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Genomic indicators for boar taint and fertility in Landrace and Large White populations

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“It always seems impossible until it’s done.”

Nelson Mandela

Für meine Familie und Torsten

Abstract

Castration of male piglets in their first week of life is a commonly used but critically reflected method to avoid boar taint. Fattening of entire males represents a long-term sustainable alternative to castration but physiological interrelations to steroid hormone synthesis have emerged as a possible conflict if breeding against boar taint. Therefore, the aim of this study was to reveal the genetic relationships (r_g) between boar taint and fertility in Landrace (LR) and Large White (LW) populations, as they represent the two most popular German nucleus dam breeds. As a first step, variance components of boar taint compounds androstenone (AND) and skatole (SKA) and male / female reproduction traits as well as their genetic relationships were estimated. Datasets of 2,729 LR and 2,908 LW animals from a commercial breeding organization were used as a basis of this analysis. Results showed moderate to high heritabilities (h^2) for AND (0.50 in LR, 0.39 in in LW) and SKA (0.52 in LR, 0.32 in LW). Genetic correlations showed an inconsistent picture of adverse effects on fertility among both breeds. The r_g between both boar taint compounds and number of piglets born alive (NBA) were unfavorable in LR (r_g between 0.18 and 0.38) and favorable in LW (r_g between -0.15 and -0.25). Genome wide association analysis (GWAS) were performed to identify genes and genomic regions with possible pleiotropic effects. Results confirmed a previously reported region for SKA metabolism at 141.6 Mb on *Sus scrofa* chromosome (SSC) 14 in both breeds.

In a following step, endocrine fertility parameters (EFP) were investigated in 500 young boars and 500 female pigs. In addition to data for boar taint from the first analysis, boar taint phenotypes from 969 boars from herd book organizations were included. Variance component estimation (VCE) revealed unfavorable r_g between AND and testosterone (TEST) / estradiol (EST) in both breeds (r_g = 0.62-0.83 for TEST, r_g = 0.46-0.49 for EST). GWAS was performed to genetically characterize the analyzed traits. GWAS for SKA in the herd book population confirmed the region on SSC 14 from the preceding analyses. A region with possible pleiotropic effects between several EFP was identified on SSC 7 between 113.1-117.9 Mb.

In general, the moderate to high h^2 of AND and SKA confirmed the genetic foundation of boar taint traits and their potential for selection. However, r_g also confirmed the physiological assumptions of a common genetic background of boar taint and reproduction / EFP. GWAS verified a previous identified candidate gene for SKA in both breeds on SSC 14 and revealed a region on SSC 7 with possible pleiotropic effects between several EFP. First results for different genomic selection (GS) scenarios showed, that a selection formula should be developed separated by breed to reach sufficient accuracies. As a consequence, implementation of boar taint in selection strategies is only advisable if intensive monitoring of fertility is taking place. Although there are no indicators for an overall negative impairment of reproduction traits, genetic relationships to EFP emphasized an unfavorable reduction of hormone concentrations of breeding against boar taint.

Zusammenfassung

Die Kastration männlicher Ferkel in ihrer ersten Lebenswoche zur Vermeidung von Ebergeruch wird hinsichtlich des Tierwohlaspektes schon lange kritisch betrachtet. Die Ebermast stellt eine langfristige und nachhaltige Alternative zur Kastration dar. Physiologische Zusammenhänge zur Steroidhormonsynthese weisen jedoch auf ein mögliches Konfliktpotential bei einer Selektion gegen Ebergeruch hin. Das Ziel dieser Arbeit war es deshalb, die genetischen Beziehungen zwischen Ebergeruch und Fruchtbarkeit in den zwei populärsten deutschen Mutterlinien Landrasse (LR) und Edelschwein (ES) zu evaluieren. Zunächst wurden Varianzkomponenten von den Ebergeruchsmerkmalen Androstenon (AND) und Skatol (SKA), sowie von Reproduktionsmerkmalen beider Geschlechter geschätzt und deren genetische Beziehung (r_g) zueinander berechnet. Grundlage dieser Analysen waren Datensätze einer deutschen Schweinezuchtorganisation mit 2.729 LR- und 2.908 ES-Tieren. Die Ergebnisse zeigten moderate bis hohe Heritabilitäten (h^2) für AND (0,50 in LR; 0,39 in LW) und SKA (0,52 in LR; 0,32 in LW). Die geschätzten r_g weisen auf Rasseunterschiede bezüglich unerwünschter Beziehungen zwischen Ebergeruchskomponenten und Reproduktions-merkmalen hin. Die r_g zwischen beiden Ebergeruchskomponenten und der Anzahl lebend geborener Ferkel (LGF) waren unerwünscht in der LR (r_g zwischen 0,18 und 0,38) und erwünscht im ES (r_g zwischen -0,15 und -0.25). Eine genomweite Assoziationsstudie (GWAS) wurde durchgeführt, um Gene oder Regionen mit möglichen pleiotropen Effekten zu identifizieren. Die Ergebnisse bestätigten eine zuvor identifizierte Region für SKA Stoffwechsel auf dem *Sus scrofa* Chromosom (SSC) 14 bei 141.6 Mb in beiden Rassen.

In einem nächsten Schritt wurden endokrine Fruchtbarkeitsparameter (EFP) in 500 Jungebern und 500 weiblichen Schweinen untersucht. Der Datensatz für Ebergeruch erweiterte sich im Vergleich zur vorherigen Studie um phänotypische und genetische Daten von 969 Ebern aus verschiedenen Herdbuchorganisationen. Eine Varianzkomponentenanalyse (VCE) ergab unerwünschte r_g zwischen AND und Testosteron (TEST) sowie Estradiol (EST) in beiden Rassen (r_g = 0,62-0,83 für TEST, r_g = 0,46-0,49 für EST). Eine erneute GWAS bestätigte die zuvor identifizierte Region für SKA auch in der Herdbuchpopulation und identifizierte auf dem SSC 7 zwischen 113.1 und 117.9 Mb eine Region mit möglichen pleiotropen Effekten zwischen mehreren EFP.

Die moderaten bis hohen rassespezifischen h^2 für AND und SKA weisen auf eine genetische Fundierung der Ebergeruchsmerkmale und deren Selektionspotential hin. Die Ergebnisse der genetischen Analysen bestätigen die physiologischen Annahmen eines Zusammenhangs zwischen Ebergeruch und Fruchtbarkeit. Die GWAS bestätigte ein bereits zuvor identifiziertes Kandidatengen für SKA auf SSC 14 in beiden untersuchten Rassen und deckte eine Region auf SSC 7 mit potenziellem pleiotropen Effekt zwischen mehreren der untersuchten EFP auf. Erste Ergebnisse von Szenarien der genomischen Selektion (GS) zeigten, dass diese nach Rasse getrennt durchgeführt werden sollte um eine zufriedenstellende Genauigkeit zu erreichen. Die Implementierung von Ebergeruch in Zuchtprogramme sollte nur unter Einhaltung eines intensiven Monitorings der Fruchtbarkeitsmerkmale stattfinden, denn obwohl es keine klaren Indikatoren für eine negative Beeinträchtigung der Reproduktionsmerkmale gibt, wiesen die genetischen Korrelationen zwischen Ebergeruch und endokrinologischen Parametern auf eine unerwünschte Reduktion der Hormonkonzentrationen hin.

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List of abbreviations

3’PUTR	3‘ prime untranslated region variant
5’PUTR	3´5‘ prime untranslated region variant
ACTH	Adrenocorticotropic hormone
ADG	Average daily gain
AND	Androstenone (5α-androst-16-en-3-one)
AI	Artificial insemination
AFI	Age at first insemination
BHZP	Bundeshybridzuchtprogramm
BMEL	Federal Ministry of Food and Agriculture
bp	Base pairs
CO_2	Carbon dioxide
CON	Sperm concentration
CORT	Cortisol
CRH	Corticotropin-releasing hormone
CV	Variation coefficient
DEG	Degradation
DGV	Downstream gene variant
DHEA	Dehydroepiandrostenone
DU	Duroc
EFP	Endocrine fertility parameter
e.g.	Exampli gratia / for example
EPW	Epididymal weight
ER	Erhuelian

ES	Edelschwein
EST	Estradiol (17β-Estradiol)
F_2	Second filial generation
FCR	Feed conversion rate
FF	Female fertility
FI	Farrowing interval
FSH	Follicle-stimulating Hormone
GC	Genomic control
G-I-FER	Genomic indicators for boar taint, fertility and robustness in Landrace and Large White populations
GnRH	Gonadotropin-releasing hormone
GRAMMAR	Genomewide rapid association using mixed model and regression
GS	Genomic Selection
GSI	Gonadosomatic index
GWAS	Genome-wide association analysis
h^2	Heritability
HA	Hampshire
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadal
HYS	Herd-year-season-effect
I	Intron variant
IB	Iberian
IG	Intergenic variant
LEI	Leicoma

LH	Luteinizing hormone
log_AND	Log-transformed concentration of androstenone
log_CORT	Log -transformed concentration of cortisol
log_EST	Log -transformed concentration of estradiol
log_FSH	Log -transformed concentration of follicle-stimulating hormone
log_LH	Log -transformed concentration of luteinizing hormone
log_SKA	Log -transformed concentration of skatole
log_TEST	Log -transformed concentration of testosterone
LR	Landrace
LW	Large White
MAF	Minor allele frequency
Mb	Mega base pairs = 1,000,000 base pairs (bp)
ME	Meishan
MET	Metabolism
MF	Male fertility
NBA	Number of piglets born alive
NBD	Number of piglets born dead
NCTEV	Non coding transcript exon variant
n.m.	Not mapped
NSC	Number of sperm cells
NWP	Number of piglets after weaning
O_2	Oxygen
OD	Optical density
OH-group	Hydroxyl group

OMIA	Online Mendelian Inheritance in Animals
PI	Piétrain
PMO	Progressive motility
PROG	Progesterone
QTL	Quantitative trait locus / loci
r	Correlation
r_g	Genetic correlation
r_p	Phenotypic correlation
SAR	Sperm abnormality rate
SC	Sperm count in billions
SD	Sperm density
SE	Standard error
SIDA-HSPM-GC/MS	Stable isotope dilution analysis-headspace solid-phase microextraction-gas chromatography/mass spectrometry
SKA	Skatole
SMO	Sperm motility
SNP	Single nucleotide polymorphism
SP	Sperm density measured by photometer
SRV	Splice region variant
SSC	*Sus scrofa* chromosome
STD	Seminiferous tubule diameter
SV	Sperm volume
SY	Synthesis
SYN	Synonymous variant
TEST	Testosterone

TMA	Total morphological abnormalities
TNB	Total number of piglets born
TRH	Thyrotropin-releasing hormone
UGV	Upstream gene variant
UTR	Untranslated region
VCE	Variance component estimation
YS	Yorkshire

Chapter 1. General introduction

1.1. The relevance of boar taint in pig breeding

Meat quality, reproduction and robustness are three essential elements of modern pig production systems for mainly economic reasons. As consumers' awareness of animal welfare and health increased in recent years, the demand of transparency for the industry is increasing, too (Bonneau and Weiler 2019). Meat production should proceed under conditions that are beneficial for all participants, including pig producer, consumer and at least the pig itself. Breeding goals have changed in the past decades from pure focus on economic production to sustainable and environmentally friendly production with a special emphasis on animal welfare (Millet et al. 2018). Nevertheless, animal welfare-related problems like boar taint have also been addressed, but no substantial change in breeding programs or selection strategies has taken place until now as no economical motivation exists.

Boar taint is the odorous and urine-like smell of pork meat from entire males that occurs, when the meat is heated. As a routine to prevent this, young male piglets were castrated within their first week of life without anesthesia or analgesia in the past. This posed a problem regarding animal welfare as a study showed that e.g. pre-weaning mortality is higher in groups of surgically castrated males compared to intact males (Morales et al. 2017). Nevertheless, in 2017, 80 % of the piglets in Germany has been castrated surgically (Backus et al. 2018). Due to a change of the German animal protection act in 2013 (Deutscher Bundestag 7/4/2013), castration without anesthesia will no longer be allowed from 2019. This legal term has been extended for two more years until 01.01.2021 as the currently available alternatives do not fulfill practical requirements (Deutscher Bundestag 11/6/2018).

One alternative is the fattening of young boars, as it is already practiced in other countries like Great Britain or Spain (Backus et al. 2018). Besides the intactness of the animal, this alternative entails additional benefits like a better commercial performance of boars resulting from more efficient feed conversion, higher lean meat content, higher meat percentage of the carcass (Bonneau and Desmoulin 1982; Walstra et al. 1999; Lundström et al. 2009) and a smaller environmental footprint (Dugué et al. 2020). However, this alternative needs to meet some requirements in the management as boars have to be kept separate to sows and the number of animals per pen has to be reduced to avoid aggressions and ranking fights (Giersing et al. 2000). Additionally, fatty acid composition of boars differs from gilts and castrates as it contains a higher percentage of polyunsaturated fatty acids (Pauly et al. 2009; Grela et al. 2013), which excludes meat from boars for the production of special convenience products like e.g. dry-cured products (Bonneau and Weiler 2019).

The main compounds responsible for boar taint, named androstenone (5α-androst-16-en-3-one) and skatole (3-Methylindole) were already identified during the late 20th century (Patterson 1968; Vold 1970). Moderate to high heritabilities in ranges of 0.25 to 0.88 for androstenone and 0.19 to 0.54 for skatole were early reviewed by Robic et al. (2008) and confirmed by more recent studies (Grindflek et al. 2011b; Strathe et al. 2013a; Baes et al. 2013; Mathur et al. 2013; Rowe et al. 2014; Parois et al. 2015). These heritabilities (h^2) clearly indicate a genetic basis of both compounds. Moreover, there is evidence of a linked hepatic metabolism via the gene CYP2E1, underlined by findings of Doran et al. (2002), Tambyrajah et al. (2004) and Zadinová et al. (2017). Its involvement in skatole metabolism in the liver by being inhibited by androstenone makes it an appealing candidate of interest.

However, breeding against boar taint is challenging, as the underlying phenotype only appears with the beginning of the puberty and is only quantifiable in male pigs after slaughter. Nevertheless, both traits are associated with candidate genes and quantitative trait loci (QTL) across the genome, including possible pleiotropic effects. Despite of the high h^2 and the underlying genetic foundation, only a few breeding organizations included boar taint in their selection strategy for sire lines, but not in dam lines, where fertility traits are more pronounced in the breeding objective. The reason for the this is the concatenated synthesis of androstenone along with androgens and estrogens, which are key hormones for fertility traits (Bonneau 1982). The impact of this commonality in the synthesis on reproduction is discussed controversially. Some studies reported negative relationships of boar taint compounds and maternal / paternal reproduction traits (Tajet et al. 2006; Mathur et al. 2013) or the physiologically linked testosterone (Grindflek et al. 2011b). In contrast to that, a study of Strathe et al. (2013a) showed favorable relationships between boar taint and semen traits. Thus, breeders are caught between the genetic improvement of the boar taint problem by selecting against androstenone and the likely undesirable consequences on reproduction traits.

1.2. Aim of this thesis

The aim of this thesis is to analyze and discuss the interactions between the boar taint compounds androstenone and skatole and the trait complexes fertility and reproduction with a particular emphasis on the genomic background of these traits and their genetic relationships. Against this background, QTL with possible pleiotropic effects should be identified to assess the opportunity of a permanent integration of boar taint in selection strategies without constraining the above-named trait complexes. A general overview of the structure of this thesis is displayed in figure 1.

Chapter 2 introduces the biological background of the boar taint compounds androstenone and skatole as well as their genetic background and possible impacts. Additionally, the physiological and genetic background of the underlying reproduction traits in male and female and robustness is introduced as well as their commonalities in synthesis and regulation regarding boar taint.

Chapter 3 discusses the genetic basis of the boar taint compounds androstenone and skatole and their genetic relationship to maternal and paternal reproduction in commercial Landrace and Large White breeds. Furthermore, genomic background of these traits is disclosed to evaluate possible future selection perspectives against boar taint without constraining reproduction traits.

In chapter 4, endocrine fertility parameters are assessed regarding their interaction with boar taint compounds by estimating variance components using hormone profiles of full-sib pairs from commercial and herd book organizations. Genome wide association analysis is performed to detect regions with effects, or regions with possible pleiotropic effects.

The final chapter, chapter 5, includes a general discussion of the impact of breeding against boar taint on fertility traits and endocrine parameters. The potential effect of changing hormone profiles on reproduction traits is briefly outlined. This is followed by an evaluation of the eligibility and extent of including boar taint into breeding programs of commercial pig breeding organizations without adversely affecting previously achieved progress in fertility traits.

