs produced from the 2 sire lines from TOPIGS in the Netherlands.

Models for genetic evaluation for ADG are often based on individual performance without considering the social environment of the pig (heritable or non-heritable). However, several studies have described inclusion of IGEs in the statistical models to account for the social heritable effect (Arango et al., 2005, Hsu et al., 2010, Chen et al., 2008; Bergsma 2008, 2013, chapter 5). These models also include non-heritable effects such as litter and pen effects. As a result of differences in modelling, heritability estimates are quite different as well. Including IGEs in the model had significant effects in most studies (Chen et al., 2008; Bergsma 2008, 2013, chapter 5), although there were some exceptions (Arango et al., 2005, Hsu et al., 2010). Even when IGEs for ADG were included results on variance components were variable.

The direct genetic effect (DGE) can be overestimated (Chen et al., 2008) when relatedness within the group is higher compared to a random group but ignored in the model (Bergsma et al., 2008). In Table 7.1 an overview for ADG is given for four studies which estimated variance components with a model including IGEs and other random effects capturing the non-heritable effect of pigs. For each study, the model with the (significantly) best log likelihood (converged) was selected to compare results between studies.

## 7 General discussion

**Table 7.1** Contribution of different random effects to the estimation of average daily gain.

	$h_D^2$	$h_I^2$	$\frac{\sigma_{litr}^2}{\sigma_P^2}$	$\frac{\sigma_{pen}^2}{\sigma_P^2}$	$\frac{\sigma_{co}^2}{\sigma_P^2}$	$\frac{\sigma_{fs}^2}{\sigma_P^2}$	$\frac{\Sigma NHE}{\sigma_P^2}$	$T^2$
Chen et al., 2008	0.20	0.001	0.08	0.13	NE	NE	0.21	0.59
Bergsma et al., 2008	0.21	0.007	0.03	0.19	NE	NE	0.22	0.71
Bergsma et al., 2013	0.22	0.002	0.04	0.09	0.14	NE	0.27	0.34
Chapter 5	0.21	0.001	0.05	0.08	0.15	0.05	0.33	0.32

$h_D^2 = \frac{\sigma_{AD}^2}{\sigma_P^2}$ ;  $h_I^2 = \frac{\sigma_{AI}^2}{\sigma_P^2}$  where  $\sigma_{AI}^2$  is the indirect genetic variance;  $\sigma_{litr}^2$  is the variance of the common litter;  $\sigma_{pen}^2$  is variance of the contemporary pen;  $\sigma_{co}^2$  is the variance of the contemporary compartment;  $\sigma_{fs}^2$  is the variance for littermates grouped together during finishing;  $\Sigma NHE$  is the sum of the non-heritable effects; and  $T^2$  is the total heritable variance over  $\sigma_P^2$ ; NE is not estimated.

The sum of the non-heritable effects (NHE: litter, contemporary group within compartment and pen, and full sibs within a group) explain 21 to 33% of the phenotypic variance for ADG, which is considerable (Table 7.1). The NHE captures early influences by the common environment of the litter, the contemporary pen during finishing, the contemporary compartment during finishing, and littermates which are penned together during finishing. These random effects all have an influence on the growth of an animal.

In Table 7.1,  $T^2$  (see section: 'Theory on IGEs for definition of  $T^2$ ') is variable between studies and besides differences in  $\sigma_{AI}^2$ , also differences in the random effects influence the  $T^2$ . Models that do not include non-heritable effects tend to overestimate the total heritable variance. For example, Bergsma et al. 2008 did not include a contemporary compartment in which pigs were housed. Inclusion of this effect in a later study (Bergsma et al., 2013) resulted in a reduction in the  $T^2$  from 0.71 to 0.34.

The heritability of the direct genetic effect ( $h_D^2$ ) is very similar and does not seem to be affected by different random effects. However, the heritability of the IGE ( $h_I^2$ ) is variable, likely due to high standard errors (SE) on the estimate.

The SE of  $\sigma_{AI}^2$  depends on the number of groups, rather than number of individuals (Bijma, 2010a). In pigs, the number of groups is often small, because the group size

is relatively large. Group sizes in the studies from Table 7.1 varied from about 8.5 in Bergsma et al., 2008 and 2013, to 11.2 in Chapter 5 and to 15 in Chen et al., 2008. Ideally groups should consist of two distinct families, to accurately estimate  $\sigma_{A_i}^2$ . The effect an individual's IGE has on its pen mates, might depend on the group size. In larger groups, the time to interact with each pen mate may be smaller (Arango et al., 2005) and might reduce the magnitude of the IGE. The dependency of IGEs on group size is referred to as dilution (Bijma, 2010b). The degree of dilution can vary between 0 and 1 and determines whether the IGE of an individual is completely diluted across  $n$  pen mates ( $d=1$ ) or is completely independent of group size ( $d=0$ ). Strong dilution ( $d=1$ ) and increasing group size will increase the SE on  $\sigma_{A_i}^2$  (Bijma, 2010a). Accurate estimation of  $\sigma_{A_i}^2$  would require large datasets or perform a designed experiment. Commercially available data are often not suitable for this purpose. Studies on the estimation of SEs for estimated genetic parameters for traits affected by IGEs using more complex family-structures are lacking at the moment, but would be very useful to manage expectations or to give direction for proper grouping and collecting commercial data.

Importance of the environment explained by IGE and NHE of the pig is often underestimated by pig breeding programs and likely also by pig farmers. However it is of great importance for pig welfare and economics. Therefore I would like to emphasize that breeding organisations should implement accurate recording of the group composition of the pig throughout its life. This will allow more accurate estimation and use of genetic variation. This will result in a better correlation between predicted and realized response to selection.

Accurate estimation of IGEs in pig breeding can be hampered by 1) improper statistical modelling and 2) improper structure of commercially available data

### 7.3.3 Use of DNA markers for IGE affected traits

In livestock breeding, DNA markers are mainly used for two reasons. One is the identification of causative mutations explaining differences in phenotypes which can be used in breeding programs, and second is the use of many genome-wide markers to increase the accuracy of estimated breeding values of selection

## 7 General discussion

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candidates (genomic selection). Both approaches can also be used for traits affected by IGEs and will be discussed in the following section.

With the commercial availability of high density SNP chips such as the 60K SNP Chip in pigs (Ramos et al., 2009), genotyping large numbers of individuals for a large number of SNPs is becoming more common. Therefore there is an increase in number of association studies in many livestock species (Zhang et al., 2012). The goal is to find causative mutations. However, as sequence information is often unavailable, association studies rely on linkage disequilibrium (LD) between the causative mutation and the SNP. The majority of the studies focus on economically important (quantitative) traits such as milk yield in dairy cattle and on the direct genetic effect of traits measured on the individual.

The first studies to map Quantitative Trait Loci (QTL) with indirect genetic effects have been conducted on laboratory species. These studies (Wolf et al., 2002; Mutic and Wolf, 2007) used interval mapping to detect QTLs with an indirect genetic effect and to determine genetic variance explained by the QTL. Both studies had a low resolution and too few individuals to fine map QTL towards candidate genes.

In livestock species, there have only been two studies which performed an association study (Biscarini et al., 2010) to map direct and indirect QTL. In these studies, the direct genetic effects of the individual's own SNPs and the indirect genetic effects of the SNPs of its pen mates were estimated. Biscarini et al. (2010) reported an association between 1,022 SNPs and feather condition score of laying hens across nine different genetic lines (N=662). There is a difference between the genetic models used by Biscarini et al. (2010) and in Chapter 4. In Chapter 4, the variance components were set to fixed values and both  $\sigma_{A_D}^2$  and  $\sigma_{A_I}^2$  were included. Biscarini et al., (2010) used a two-step method. In the first step, no genetic effects (except for the SNP) were included in the model while in the second step the significant SNPs were included in an animal model without a variance component for the IGE. Since the indirect variance component was not included, inflation of significant SNPs with indirect genetic effects could be expected.