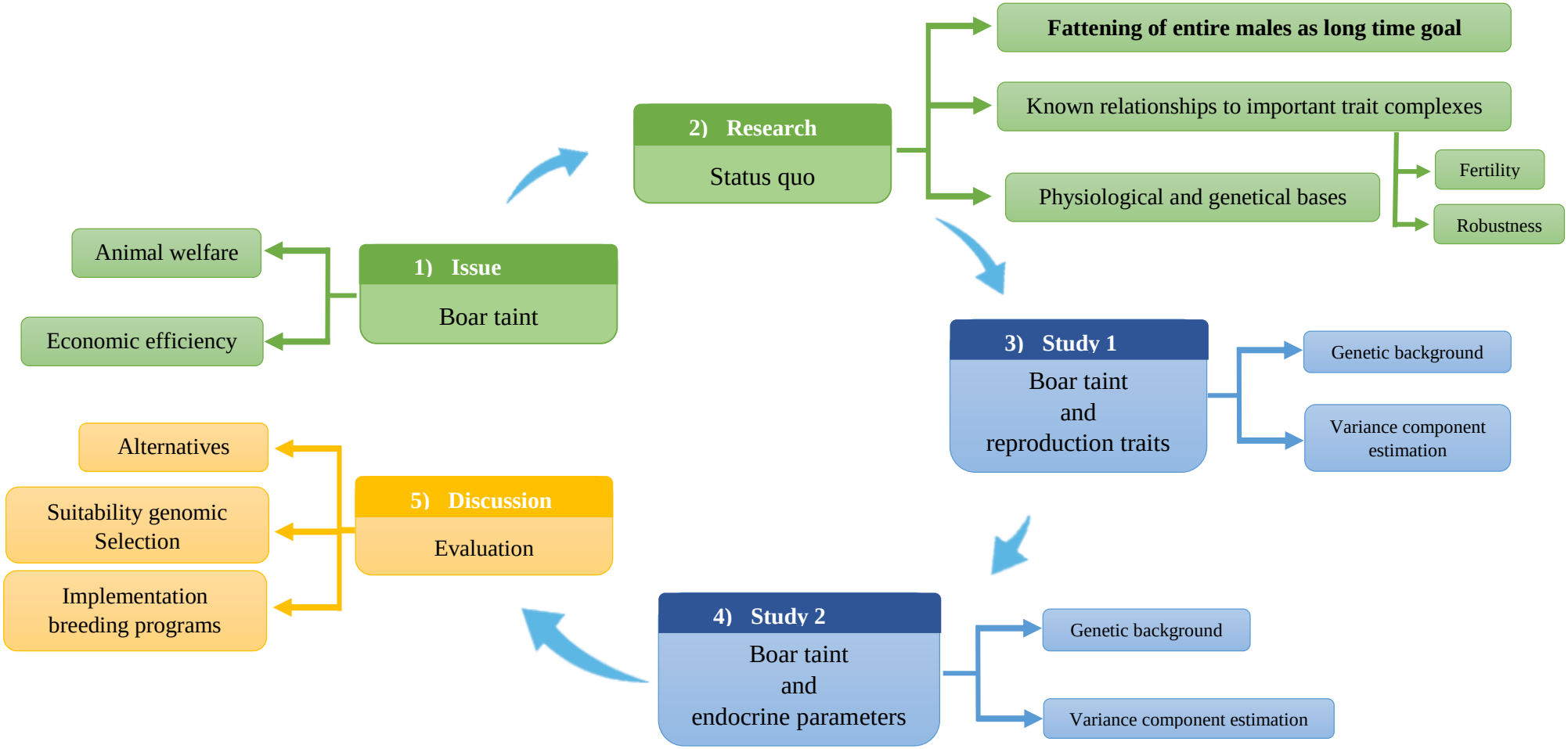

Figure 1: Workflow of this thesis.

Chapter 2. Literature review

2.1. Boar taint

Boar taint is the odorous smell of heated meat from young uncastrated boars and the reason for surgical castration of young male piglets in their first week of life. It is mainly influenced by the compounds androstenone, skatole and indole and occurs with the beginning of puberty by sexual maturation. As androstenone and skatole are considered as the main compounds, indole will not be described in the following chapters as it is highly correlated to skatole and follows the same synthesis and metabolism pathways.

2.1.1. Androstenone

Androstenone (5α-androst-16-en-3-one, AND) is a pheromone which was the first compound identified to be responsible for boar taint (Patterson 1968). It belongs to the group of androgens and is synthesized in the Leydig cells of the boars' testis. Nevertheless, small concentrations of AND measured in gilts and castrates indicate possible synthesis in ovary and adrenal cortex (Claus et al. 1971). The main synthesis of AND is part of the synthesis of sex steroid hormones and is therefore initiated at the beginning of puberty as this remarks the start of sexual maturation (Gower 1972; Bonneau 1982). Precursor of all hormones formed in this pathway is cholesterol, which is transformed in a first step to pregnolone by the enzyme CYP11A1. From there on, possible pathways over 5,16 Androstadien-3βol or progesterone and 4,16 Androstadien-3one lead to the formation of AND (figure 2).

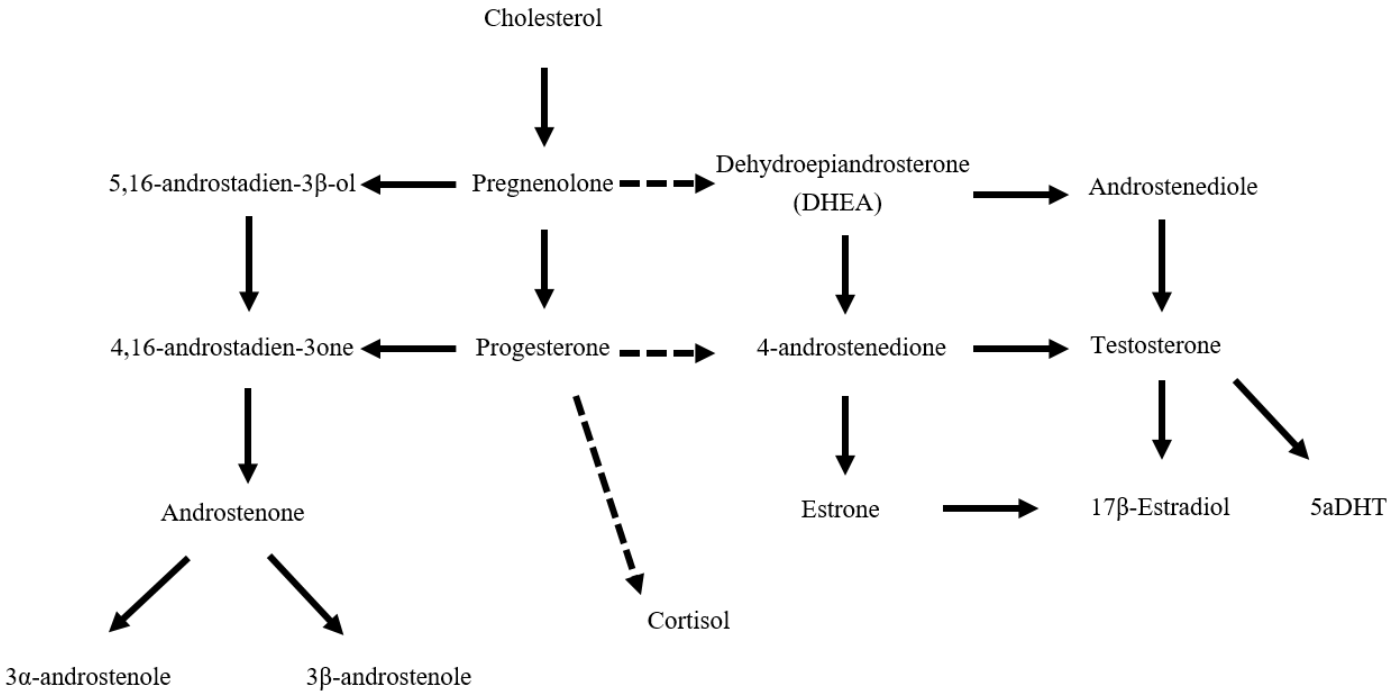

Figure 2: Pathway of AND synthesis. Modified according to Weiler and Wesoly (2012). Solid arrows: single reaction steps, broken arrows: multiple reaction steps.

The enzymes involved in the AND synthesis pathway (figure 2) are regulated by neuroendocrine mechanisms. These mechanisms are also regulating the synthesis of other testicular steroids and are stimulated by the luteinizing hormone (LH) or are at least LH dependent (Weiler and Wesoly 2012). Cholesterol side-chain cleavage enzyme (CYP11A1) (Quintanilla et al. 2003) and cytochrome b5 (CYB5A) (Davis and Squires 1999) are two enzymes known to be involved in AND synthesis. High levels of expression in CYB5A therefore lead to higher synthesis in the testis, which makes it a target gene when trying to reduce AND synthesis in boars (Robic et al. 2008). Produced AND is released into the blood by the spermatic vein and circulating AND in the blood can be processed in three different ways (figure 3).

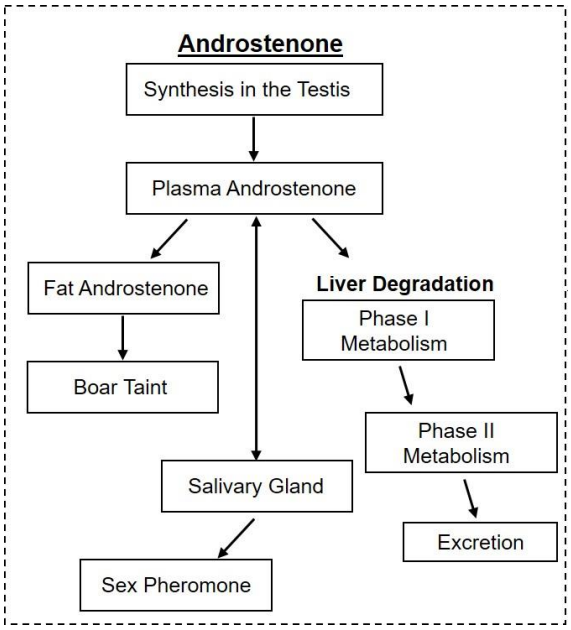

Figure 3: Summary of the metabolic pathways of androstenone. Modified according to Zamaratskaia and Squires (2009).

One way is the release of AND via the saliva as a pheromone by the volatile alcohols 3α-androstenole and 3β-androstenole. This transformation happens by replacing the keto group of AND with an OH-group in the so-called oxidative phase (Phase I Metabolism in figure 3). Phase I metabolism is controlled by the enzyme 3β-Hydroxysteroid dehydrogenase (HSD3B) (Robic et al. 2008). Together with AND the alcohols form the group of Δ16-steroids (Weiler and Wesoly 2012). The salivary glands of a boar have the highest concentration of these Δ16-steroids compared to other tissues or blood level (Babol et al. 1996). The release of these pheromones by the boar is an important part of social interaction regarding ranking and sexual behavior of animal individuality as every animal has its own composition of these Δ16-steroids (Giersing et al. 2000). In sows, these pheromones are stimulating earlier puberty and causing

the standing reflex (Claus and Hoffmann 1980). Another metabolism pathway of AND is the degradation by the liver in a two-phase metabolism. As described before, metabolism starts with the oxidative phase followed by the conjugation (Phase II Metabolism in figure 3). This phase allows the attachment of hydrophilic molecules like glucoronic acid to create water soluble compounds that are more hydrophilic to avoid diffusion into the fat (Weiler and Wesoly 2012). The resulting glucuronides are excreted by the kidneys through urine. The proportion of AND that is not conjugated as described before, remains lipophilic and diffuses into the fat tissue, where it accumulates.

The amount of produced AND in the testis is depending on the age of the individual animal and its stage of maturity (Bonneau 1982; Babol et al. 2004), whereas the amount of accumulation in the fat is primarily influenced by the production rate of AND in the testis, salivary gland storage, productivity of liver metabolism and excretion with urine (Babol et al. 1999; Robic et al. 2008). Concentrations of AND in fat and circulating AND in plasma are highly correlated (correlation (r) = 0.58) (Whittington et al. 2004).

The individual potential of a boar to produce and accumulate AND is mainly determined by genetics. Xue et al. (1996) showed breed differences between tissue concentrations of AND in salivary glands and fat tissue in Duroc and Hampshire breeds compared to Yorkshire and Landrace. The beginning of puberty is an important trigger for the beginning of AND production and testicular synthesis. It is affected by individual weight and growth rate (Anderson 2009) and shows genetic differences as some breeds are earlier maturating (e.g. Meishan) than others (Ding et al. 2016).

Social environment factors like the hierarchy in the pen are discussed to have an effect on the production of AND (Giersing et al. 2000). Entire males kept in changing groups showed higher AND concentrations than animals in social isolation as well as in socially stable groups. (Rydhmer et al. 2006; Fredriksen et al. 2008). Furthermore, entire males kept in stable groups showed less mounting behavior and skin lesions at slaughterhouse (Rydhmer et al. 2013). Age and weight at slaughter as well as season of slaughtering seems to have an impact on the boar taint exposure of the carcass (Babol et al. 2004; Fredriksen et al. 2006; Fàbrega et al. 2011; Frieden et al. 2014; Thomsen et al. 2015b).

2.1.2. Skatole

Skatole (3-Methylindole, SKA) is the second of the two major compounds responsible for boar taint. Unlike AND, SKA is not a hormone, but results from the degradation of the amino acid L-tryptophan by microbial activity in the large intestine of the pig (Yokoyama and Carlson 1979). Therefore, it is also measurable in similar amounts in female pigs and castrates (Weiler et al. 2000; Wesoly and Weiler 2012). In contrast to gilts and castrates, boars accumulate more SKA in backfat due to the presence of sex steroids. Additionally, AND inhibits the degradation of SKA in the liver (Weiler and Bonneau 2019).

In the formation process, L-tryptophan, which mainly originates from the turnover of gut mucosa cells (Lanthier et al. 2006), is degraded in two steps: I) transformation to 3-indolacetic acid by *Escherichia coli* and *Clostridium* spp. and II) conversion to SKA by *Clostridium* and *Lactobacillus* (Jensen et al. 1995). SKA production is limited by the amount of available tryptophan and influenced by composition of the microbiome in the gut (Claus et al. 1994).

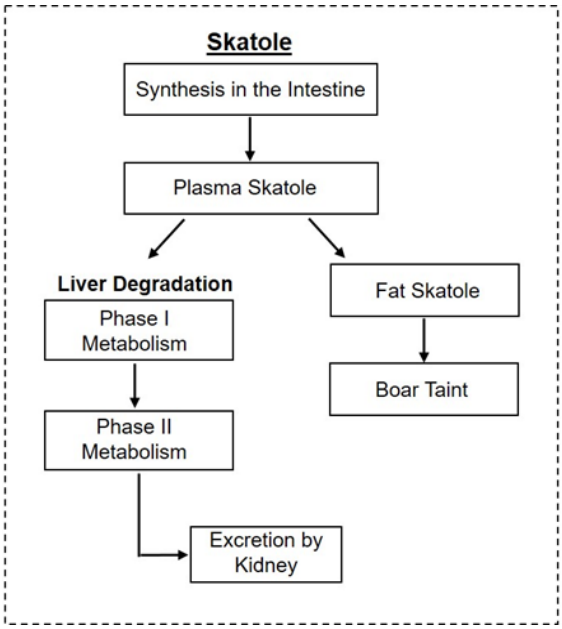

Figure 4: Summary of the metabolic pathways of SKA. Modified according to Zamaratskaia and Squires (2009).

From the large intestine, SKA diffuses into the portal vein (*V. porta*), from where it is transported to the liver. Here, a major part of SKA is already metabolized and excreted via urine or faeces (Zamaratskaia and Squires 2009). Steps I and II of metabolization (Phase I: oxidation; Phase II: conjugation) are similar to the AND metabolism (figure 4), even though the enzymes involved differ. Enzymes with a confirmed role in SKA metabolism include cytochrome P450 2E1 (CYP2E1) for phase I and sulfotransferase 1A (SULT1A) for phase II (Robic et al. 2008).

Both enzymes are more strongly expressed in gilts and castrates, which means that higher expression levels lead to lower SKA levels. Metabolization of SKA in the liver is also influenced by AND, which will be explained in detail in section 2.1.3 “Relationship between androstenone and skatole”. A smaller part of the produced SKA in the large intestine directly diffuses into the blood via the inferior vena cava (*V. cava caudalis*) and accumulates in the fat.

In contrast to AND, the amount of SKA production is affected by environmental factors more than from genetic factors (Bonneau 1982). Zamaratskaia and Squires (2009) reviewed that three of the four main influence factors for the accumulation of SKA in fat tissue can be affected by the diet, which are the rate of SKA production, the average stay of SKA in the intestine and the rate of intestinal absorption. As the availability of L-tryptophan is one of the limiting factors for SKA production, studies have shown that adding carbohydrates like sugar beet pulp, chicory inulin or raw potato starch to the diet or changing the diet composition can contribute to reduce the amount of SKA excreted in the faeces (Hawe et al. 1992; Claus et al. 2003; Rideout et al. 2004).

Although the beginning of puberty does not seem to have an influence on an increasing production of SKA, Zamaratskaia and Squires (2009) have reported that younger pigs seems to have a different SKA metabolism compared to mature pigs due to differences in gene expression of hepatic enzymes. In contrast to that, an earlier study has been shown that SKA concentrations in blood are correlated with the weight of the boar, which is linked to puberty (Zamaratskaia et al. 2004).

Studies which analyze the influence of hygienic pen conditions on SKA backfat accumulation provide contrary results. An early study has reported an increase of SKA in adipose tissue of pigs which are kept in soiled or less slatted pen floors (Hansen et al. 1994). Similarly, a study of Thomsen et al. (2015a) indicated that AND as well as SKA was accumulated within backfat with increased soiling degree of the pig. The authors have concluded that SKA from the manure is contaminating the pig via absorption through skin or lungs. In contrast, no relationship between the degree of soiling and SKA deposition was found in laboratory analyses in a more recent study of Aluwé et al. (2011).

2.1.3. Relationship between androstenone and skatole

Both boar taint compounds, AND and SKA, have been early identified to be responsible for boar taint (Patterson 1968; Vold 1970). However, in these studies mainly AND was charged to be responsible for the off-odour (reviewed by Bonneau (1982)). In newer studies of Wesoly and Weiler (2012) as well as Mörlein et al. (2016), SKA is discussed as relatively more important for the olfactory perception of boar taint, compared to AND.