Ideally studies which aim to detect genomic regions associated with IGEs or predict breeding values should use genome-wide SNP markers and a complete model. The complete model should include all fixed and random effects including all SNPs simultaneously. For genome-wide association studies, fitting of all markers simultaneously is advantageous for two reasons. First, the multiple testing problem is overcome and second, as large number of SNPs can be in LD with the QTL and it is difficult to define the region containing the causal mutation (Gondro et al., 2013).

Therefore, similar to genomic prediction, the best models are models including all SNPs simultaneously.

Methods for genomic prediction and GWAS such as SNPBLUP (equivalent to GBLUP) or nonlinear models such as Bayes methods need to be adapted estimate SNP associations with respect to direct and indirect genetic effects. Following model including these effects is suggested:

$$\mathbf{y} = \mathbf{1}_n \mu + \mathbf{X}\mathbf{b} + \mathbf{Z}_D \mathbf{a}_D + \mathbf{Z}_I \mathbf{a}_I + \dots + \mathbf{e} \quad 7.6$$

where  $\mathbf{y}$  is a vector of phenotypic observations;  $\mu$  is the mean of the populations and  $\mathbf{1}$  a vector of ones;  $\mathbf{X}$  is the design matrix for the fixed effects (sex and herd-year-season of birth);  $\mathbf{b}$  is a unknown vector of fixed effects;  $\mathbf{a}_D$  is a vector of direct SNP effects for each marker from the focal individual and  $\mathbf{Z}_D$  is a design matrix of which the entries are SNP genotypes coded as the count of a given allele. The sum  $\mathbf{Z}_D \mathbf{a}_D$  over all markers is assumed to equal the vector of direct breeding values. Vector  $\mathbf{a}_I$  contains the indirect genetic effects for each marker from the it's pen mates ( $\sum_j SNP$ ) and  $\mathbf{Z}_I$  is a design matrix of which the entries are regressors

calculated from the sum of the marker genotypes of  $j$  pen mates coded as the count of a given allele. The sum  $\mathbf{Z}_I \mathbf{a}_I$  over all markers is assumed to equal the vector of indirect breeding values. The SNP effects both for  $\mathbf{a}_D$  and  $\mathbf{a}_I$  can be derived from a normal distribution e.g. in SNPBLUP, where  $\mathbf{a} \sim N(0, \mathbf{I}\sigma_g^2)$ , where  $\mathbf{I}$  is an identity matrix and  $\sigma_g^2$  due to a single SNP (Meuwissen et al., 2001). Alternatively, different prior assumptions for the SNP effects can be assumed f.e. Bayesian methods (Habier et al., 2011). For GWAS, direct and indirect SNP effects from the model can be plotted by chromosomal position to identify genomic regions of interest. Of special interest would the results from the indirect SNP effect be, to find genomic regions affected by IGEs. Possible candidate genes could be involved in processes regulating stress, activity, or social behaviour; although no candidate genes for IGEs have been described so far.

Using genomic selection for traits affected by IGEs could, similar to other quantitative traits, be improved by higher accuracy of breeding values.

In the future it is likely that the number of studies performing association studies on traits with IGE will increase, as the number of genotyped individuals is rapidly increasing and the abundance of DNA markers that is commercially coming available is also increasing. This could enhance our knowledge which processes are underlying IGEs.

Application of genomic selection including indirect genetic effects in livestock species requires genotyping of (almost) complete pens, which is not common practice at present. For example in pig breeding programs which apply genomic selection an average of 30% pig in a pen are genotyped to minimize genotyping costs. The expectations are that in the near future, all animals within a pen in testing stations will be genotyped due to decreasing genotyping costs (Personal communication Egiel Hanenberg; TOPIGS). This would allow for genomic prediction of the direct and indirect breeding values and increase the accuracy of selection.

Genomic prediction for the direct and indirect genetic effects should be implemented in pig breeding programs to increase the accuracy of prediction. Efforts should be made to genotype all pen mates.

### 7.3.4 Dependency of IGEs on relatedness

It is evident from the results in Chapter 5, that the inclusion of a random effect for the interaction of group by full-sib family in the statistical model (to account for non-genetic kin effects) was a significant improvement of the model, even though a common litter effect, pen effect and IGEs were fitted also. The effect on the estimation of IGEs when related animals respond differently on each other compared to unrelated animals is discussed in this section.

To account for a possible non-genetic effect of relatedness between pen mates, an extra random effect was included in the statistical model in Chapter 5, which was an interaction of group by full-sib family (referred to as full-sib group) which were penned together during the finishing phase. Although a common litter effect and pen effect were already in the model, the addition of full-sib group effect significantly improved the model (Table 7.2) and explained 5 % of the phenotypic variance. Significance of full-sib group indicates that full-sibs interact differently with each other than with unrelated pen mates.

Commercial piglets are likely to be reared with full-sibs for the first few weeks, although a small percentage will be cross-fostered. Piglets which were reared apart tend to fight more with each other than piglets reared together, independent whether they were genetically related (Stookey and Gonyou, 1998). More studies have focused on the effect of mixing at the start of the finishing phase on aggression in pigs (Meese and Ewbank, 1973; Puppe et al., 2008; Li and Johnston, 2009). Higher levels of aggression during the first 24 – 48 hours post-mixing are found in newly mixed pens compared to non-mixed pens. This resulted in a reduced growth performance for the first period, though overall growth

performance was not affected (Hyun et al., 1998; Li and Johnston, 2009). In addition to the effect of familiarity, the effect of relatedness between pen mates seems to influence growth moderately as Bergsma et al., 2008 showed that pens which consisted of full sibs had a 15 g/day higher growth than pens with unrelated pen mates ( $\sim 0.2 \sigma_p$ ).

**Table 2.** Difference in Log Likelihood due to inclusion of relatedness

Models compared	LRT <sup>1</sup>	P-value	Df <sup>2</sup>
C vs. IGE	10	0.007	2
C vs C+FS	94	<0.001	1
C vs IGE+FS	104	<0.001	3

C=classical model, IGE=model including indirect genetic effects, FS=including an extra random effect to account for a group by full-sib family interaction.

<sup>1</sup>LRT=chi-square test statistic for the likelihood-ratio test ( $-2(\text{LogL}_{\text{reduced model}} - \text{LogL}_{\text{full model}})$ ).

<sup>2</sup>Degrees of freedom for the chi-square test statistic defined as the difference in number of (co)variances fitted for the two models.

Full sibs are likely to behave more similar because they grew up in the same litter (environment). Kin selection theory predicts that sibs are more cooperative towards each other and might have inclusive fitness benefits (Hamilton, 1964). For estimating IGEs, this might need an extra effect in the statistical model, where IGEs are split in IGEs expressed on similar related animals and IGEs expressed on unrelated animals (Ellen, 2009):

$$A_{T,i} = A_{D_i} + f_k(n-1)A_{I_{k,i}} + f_{nk}(n-1)A_{I_{nk,i}}$$

The total breeding value ( $A_{T,i}$ ) then becomes the sum of the individual's direct breeding value ( $A_{D_i}$ ), the indirect breeding value of individual  $i$  on its kin ( $A_{I_{k,i}}$ ) and the indirect breeding value of individual  $i$  on its non-kin ( $A_{I_{nk,i}}$ ),  $f_k$  and  $f_{nk}$  are the fractions of kin and non-kin within the group.

The estimation of variance components becomes more complex by including kin and non-kin effects and puts an even higher demand on the data structure. Three variance components and three genetic correlations need to be estimated, which was modelled in a simulation study by Alemu et al. (2014a). In an optimum design to estimate IGEs, where each group consist of two distinct families (Bijma, 2010a) and in the presence of kin, Alemu et al. (2014a) show that all six genetic parameters are not identifiable. When group composition is same for all groups, the DGE is fully confounded with kin IGE, irrespective of the composition of groups.

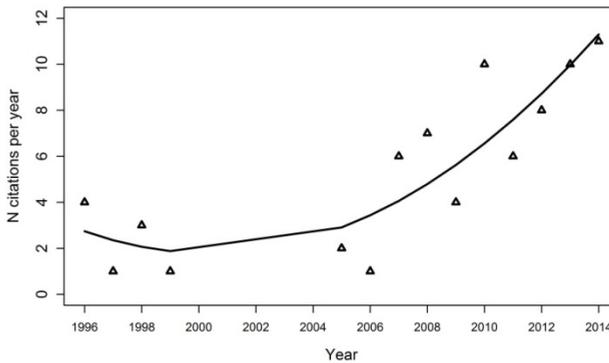
A possible solution is cross-fostering where groups consist of a mix of full sibs, half sibs and unrelated individuals.

Although empirical results on kin and non-kin IGEs have not been published yet, contribution of non-genetic kin effects (group by family interaction) has also been found in Tilapia (Khaw et al., submitted) and mink (Alemu et al., 2014b). The addition of a non-genetic kin effect by Kwah et al. (submitted) was highly significant, and comparable to results shown in Table 7.2. In the study by Alemu et al. (2014b), mink were penned with two full sib families which were completely confounded with sex. Inclusion of a cage\*sex interaction was significant and, at least partly accounted for non-genetic-IGEs that depend on relatedness. Together with the results from Chapter 5, there is a clear indication that familiarity is important to consider in statistical models with IGEs. To test whether kin and non-kin IGEs exist, large datasets from pig breeding organisations could provide insight as cross-fostering is common practice. However, other factors such as pen size are more variable, which might complicate estimation again.

Group by family interaction should be included for estimation of IGEs. Group selection based on family groups in the presence of kin and non-kin IGEs will result in higher response in the desirable direction compared to random groups.

### 7.3.5 Validation of IGEs

The number of studies referring to the paper published by Bruce Griffing in 1967 which is one of the founding papers on the theory of IGEs, is rapidly increasing (Figure 7.3). Empirical estimates of IGEs have mainly been published on livestock species and summarized by Ellen et al (submitted), Table 4. However, studies on validation of the IGE models are very limited. In this section, I will discuss several studies that conducted a validation and provide insight in the underlying factors which can be crucial for a successful validation.



**Figure 7.3** Number of scientific papers referring to Griffing, 1967. Results are from Scopus database, visited on 05-06-2014.

Validation of models can be done using two methods: a specifically designed selection experiment or prediction of phenotypes in an existing dataset. Selection experiments have been conducted in chicken and quail and pigs, showing promising results. Craig and Muir (1996) and Muir (1996) showed that group selection in layers was successful in reducing mortality. They showed that when IGEs are important, response to selection is large when group selection with related group members is applied, which is according to theoretical expectation (Griffing, 1976; Bijma, 2013). A rather anonymous pig experiment where group selection was implemented in a sire line for multiple generations showed an increase in phenotypic trend for growth after the group selection (half sibs within the group) protocol was implemented. Although the conference proceeding does not report any statistics, the selection method seemed to be working (Gunsett, 2005). A 18-generation selection experiment in Japanese quail also showed the benefit of using kin groups compared to random groups (Muir et al., 2013) in the response to selection for mortality and weight gain. Expected results based on theoretical derivations were not significantly different from observed results, though results were still variable between generations. Overall, they showed that multi-level selection in kin groups effectively reduced mortality and increased weight gain. Similar conclusions can be drawn from a six-generation selection experiment against mortality in laying hens (Ellen et al., 2014). The individually housed selection candidate had 4 or 5 sibs housed in family groups and selection was based on their survival time. Results between generations were variable, but the general trend for the 6 generations was that selection using IGEs was successful in reducing mortality.

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In pigs, a one-generation selection experiment was conducted at Wageningen University. Even though power calculation indicated that the contrast between high and low IGE pigs should result in a significant difference in growth performance, this was not detected (Camerlink et al., 2014). Animals within the experiment were housed in other management and environmental conditions compared to the commercial farms, in which the breeding values were estimated. As shown in multi-generational selection experiments in chicken (Ellen et al., submitted) and quail (Muir et al., 2013), results between generations were variable. A one-generation experiment in pigs might therefore not be sufficient to conclude that selection using a model including IGEs would outperform a classical animal model.

The second method to validate whether models including IGEs will outperform classical animal models in breeding value estimation is by predicting 'blinded' phenotypes as shown in Chapter 5 and Ellen et al. (2010). Results indicate that growth in pigs is a multi-factorial trait where management and environmental conditions influence the trait.

However, I am convinced when a multi-generational selection experiment would be conducted in comparable circumstances to the environment in which genetic parameters were estimated, similar results can be achieved as reported for layers and quail. To realize this additional response to selection in growth and welfare, breeding organisations need to invest in accurate pen recording and a good penning strategy (no regrouping and ideally two distinct families per group) to be able to model the effect of pen mates throughout the finishing period.

Properly designed experiments including multiple generations of selection including IGEs should be conducted in pigs. These should then result in similar significant improvements in social interactions and production as in chicken and quail.

### 7.3.6 Conclusions

To summarize, IGEs is a thriving field of research. A combination of suboptimal data structure, development of statistical models and not yet abundant available genotypes, makes implementation of IGEs challenging in commercial pig breeding programs. Though, increasing number of available (accurate) phenotypic data (including pen information) and increasing number of genotyped animals will soon be available and will no longer hamper implementation of IGEs in pig breeding programs.

#### **7.4 Involvement of stakeholders in animal welfare related discussions**

One of the goals of the research project 'Seeking sociable swine' is to incorporate societal concern expressed by stakeholders into the project by creating a feedback-loop in which stakeholders and researchers share their disciplinary and experiential knowledge. This was facilitated and studied by Marianne Benard, PhD Student VU University. In this section, this process and outcomes of the process are placed in a broader perspective not by repeating results from Marianne Benard, but based on personal observation and experience.

Prior to the start of the project, involvement of stakeholders sounded as an extra obligation in addition to scientific publications. There are no guidelines how to communicate research results to the more 'general' public when projects are related to ethical issues in livestock production. However, I would argue that it would be worthwhile including stakeholders in a project. In the next section, I will outline how the involvement of stakeholders enhanced the project outcome.

Tail biting was identified as one of the most 'trending topics' in discussions on pig welfare with stakeholders (representatives of pig farmers, slaughter house, breeding company, animal protection association and scientists). The Dutch government aims to abolish all interventions on animals by 2023 (LNV, 2007) and tail docking is one of these interventions. Welfare of pigs is impaired by the pain which piglets experience during the act of tail docking and shortly after. On the other hand, it is argued that avoiding tail docking can lead to injuries to the intact tail by tail biting, resulting in pain, increased chance of infections and even early death (EFSA, 2007). A symposium entitled 'The effect of social pig behaviour on pig production, welfare and health', was organized by the four PhD students from this project, to convey research results on tail biting to mainly pig farmers (N=37). Results from different fields of research were orally presented and followed-up by a workshop where participants were asked to identify bottlenecks and put forward solutions without any barriers. The responses on the presentations were critical and had a great degree of reservation (Benard et al. 2013). Reliability and practicality of the scientific results were often doubted by the farmers and discussion between farmers and scientists ended in deadlocks.