Against this background Wesoly and Weiler (2012) pointed out that boar taint is mostly occurring in boars, but not in gilts or castrates although they also produce SKA in their large intestine. This apparent dissent can be explained by Rasmussen et al. (2011) who demonstrated that a lack of AND as a consequence of no testosterone production in gilts and castrates results in a higher metabolic clearance rate and an unhindered degradation of SKA. As a consequence, gilts and castrates do not accumulate SKA in an olfactory relevant concentration in their adipose tissue.

Additionally, there seems to be an interrelation between estradiol and SKA, as boars have higher estradiol concentrations compared to gilts and castrates. This leads to an inhibited CYP2E1 activity and consequently to a decrease of SKA metabolism in the liver (Babol et al. 1999; Zamaratskaia and Squires 2009).

The physiological dependency of AND and SKA is confirmed by the positive genetic correlation (r_g) which is reviewed in a range between 0.36 and 0.62 (Robic et al. 2008). Doran et al. (2002), Tambyrajah et al. (2004) and Windig et al. (2012) have reported that the presence of androstenone inhibits the enzymes that are responsible for skatole metabolism. As has been shown by Babol et al. (1999) gene expression of CYP2E1 is high in animals with low androstenone levels, as CYP2E1 is involved in liver metabolism of skatole. In addition, they have proven that concentrations of CYP2E1 in liver, skatole in fat and estrone sulfate in plasma are correlated to androstenone levels in fat and plasma. Thus, high amounts of androstenone are inhibiting CYP2E1 expression and are therefore consequently inhibiting the skatole metabolism which leads to high skatole levels.

Besides the physiological point of view interaction between AND and SKA, also the olfactory point of view is of major interest. Regarding off-odor, Mörlein et al. (2016) have proven in their sensory study that not only the additive effects of the compounds AND and SKA itself, but also their interaction is responsible for boar taint. Their results have shown, that the influence of high AND levels on off-odor can be compensated by very low levels of SKA and *vice versa*.

2.1.4. Genetic background of boar taint compounds

Genetic parameters

In general, Windig et al. (2012) have ranked the concentrations of boar taint with dam lines as the highest, followed by crosses and sire lines. Different studies have shown the genetic foundation of AND and SKA as a result of variance component estimations in several breeds (Robic et al. 2008; Duijvesteijn et al. 2010; Le Mignon et al. 2011; Grindflek et al. 2011a; Robic et al. 2011; Gregersen et al. 2012; Windig et al. 2012; Lukić et al. 2015). Heritabilities (h^2) of AND and SKA measured in back fat tissue are moderate to high. A review of Robic et al. (2008) showed h^2 in a range of 0.25-0.88 for AND and h^2 of 0.19-0.54 for SKA in different pig populations. A similar large range of h^2 estimates were also found in recent studies (Grindflek et al. 2011b; Strathe et al. 2013a; Baes et al. 2013; Mathur et al. 2013; Rowe et al. 2014; Parois et al. 2015). Taking into account the large range of h^2 estimates it would be interesting to know, whether these differences can be linked to breed specific characteristics. However, because of differences in the underlying ages or weights of the animals and the applied statistical approaches, it is hardly possible to rank the breeds regarding h^2 of boar taint compounds.

As h^2 showed the potential of breeding against AND and SKA, the r_g to other production traits are also mostly favorable. Strathe et al. (2013a), Haberland et al. (2014) and Dugué et al. (2020) have reported mostly favorable genetic relationships between the feed conversion rate (FCR) and AND in a range from 0.06 to 0.47 in different breeds like Piétrain (PI), Danish LR or crossbreds. Furthermore, r_g between AND and average daily weight gain (ADG) showed no effect or a small favorable relation (r_g between 0.03 and -0.16) in different breeds (Strathe et al. 2013a; Haberland et al. 2014; Dugué et al. 2020). If unfavorable r_g to production traits were found (Sellier et al. 2000), they were mostly low so that a balanced selection between genetic progress and costs are likely.

The genetic relationship among boar taint compounds and male as well as female fertility will be described in section 2.3.

QTL

For the clarification of the genetic background, it is of major interest to identify candidate genes and / or QTL regions with an impact on formation, metabolism or degradation of AND and SKA. Information about that can be used for development of further selection strategies by considering genomic information. Newer GWAS and QTL studies from 2000 until now showed

genes and regions associated with AND spread across the whole genome, except SSC 16 and SSC Y, as summarized by Große-Brinkhaus et al. (2015) (figure 5).

In total, findings of 62 QTL and significant associations for AND distributed across the genome lead to the assumption of a polygenetic inheritance of AND, whereas SKA shows a more monogenetic inheritance with more punctual QTL on specific chromosomes (figure 5).

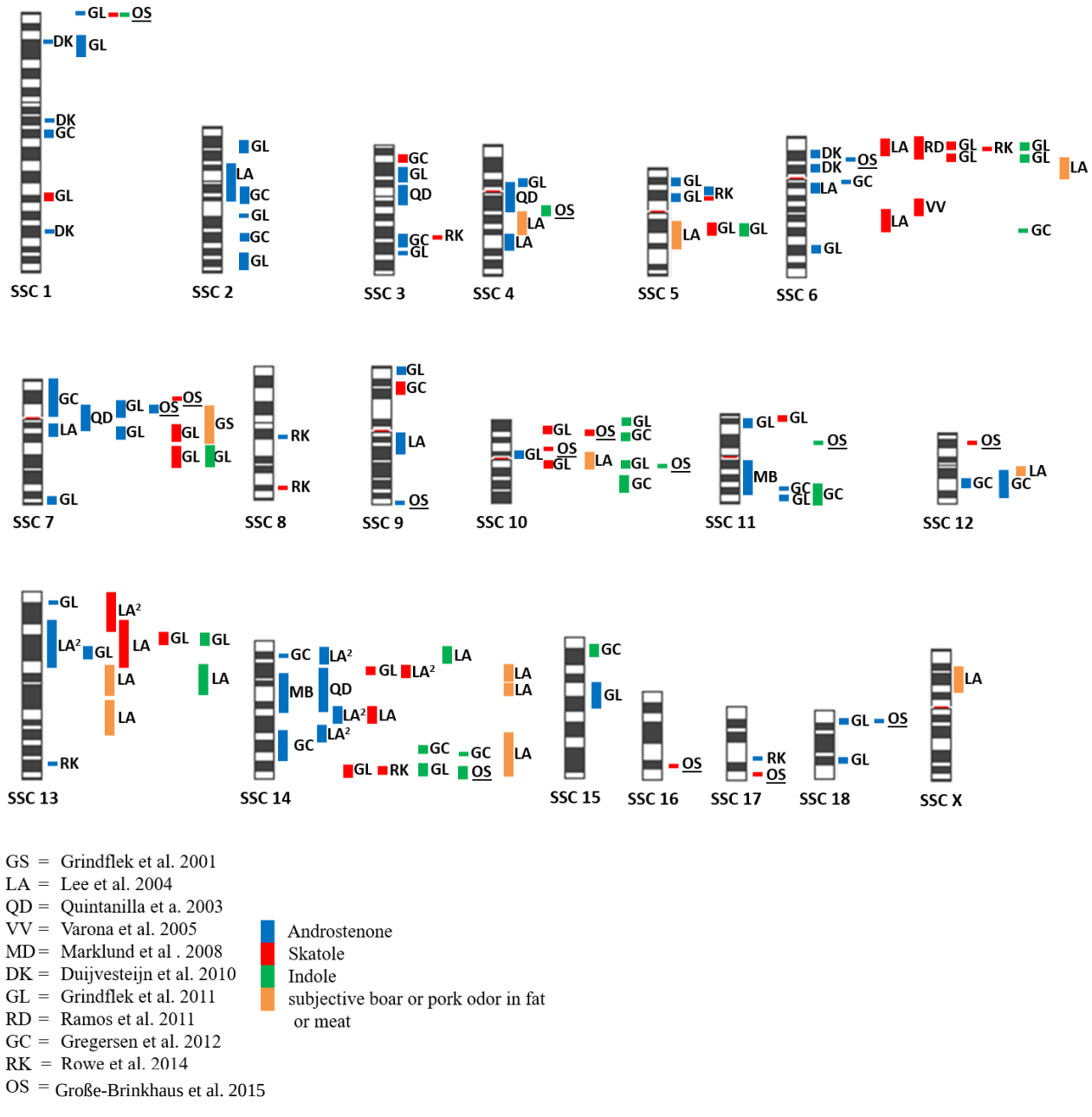

Figure 5: Overview about identified QTL for boar taint components. Modified according to Große-Brinkhaus et al. (2015).

An overview of the investigated candidate genes for AND and SKA is given in table 1. A lot of these studies are based on F_2 crossbred populations like e.g. F_2 Meishan × LW (Quintanilla et al. 2003) or F_2 Duroc × Norwegian LR (Grindflek et al. 2001). Most of the candidate genes that are discussed until now, are involved in AND synthesis and are members of the cytochrome P450 family (CYP), e.g. CYP11A1 (cytochrome P450, family 11, subfamily A, polypeptide 1) and CYP17A1 (cytochrome P450, family 17, subfamily A, polypeptide 1) (Moe et al. 2007; Grindflek et al. 2010; Leung et al. 2010; Gunawan et al. 2013). These genes were upregulated in animals with high AND levels. Another important gene that is involved in AND synthesis is CYB5A (Cytochrome B5 Type A). Several studies have shown higher AND levels in animals with an up regulated expression of CYB5A in the testis (Davis and Squires 1999; Moe et al. 2007; Grindflek et al. 2010; Leung et al. 2010). CYB5A is therefore described as a possible target gene for regulations of boar taint by Robic et al. (2008) and Peacock et al. (2008). Moreover, results of Sinclair et al. (2006) have shown that animals with a high expression of SULT2A1 (Sulfotransferase family 2A member 1) in the testis are showing highest AND levels in plasma. Therefore, SULT2A1 is identified as a key enzyme for the metabolism of 5-α-androstenone in the testis.

The expression of other members of the CYP family seems to be indicative not only for AND synthesis but also for the metabolism in liver tissue: CYP2E1 and CYP2A6. These genes were down regulated in animals with higher AND levels (Moe et al. 2008). Simultaneously, CYP2E1 is known to be up regulated in animals with low SKA levels (Doran et al. 2002; Whittington et al. 2004) which is an indicator for their common genetic regulation as it has been described in the subsection "Relationship between androstenone and skatole". Moreover, Robic et al. (2008) attributed genes from the 3β-HSD (*3β-Hydroxysteroid dehydrogenase*) (HSD3B1, HSD3B2) complex to be involved in AND degradation. Doran et al. (2004) found an association between animals with a low AND level and high expressions of 3β-HSD and 17β-HSD enzymes in the liver. High AND levels along with low SKA levels were associated with a down regulated gene expression of HSD17B2 in the liver (Moe et al. 2008) and an up regulated gene expression of HSD17B4 (Moe et al. 2007) in the testis.

Regarding the candidate genes that are involved in formation of SKA, little is known. The major impact on the formation rate of SKA is owed by environmental factors like management, nutrition and housing as described in the subsection above.

It is noteworthy, that all candidate gene complexes that are described above for AND and SKA (CYP-family, CYB5A, SULT2A1 and 3β-HSD complex) are also members of the steroid

hormone synthesis pathways (Sinclair et al. 2006), such as e.g. mutations of CYB5A. These mutations are also described to be involved in an modified conversion of pregnolone to 17α-hydroxypregnolone (Squires et al. 2019). This could be of particular relevance regarding the relationships between boar taint and fertility as it will be further described in subsection 2.3. Nevertheless, the complete regulation of genes that are involved in AND synthesis is not fully uncovered so far as different studies have shown breed differences (reviewed by Robic et al. (2008)).

Table 1: Overview about candidate genes for AND and SKA from literature

Gene*	SSC	Location (Mb)	Trait	Population	Reference
CYB5A	1	149.7	AND SY	YS DU, Norwegian LR DU, Norwegian LR YS	Davis and Squires (1999) Moe et al. (2007) Grindflek et al. (2010) Leung et al. (2010)
CGA	1	55.5	AND	Commercial DU	Duijvesteijn et al. (2010)
ESR1	1	14.2 - 14.6	AND, SKA	PI × (LW(LEI × GL))	Neuhoff et al. (2015)
HSD17B4	2	123.3 - 123.4	AND SY	DU, Norwegian LR	Moe et al. (2007)
FMO5	4	100.3	AND	PI × (LW(LEI × GL))	Neuhoff et al. (2015)
HSD3B1	4	101.5	AND DEG	-	Robic et al. (2008)
HSD17B2	6	6.2 - 6.3	AND DEG SKA DEG	DU, Norwegian LR	Moe et al. (2008)
CYP2A6 / CYP2A19	6	49.0	AND MET SKA DEG	DU, Norwegian LR Commercial DU LW × LR ME ×LW LR	Moe et al. (2008) Duijvesteijn et al. (2010) Doran et al. (2002) Whittington et al. (2004) Varona et al. (2005)
SULT2A1	6	53.5	AND MET	Commercial DU YS	Duijvesteijn et al. (2010) Sinclair et al. (2006)
HSD17B14	6	54.1	AND	Commercial DU	Duijvesteijn et al. (2010)
LHB	6	54.2	AND	Commercial DU	Duijvesteijn et al. (2010)
CYP21A2	7	24.0	AND	F_2 ME × LW PI × (LW(LEI × GL))	Quintanilla et al. (2003) Neuhoff et al. (2015)
CYP11A1	7	59.1	AND SY	F_2 ME ×LW DU, Norwegian LR DU, Norwegian LR YS DU × ((LEI × LR) × LW)	Quintanilla et al. (2003) Moe et al. (2007) Grindflek et al. (2010) Leung et al. (2010) Gunawan et al. (2013)

Gene*	SSC	Location (Mb)	Trait	Population	Reference
CYP17A1	14	113.8	AND SY	YS DU, Norwegian LR DU, Norwegian LR YS DU × (LW(LEI × LR))	Davis and Squires (1999) Moe et al. (2007) Grindflek et al. (2010) Leung et al. (2010) Gunawan et al. (2013)
CYP2E1	14	141.6 - 141.7	AND MET SKA MET SKA	DU, Norwegian LR LW × LR ME × LW	Moe et al. (2008) Doran et al. (2002) Whittington et al. (2004)

SSC = *Sus scrofa* chromosome, AND = androstenone, SKA = skatole, DEG = degradation, SY = synthesis, MET = metabolism, *The declaration of gene symbols can be obtained from Ensembl orhttp://www.ncbi.nlm.nih.gov/gene

2.2. Reproduction traits and fertility

Within commercial pig production herds and artificial insemination (AI) stations, maternal and paternal fertility is crucial for the profitability of these enterprises. From the perspective of a breeding company, establishing an efficient AI system is important for their breeding program because one single boar can have a huge impact on many sow populations (Zak et al. 2017). Combined with genomic selection, AI shortens the generation interval and helps to reach new selection goals earlier and more efficient. In piglet production herds as well as in nucleus farms, reproduction and fertility are mandatory for successful breeding (Rothschild 1996). In conclusion, paternal and maternal fertility traits are important for genetic progress and profitability which leads to an undoubtedly high motivation to genetically improve these traits.

Against this background, it is important to known the underlying candidate genes for male and female fertility to be able to identify animals with a low fertility and reproduction potential early in the selection process. To identify and exclude these animals from the nucleus herd is one of the most decisive factors for economic success of AI stations.

Particularly in dam breeds, focusing on reproduction traits like total number of piglets born or age at first insemination has led to an increased litter size in the last decades (Oliviero et al. 2019). Nevertheless, these high demands on reproduction did not remain without consequences. High litter sizes are accompanied by heterogeneous litters and increased piglet mortalities at birth as well as during the suckling period (Edwards 2002; Baxter et al. 2013; Rutherford et al. 2013).

In general, heritabilities of maternal reproduction traits are usually low to moderate (Zak et al. 2017; Mencik et al. 2019), which can be explained by the sensitivity of these traits for environmental impacts induced by various management factors. (Baxter et al. 2013). Although h^2 are on a marginal higher level in males, this statement can also be applied for paternal reproduction traits (Smital et al. 2005). In order to get greater insights into the complex architecture of fertility traits, the following sections 2.2.1 and 2.2.2 summarize known important physiological and genetic determinants for these traits.

2.2.1. Male fertility

Reproduction traits

In pig breeding, male fertility is most important for the profitability of AI stations. Until now, in sire, breeds the breeders' focus is mainly on production traits whereas sperm quality parameters play an inferior role (Diniz et al. 2014). Nevertheless, the fertilization capability of boars is the most important factor for the decision if an AI boar stays in station or will be culled. Fertility itself can be affected by many factors like the individual potential of the animal, breed, management, environment, age and weight.

The efficiency of a boar is determined by the semen quantity and quality as this affects the amount of sows that can be fertilized by one ejaculation (Zak et al. 2017). Phenotyping of selected boars in AI stations differs depending on the available techniques and systems. Whereas some AI stations are evaluating the sperm samples manually, others are using the automated systems like the SCA® CASA system. This leads to differences in the definition and accuracy of recorded traits which results in difficulties when comparing sperm quality parameters of AI boars from different stations.