After the symposium, several new initiatives were launched within the project to (1) investigate the reason for differences in perspectives of stakeholders and (2) give practical advice to pig farmers on reducing tail biting. These efforts were combined in a 2<sup>nd</sup> symposium named 'interactive masterclass: insight into tail biting', which will be discussed in the following two sections.

### 7.4.1 Differences in perception between stakeholders

After the first symposium, we decided that we needed to further investigate differences between farmers and scientists to better understand the causes of some of the misunderstandings.

We wanted to investigate differences in perception of farmers and scientists using Qualitative Behaviour Assessment (QBA) as a tool. Three stakeholder groups (pig farmers, scientists and citizens) were asked to assess a set of videos of pigs and score them on a set list of 21 terms defining the animal's mood, to address differences in observation between the observers.

Results from the study indicated that the pig farmers observed pig behaviours more positively than the animal scientists and the urban citizens. Pig farmers had higher scores on the positive terms (such as 'satisfied' and 'enjoying') used to describe the pig behaviours (Duijvesteijn et al., in press). Given differences between the pig farmers and scientists, a different approach for the 2<sup>nd</sup> symposium was taken. The first part of the afternoon was intended to have discussion between different stakeholder groups in a small setting (60 participants: farmers, scientists, veterinarians, feed industry and journalists divided in five groups). Video fragments on tail biting were shown, and discussions on the video were facilitated to stimulate multi-stakeholder learning processes. Contrary to the first symposium (Benard et al., 2013), a constructive in-depth discussion was established. This way of organizing a workshop was received positively by the participants. By confronting participants with their differences in observation, the differences became explicit and inescapable. Careful facilitation of the dialogue should result in "calibration" of the perceptions.

Organization of a symposium for stakeholders is not difficult in itself. Achieving a constructive discussion is. Natural scientists focus more on explaining and predicting nature's phenomena. Organization of a symposium by natural scientists will therefore often result in a one-sided transfer of knowledge on the subject under study (first symposium). Social scientists, on the other hand, focus more on humans and experiences. A combination of both social and natural scientists in a symposium should provide both scientific and social points of views. Therefore, if a constructive dialogue between stakeholders is the goal of a symposium, the organization should incorporate both natural and social scientists for a balanced program and interactive discussions. Organizing the two symposia together with the PhD team was a good learning experience. Therefore I would advise PhD students also involved in sensitive discussions with stakeholders to find a partner in social sciences for the organization of a symposium. I would suggest discussing the

goal of the symposia and organizing an interesting symposium with an evaluation at the end. It will help in getting the research closer to the users and helps in developing communication skills.

### 7.4.2 Practical advice to pig farmers on reducing tail biting

The second part of the symposium ‘interactive masterclass: insight into tail biting’, focused on providing comprehensible results from the research, to give pig farmers tools to prevent or reduce tail biting problems. Instead of summing up scientific knowledge, an interview with a pig farmer who was experimenting with reduced tail docking was shown. Practical tips and tricks were greatly appreciated by the participants and accepted as possible solutions. Furthermore a (rather) simple trial was conducted to investigate the effect of a jute sack on damaging behaviours. This trial was not planned, but input in the project from stakeholders indicated the project would benefit from a comprehensible trial on tail biting. The trial was conducted at the farm of a pig breeder where a jute sack (see Figure 7.4) was (or wasn't) provided to piglets from birth until 13 weeks of age. Jute sacks significantly reduced tail and ear damages in the pre- and post-weaning phase with a 5-fold reduction of the proportion of tail wounds (Ursinus et al., submitted). These results are comprehensible and performed given commercial circumstances. Therefore, articles in magazines which have farmers as readers were approached to publish these results (Varkens, 2013a, b).



**Figure 7.4** Jute sack provided as environmental enrichment to reduce tail biting.

Actively approaching journalists that write articles for magazines read by farmers, was a good method to distribute results from the project to our users (farmers, veterinarians, breeders etc.). National (The Netherlands) but also international

articles were published and well received (overview can be seen on the project website: [www.sociableswine.nl](http://www.sociableswine.nl)).

Smaller side-steps in a project which at first sight might not seem scientifically challenging, but will allow to gain trust from stakeholders and especially farmers as they feel and see that their input is taken seriously. Eventually this type of approach will also lead to better acceptance of the 'other' scientific results, because of the trust built up with the stakeholders. As research on livestock production in an applied field of research, not only scientific outcomes of research gaining new insights in biological mechanisms or better statistical modelling is important, but also the application of these outcomes is of vital significance.

### **7.5.3 Conclusions**

Successful communication of results to stakeholders involved in a research project is more than giving scientific information. Understanding differences in perception of different stakeholders is essential for a good communication between them. For PhD projects which link to sensitive topics such as animal welfare, extra attention should be given to the communication with and between stakeholders. My advice would be to cooperate with a social scientist, preferably also a PhD student, to determine the best strategy of communication. This could be the organization of a symposium, but other ways are also possible. Besides personal development, it will help in getting more involvement and better understanding of the different points of view from stakeholders.

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## **Summary**



New legislative measures to ban interventions such as castration of male piglets and tail docking, can change the social environment of a pig. A change in the social environment will result in more or different social interactions between pigs. It is therefore desirable that pigs will fit in their new environment by e.g. showing wanted social behaviour. Social interactions between pigs can be genetically determined and then are referred to as indirect genetic effects (IGE), meaning that a pig can influence the trait value of a pen mate genetically.

Traditional breeding has selected on the individual performance without considering the IGE, which an individual has on its pen mates. Potentially it underestimates the heritable variation which could be used for genetic improvement. The aim of this thesis is to investigate the contribution of IGEs to selection traits in pig breeding programs. This was investigated by incorporating IGEs in quantitative genetic frameworks, or by using genomic information to detect associations between the phenotype and the direct and indirect genetic effects.

Chapters 2,3 and 4 focus on the trait androstenone. Castration of boars will be prohibited from January 2018 in the EU for welfare reasons. Pork from some entire males could have 'boar taint'; an off-flavour and odour when cooked or heated. One of the components causing boar taint is androstenone.

Chapter 2 describes a high-density genome-wide association study (GWAS) to identify Single Nucleotide Polymorphisms (SNPs) associated with androstenone levels in a commercial sire line of pigs. Androstenone levels of 987 pigs were associated with 47,897 SNPs. On pig chromosomes 1 and 6, 37 SNPs were significantly affecting the androstenone levels. Five most significant SNPs explained almost 14% of the genetic variance. On SSC6, a larger region of 10 Mb was shown to be associated with androstenone covering several candidate genes potentially involved in the synthesis and metabolism of androgens. In the region of SSC6 were found, among known candidate genes e.g. *CYP2A19*, *SULT2A* and *SULT2B1*, also new candidate genes such as members of the cytochrome P450 *CYP* gene subfamilies and of the hydroxysteroid-dehydrogenases (*HSD17B14*).

In Chapter 3, the social environment is also considered, as androstenone is a pheromone released from the saliva and can be spread through contact between pigs. In the presence of a sow, androstenone is released to attract her and induce a standing response. The contribution of social interactions between group-housed boars to the expression of androstenone was unknown. We estimated the contribution of IGEs, and also the non-heritable contribution of the pen and compartment are included in the model to estimate variance components for androstenone. The model including IGEs, a random pen and compartment effect fitted the androstenone data best. The IGE explained 11.7% of the total genetic variance. For selection against boar taint and, therefore also against androstenone, it can be recommended that at least the social environment of the boars should be considered.

In Chapter 4, the knowledge of Chapter 2 and 3 is used to accurately model androstenone in an association study where direct SNP effects and indirect SNP

effects are estimated. The dataset consisted of 1,282 non-castrated boars (993 boars genotyped), from 184 groups of pen members and 46,421 SNPs were included in the analysis. Both the direct SNP-effect of the individual itself and the indirect SNP-effects of its pen mates were model in a single-SNP analysis. None of the SNPs (direct or indirect) were found genome-wide significant. One Quantitative Trait Loci (QTL) on SSC6 was chromosome-wide significant for the direct effect which was the same region as described in Chapter 2. A single SNP on SSC9 and two regions and a single SNP on SSC14 were found for the indirect effect. In addition, to the newly discovered QTL and the confirmation of known QTL, this study also presents a methodology to model SNPs for indirect genetic effects.

Chapter 5 describes a validation study of indirect genetic effects for average daily gain (ADG) in pigs. The first objective was to estimate direct effects and IGEs for ADG. The second objective was to validate the IGEs by comparing the predictive ability of a classical animal model to a model including IGEs based on correlations between predicted and observed phenotypes. Two purebred sire lines were used with 41,144 records on ADG. Models including IGEs fitted the data significantly better than a classical animal model. However, no significant improvement was observed in ability to predict observed phenotypes between a classical animal model and a model including IGEs. Results differed among sire lines, validation years and farms, and were non-conclusive. Further research with larger dataset, including more sires and more groups, and with closely monitored pen recording is suggested to investigate the additional benefits of genetic evaluation models including IGEs in pig breeding programs.

In Chapter 6 describes the possible differences between stakeholders in the perception of animal behaviour and welfare. Debate on animal welfare in pig production is often led by differences in values, forms, convictions, interests and knowledge among the stakeholders. Difference in perception between stakeholders was explored using Qualitative Behaviour Assessment (QBA). Participating stakeholder groups were pig farmers (N=11), animal scientists (N=18) and urban citizens (N=15). Different stakeholders were asked to define an animal's mood by using descriptive terms such as 'active', 'happy' or 'irritated' by viewing nine video fragments showing different pig behaviours. Results from the QBA showed that the pig farmers observed the behaviour of pigs more positively than the urban citizens and the animal scientists. This was evident from the consistently higher scores on the positive terms to assess pig behaviour. In a follow-up stakeholder workshop, which focussed on differences in observation, QBA showed to be an effective tool to stimulate mutual learning among stakeholders, which is necessary to find shared solutions.

In the final chapter, Chapter 7, results from previous chapters are discussed in a broader sense, focusing on three main topics: raising entire males, indirect genetic effects applied and involvement of stakeholders in research projects related to animal welfare.

The first part of the general discussion describes the consequences of stopping castration which is inescapable in the near future in the EU. Increased aggression levels, compared to castrates and gilts, as well as boar taint in the meat are both risks for raising entire males. Breeding could provide solution to reduce those problems. Boar taint needs to be incorporated in breeding goals of the sire lines and dam lines to reduce the risk of tainted carcasses reaching the consumer. The use of SNPs to increase the accuracies of estimated breeding values of boars for boar taint related phenotypes needs to be implemented to achieve the desired direction of the change. Farmers need to pay extra attention to the penning strategy to reduce aggressive behaviours in entire males e.g. single-sex pens and stable pen composition throughout life. From a breeding perspective, unwanted social behaviour between the pigs, and especially entire males, can be beneficially improved by selecting on high IGEs for average daily gain, resulting in a good economic performance and better welfare.

The second part of the general discussion concentrated on the application of indirect genetic effects. Implementation of IGEs in pig breeding programs at the moment is hampered by 1) improper statistical modelling and 2) improper structure of commercially available data. Improper statistical modelling could result in an over- or underestimation of the contribution of IGE to traits affected by IGEs and, therefore, prediction of the response to selection can deviate from the observed response to selection. Improper structure of commercially available data in pigs is due to relatively large group sizes, variable group sizes and lacking of family structure within groups. Genomic prediction for the direct and indirect genetic effects should be implemented in pig breeding programs to increase the accuracy of prediction. Therefore, all pen mates need to be genotyped and with the increasing number of genotyped animals, this should be possible in the near future. The final part of the general discussion focusses on the involvement of stakeholders in sensitive discussions such as animal welfare. Differences in perspectives of stakeholders could stimulate an in-depth discussion, while confronting stakeholders with their differences in perceptions resulted in mutual learning. During the process of involving stakeholders in the research program 'Seeking Sociable Swine' the experience was that communication between and among stakeholders required special attention. A communication strategy was necessary; to achieve this the input from social sciences was essential. As not only scientific outcomes of research gaining new insights in biological mechanisms or better statistical modelling is important, also the application of these outcomes is of vital significance. Therefore, natural scientists and social scientists could combine efforts to achieve a better communication between stakeholders and help in getting the research closer to the users.



## **Samenvatting**



Nieuwe regelgeving om ingrepen aan varkens te verbieden, zoals castreren en staart couperen, kunnen leiden tot een verandering van de sociale omgeving van het varken. Die verandering van de sociale omgeving kan leiden tot meer of andere sociale interacties tussen varkens. Het is dus wenselijk dat varkens goed passen binnen deze vernieuwde omgeving onder andere doordat ze gewenst sociaal gedrag vertonen. Sociale interacties tussen varkens kunnen genetisch bepaald zijn en worden dan aangeduid als indirecte genetische effecten (IGE). Dit houdt in dat een individu een erfelijke aanleg heeft om de (productie) prestatie van hokgenoten, zoals groei, te beïnvloeden.

Traditionele fokkerij heeft dieren geselecteerd op basis van individuele prestatie zonder IGE mee te nemen. In potentie zou dit de erfelijke variatie die kan worden gebruikt voor genetische verbetering onderschatten. Het doel van dit proefschrift is om de bijdrage van IGE voor kenmerken welke opgenomen zijn in varkensfokkerijprogramma's te onderzoeken. Dit is onderzocht door IGE op te nemen in kwantitatieve genetische modellen of door DNA-informatie te gebruiken om associaties te vinden tussen het fenotype en directe en indirecte genetische effecten.

Hoofdstukken 2,3 en 4 focussen op het kenmerk androstenon. Castratie van beren zal verboden worden per januari 2018 in de EU om dierenwelzijnredenen. Varkensvlees van sommige intacte beren kan 'berengeur' hebben: een onaangename geur en smaak welke vrijkomt bij het koken of verhitten van het varkensvlees. Een van de componenten welke berengeur veroorzaakt is androstenon.

Hoofdstuk 2 beschrijft een genomwijde associatiestudie om Single Nucleotide Polymorphisms (SNPs) te vinden die geassocieerd zijn met androstenonniveaus in een commerciële berenlijn. Androstenonniveaus van 987 varkens zijn geassocieerd met 47,897 SNPs. Op varkenschromosoom 1 en 6 waren 37 SNPs significant geassocieerd met androstenonniveaus. De vijf meest significante SNPs verklaarde bijna 14% van de genetische variatie. Op chromosoom 6 is een grotere regio gevonden van 10 Mb waar een aantal kandidaatgenen zitten die betrokken zijn in de synthese en metabolisme van androgens. In de regio gevonden op chromosoom 6 zitten enkele bekende kandidaatgenen e.g. *CYP2A19*, *SULT2A* en *SULT2B1*, maar ook enkele nieuwe kandidaatgenen zoals cytochrome P450 *CYP2* en *HSD17B14*.