Moreover, it has to be considered that boars which are phenotyped in AI stations are already pre-selected. This leads to a lack of genetic variation because only the best boars are passing the selection process which makes it more difficult to analyze the underlying genes. In literature the traits sperm volume (SV), sperm density (SD), sperm motility (SMO) and sperm abnormality rate (SAR) are analyzed most frequently. The sperm quality strongly depends on the spermatogenesis, that is controlled by several endocrine factors.

Endocrinological traits for male fertility

Endocrine parameters like hormones are the basis for sexual maturation and also for sexual behavior. Important hormones for male fertility are among others: Testosterone (TEST), 17-β estradiol (EST) and the regulatory hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

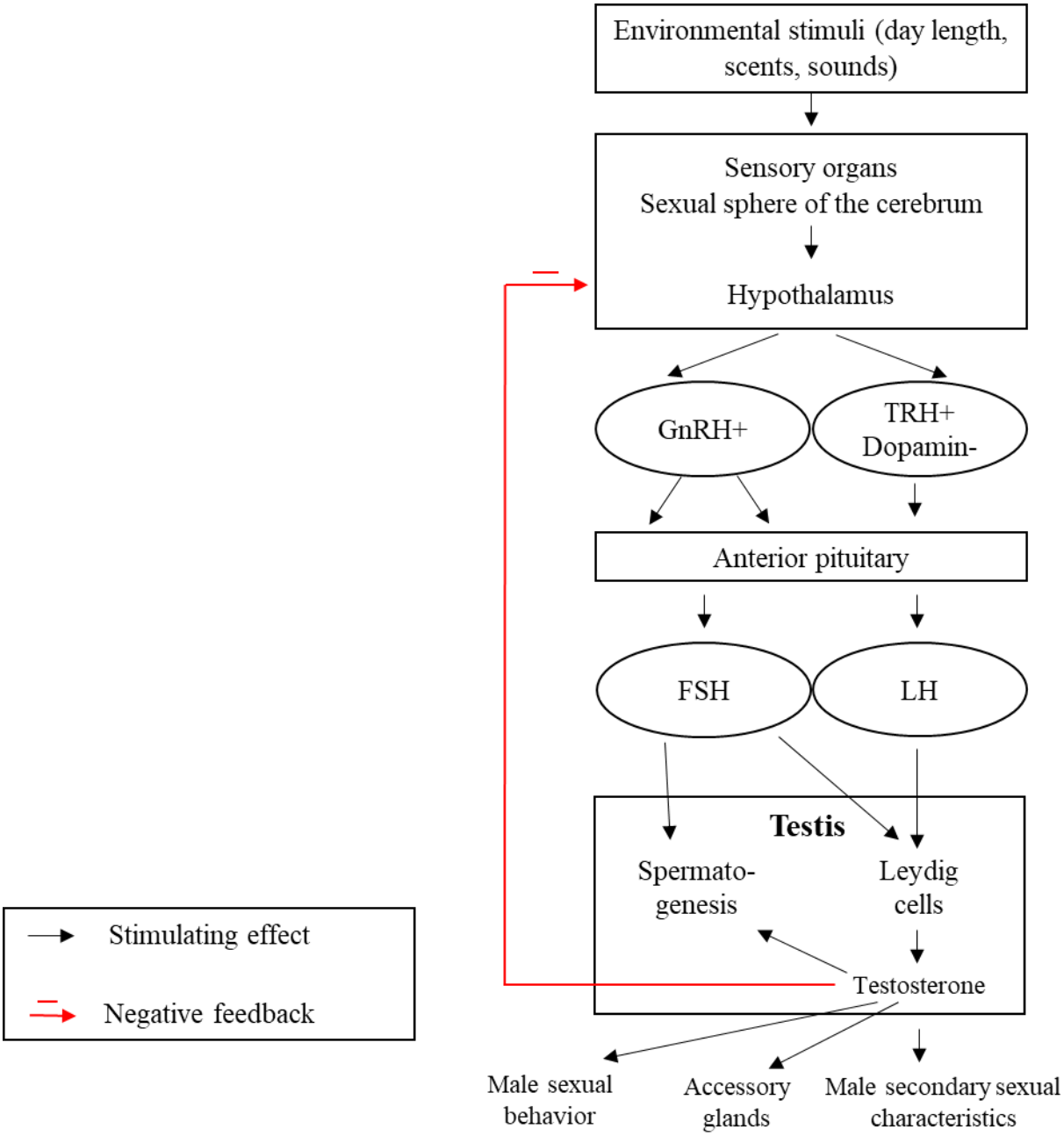

Figure 6: Management of male fertility by hormones. Modified according to Weiß (2005). GnRH = Gonadotrophin-releasing hormone, TRH = Thyrotropin-releasing hormone, FSH = Follicle-stimulating hormone, LH = Luteinizing hormone.

As presented in figure 6, TEST is responsible for male sexual behavior and the development of accessory glands and male secondary characteristics as well as for spermatogenesis. Although TEST is known as the most important hormone for male fertility, a study of Walker et al. (2004) showed no correlation between testosterone concentration and sperm production. However, the same study showed that TEST concentration seems to be of importance for the prenatal development of the epididymis and its growth during puberty as boars with higher TEST concentration showed larger epididymis than those with lower TEST concentration (Walker et al. 2004). TEST is underlying a circadian or diurnal rhythm (Claus and Hoffmann 1980).

LH and FSH as regulatory hormones are involved in the stimulation of the development of the Leydig cells and the spermatogenesis (figure 6). LH is stimulating TEST synthesis in the leydig cells whereas FSH stimulates cell differentiation in the Sertolli cells before puberty. This determines the size of the testis and has therefore an impact on the amount of produced spermatozoa (França et al. 2000). Furthermore, LH as well as FSH are involved in the early neonatal stimulation of Leydig and Sertolli cell proliferation (Wells et al. 2013). Both of them are regulated indirectly by environmental stimuli via gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) (figure 6).

Although EST is not included in figure 6, it is produced by aromatization of *inter alia* TEST in the Leydig cells (Mutembei et al. 2005). In contrast to other mammalian species, boars produce more estrogens in the testis than other animals do (Booth 1980b; Mutembei et al. 2005) so that high EST concentration can be found in the semen (Hess 2003). Furthermore, EST levels in a boar can be higher than in a sow during oestrus (Claus and Hoffmann 1980). EST is hypothesized to play a role in male fertility (Hess 2003), especially in the sexual behavior (Joshi and Raeside 1973) and the function of the accessory glands (Claus and Hoffmann 1980). Bilić-Šobot et al. (2014) stated, that the major function of "EST in the testes is the production of spermatozoa which are under control of LH and [..] FSH." Moreover, the lack of the estrogen receptor ESR1 leads to impaired male fertility, especially to reduced spermatogenesis and reduced fertilizing ability (Gunawan et al. 2011; Gunawan et al. 2012). Claus and Hoffmann (1980) described estrogens as "necessary to produce an anabolic effect in boars which result in the typical body shape for the male animal". Similarly to TEST, EST is also underlying a circadian or diurnal rhythm (Claus and Hoffmann 1980).

Genetic background of male fertility

In general, selection for male reproduction traits within many AI stations is only performed by phenotypic selection (Schulze et al. 2014). This practice is somewhat surprising because sperm quality parameters have moderate h^2 (Smital et al. 2005) but in comparison to production traits they are still low. Previous studies estimated h^2 in a range from 0.20 to 0.25 in purebred Czech LR and LW (Wolf 2010) and between 0.23 to 0.31 for purebred Danish LR (Strathe et al. 2013b). For a PI × F_1 cross, h^2 was higher ($h^2 = 0.56$) as described by Frieden et al. (2014). Strathe et al. (2013b) also mentioned that the h^2 of SV in their study was increasing simultaneously to the sexual maturation of the boar, whereas SC was unaffected by age. For the sperm concentration, h^2 were previously estimated in a range from 0.18 to 0.26, depending on the breed whereas estimators for Czech LR and LW boars were in general lower than those for Danish LR boars (Wolf 2010; Strathe et al. 2013b). Furthermore, Wolf (2010) and Strathe et al. (2013b) have shown h^2 in a range between 0.10 and 0.20 for SC.

Nevertheless, decisive reproduction traits like sperm characteristics are selection responsive (Zak et al. 2017). One obstacle for the conventional phenotypic selection for sperm quality parameters in male is that most of the spermatozoa traits can only be measured after beginning of puberty, similar to boar taint compounds. Moreover, collected phenotypes differ depending on the used phenotyping system. Genomic selection and identification of responsible candidate genes could help to overcome this obstacle as identification of candidate genes, that are responsible for male fertility could help to improve costly and long selection processes.

Therefore, several associations studies have been performed which resulted in a variety of possible QTL regions all over the genome. An overview about identified candidate genes in literature for several sperm quality traits in different sire breeds is presented in table 2. Male reproduction traits analyzed in this thesis are sperm volume (in ml), sperm density (measured by optical sensors) and sperm count (in billions).

As can be seen in table 2, identified genes are across the whole genome and QTL regions for different traits are often overlapping. This can be an indicator for pleiotropic effects of single candidate genes.

Table 2: Overview about candidate genes for male fertility traits from literature

Gene*	SSC	Location (Mb)	Trait	Population	Reference
AKAP4	X	43.8	SMO	DU	Gao et al. (2019)
RNASET2	1	2.1	SMO	DU	Gao et al. (2019)
ESR1	1	14.2 - 14.6	SMO	PI and PI × HA	Gunawan et al. (2011)
MTFMT	1	106.9 - 107.0	SMO SMO	Commercial LR, LW LR type and LW type	Diniz et al. (2014) Marques et al. (2018)
ESR2	1	193.8 - 193.9	SMO	PI and PI × HA	Gunawan et al. (2012)
DNM1	1	286.6 - 286.7	PMO	DU	Gao et al. (2019)
LCN2	1	286.6	PMO	DU	Gao et al. (2019)
NME5	2	140.1	NSC	LR type and LW type	Marques et al. (2018)
PPP2R2B	2	147.9 - 148.4	SMO	DU	Gao et al. (2019)
SPINK1	2	149.0	SMO	DU × ER F_2	Zhao et al. (2016)
SH2B1	3	18.5	PMO	DU	Gao et al. (2019)
TIMP3	5	12.1 - 12.2	NSC	DU	Gao et al. (2019)
SCN8A	5	16.9 - 17.1	SMO, PMO	LR type and LW type	Marques et al. (2018)
PLC*z*	5	54.2 - 54.4	CON MF, FF	PI and a PI × HA DU	Kaewmala et al. (2012) Tremoen et al. (2019)
CD9	5	64.4	SMO	PI and a PI × HA	Kaewmala et al. (2011)
VWF	5	64.5 - 64.6	MF	DU	Tremoen et al. (2019)
VPS4A	6	17.6	PMO	DU	Gao et al. (2019)
AZIN2	6	89.3	TMA	LR type and LW type	Marques et al. (2018)
APN	7	55.3	EPW	DU × ER F_2	Zhao et al. (2016)
METTL3	7	77.6	SMO, PMO	LR type and LW type	Marques et al. (2018)
TEP	7	78.4 - 78.8	CON	DU × ER F_2	Zhao et al. (2016)
PARP2	7	78.5	CON	DU × ER F_2	Zhao et al. (2016)
SPATA7	7	110.3	SMO, TMA	LR type and LW type	Marques et al. (2018)
BMPR1B	8	124.5 - 125.0	MF, FF	LR	Tremoen et al. (2019)

Gene*	SSC	Location (Mb)	Trait	Population	Reference
HPGDS	8	125.3 - 125.4	PMO	LR type and LW type	Marques et al. (2018)
ZNF215	9	2.5 - 2.6	STD	DU × ER F_2	Zhao et al. (2016)
LAMB1	9	107.6 - 107.7	PMO	DU	Gao et al. (2019)
TDRD5	9	121.2 - 121.3	NSC	DU	Gao et al. (2019)
QSOX1	9	121.7 - 121.8	NSC	DU	Gao et al. (2019)
COX-2 / PTGS2	9	- 127.8	PMO MF, FF	PI and a PI × HA, LR type and LW type LR	Kaewmala et al. (2012) Marques et al. (2018) Tremoen et al. (2019)
PLA2G4A	9	127.8 - 128.1	PMO	LR type and LW type	Marques et al. (2018)
NEK2	9	131.5	SMO	DU	Gao et al. (2019)
SLC16A5	12	6.1 - 6.2	TMA	DU	Gao et al. (2019)
DNAI2	12	6.8	SMO TMA	LR type and LW type DU	Marques et al. (2018) Gao et al. (2019)
ZPBP1	12	22.1 - 22.5	NSC	DU	Gao et al. (2019)
THRA	12	22.2	NSC	DU	Gao et al. (2019)
CSF3	12	22.3	NSC	DU	Gao et al. (2019)
SPAG9	12	27.2 - 27.3	PMO	DU	Gao et al. (2019)
IQCG	13	134.5	SMO, PMO	LR type and LW type	Marques et al. (2018)
ADAM7	14	8.4 - 8.5	SMO	DU	Gao et al. (2019)
BLK	14	146.8 - 147.4	NSC PMO	DU DU	Gao et al. (2019)
SKP2	16	21.4 - 21.5	SMO	DU	Gao et al. (2019)
GHR	16	27.1 - 27.4	TMA	DU	Gao et al. (2019)
SELENOP	16	27.5	TMA	DU	Gao et al. (2019)
MTRR	16	74.2	STD	DU × ER F_2	Zhao et al. (2016)
PDE1C	18	40.8 - 41.4	STD	DU × ER F_2	Zhao et al. (2016)

SSC = *Sus scrofa* chromosome, CON = Sperm concentration, NSC = Number of sperm cells, SMO = Sperm motility, PMO = progressive motility, TMA = Total morphological abnormalities, EPW = Epididymal weight, STD = seminiferous tubule diameter, MF = male fertility defined as sire of the litter (estimated breeding value based on total number of piglets born), FF = female fertility defined as fertility of the boars' daughter (estimated breeding value

based on total number of piglets born). *The declaration of gene symbols can be obtained from Ensembl orhttp://www.ncbi.nlm.nih.gov/gene

The three most common identified candidate genes for sperm quality parameters were:

- Phospholipase C zeta (PLC*z*): is involved in prostaglandin synthesis and effects Ca^{2+} oscillations in pigs for successful fertilization (Kaewmala et al. 2012),
- Cyclooxygenase isoenzyme type 2 (COX-2): is involved in prostaglandin synthesis (Kaewmala et al. 2012; Tremoen et al. 2019) and
- Mitochondrial Methionyl-TRNA Formyltransferase (MTFMT): seems to have an effect on protein in sperm cells (Marques et al. 2018).

Kaewmala et al. (2012) reviewed that prostaglandin was already identified to play an important role in the process of spermatogenesis in hamster, rat and human. Associations with COX-2 for male fertility (measured as number of piglets born alive per boar) were also confirmed by a newer study (Tremoen et al. 2019).

2.2.2. Female fertility

Reproduction traits

Breeding for maternal productivity was the main goal of selection in the past 30 years (Kemp et al. 2018), especially in dam breeds. In nucleus herds, this selection is focused on the phenotypes number of piglets born in total (NBT), number of piglets born alive (NBA) and number of piglets born dead (NBD) (Tribout et al. 2008). Although litter size traits are characterized by a low h^2 in general (Zak et al. 2017), selection programs led to an increase of one piglet per litter within three years (van Engen et al. 2010). However, number of weaned piglets did not increase the same amount as NBA (Prunier et al. 2010; Kemp et al. 2018). Edwards (2002) and Heuß (2020) explained this by an increase of NBD and a decreased piglet survival.

Similar to boar taint, public controversial discussions in most recent years led to important changes in the breeding goal of many pig breeding companies with more focus on the reduction of piglet losses. Further, breeding organizations also included the longevity of a sow to the breeding strategy to balance efficiency and sustainability. This includes a change from the dominating breeding target “maximized litter sizes” towards well-balanced and more homogenous litters (Merks et al. 2012).

Endocrinological traits for female fertility

Important hormones for female fertility are *inter alia*: 17 β-estradiol, LH, FSH and progesterone. Additionally, the hormones prolactin and oxytocin are mainly involved in mammary gland development and milk production (Weiß 2005).