In hoofdstuk 3 is de sociale omgeving van de beren ook meegenomen, omdat androstenon een feromoon is welke vrijkomt uit speeksel en kan verspreid worden door contact tussen varkens. In het bijzijn van een zeug wordt androstenon vrijgelaten om haar aan te trekken en het kan een sta-respons opwekken bij de zeug. De bijdrage van sociale interacties aan de expressie van androstenon tussen beren die in groepen zijn gehouden was onbekend. We hebben de bijdrage van IGE onderzocht en ook de niet-genetische bijdrage van het hok en de stal zijn in het model opgenomen om variantiecomponenten te schatten voor androstenon. Het model met IGE en effect voor hok en stal was het beste model. De IGE verklaarde 11.7% van de totale genetische variatie. Voor selectie tegen berengeur, en dus ook

tegen androstenon, is het aanbevolen om de sociale omgeving van de beren mee te nemen in de modellering.

In hoofdstuk 4 is de kennis van hoofdstuk 2 en 3 gebruikt om met het meest accurate model voor androstenon, een associatiestudie uit te voeren waarbij directe en indirecte SNP effecten zijn geschat. De dataset bestond uit 1,282 beren (993 beren gegenotypeerd), uit 184 groepen van hokgenoten en 46,421 SNPs waren in de analyse opgenomen. Zowel het direct SNP effect van een individu, als het indirect SNP effect van de hokgenoten zijn opgenomen in een single-SNP analyse. Geen van de SNPs was genomewijd significant. Één Quantitative Trait Loci (QTL) op chromosoom 6 was chromosomewijd significant voor het directe effect hetgeen dezelfde regio was die in hoofdstuk 2 is beschreven. Een enkele SNP op chromosoom 9 en twee regio's en een enkele SNP op chromosoom 14 zijn chromosomewijd significant gevonden voor het indirecte effect. Naast het beschrijven van een nieuwe QTL en de bevestiging van een bekende QTL, beschrijft deze studie ook een methodologie hoe SNPs voor indirecte effecten gemodelleerd kunnen worden.

Hoofdstuk 5 beschrijft een validatie studie van indirecte effecten voor groei in varkens. Het eerste doel was om variantiecomponenten voor directe en indirecte genetische effecten te schatten. Het tweede doel was het valideren van IGE door middel van het voorspellen van groei met een klassiek model of met een model waar IGE zijn meegenomen. De voorspelde groei werd gecorreleerd aan de gemeten groei om zo te bepalen welk model beter was. Twee zuivere berenlijnen met 41,144 groeigegevens zijn gebruikt. Modellen met IGE waren significant beter dan modellen waarbij IGE niet waren opgenomen (klassiek model). Er werd echter geen significante verbetering waargenomen in een beter voorspellend vermogen tussen het klassieke model en het model waarbij IGE waren opgenomen. Resultaten verschilden tussen berenlijn, validatiejaar en bedrijven en waren niet overtuigend. Meer onderzoek met een grotere dataset, met meer vaders, meer groepen en een goed gemonitorde hokregistratie, wordt geadviseerd om de toegevoegde waarde van IGE in varkensfokprogramma's te kunnen kwantificeren.

Hoofdstuk 6 beschrijft de mogelijke verschillen tussen stakeholders in de perceptie van gedrag en dierenwelzijn. Het debat over dierenwelzijn in de varkenshouderij wordt vaak gevoerd terwijl er op de achtergrond verschillen in waarden, normen, overtuigingen, interesses en kennis tussen de stakeholders meespelen. Verschillen in perceptie tussen stakeholders werd onderzocht met behulp van een Qualitative Behaviour Assessment (QBA). De deelnemende stakeholdergroepen waren varkenshouders (N=11), dierwetenschappers (N=18) en stedelingen (N=15). De verschillende stakeholders werden gevraagd om de gemoedstoestand van een varken te omschrijven gebruikmakende van beschrijvende termen zoals 'actief', 'gelukkig' of 'geïrriteerd'. Zij hebben dit voor negen videofragmenten gedaan waar verschillende varkensgedragingen te zien waren. Resultaten van de QBA laten zien dat varkenshouders het gedrag van varkens positiever inschatten dan stedelingen en dierwetenschappers. Dit was duidelijk door het consequent hoger scoren van

positieve termen om het gedrag te beoordelen. In een vervolg stakeholderworkshop welke focuste op verschillen in observatie, liet zien dat QBA een effectief middel kan zijn om wederzijds leren tussen stakeholders te stimuleren wat nodig is om tot gezamenlijke oplossingen te komen.

In hoofdstuk 7 zijn de resultaten van de voorgaande hoofdstukken bediscussieerd en in een breder perspectief geplaatst met focus op drie onderwerpen: het houden van beren, de toepassing van indirect genetische effecten en de betrokkenheid van stakeholders in onderzoeksprojecten gerelateerd aan dierenwelzijn.

In het eerste deel van de algemene discussie worden de consequenties beschreven van het stoppen met castreren, hetgeen gaat gebeuren in de nabije toekomst in de EU. Verhoogde agressie, ten opzichte van gelten en borgen, en berengeur zijn beide risico's van het houden van beren. Fokkerij kan een oplossing zijn om deze problemen te verminderen. Berengeur moet als kenmerk in de fokdoelen meegenomen worden, zowel in zeugen- als in berenlijnen, om het risico van varkensvlees met berengeur te verminderen. Het gebruik van SNPs om de nauwkeurigheid van de fokwaarde voor berengeurkenmerken te verhogen, moet worden geïmplementeerd in fokprogramma's. Varkenshouders zouden meer aandacht moeten besteden aan een goede hokstrategie om de agressie bij beren te verminderen zoals hokken met hetzelfde geslacht en een stabiele hoksamenstelling gedurende het leven. Vanuit fokkerijperspectief, kan ongewenst sociaal gedrag tussen varkens, en vooral tussen beren, positief verbeterd worden door te selecteren op hoge IGE voor groei; wat zou resulteren in een goede economische prestatie en een beter dierenwelzijn.

Het tweede deel van de algemene discussie concentreert zich op de toepassing van IGE. Implementatie van IGE in varkensfokprogramma's is op dit moment gehinderd door: 1) onjuiste statistische modellen en 2) onjuiste structuur van commerciële beschikbare data. Onjuiste statistische modellen kunnen resulteren in een over- of onderschatting van de bijdrage van IGE aan kenmerken die beïnvloed worden door IGE met als gevolg dat de voorspelde respons op selectie kan afwijken van de daadwerkelijke respons op selectie. Onjuiste structuur van commercieel beschikbare data in varkens komt door de relatief grote groepen, variabele groeps grootte en een gebrek aan duidelijke familiestructuur in groepen. Genomische fokwaardes voor het direct en indirecte genetische effect zou geïmplementeerd kunnen worden in varkensfokprogramma's om de betrouwbaarheid van de voorspelling te verbeteren. Daarvoor moeten wel alle hokgenoten gegenotypeerd worden en met het toenemende aantal gegenotypeerde dieren zou dit in de toekomst mogelijk moeten zijn.