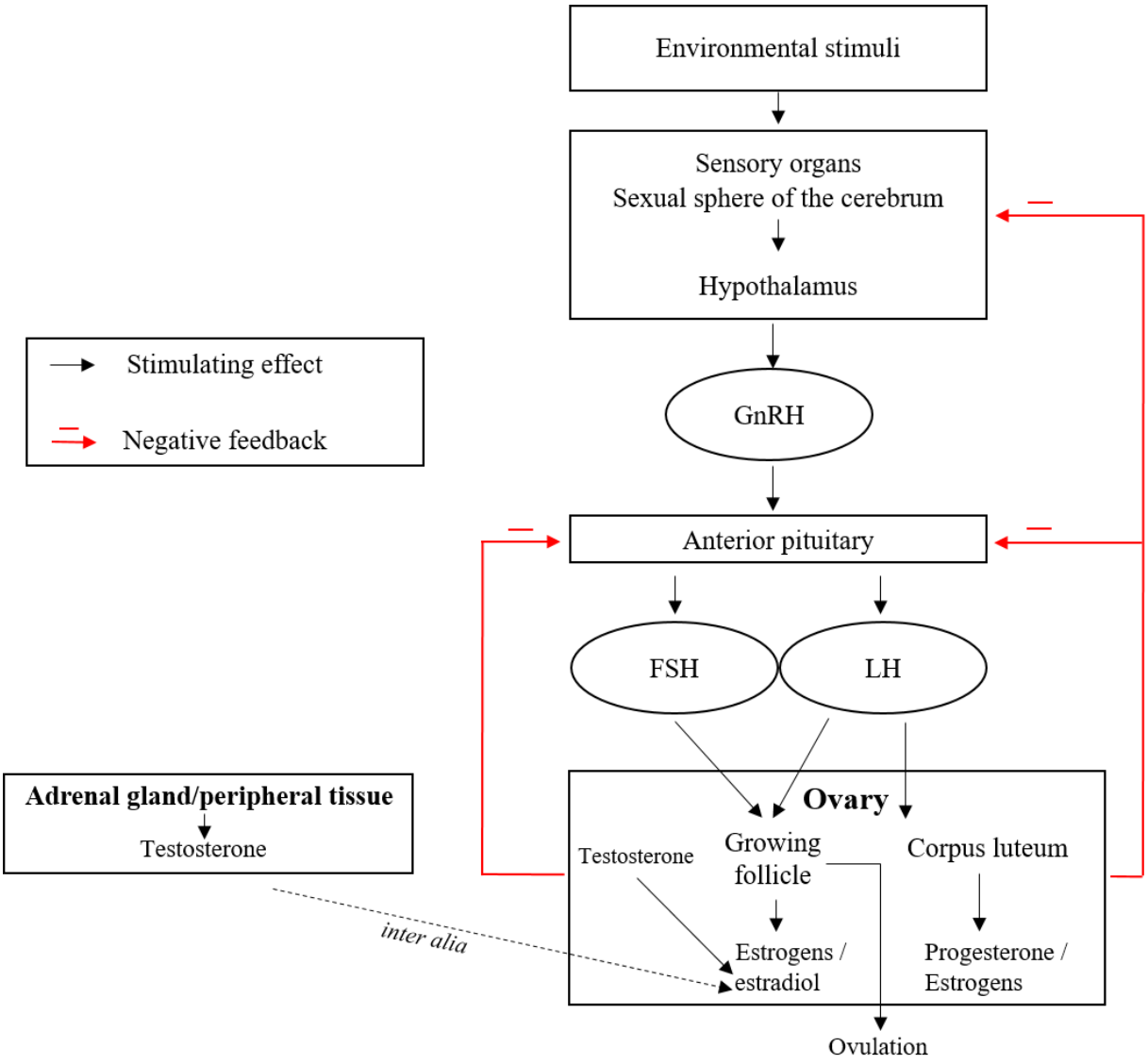

Figure 7: Management of female fertility by hormones. Modified according to Prunier and Quesnel (2000), Weiß (2005) and Sun et al. (2013). GnRH = Gonadotrophin-releasing hormone, FSH = Follicle-stimulating hormone, LH = Luteinizing hormone.

LH and FSH are both released by the anterior pituitary under the control of the GnRH and are targeting the gonads. By underlying the same regulatory feedback mechanism as in males, both are regulating the synthesis of steroid hormones (Ohlsson 2016).

Furthermore, FSH has a direct influence on the folliculogenesis in the ovary and is involved in the estrogen production in ovarian granulosa cells (Ohlsson 2016; Kumar 2018) (figure 7). LH is responsible for late maturation of follicles and is involved in the ovulation as well as in formation and maintenance of new corpora lutea from preovulatory follicles (Ziecik et al. 2018).

17 β-estradiol, also known as E2 or estradiol, is the most important hormone for the female fertility and is mainly produced in ovary, corpus luteum and in pregnant individuals also in the placenta and is involved in "development, maturation and functioning of the female reproductive tract" (Kumar et al. 2018). Beneath effects on sexual behavior it is also involved in the development of mammary tissue during puberty (Kumar et al. 2018) and in the expression of female secondary sex characteristics. Moreover Robic et al. (2014) described estradiol as affecting body weight and body composition of the progeny.

In females, progesterone production is primarily induced by LH. Due to the negative feedback regulation (figure 7), an increasing progesterone concentration leads to a lower LH surge. Progesterone is mainly produced by the corpus luteum during estrus and by the placenta in case of pregnancy (Spencer et al. 2004). A low amount is also synthesized by the adrenal glands (Holzbauer and Newport 1969). Its function is to establish and maintain early pregnancies (Spencer et al. 2004; Waclawik et al. 2017). Therefore, its concentration is expected to be low in prepubertal animals.

Testosterone is only playing a tangential role in female animals. Nevertheless, it is a precursor of estrogen, which means that it is synthesized in female organism but not transported or accumulated in blood or other organs. It is principally synthesized from androgen precursors in liver, adipose tissue and skin by conversion (Walters 2015) from e.g. dehydroepiandrostenone (DHEA).

Genetic background of female fertility

The most important traits to characterize the female reproduction are: Total number of piglets born (TNB), number of piglets born alive (NBA), number of piglets born dead (NBD), age at first insemination (AFI), farrowing interval (FI) and the number of piglets after weaning (NWP). Together with the longevity of the sow, these measurements determine the profitability of piglet production. In comparison to male reproduction traits, female reproduction traits often have lower h^2 (Smital et al. 2005), e.g. h^2 for NBA is estimated in a range between 0.10 and 0.12 in German LR and LW (Hellbrügge et al. 2008; Heuß 2020) and as 0.08 in a purebred French LW population by Canario et al. (2006). h^2 for NBD is in general lower and ranges between 0.05 and 0.08 in the previous mentioned studies of German LR and LW and French LW (Canario et al. 2006; Hellbrügge et al. 2008; Heuß 2020). For AFI, Mathur et al. (2013) estimated a higher h^2 in PI-derived sire line ($h^2 = 0.34$) compared to a LR × YS crossbred ($h^2 = 0.27$).

Although h^2 for female reproduction traits are generally low (Zak et al. 2017; Mencik et al. 2019) and expression is sex-limited (Uimari et al. 2011), an accelerated genetic progress was realized in the last recent years. Along with high selection intensities, modern breeding tools like genomic selection obviously allow breeding organizations to utilize the available genetic variation for important fertility traits to a high extent. Although a polygenetic additive inheritance mode is most likely for traits like NBA, NBD and AFI, a long list of QTL (table 3) is the result of many GWAS studies performed in the last decades. These QTL are the expression of the available genetic variation of these traits. Moreover, the number of QTL reflect the presumably economically driven interest in the genetic foundation of maternal fertility traits. For reasons of legibility and development of research, table 3 only shows an extract of the most recent published and most important results of QTL studies. A more detailed list can be found in the database "Online Mendelian Inheritance in Animals" (OMIA) (OMIA 2020).

Table 3: Overview about candidate genes for female fertility traits from literature

Gene*	SSC	Location (Mb)	Trait	Population	Reference
ESR1	1	14.2 - 14.6	NBA	LR × LW ME × synthetic ME	Mencik et al. (2019) Rothschild et al. (1996)
ALDH1A2	1	113.9 - 114.0	NBA	LR	Wu et al. (2018)
MEF2C	2	96.1 - 96.2	NBA	LW × (LR × LW)	Onteru et al. (2012)

Gene*	SSC	Location (Mb)	Trait	Population	Reference
SLC22A5	2	134.6 - 134.8	NBA	LW	Sato et al. (2016)
NPHP1	3	46.3	NBD	LW	Verardo et al. (2016)
PPARα	5	3.3	NBD	LW	Bergfelder-Drüing et al. (2015)
FBLN1	5	3.8 - 3.9	NBD	LW	Bergfelder-Drüing et al. (2015)
KCNC2	5	38.3 - 38.5	NBA	LW	Sato et al. (2016)
ARID1A	6	84.0 - 84.1	NBA	DU	Chen et al. (2019b)
PTP4A2	6	88.4	NBD	LW	Verardo et al. (2016)
LEPR	6	146.8	NBA	LW	Wu et al. (2018)
PPARD	7	31.2	NBA	LW	Spötter et al. (2010)
NUBPL	7	67.7 - 68.0	NBD	LR, DU, YS	Schneider et al. (2015)
NFATC4	7	74.9	NBD	DU	Chen et al. (2019b)
C4orf19	8	29.0 - 29.1	NBD	LW × (LR × LW)	Onteru et al. (2012)
RELL1	8	29.1	NBD	LW × (LR × LW)	Onteru et al. (2012)
KDR	8	41.8	NBA	LW	Spötter et al. (2010)
EGF	8	112.2 - 112.3	NBA	Hungarian LW, DU, PI LW	Hunyadi-Bagi et al. (2016) Sato et al. (2016)
HNRNPD	8	135.8	NBD	LW × (LR × LW)	Onteru et al. (2012)
SLC9A3R1	12	6.4	NBA	IB × ME F_2	Fernández-Rodríguez et al. (2010)
MAP3K3	12	15.2	NBA	LW	Spötter et al. (2010)
NOS2	12	44.1 - 44.2	NBA	IB × ME F_2	Fernández-Rodríguez et al. (2010)
PLSCR4	13	86.5	NBA	LW × (LR × LW)	Onteru et al. (2012)
PLSCR5	13	86.9	NBA	LW × (LR × LW)	Onteru et al. (2012)
MBL2	14	97.1	NBA	LW	Sato et al. (2016)
RBP4	14	105.0	NBA	LR × LW Crossbred	Mencik et al. (2019) Rothschild et al. (2000)
DPP10	15	21.3 - 22.0	NBD	LR, DU, YS	Schneider et al. (2015)
NOSTRIN	15	75.2 - 75.3	NBD	LR, DU, YS	Schneider et al. (2015)

Gene*	SSC	Location (Mb)	Trait	Population	Reference
FBXL7	16	4.7 - 5.1	NBA	LW	Wu et al. (2018)
PRLR	16	20.6	NBA	LR and LW	Tribout et al. (2008)
ERBIN	16	44.4 - 44.5	NBA	LW	Spötter et al. (2010)
CYP24A1	17	55.1	NBD	LW	Verardo et al. (2016)

SSC = *Sus scrofa* chromosome, NBA = number of piglets born alive, NBD = number of piglets born dead. *The declaration of gene symbols can be obtained from Ensembl or http://www.ncbi.nlm.nih.gov/gene

To our knowledge, in contrast to NBA and NBD, information about candidate genes for AFI is limited although AFI is a critical factor regarding the lifetime sow performance and therefore also important for the economic success of a pig breeder (Malanda et al. 2019). Reasons for the limited research could be that AFI is strongly influenced by management strategies (King 1989; Prunier 1991; Le Cozler et al. 1998). Most important for the AFI are weight and age of gilts (Engblom et al. 2007; Quinn et al. 2015). However, the applied time or weight depending thresholds are defined differently within breeds (Zak et al. 2017), breeding companies or even within nucleus herds. Moreover, under practical conditions it is difficult to record the first estrus of a sow, which would be a more exact indicator of sexual maturity of gilts than AFI (Lee et al. 2019). Under these conditions it is difficult to resolve the genetic foundation of AFI from environmental management effects. This might be an explanation for the reduced number of QTL results for AFI found in literature.

2.3. Interactions between boar taint and fertility

To establish fattening of entire males as a sustainable and long-term alternative to surgical castration, it is necessary to supervise possible unfavorable relationships to other economically important trait complexes. Previous studies did not reach a consensus regarding the adverse effect of selection against androstenone on reproduction.

Physiological causes for the relationship between boar taint and reproduction

The synthesis of the boar taint compound androstenone is closely linked to the synthesis of other important sex steroids like testosterone, that are crucial for fertility and reproduction. Moreover, characterization of endocrine parameters showed that synthesis of sex steroids and production of androstenone are both affected by LH (Weiler and Wesoly 2012; Ohlsson 2016).

Physiologically expected relationships between boar taint compounds and sex steroids as described above are known and are confirmed in several earlier studies. A study by Willeke et al. (1987) showed a delayed puberty in sows of a "low androstenone" line. Squires et al. (1991) postulated a positive correlation between plasma levels of testosterone and levels of androstenone in fat. Additionally, results showed positive correlations between fat levels of androstenone and skatole and estrone sulfat as well as between levels of estrogens and levels of androstenone in salivary glands (Squires et al. 1991). Furthermore, a study of Babol et al. (1999) showed a correlation (r) of 0.75 ($p < 0.001$) between the rate of androstenone synthesis and the rate of sex steroids. This correlation was also observed between the rate of sex steroid synthesis and skatole levels in fat ($r = 0.74$, $p < 0.001$) (Babol et al. 1999). Nevertheless, there seems to be no clear relation between testosterone in plasma and testis morphology (Lervik et al. 2013)

Genetic background of the relationships between boar taint and reproduction

In general, the observed physiological correlations are partly confirmed by genetic analyses. Regarding androstenone and endocrine parameters, Grindflek et al. (2011a) analyzed that most of the QTLs that were found to be significant for androstenone were also affecting estrogen levels. A current study of Dugué et al. (2020) showed that selection against androstenone or estradiol have adversely effects on testosterone which could result in a restricted reproduction potential in both sexes.

Regarding the reproduction traits itselves, studies of Strathe et al. (2013b) and Hidalgo et al. (2014a) showed favorable r_g between boar taint compounds and different semen traits in Danish LR, Dutch LR and LW, whereas analyses of Tajet et al. (2006) resulted in unfavorable

relationships between boar taint and the length of the bulborethral gland as an indicator for sexual maturity in boars. Bernau et al. (2018) and Needham et al. (2020) have reported higher testis volume in boars with a higher AND concentration compared to boars with low AND concentration. This is in accordance with results from Frieden et al. (2014) who showed r_g in a range of 0.45 to 0.54 between AND and testicular parameters like testis wide, weight and length. For SKA, the genetic estimates for these testicular parameters were lower between 0.12 and 0.30 (Frieden et al. 2014).

Regarding the antagonistic relationship between boar taint and maternal reproduction traits, results are contrary, too. Whilst some studies showed that reducing androstenone levels will not adversely affect maternal reproduction traits (Moe et al. 2009; Mathur et al. 2013; Hidalgo et al. 2014a), there are also studies that showed the opposite (Bonneau 1982; Babol et al. 2004; Frieden et al. 2014).

Moreover, gene expression patterns and genome wide analyses confirmed the interrelation between endocrine fertility parameters and boar taint compounds. Several analyses showed, that there are certain genes, like e.g. CYB5A, SULT2A1 and ESR1 which are associated with boar taint compounds as well was with pathways of sex steroid synthesis (Sinclair et al. 2006; Squires et al. 2019).

Consequences for breeding against boar taint

To establish fattening of entire males as a suitable alternative, it is mandatory to reduce the amount of odorous carcasses at slaughterhouse by implementing breeding against boar taint in sire and dam lines. To reach this goal, this thesis was part of a project called G-I-FER ("Genomic indicators for boar taint, fertility and robustness in Landrace and Large White populations"), which aimed to genetically improve boar taint in maternal breeds in consideration of fertility and robustness. h^2 for AND and SKA as described in the subsection 2.1.4 "Genetic background of boar taint compounds" are promising for breeding against boar taint but previous studies are anticipating unfavorable physiologic and genetic relationships to the important complex of reproduction traits (Gower 1972; Sellier et al. 2000; Grindflek et al. 2011b; Frieden et al. 2014; Parois et al. 2015). If breeding against boar taint in general, or against androstenone, it has to be ensured, that these pathways are not affected by this selection. The overall goal for breeding should be to identify animals with a low potential for androstenone synthesis / accumulation or rather a high rate of androstenone metabolism that are simultaneously producing a consistent concentration of steroid hormones for reproduction (Zamaratskaia and Squires 2009). In chapter 5 "General discussion", consequences for breeding

against boar taint based on results of this thesis will be debated, followed by the evaluation of including boar taint into realizable breeding programs.

Chapter 3. Genomic background and genetic relationships between boar taint and fertility traits in German Landrace and Large White

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3.1. Abstract

Background: Due to ethical reasons, surgical castration of young male piglets in their first week of life without anesthesia will be banned in Germany from 2021. Breeding against boar taint is already implemented in sire breeds of breeding organizations but in recent years a low demand made this trait economically less important. The objective of this study was to estimate heritabilities and genetic relationships between boar taint compounds androstenone and skatole and maternal / paternal reproduction traits in 4'924 Landrace (LR) and 4'299 Large White (LW) animals from nucleus populations. Additionally, genome wide association analysis (GWAS) was performed per trait and breed to detect SNP marker with possible pleiotropic effects that are associated with boar taint and fertility.

Results: Estimated heritabilities (h^2) were 0.48 (±0.08) for LR (0.39 ±0.07 for LW) for androstenone and 0.52 (±0.08) for LR (0.32 ±0.07 for LW) for skatole. Heritabilities for reproduction did not differ between breeds except age at first insemination (LR: h^2 = 0.27 (±0.05), LW: h^2 = 0.34 (±0.05)). Estimates of genetic correlation (r_g) between boar taint and fertility were different in LR and LW breeds. In LR an unfavorable r_g of 0.31 (±0.15) was observed between androstenone and number of piglets born alive, whereas this r_g in LW (-0.15 (±0.16)) had an opposite sign. A similar breed-specific difference is observed between skatole and sperm count. Within LR, the r_g of 0.08 (±0.13) indicates no relationship between the traits, whereas the r_g of -0.37 (±0.14) in LW points to an unfavorable relationship. In LR GWAS identified QTL regions on SSC5 (21.1- 22.3Mb) for androstenone and on SSC6 (5.5-7.5Mb) and SSC14 (141.1-141.6Mb) for skatole. For LW, one marker was found on SSC17 at 48.1Mb for androstenone and one QTL on SSC14 between 140.5Mb and 141.6Mb for skatole.

Conclusion: Knowledge about such genetic correlations could help to balance conventional breeding programs with boar taint in maternal breeds. QTL regions with unfavorable pleiotropic effects on boar taint and fertility could have deleterious consequences in genomic selection programs. Constraining the weighting of these QTL in the genomic selection formulae may be a useful strategy to avoid physiological imbalances.

Keywords: boar taint, reproduction, pigs, genome wide association analysis, androstenone, skatole

3.2. Introduction

Boar taint is described as an unpleasant smell of the meat from entire male pigs (Moe et al. 2009), which occurs as soon as the young pigs reach puberty. There are two main compounds which are responsible for boar taint. The first one is androstenone (5α-androst-16-en-3-one) (Patterson 1968), a steroid hormone which is built in the Leydig cells of the testis. The second one is skatole (3-methyindole) which results from the degradation of the amino acid tryptophan in the colon (Zamaratskaia and Squires 2009). Both compounds can be affected by genetics and environmental factors whereas skatole is more sensitive to housing conditions and nutritional management (Squires 2006; Weiler and Wesoly 2012). Currently, surgical castration without anesthesia is performed on young male piglets in their first week of life to prevent that odor, which represents a strong contrast to the increasing role of animal welfare in consumer acceptance. Due to a modification of the German animal protection law in 2013, castration without anesthesia should have been banned in Germany from 2019 but disagreement about alternatives lead to an extension of the deadline for the ban for two more years until 2021 (Deutscher Bundestag 11/6/2018).

When it comes to the integrity of the animal, fattening of entire boars is a suitable option to replace surgical castration. Furthermore, raising of entire males can be more sustainable regarding feed conversion, carcass composition (Lundström et al. 2009) and carbon footprint (Stefanski et al. 2018). To establish this method as a long-term alternative, it is necessary to reduce the percentage of odorous boars at slaughterhouse. This can be achieved by breeding against boar taint, as previous reported h^2 showed a genetic potential of both compounds (Robic et al. 2008). As has been suggested by some breeding organizations, boar taint is included into the breeding goal of selected sire breeds (BHZP GmbH; Sauter 2012; Schrade 2013). Information about an implementation of boar taint into breeding objectives of maternal nucleus populations cannot be found which indicates that there have been no activities in selection against boar taint.

Due to high genetic correlations between the boar taint compound androstenone and steroid hormones like testosterone, estrone sulfate and 17β-estradiol (Sellier et al. 2000; Grindflek et al. 2011b; Parois et al. 2015) antagonistic relationships between boar taint and fertility traits have to be expected. This is supported by common physiological pathways of androstenone and steroid hormone synthesis (Brooks and Pearson 1986). As reproduction represents an economically important trait, especially in maternal nucleus populations, breeding against boar taint could lead to a deterioration of traits from recent breeding goals in female reproduction

traits like the number of piglets born alive or age at first insemination as well as in male reproduction traits (Mathur et al. 2013). Negative relationships between boar taint and paternal fertility traits like the length of bulbourethral gland as an indicator for sexual maturation in boars has been reported by Tajet et al. (2006). Additionally, high correlations between androstenone and physiologically linked sex hormones like testosterone were found by Grindflek et al. (2011b) which indicate possible antagonisms to paternal fertility. However, in contrast to these results Strathe et al. (2013b) have estimated favorable genetic correlations between boar taint compounds and different semen traits. In a similar way impact breeding of against boar taint compounds on maternal fertility is still under discussion due to controversial results (Moe et al. 2009; Mathur et al. 2013; Hidalgo et al. 2014b). As common synthesis and high correlations affirm an interrelated control by genomic regions (Parois et al. 2015), it is important to identify genes or regions with a stimulating influence on androstenone / skatole degradation without adverse effects on both, male and female fertility (Grindflek et al. 2011b).

Therefore, the aim of this study was to investigate the relationship between boar taint compounds and reproduction traits by estimating genetic correlations and heritabilities in Landrace (LR) and Large White (LW) populations. Additionally, genome wide association analysis (GWAS) was performed per trait and breed to detect SNP marker with possible pleiotropic effects that are associated with boar taint and fertility.

3.3. Material and Methods

Phenotypes

All phenotypes related to boar taint, maternal and paternal reproduction traits were recorded within a LR and LW nucleus population of a commercial breeding organization, respectively. Pedigree information was available for all animals up to 18 generations in both breeds. The LR pedigree contained 3'331 males and 3'967 females with an average inbreeding coefficient of 0.019. The LW pedigree contained 2'410 males and 3'122 females with an average inbreeding coefficient of 0.021.

Boar taint

Purebred LR- and LW-boars were raised under the same conditions in a central testing station. A total of 1'410 LR and 1'396 LW boars was slaughtered at a constant age of 160 days in the routine process of a commercial EU-certificated abattoir. Animals were anesthetized using a 92% CO_2 atmosphere and bled by cutting the main arteries closer to the heart. Tissue samples were collected at birth for DNA extraction and genotyping. Adipose tissue samples were collected post-slaughter from the neck area at slaughterhouse and stored at -20°C until analysis. Androstenone (AND) and skatole (SKA) concentration in adipose tissue was analyzed in all samples by using a standardized stable isotope dilution analysis-headspace solid-phase microextraction-gas chromatography / mass spectrometry (SIDA-HSPM-GC/MS) (Fischer et al. 2011). Because of the skewness of AND and SKA, concentrations were log-transformed into log_AND and log_SKA for all statistical analyses. Estimated heritabilities and GWAS regarding boar taint are based on these log-transformed values.

Maternal reproduction

Maternal reproduction traits included information about number of piglets born alive (NBA), number of piglets born dead (NBD) and age at first insemination (AFI) and was routinely collected from 2'049 (LR) and 2'096 (LW) sows in 4'519 (LR) and 5'205 (LW) litters. Information about AFI was provided for 1'529 LR and 1'866 LW sows.

Paternal reproduction

Paternal reproduction information comprised the traits sperm volume (SV), sperm density measured by photometer (SP) and sperm count in billions (SC) and was collected from 1'465 (LR) and 807 (LW) boars with 41'104 (LR) and 21'935 (LW) manual observations at insemination stations.

Animal care within all herds followed the general guidelines outlined in the European animal welfare regulations.

Variance component estimation

Variance components were estimated with a multivariate approach using ASReml ® (Gilmour et al. 2015). Analyzed traits log_AND, log_SKA, NBA, NBD, AFI, SV, SC and SP were evaluated in a full multiple eight trait model in combination with the pedigree information. Residual covariance between traits that cannot be measured in the same individual like paternal and maternal fertility were fixed to 0. Breeds were analyzed separately.

Variance components were estimated by using the following polygenetic model for the boar taint compounds log_AND and log_SKA:

$$y = X\beta + Z_1u + Z_2w + e \quad (1)$$

where y contains the observed traits. The generalized linear mixed model (Eq. 1) was fitted to log_AND and log_SKA and consisted of year-season of slaughter (37 levels in LR and LW) as fixed environmental effect denoted by the vector β and animal, pen and error as random effects, represented by the vectors u, w and e, respectively. Weight and age at slaughter were used as covariates in this model. e is the vector of random residual effects. X, $Z1$ and Z_2 were the corresponding incidence matrices for the fixed effects in β and the random effects u and w, respectively.

Reproduction traits with repeated measurements are estimated by using a polygenetic model including the repeated measurements (*pe*) as a random effect:

$$y = X\beta + Z_1u + Z_3pe + e \quad (2)$$

Equation 2 for the maternal reproduction traits consisted of herd-year-season (130 levels in LR, 44 levels in LW) of litter as a fixed environmental effect represented by vector β and animal (u) and error (e) as random effects. Additionally, for the traits NBA and NBD litter number was included as a fixed effect in the model. Repeated measurements per sow were considered as a random effect for NBA and NBD in vector *pe*.

Equation 2 for the paternal reproduction traits consisted of herd-year-season of sperm sample date (58 levels in LR and LW) and station (three levels in LR and LW) as fixed environmental effects and animal as a random effect. Age of the boar at sample date was used as covariate in the model. Repeated measurements per boar were included as an additional random effect (*pe*).

For Eq. 2, X, Z_1 and Z_3 were handled as the incidence matrices for the fixed effects in β and the random effects u and pe, respectively.

Genotype data

A total of 2’729 (LR) and 2’908 (LW) pigs were also genotyped by the Illumina PorcineSNP60 BeadChip (Illumina, San Diego, CA, USA). Details about the number of genotyped animals per breed, trait and sex are reported in table 4. This data was used to perform a GWAS for boar taint compounds and reproduction traits, separated by trait and line.

Table 4: Number of genotyped animals for GWAS per trait and breed

Complex	Trait	Number of animals	Number of observations	Markers	Breed
Boar taint	log_AND, log_SKA	1’293	1’293	38’411	LR
		1’317	1’317	39’302	LW
Female reproduction	NBA, NBD	1’083	2’932	38’532	LR
		1’282	3’476	39’442	LW
Female reproduction	AFI	961	961	38’504	LR
		1’267	1’267	39’450	LW
Male reproduction	SV, SC, SP	353	11’675	37’991	LR
		309	6’913	39’089	LW

log_AND = log-transformed androstenone, log_SKA = log-transformed skatole, NBA = number of piglets born alive, NBD = number of piglets born dead, AFI = age at first insemination, SV = sperm volume, SC = sperm count in billions, SP = sperm density measured by photometer

SNPs and individuals with a call-rate of less than 0.95 and SNPs with a minor allele frequency (MAF) less than 0.05 were excluded from further analysis. The quality control was conducted with PLINK (Purcell et al. 2007). For further analysis, 2’729 LR and 2’908 LW pigs with a marker amount between 37’991 and 39’450 SNPs, depending on the trait were available. Information about the number of animals and markers per trait that was available for GWAS after quality control are shown in Table 4.

GWAS

GWAS was performed with the R-package GenABEL (Aulchenko et al. 2007b). Within the GWAS log-transformed concentrations were regarded as a phenotype for AND and SKA. Because GenABEL (Aulchenko et al. 2007b) allows only one record per animal, we have calculated an adjusted mean per sow / boar for the reproduction traits with repeated measurements (NBA, NBD, SV, SC, SP). This calculation was performed by using Model II, excluding the additive genetic effect. The resulting pe-effects of the sows / boars were interpreted as such an adjusted mean per sow / boar and were used as a new phenotype for GWAS analysis. For AFI, the raw phenotype was used.

Due to the recording and selection scheme, the sample size and structure for the trait complexes boar taint and reproduction differ. As a result, different levels of population stratification within these datasets can be observed. For AND and SKA all analyzed animals were randomly selected from the population. In both resulting LR / LW datasets, population stratification was unexplainable moderate to high as indicated by λ-values > 2.5. In order to correct for this detrimental effect the GRAMMAR approach (Aulchenko et al. 2007a) was applied. After correction, the λ-values were in an acceptable range between 1.0 and 1.05. As a first step of the GRAMMAR approach, phenotypic data was corrected as described in Model I under consideration of genomic kinship matrix. Genomic kinship was estimated by implemented functions in the GenABEL package (Aulchenko et al. 2007b). Resulting residuals from this model can be used as new phenotypes for the following association studies.

The reproduction traits were displayed by animals from the nucleus population, which represents a preselected sample set. Within these data sets the λ-values were low to moderate (< 1.5). In this situation, the genomic control (GC) approach by Devlin and Roeder (1999) was regarded as sufficient to correct for the population stratification. The following formula was applied:

$$T_{corrected} = \frac{T^2}{\lambda},$$

whereas T^2 is the empirical test statistic for each locus by a fast score test or t-test and λ is the value of population stratification. Resulting p-values were transformed by Bonferroni correction to avoid error accumulation by multiple testing. Markers with an adjusted p-value < 0.05 were handled as genome wide / chromosome wide significant. Additionally, the variance explained by the single SNP was calculated according to the transformation of the student's *t*-distribution into a *z*-distribution (Kendall et al. 1977) using following formula:

$$\text{Var [\%]} = \frac{\chi^2_{1df}}{\text{N-2+}\ \chi^2_{1df}},$$

whereas χ^2_{1df} is the test statistic of each SNP from GWAS and N the number of animals. Locations of SNPs for the analysis are in accordance with the recent pig genome sequence SusScrofa 11.1, variants are identified according to Ensembl release 95 (Zerbino et al. 2018).

3.4. Results

The number of animals, overall means and standard deviations of raw phenotypes and log-transformed data are shown in Table 5 for LR and LW, respectively. Animals were slaughtered at a mean age of 163.6 days (LR) and 165.2 days (LW). The average slaughter weight was 94.5 kg for LR and 88.9 kg for LW.

Table 5: Descriptive statistics of the analyzed traits

Trait	LR			LW		
	N	Mean	SD	N	Mean	SD
AND (ng/g in fat)	1’410	1883.72	1269.90	1’396	1284.90	1021.87
log_AND	1’410	7.32	0.69	1’396	6.90	0.73
SKA (ng/g in fat)	1’410	183.89	156.80	1’396	82.10	89.96
log_SKA	1’410	4.88	0.82	1’396	4.10	0.72
NBA	2’049	14.75	3.24	2’096	14.52	3.74
NBD	2’049	1.48	1.68	2’096	0.89	1.44
AFI (days)	1’529	254.71	13.22	1’866	274.75	53.39
SV (ml)	1’465	209.68	77.69	807	237.09	76.94
SC (count in billions)	1’465	62.91	22.67	807	62.66	22.60
SP (OD)	1’465	394.94	143.34	807	340.68	113.54

AND= androstenone, log_AND= log-transformed androstenone, SKA= skatole, log_SKA= log-transformed skatole, NBA= number of piglets born alive per litter, NBD= number of piglets born dead per litter, AFI= age at first insemination, SV= sperm volume, SC= sperm count in billions, SP = density of sperm measured by photometer (SP) in optical density (OD)

Variance component estimation

In general, estimated heritabilities and genetic correlations in this study are based on the log-transformed value of AND and SKA and were not transformed in its original scale. Variance component estimation (table 6) showed moderate to high h^2 of 0.50 for log_AND in LR (h^2 = 0.39 in LW) and of 0.52 for log_SKA in LR (h^2 = 0.32 in LW). Phenotypic correlations (r_p)

between log_AND and log_SKA were similar (r_p = 0.30) in both breeds whereas genetic correlations (r_g) were slightly different (r_g = 0.29 in LR and r_g =0.41 in LW).

Table 6**:** h^2, r_g and r_p for boar taint compounds and maternal reproduction traits (LR and LW)

	log_AND	log_SKA	NBA	NBD	AFI	Breed
log_AND	**0.50 (0.08)**	0.29 (0.12)	0.31 (0.15)	0.00 (0.16)	-0.10 (0.15)	LR
	0.39 (0.07)	0.41 (0.14)	-0.15 (0.16)	0.15 (0.19)	0.01 (0.14)	LW
log_SKA	0.32	**0.52 (0.08)**	0.18 (0.15)	0.04 (0.16)	0.36 (0.14)	LR
	0.25	**0.32 (0.07)**	-0.25 (0.16)	0.06 (0.21)	-0.34 (0.14)	LW
NBA	0.61	0.47	**0.12 (0.03)**	0.34 (0.14)	0.16 (0.13)	LR
	0.19	0.15	**0.14 (0.03)**	0.36 (0.13)	0.06 (0.10)	LW
NBD	0.14	0.12	0.14	**0.09 (0.02)**	0.14 (0.14)	LR
	0.12	0.09	0.00	**0.07 (0.02)**	0.38 (0.13)	LW
AFI	-0.04	0.13	0.01	0.02	**0.27 (0.05)**	LR
	0.00	-0.11	0.00	-0.01	**0.34 (0.05)**	LW

h^2 (± standard error) on the diagonal, r_p = phenotypic correlation under the diagonal, r_g =genetic correlation above the diagonal, log_AND = log-transformed androstenone, log_SKA = log-transformed skatole, NBA= number of piglets born alive per litter, NBD= number of piglets born dead per litter, AFI= age at first insemination

Heritabilities for NBA and NBD were in a range of 0.07 to 0.14 in both breeds (table 6). For AFI, h^2 was 0.27 for LR and 0.34 for LW. Genetic correlations between NBA and NBD and NBA and AFI did slightly differ between the breeds. In contrast to that, the r_g of NBD and AFI was nearly three times higher in LW (r_g = 0.38) than in LR (r_g = 0.14) with high standard errors in both breeds. The permanent environmental effect (pe^2) of the sow was low with 0.10 for NBA in LR (pe^2 =0.04 in LW) and 0.05 for NBD in LR (pe^2 = 0.04 in LW).

Heritabilities for sperm quality traits were mainly high in a range from 0.39 to 0.48 in both breeds (table 7). High positive r_g between SV and SC of 0.51 in LR and 0.54 in LW showed that an increase in sperm volume would result in an increase of sperm count. The sperm density was genetically highly positive correlated with the sperm count in both breeds. An increase in sperm count would hence result in a higher density of the ejaculate.