Het laatste deel van de algemene discussie concentreert zich op de betrokkenheid van stakeholders in gevoelige discussie zoals dierenwelzijn. Verschillen in perspectief tussen stakeholders kunnen een discussie stimuleren, en het confronteren van stakeholders met de verschillen in perceptie kunnen resulteren in gezamenlijk leren. Tijdens het proces van het betrekken van stakeholders in het onderzoeksprogramma 'Seeking Sociable Swine' was de ervaring dat communicatie

tussen en met stakeholders speciale aandacht behoefte. Een communicatiestrategie bleek nodig; om hiertoe te komen was de input van gammawetenschappers essentieel. Omdat niet alleen de uitkomst van het onderzoek belangrijk is, welke vaak leidt tot nieuwe biologische inzichten of betere statistische modellen, ook de toepassing van de uitkomst is van groot belang. Daarom zouden beta- en gamma-wetenschappers meer samen moeten werken om een goede communicatie tussen stakeholders te bewerkstelligen en daarbij helpen om onderzoek dichterbij de gebruiker te krijgen.

# **Curriculum Vitae**



**About the author**

Naomi Duijvesteijn was born on 28 March 1984 in Boxmeer and raised in Standdaarbuiten, the Netherlands. In 2002 she graduated from high school 'de Markenlanden', Oudenbosch and started her bachelor study Animal Sciences at Wageningen University. For her bachelor thesis she wrote a review and conducted a data analysis on cannibalism and feather pecking in layer hens and graduated in 2005. She continued with a Master study with the specialization Animal Breeding and Genetics. In 2009 she went five months to Uppsala, Sweden for an Erasmus Mundus exchange program to study molecular genetics at the SLU (Swedish University of Agricultural Sciences). In 2008 she went on an six month internship to LIC (a dairy breeding organization) in New Zealand to perform a QTL analysis on once-a-day milking. For her major thesis she performed a simulation study on the optimization of breeding programs for traits affected by social interactions in pigs. In January 2008 she finished her master after which she started working at TOPIGS Research Center IPG where she conducted several different studies for TOPIGS, a Dutch pig breeding organization. In March 2010 she combined work at TOPIGS Research Center IPG, with a PhD at the Animal Breeding and Genomics Centre (ABGC) at Wageningen University to investigate the prospects of including indirect genetic effects in pig breeding programs of which the results are described in this thesis. This research was part of two larger research projects: "Genetics of social interactions in livestock: Improving health, welfare, and productivity in laying hens and pigs" and "Seeking sociable swine? Incorporating social genetic effects into pig breeding programs to achieve balanced improvement in productivity and welfare". Since September 2014, Naomi has a position as research manager at Topigs Norsvin, Beuningen, the Netherlands.

### **Over de auteur**

Naomi Duijvesteijn werd geboren op 28 maart 1984 te Boxmeer en groeide op in Standdaarbuiten. In 2002 is ze geslaagd voor haar VWO diploma aan 'de Markenlanden' te Oudenbosch en is zij begonnen aan haar bachelor Dierwetenschappen aan Wageningen Universiteit. Voor haar bachelor thesis heeft ze een review geschreven en data analyse uitgevoerd over kannibalisme en verenpikken bij leghennen en in 2005 is zij geslaagd voor haar bachelor. Ze is doorgegaan met een master studie met de specialisatie fokkerij en genetica. In 2009 is ze vijf maanden naar Uppsala, Zweden geweest met een Erasmus Mundus uitwisselingsprogramma om moleculaire genetica te studeren aan de SLU (Swedish University of Agricultural Sciences). In 2008 heeft ze zes maanden stage gelopen bij LIC (een melkveefokkerij-organisatie) in Nieuw Zeeland en heeft ze een QTL analyse uitgevoerd voor 1-keerdaags melken. Voor haar major thesis heeft ze een simulatie studie uitgevoerd voor de optimalisatie van varkensfokprogramma's voor kenmerken waar sociale interacties een rol spelen. In januari 2008 heeft ze haar master studie afgerond en is zij begonnen als onderzoeker bij TOPIGS Research Center IPG waar zij verschillende onderzoeken heeft verricht voor TOPIGS (een Nederlandse varkensfokkerij-organisatie). In maart 2010 heeft ze haar werk bij TOPIGS Research Center IPG gecombineerd met een PhD bij de vakgroep Animal Breeding and Genomics Centre van Wageningen Universiteit. Hier heeft zij onderzoek gedaan naar de mogelijkheden van het opnemen van indirecte genetische effecten in varkensfokprogramma's, waarvan de resultaten zijn beschreven in deze thesis. Dit onderzoek is onderdeel van twee grotere onderzoeksprogramma's: "Genetics of social interactions in livestock: Improving health, welfare, and productivity in laying hens and pigs" en "Seeking sociable swine? Incorporating social genetic effects into pig breeding programs to achieve balanced improvement in productivity and welfare". Sinds September 2014 is Naomi werkzaam als research manager bij Topigs Norsvin, Beuningen.

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## **Training and Supervision plan**



**Training and supervision plan**



**Basic package (2 ECTS)**

WIAS introduction course	2010
Ethics and philosophy of animal science	2010

**Scientific exposure (12 ECTS)**

*International conferences*

WCGALP, Leipzig, 1-8 August	2010
EAAP, Stavanger, August 29- September 1	2011
ICQG, Edinburgh, 17-22 June	2012

*Seminars and workshops*

QTLMAS workshop, Poznan, Poland	2010
Seminar 'Friends or Fiends? Consequences of social interactions for artificial breeding programs and evolution in natural populations', Wageningen	2009
Seminar 'Developments in genome-wide evaluation and genomic selection', Wageningen	2009
F&G connection days, Vught	2010
WIAS Science day, Wageningen	2012
WIAS Science day, Wageningen	2013
Seminar 'Genetics of social life: Agriculture meets evolutionary biology', Wageningen	2013

*Presentations*

Optimizing breeding programs including social interactions in pigs, EAAP, oral	2008
Parental Identification in pigs using SNPs, WCGALP, oral	2010
Direct and associative effects for androstenone in entire boars, EAAP, oral	2011

## Training and supervision plan

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A search for indirect genetic effects and genomic regions for boar taint, ICQG, poster 2012

Androstenone-levels in entire male pigs: a GWAS for direct and indirect genetic effects , EAAP, poster 2013

### **In-depth studies (13 ECTS)**

#### *Disciplinary and interdisciplinary courses*

Quantitative genetics, within focus on selection theory 2010

Introduction into ASREML 2010

Genomic Selection in Livestock 2011

Quantitative genetics with integration of genomic selection 2011

Education program NWO "waardering van dierwelzijn" 2010-2013

#### *PhD students' discussion groups*

Quantitative genetics discussion group 2010-2014

### **Professional Skills Support Courses (4 ECTS)**

Our future leaders program (STW) 2012

Techniques for writing and presenting a scientific paper 2012

Project management based on Prince2 and IPMA 2012

### **Didactic Skills Training (5 ECTS)**

#### *Supervising theses*

Supervising MSc minor 2011

Supervising MSc major (second supervisor) 2012

Supervising MSc major 2013

### **Education and training total: 35 ECTS**

## **Dankwoord**



‘Weet ik niet’ zijn de woorden die ik het meeste heb gebruikt toen ik een kleine Naomi was. Op de vragen: ‘wat wil je worden?’ of ‘wat wil je doen’ kwam standaard het antwoord: ‘weet ik niet’. In 2002 heb ik toch gekozen om dierwetenschappen te gaan studeren in Wageningen en dat bleek een goede keuze. Ik wist het! Na een prachtige studententijd van 5,5 jaar met een onderzoekje hier en een biertje daar, ga je toch weer nadenken: wat wil ik hierna; wel of geen PhD? Al snel kwam ik erachter dat ik het wel wilde, maar het liefst vanuit een bedrijf en al snel kwam die mogelijkheid bij TOPIGS Research Center IPG. Uiteindelijk zijn twee gesubsidieerde projecten (NWO en STW) gehonoreerd en kon er begonnen worden aan de uitdaging. Parttime PhD en parttime onderzoeker bij TOPIGS Research Center voelde soms als jongleren, maar na een jaar was iedereen op de hoogte van de weekindeling en verliep het toch vrij soepel met het beoogde resultaat. Dit was niet alleen mijn verdienste, zonder de hulp van een aantal mensen was dit niet gelukt en die wil ik graag even bedanken.