Table 7: h^2, r_g and r_p for boar taint compounds and paternal reproduction traits (LR and LW*)*

	log_AND	log_SKA	SV	SC	SP	Breed
log_AND	**0.50 (0.08)**	0.29 (0.12)	-0.18 (0.13)	-0.17 (0.14)	0.03 (0.03)	LR
	0.39 (0.07)	0.41 (0.14)	-0.25 (0.14)	-0.19 (0.15)	0.04 (0.15)	LW
log_SKA	0.32	**0.52 (0.08)**	0.04 (0.13)	0.08 (0.13)	0.06 (0.13)	LR
	0.25	**0.32 (0.07)**	0.08 (0.14)	0.37 (0.14)	0.32 (0.14)	LW
SV	0.16	0.21	**0.46 (0.01)**	0.51 (0.02)	-0.55 (0.02)	LR
	0.22	0.32	**0.44 (0.02)**	0.54 (0.03)	-0.44 (0.04)	LW
SC	-0.05	0.05	0.57	**0.43 (0.01)**	0.43 (0.03)	LR
	0.06	0.25	0.59	**0.39 (0.02)**	0.50 (0.04)	LW
SP	0.63	0.51	-0.40	0.60	**0.45 (0.01)**	LR
	0.83	0.84	-0.31	0.69	**0.48 (0.02)**	LW

h^2 (± standard error) on the diagonal, r_p = phenotypic correlation under the diagonal, r_g =genetic correlation above the diagonal, log_AND = log-transformed androstenone, log_SKA = log-transformed skatole, SV= sperm volume, SC = sperm count in billions, SP = sperm density (measured by photometer)

As shown in table 6 genetic correlation between log_AND and NBA is moderate to low in LR (r_g = 0.31) and LW (r_g = -0.15) but different in the sign. As a consequence, breeding against AND would result in a lower NBA in LR and a higher NBA in LW. The r_g between log_SKA and AFI shows another distinct difference between the breeds. While breeding against SKA seems to extend the AFI in LW (r_g = -0.34), this is the opposite in the LR breed where the correlation is moderately positive (r_g = 0.36).

Favorable genetic relationship was observed between log_AND and SV within both breeds (LW: r_g = -0.18, LW r_g = -0.25). In contrast, regarding the r_g between log_SKA and SC breeding against SKA might have unfavorable consequences for paternal fertility. However, the undesired outcomes for SC are more relevant within the LW (r_g = 0.37) than within the LR breed (r_g = 0.08). Similar results are observed in the r_g between log_SKA and SP, where the r_g of 0.32 in LW points to an unfavorable consequence for paternal fertility, whereas the r_g between these traits in LR is near 0 (r_g = 0.06).

Besides the genetic correlation between boar taint and fertility traits some other relationships between paternal and maternal fertility traits are worthwhile to mention (table 8). While r_g between SC and AFI is close to zero in LR (r_g = 0.09), these traits are moderately negative correlated in LW (r_g = -0.34). Another noticeable breed difference is observed regarding the

genetic correlation between SP and AFI. These estimates suggest that (indirect) breeding against sperm count or sperm density result in a later AFI in LW, whereas it shortens the AFI in LR. Genetic correlation between SV and NBD also indicate breed differences. Indirect breeding against SV could result in a lower NBD (r_g = 0.21) in LR, whereas no consequences in the LW can be expected as indicated by the estimated genetic correlation coefficient (r_g = - 0.07).

Table 8: h^2, r_g and r_p for paternal and maternal reproduction traits (LR and LW)

	SV	SC	SP	NBA	NBD	AFI	Breed
SV	**0.46(0.01)**	0.51(0.02)	-0.55(0.03)	-0.14 (0.12)	0.21 (0.14)	-0.11(0.12)	LR
	0.44(0.02)	0.54(0.03)	-0.44(0.04)	-0.26 (0.11)	-0.07 (0.15)	-0.05(0.12)	LW
SC	0.57	**0.43(0.01)**	0.43(0.03)	0.27 (0.13)	0.26 (0.15)	0.09(0.12)	LR
	0.59	**0.39(0.02)**	0.50(0.04)	0.04 (0.12)	0.11 (0.16)	-0.34(0.12)	LW
SP	-0.40	0.60	**0.45(0.01)**	0.40 (0.12)	0.06 (0.14)	0.26(0.12)	LR
	-0.31	0.69	**0.48(0.02)**	0.53 (0.10)	0.27 (0.15)	-0.25(0.12)	LW
NBA	0.05	0.20	0.23	**0.12 (0.03)**	0.34 (0.14)	0.16(0.13)	LR
	0.01	0.09	0.28	**0.14 (0.03)**	0.36 (0.13)	0.06(0.10)	LW
NBD	0.15	0.09	0.25	0.14	**0.09 (0.02)**	0.14(0.14)	LR
	0.13	0.10	0.38	0.00	**0.07 (0.02)**	0.38(0.13)	LW
AFI	-0.03	0.03	0.13	0.01	0.02	**0.27(0.05)**	LR
	-0.01	-0.12	-0.09	0.00	-0.01	**0.34(0.05)**	LW

h^2 (± standard error) on the diagonal, r_p = phenotypic correlation under the diagonal, r_g =genetic correlation above the diagonal, SV= sperm volume, SC = sperm count in billions, SP = sperm density (measured by photometer), NBA = number of piglets born alive, NBD = number of piglets born dead, AFI = age at first insemination

GWAS

A summary of significant associated markers per trait along with their position are presented in Additional file 1 for LR (see Additional file 1) and Additional file 2 for LW (see Additional file 2). In total, 28 markers in LR and 18 markers in LW were found to be significantly associated with log_AND, log_SKA, AFI and NBD. For all other reproduction traits, no significant markers were identified.

Androstenone

Androstenone in LR was found to be significantly associated with nine genome wide significant markers (figure 8). Additionally, 5 markers were also chromosome wide significant. Two of these markers were not mapped until now. The most important region was identified on *Sus scrofa* chromosome (SSC) 5 and is ranging from 20.9 Mb to 22.9 Mb. It contains 12 significant SNPs of which five were intron variants, one was an upstream gene variant, one was a downstream gene variant and one was a splice region variant as well as one synonymous, one 3' prime untranslated region (3'PUTR) variant and two intergenic variants. Phenotypic variance explained by a significant SNP in this region varied between 1.3% and 3.1%.

In LW one marker was found to be chromosome wide significant associated for log_AND at 48.1 Mb on SSC 17. This marker is a 3' prime untranslated region (3'PUTR) variant, explaining 1.3 % of the phenotypic variance.

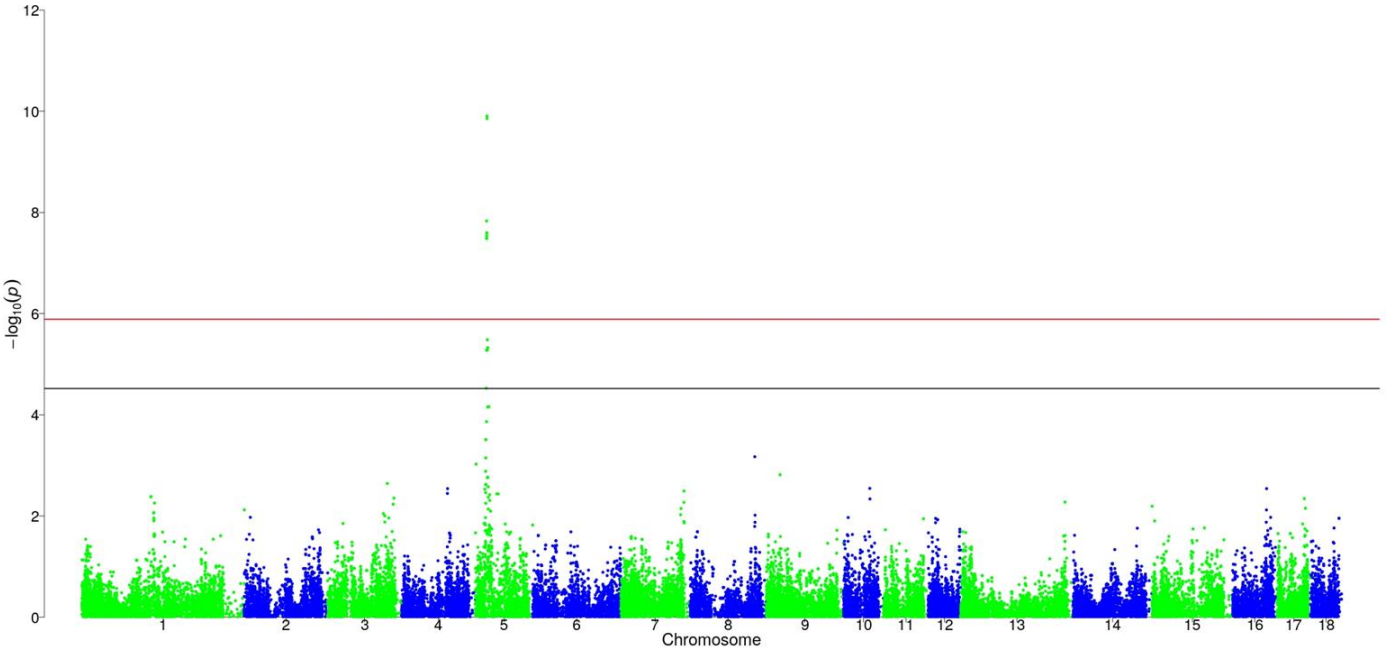

Figure 8: Distribution of SNPs for log-transformed androstenone in Landrace. Black line corresponds to the threshold of chromosome wide significance; red line corresponds to the threshold of genome wide significance.

Skatole

GWAS for log_SKA revealed two chromosome wide associations with markers on SSC 14 in LR (17 markers in LW). Both markers in LR and four markers in LW were also genome wide significant.

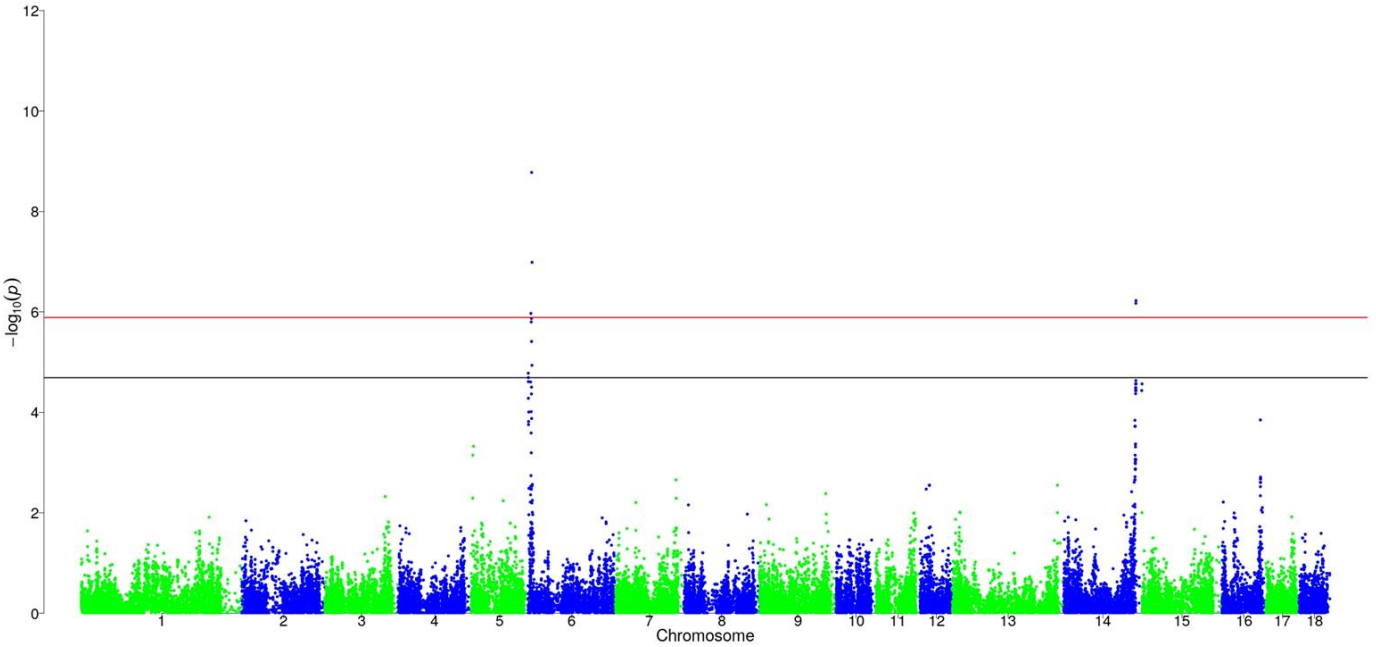

Figure 9: Distribution of SNPs for log-transformed skatole in Landrace. Black line corresponds to the threshold of chromosome wide significance; red line corresponds to the threshold of genome wide significance.

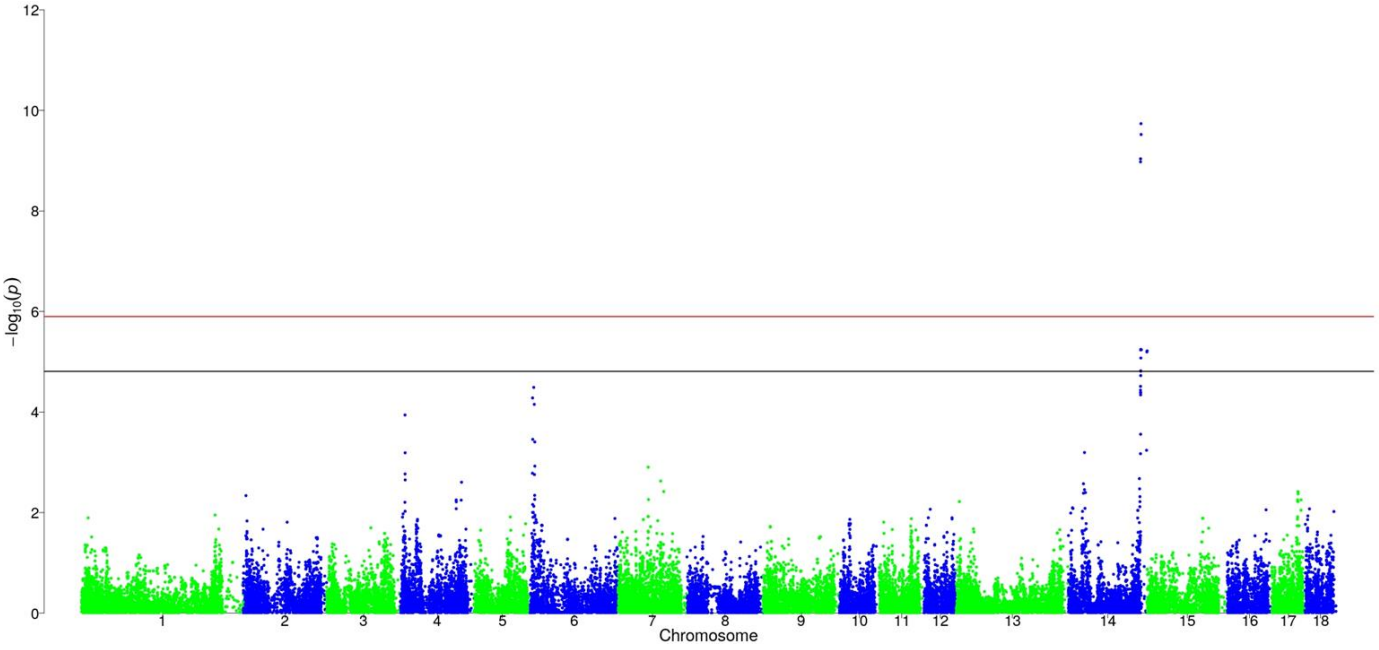

Figure 10: Distribution of SNPs for log-transformed skatole in Large White. Black line corresponds to the threshold of chromosome wide significance; red line corresponds to the threshold of genome wide significance.

All significant markers for both breeds on SSC 14 are located in a region from 140.5 Mb to 141.6 Mb (figures 9, 10), except for two markers in LW that were located around 153 Mb. An upstream gene variant of SNP *SIRI0000194* on SSC 14 was found to be genome wide

significant for both breeds as well as an intergenic variant (*ASGA0068311)*. The variance explained by a significant SNP varied between 1.5% and 2.7%.

Additionally, nine markers were found to be chromosome wide significant associated with log_SKA in LR on SSC 6 (three of them were also genome wide significant) (figure 9). These QTL were located in two delimitable regions. The first region is ranging from 0.3 Mb to 0.4 Mb containing 2 markers and the second region is ranging from 5.5 Mb to 7.5 Mb, which includes 7 markers. Explained variance by SNP in these regions was ranging from 1.3% to 2.7%.

Maternal reproduction traits

In maternal reproduction traits, significant associations were only found for NBD and AFI in LR.

For NBD one marker was identified as chromosome wide significant on SSC 1. It is an intron variant around 92.1 Mb which explains 2.9% of phenotypic variance.

GWAS for AFI revealed two chromosome wide significant markers, one on SSC 1 and one on SSC 2. The marker on SSC 1 is at 0.4 Mb and thus, is not overlapping with the detected one for NBD. The variance explained by this significant SNP was 4.1%. The significant marker on SSC 2 is located at 11.7 Mb and its variance explained by this SNP was 2.8%.

3.5. Discussion

The importance of animal welfare in pig production systems has increased which has led to a ban of surgical castration from 2021 in Germany. To achieve this ban, it is necessary to face alternatives like fattening of entire male pigs.

This alternative is only feasible if the amount of tainted carcasses of entire boars will be reduced close to zero. Hence, breeding against boar taint is an important and sustainable tool to reach this goal. However, unfavorable relationships between boar taint and fertility can be expected due to common endocrinological synthesis (Gower 1972). This study aims to reveal these relationships as well as identify genes or QTLs with possible pleiotropic effects on boar taint and fertility.

The descriptive data showed that the concentrations of AND and SKA in fat were on average much greater in LR (2’062 ng/g for AND, 188.5 ng/g for SKA) compared with LW (1’422 ng/g for AND, 77.5 ng/g for SKA).