Ik herinner me nog de eerste keer dat we een afspraak hadden in het ‘oude oude’ Zodiac waar jij (Egbert Knol) mij het principe van de varkensfokkerij hebt uitgelegd. Het was even wennen aangezien je graag in metaforen spreekt, maar de hoeveelheid kennis die jij bezit verbaast me nog steeds elke dag! Soms was het (helaas) noodzakelijk om de voortgang in het onderzoek te bespreken tijdens een vrijdagmiddaglunch. Deze hebben zeker geholpen om weer prioriteiten kunnen te stellen en statistische problemen op te lossen. We blijven voorlopig ook samenwerken, misschien nog wel meer dan eerst, en dat gaat helemaal goed komen!

Niet alleen was je (Piter Bijma) begeleider tijdens mijn PhD thesis, maar ook van mijn bachelor thesis en master thesis! De vraag is of je nu echt van me af bent :-p. Zo’n traject kan je alleen maar samen doorlopen als het goed zit. Je kritische blik op alle analyses die gedaan zijn, hebben zeker gezorgd voor het beter doorgronden van de data en meer kennis over genetica. Structuur in het hoofd en structuur op papier zijn twee hele verschillende dingen, maar samen zijn we er altijd uitgekomen. Hopelijk gaan we nog jaren door met onze samenwerking!

In januari 2008 reikte je (Johan van Arendonk) mijn MSc. titel uit in de aula met de woorden: ‘Als het goed is zie ik je weer terug op deze plek in een jaartje of vier’. Alhoewel het wat langer heeft geduurd, is het toch gelukt. Bedankt dat je mij daar altijd in hebt gesteund. Je hebt duidelijke doelen gesteld en me altijd gestimuleerd om ook ‘andere’ wetenschap te bedrijven zoals het QBA hoofdstuk in deze thesis. Daarnaast kan je ook aardig fietsen net zoals Piter trouwens ;-).

Mogelijkheden zijn er altijd, maar er in geloven en er ruimte aan geven is een tweede en dat hebben Jan Merks en Hans Olijslagers wel gedaan. Ik ben jullie als

werkgever (TOPIGS Research Center IPG) erg dankbaar voor de ruimte die is gegeven.

The good thing of having two offices is that you have double the colleagues. A room @ TOPIGS Research Center with Jascha and Marcos and later Claudia. Great discussions on holiday destinations, parties, life in general and some work related discussions as well. Marcos, een echte vriend voor het leven, de vraag is wel wanneer ik mijn Portugees kan oefenen in Brazilië... And for some relaxation we (Marcos, Claudia, Jascha, Bruno, Sharonne, Gerda, Roos, Annemieke, Juanma and Susan) could release some energy at the soccer table during coffee breaks. All the colleagues @ TOPIGS Research Center a great thanks for the nice working environment. Extra thanks to Pramod, who helped me out with my writing the last months before handing in my thesis.

At the 'old-old' Zodiac, the 'old' Zodiac and the 'new' ABGC we were never separated: the social girls. Ewa and Katrijn, you girls made the PhD period a great pleasure. Secret coffee breaks (with home-made cakes), but also discussions on complex formulas (Katrijn) and (the) use of English (Ewa) really made a difference. Even though the working environment changed, the social girls will always remain. Big thanks for all the diners, drinks etc. we had together among PhD students (Andre, Big G, Sandrine, Gus, Hamed, Ilse, and all the others I forgot to mention). All colleagues @ ABGC also a big thanks for the enjoyable conversation during tea/coffee breaks. Also a great thank for the secretary from ABGC, I would be so lost without you!

Then, when you think there is no more room for more colleagues, there were the sociable swine girls. Irene, Inonge and Marianne, we managed to all defend our theses with great results and even with a publication with just the four of us. Who would have thought in the beginning when we were struggling... Also we followed the courses of the research program: 'The value of animal welfare' together which increased our collaboration (and with the other research programs), but was it because of the content or because of the evenings? I also really appreciated the collaboration with the Adaptation Physiology group with special thanks to Nanda, Liesbeth and Fleur.

En dan had ik ook nog tijd voor wat sociale activiteiten naast werk. Cqqa zal altijd blijven tot aan het bejaardentehuis, daar ben ik van overtuigd. Gezellige weekendjes, (winterport) vakanties, concerten, avondjes met een zakje bugles op de bank, jullie hebben altijd een luisterend oor en uiteraard is er altijd genoeg plek voor flauwe grappen ;-). In 1 woord: BANAAAN.

Van het veetelersjaar 2002 hebben 'mensen' zich wel eens afgevraagd of dat wel wat zou worden (verhouding feestjes-studieprestatie was soms wat ver te zoeken).

Samen hebben we in ieder geval heel veel lol samen met het jaarlijkse weekendje weg (sorry dat ik het dit jaar moest missen), 5 mei en Koninginnedag (Koningsdag).

En uiteindelijk zijn we allemaal goed op onze plek terecht gekomen lijkt me zo!

Een bijzonder plekje heb ik voor jou Nonja. Vriendschap was er vanaf dag 1 en die zal nooit overgaan. Altijd sta je voor me klaar, maken we tijd voor elkaar en kunnen het over alles hebben, dat is toch het belangrijkste in een vriendschap.

Alhoewel ze als laatste worden opgenoemd is familie het belangrijkste van alles. Nu is dit schrijf denk ik terug aan mijn afstudeerfeest in 2008, het laatste grote feest waar mijn vader nog bij was. Goh, wat zou je trots geweest zijn als je hier was geweest, daarom is dit boekje voor jou. Mam ik ben echt trots op je dat je je leven zo goed hebt opgepakt en ik vind het echt mooi om je weer gelukkig te zien. Daarnaast ben ik jullie ook dankbaar voor alle steun (in welke vorm dan ook) tijdens mijn studie, daarvoor en daarna. Maik, mijn broer, sinds ik het huis uit ben zijn we minder gaan vechten en meer gaan delen. We zijn uit hetzelfde hout gesneden, dat is duidelijk! Oma, wat ben ik blij dat u er bent en zo geniet van het leven, die 100 gaat u gewoon redden, want wie weet komen er nog meer kleine kleinkinderen. Isolde, Jan, Miep, Judith, Menke, Anne-Jan en Karin; familie uitjes zijn van groot belang voor ontspanning! Bedankt voor de leuke gesprekken en ik kijk uit naar what's yet to come.

Loren, moppie, hopelijk lees je dit een keer als je groter bent. Jij bent echt een zonnetje, zo vrolijk en zo leuk! Wel lief blijven voor papa en mama he ;-).

Leendert, mopie, wij hebben al aardig wat jaartjes er samen op zitten. Ik geniet van je humor, kennis, rariteiten en liefde. Daarnaast delen we dezelfde passie voor sport: Alpe d'Huzes of fietsen naar de olympische spelen zijn activiteiten waar wij blij van worden (andere vaak niet). Samen kunnen we veel maken (Loren bijvoorbeeld), delen (M&Ms) en vooral samen genieten. Daar gaan we nog vele jaren voor!

**Naomi, Nemo, #5**



## **Colophon**

### **Colophon**

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