These findings contrasts with results of Xue et al. (1996) who reported higher AND concentrations in LW than in LR. Newer studies describe LR as a breed with a high AND potential (Zamaratskaia and Squires 2009), which can be due to the breeding history of both breeds in the past 20 years. Due to e.g. individual sensitivity or product type perception thresholds of the safe box, which indicates an acceptable low risk of boar taint can vary between < 1’500 to < 3’000 ng/g for AND and < 150 to < 250 ng/g for SKA (Aluwé et al. 2018). Applying the lowest thresholds of 1’500 ng/g AND and 150 ng/g SKA, 66.2% of all LR and 33.8% of all LW boars would be classified as conspicuous. By taking into account that SKA could have a bigger impact on the perception of boar taint than AND (Bonneau et al. 2000), limiting the thresholds of only SKA to 150 ng/g and disregarding AND limits would result in a proportion of rejected carcasses of 41.1% in LR and 10.9% in LW.

Genetic background for boar taint compounds

The heritabilities in the present study for log_AND (0.50 in LR; 0.39 in LW) and log_SKA (0.52 in LR; 0.32 in LW) are in accordance to reviewed ranges in the literature of 0.25 to 0.88 for AND and 0.19 to 0.54 for SKA (Sellier et al. 2000; Engelsma et al.; Robic et al. 2008; Strathe et al. 2013a). This wide range is caused by genetically determined differences between breeds due to growth rate, backfat thickness and sexual maturation. Further development of technique and methods of the quantification of AND and SKA could play an additional role in the estimation of h^2.

The genetic correlation between log_AND and log_SKA was r_g = 0.29 in LR and around r_g = 0.41 in LW. The findings for LR are close to reported values of 0.35 and 0.36 (Tajet et al. 2006; Strathe et al. 2013a). The genetic correlation between log_AND and log_SKA is already physiologically explained by Doran et al. (2002) who described that the induction of the gene *Cytochrome P450 2E1* (CYP2E1), which is involved in the skatole metabolism can be blocked by high concentrations of AND in pig hepatocytes. As a consequence, an increasing AND concentration leads to an increasing SKA concentration, because SKA cannot be degraded by the liver anymore and accumulates in fatty tissue like backfat.

As the heritabilities of log_AND and log_SKA showed a high breeding potential for breeding against these boar taint compounds, possible negative relationships to reproduction traits have to be considered due to similar synthesis pathways (Brooks and Pearson 1986). To ascertain the extent of these possible unfavorable consequences, r_g were determined between maternal reproduction traits and boar taint compounds.

Boar taint and maternal fertility

Low heritabilities for NBA and NBD in LR are consistent with what has been reported in the literature for LR and LW (Canario et al. 2006; Hellbrügge et al. 2008). Furthermore, in LR h^2 for AFI (h^2 = 0.27) is in accordance with the reported h^2 of Mathur et al. (2013) (AFI = 0.27). The high h^2 of AFI in LW in this study (0.34) is more comparable with the h^2 of Piétrain breed (h^2 AFI = 0.34), which was also reported by Mathur et al. (2013). Some of the genetic correlations between boar taint compounds and fertility were favorable or close to zero in both breeds, like the r_g between log_AND and NBD or between log_AND and SP. However, some genetic correlations between boar taint compounds and fertility showed a non-consistent picture but indicated that there could be unfavorable relationships. For example, the r_g between log_AND and NBD in LW is unfavorable (r_g = 0.15) whereas in LR it is close to zero which is comparable to the correlation of 0.04 as reported by Mathur et al. (2013). Similar unfavorable genetic relationships are observed between log_AND and SC in both breeds or log_SKA and SC in LW which is in contrast to the results of Strathe et al. (2013b) who observed favorable relationships between boar taint compounds and semen traits.

The negative genetic correlation of -0.34 between AFI and log_SKA in LW represent the well-known unfavorable relationship between the onset of puberty and boar taint risk (Bonneau 1982; Frieden et al. 2014), however the high standard error (SE) has to be considered in the interpretation of this result. Previous reported unfavorable relationships between log_AND and AFI (Bonneau 1982; Frieden et al. 2014) were not confirmed. Genetic correlation between

log_AND and NBD in LR is zero and slightly comparable to the correlation of 0.04 between log_AND and number of stillborn as reported by Mathur et al. (2013).

Boar taint and paternal fertility

The shared synthesis pathway of AND and sex steroid hormones like testosterone may also have consequences for paternal fertility traits (Grindflek et al. 2011b). Thus, testosterone as a precursor of AND is a sex hormone which is necessary for spermatogenesis in boars (Walker 2010) due to its regulatory function on the *gonadotropin-releasing hormone* (GnRH) pulse frequency (Walker et al. 2004). In the HPA axis the GnRH pulse frequency influences the release of the *luteinizing hormone* (LH) which is required for the development of paternal and maternal maturity (Walker et al. 2004). By analyzing sperm quality parameters it has to be taken into account, that these traits are influenced to a large extent by environmental effects as age of the boar or frequency of sperm collecting (Marques et al. 2017).

Moreover, different techniques were used in the artificial insemination stations (AI-stations) to measure sperm quality parameters. As a consequence, results of the different AI-stations might have an impact on the expression of these traits. In our study estimated h^2 for paternal reproduction traits were mainly high in a range of 0.39 to 0.48. These h^2 are higher than the results of Wolf (2010) and Strathe et al. (2013a) who estimated values between 0.08 and 0.20 within the purebred Czech LR and LW pig populations (Wolf 2010) and between 0.17 and 0.31 in Danish LR boars (Strathe et al. 2013a). High h^2 for paternal reproduction traits are observed in a Piétrain crossbred study by Frieden et al. (2014). Genetic parameters between SV and SP estimated in our study indicate a distinct antagonistic genetic relationship, which is in accordance with observations in the Czech purebred pendants in the study of Wolf (2010).

In the current study, r_g between log_AND and sperm quality parameters do not seem to be unfavorable related in both breeds, as all correlations are moderate favorable or close to zero. That means that breeding against log_AND would not result in lower SV, lower SP or lower SC. Within the LR breed the low r_g between log_SKA and sperm quality parameters leads to the same conclusion as Strathe et al. (2013a) that breeding against SKA would not impair paternal fertility traits. The opposite can be observed regarding SKA and sperm quality parameters within the LW breed. Here, the genetic relationships between log_SKA and paternal reproduction traits are moderate to high unfavorable, which means that breeding against SKA could lower the genetic potential of SV, SC and SP.

However, the high SEs of all genetic correlations between boar taint compounds and paternal fertility limit the significance of our study. In addition, it should be taken into account that our

dataset does not include AI-boars with extreme negative sperm quality parameters as these boars were preselected by the AI-station.

Maternal and paternal fertility

Estimation of genetic parameters between paternal and maternal reproduction traits like SV and NBA showed a r_g of -0.14 in LR and a r_g of -0.24 in LW. These findings are in contrast to previously reported correlations in an earlier study in Czech LR and LW (Wolf 2010) which showed an r_g of -0.01 between SV and NBA in LR and an r_g of 0.21 for LW.

GWAS

Quantitative analyses showed the genetic background of the analyzed trait. Additionally, GWAS was performed to reveal possible candidate genes or genes with possible pleiotropic effects on boar taint compounds and fertility. In the present study, univariate GWAS per trait and breed showed 25 (14) markers in LR and 18 (4) markers in LW which were found to be chromosome wide (genome wide) significantly associated with one of the boar taint traits.

In LR an important region which contained 12 significantly associated markers with log_AND was identified on SSC 5 ranging from 20.9 Mb to 22.9 Mb. One of these associated markers (*ASGA0103650*) was a downstream gene variant of the gene *tachykinin 3* (TAC3). Although this gene seems to have a regulatory function in reproduction, it was excluded as a candidate gene by van Son et al. (2017) because amino acid changes did not seem to have an effect on the protein function of TAC3. Nevertheless, significant associations with markers in this QTL and log_AND in fat were already described earlier in the study of Grindflek et al. (2011a) in Duroc. Close to this region Rowe et al. (2014) reported a QTL for Danish Landrace boars for AND. Additionally, a QTL in this region was identified for testicular length and gonadosomatic index (GSI) by Große-Brinkhaus et al. (2015). It is described as an interesting, gene enriched region with possible candidate genes for AND biosynthesis (van Son et al. 2017).

In LW, one marker was found to be chromosome wide significantly associated with log_AND at 48.1 Mb on SSC 17. This variant is a 3’ prime untranslated region variant in a transcript region of the protein coding gene *PDX1 C-terminal inhibiting factor 1* (PCIF1). Until now, there are no information provided about this gene regarding consequences of mutations in pigs. Next to this region, significantly associated markers were found for AND (Rowe et al. 2014) and SKA (Große-Brinkhaus et al. 2015). However, a few studies identified significant associations on SSC 17 in other regions for traits like average daily gain (ADG) in Italian LW pigs (Fontanesi et al. 2014) or backfat thickness in LW and French LR populations (Tribout et

al. 2008). In this study the LR breed showed more significant associations with log_AND than LW. Boars of both breeds were tested in the same age-dependent performance testing scheme (160 days) of the breeding company. However, due to the higher average daily gain (ADG) of 118.5 g/day of the LR pigs, sexual maturity within this breed was more expressed. This hypothesis is in accordance with the findings of Babol et al. (2004) who proved the close relationship between ADG and begin of puberty. Beside this explanation, the higher amount of QTLs found for log_AND can be the result of breed differences, which were also postulated by Babol et al. (2004).

In combination with the moderate to high h^2 GWAS results confirmed the potential of breeding against AND, especially in LR. The region on SSC 5 seems to be important as has been shown by several authors (Walker et al. 2004; Grindflek et al. 2011a; Frieden et al. 2014; Rowe et al. 2014; Große-Brinkhaus et al. 2015; van Son et al. 2017). Within this region no pleiotropic effects on maternal and paternal fertility can be found. Although GWAS did not show any regions for log_AND or log_SKA with pleiotropic effects on maternal and paternal fertility, results of variance component estimation indicate, that there is a common genetic background of the trait complexes boar taint and fertility.

For log_SKA there are significantly associated markers in both breeds that are located close to each other in a region on SSC 14 between 140.5 Mb and 141.6 Mb. One marker (*SIRI0000194*) was shared by both breeds as a genome wide significant upstream gene variant at position 141’690’183. This marker was also identified as the most significant SNP effect on SSC 14 for SKA in a study from Rowe et al. (2014), although they used a prior version of the reference genome (Sus Scrofa 10.2). The identified shared region lies within the promoter region of the CYP2E1 gene, which is described to be involved in the SKA metabolism in several crossbred and purebred lines (Squires and Lundström 1997; Skinner et al. 2005; Moe et al. 2009; Mörlein et al. 2012; Wiercinska et al. 2012). Although there is no indicator that CYP2E1 is involved in pathways linked to reproduction traits, CYP2E1 seems to be a promising across-breed candidate gene for enhancing the SKA metabolism.

Furthermore, nine chromosome wide significant markers for log_SKA were identified only for LR on SSC 6 between 0.3 Mb to 0.4 Mb and 5.5 Mb to 7.5 Mb. Within the last-named larger region, Ramos et al. (2011) identified markers that were significantly associated with SKA. Furthermore, Grindflek et al. (2011a) characterized a breed specific QTL for SKA and Indole in Norwegian LR at the interval of 3.7-5.0 Mb on SSC 6. Additionally, several studies identified significant markers on this chromosome for AND (Grindflek et al. 2011b; Grindflek et al.

2011a; Duijvesteijn et al. 2014; Große-Brinkhaus et al. 2015). Grindflek et al. (2011b) identified a QTL for AND in Duroc on the same chromosome but in another region.

Other previously identified QTL regions for SKA or AND on SSC 6 in earlier studies (Varona et al. 2005; Grindflek et al. 2011b; Wiercinska et al. 2012; Duijvesteijn et al. 2014; Große-Brinkhaus et al. 2015) could not be confirmed by this study.

For paternal reproduction traits, no significant markers were identified. Taking into account the high h^2 of these traits this result is somewhat unexpected and can be explained by a pure polygenetic inheritance of paternal fertility traits. But as has been mentioned above, boars with extremely negative fertility are not included within the data set. Along with the limited size of the genotype data set this could serve as a further explanation of the result of our study.

For maternal reproduction traits, GWAS identified significant markers for NBD and AFI in LR. The identified marker for NBD is an intron variant around 92.1 Mb on SSC 1 in a transcript of the protein coding gene *CD109 molecule* (CD109). As there is no link to fertility or boar taint for this gene, it can be excluded as a candidate gene. The marker which was significantly associated with AFI on SSC 1 is located at 0.4 Mb. This locus does not contain any gene. Another marker on SSC 2 was significantly associated with AFI in LR and is located at 11.7 Mb within the region of the gene *syntaxin 3* (STX3), which can be excluded as a candidate gene for AFI due to his functions and pathways.

In general, GWAS showed significant regions, which differed per breed, except for the shared region for log_SKA on SSC 14. Variance component estimation as well as GWAS indicated breed differences between LR and LW population. Variance component estimation showed that unfavorable relationships between boar taint and fertility could be possible. Multivariate approaches could be an appropriate tool to further investigate possible pleiotropic effects between boar taint compounds and maternal as well as paternal fertility.

3.6. Conclusion

In conclusion, the results of the study showed contrary results for antagonistic relationships between boar taint and fertility in LR and LW breed. Therefore, the results could not serve as clear evidence that breeding for boar taint has relevant negative consequences for fertility traits in maternal breeds. In order to reduce boar taint, genomic selection in dam breeds for AND and SKA seems to be beneficial. Because no clear pleiotropic effects between boar taint and fertility were detected, this strategy is advisable without constraining effects of possible pleiotropic QTLs. However, detected antagonistic r_g between both trait complexes underline the necessity of a close monitoring of genetic changes. In case of unexpected genetic progress, selection intensity against boar taint should be lowered.

Chapter 4. Endocrine fertility parameters – genomic background and their relationship to boar taint in German Landrace and Large White

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4.1. Simple summary

Breeding against boar taint compounds androstenone and skatole can be an efficient alternative for surgical castration of young piglets during their first week of life to avoid boar taint. Physiogical links of androstenone to steroid hormones in the synthesis pathway are documented and have to be analyzed for their genetic effects on reproduction and fertility. Using boar taint and hormone data from Landrace and Large White pigs (commercial nucleus populations and herd book populations), effects of breeding against androstenone on fertility were evaluated. Moreover, the genetic foundation of the chosen hormones testosterone, 17-β estradiol, luteinizing hormone, follicle stimulating hormone and progesterone was analyzed and checked for possible pleiotropic effects with boar taint compounds. Results showed consistent unfavorable side effects of breeding against androstenone on testosterone and 17- β estradiol in both breeds. The other hormones showed contrary results regarding unfavorable relationships between boar taint and endocrine fertility parameters. Genetic foundation showed a high potential of breeding against boar taint but the impact on fertility potential should be supervised.

4.2. Abstract

Surgical castration of young male piglets without anesthesia will no longer be allowed in Germany from 2021. One alternative is breeding against boar taint but the shared synthesis pathway of androstenone (AND) and several endocrine fertility parameters (EFP) indicate a risk of decreasing fertility. The objective of this study was to investigate the genetic background between AND, skatole (SKA) and six EFP in purebred Landrace (LR) and Large White (LW) populations. Animals were clustered according to their genetic relatedness because of their different origins. Estimated heritabilities (h^2) of AND and SKA were ranged between 0.52 and 0.34 in LR and LW. For EFP h^2 differed between the breeds except for FSH (h^2: 0.28-0.37). Both breeds showed unfavorable relationships between AND and testosterone, 17-β estradiol and FSH. Genetic relationships between SKA and EFP differed between the breeds. A genome-wide association analysis revealed 48 significant associations and confirmed a region for SKA on SSC14. For EFP, results differed between the clusters. In conclusion, genetic correlations partly confirmed the physiologically expected antagonism between AND and sex steroid hormones. Particular attention should be spent on fertility traits based on EFP when breeding against boar taint to balance the genetic progress in both trait complexes.

Keywords: boar taint, fertility, hormones, pigs, androstenone, skatole, cortisol, estradiol, testosterone, luteinizing hormone, follicle stimulation hormone

4.3. Introduction

Breeding objectives of most pig breeding organizations try to balance economically important production, reproduction and fitness traits including animal welfare and meat quality aspects. In the past (< 2010) these breeding goals were dominated by traits like fat and carcass composition characteristics as well as litter size (Merks et al. 2012). As a consequence of intensive discussion with society and consumers, increased attention has been paid to animal welfare aspects, recently. This leads to changes in the breeding objective in favor of traits like prenatal survival, vitality, uniformity of the litter and robustness, which are getting as important as litter size (Merks et al. 2012).

According to the German animal protection law, castration of piglets without anesthesia is banned from year 2021 (Deutscher Bundestag 7/4/2013). Due to this legal regulation, fattening of entire boars has been an attractive alternative, not only because of animal welfare reasons but also because of improved sustainability of pig production regarding feed conversion rate, carcass composition (Lundström et al. 2009) and carbon footprint (Stefanski et al. 2018). However, piglets were castrated because of the risk of boar taint, an odorous smell of heated pork meat due to the onset of puberty. In order to lower the hazard of tainted carcasses some breeding organizations have extended their performance recording scheme and their breeding goals by boar taint traits. One main cause for the occurrence of boar taint are the sex steroid hormone androstenone (AND), which is built in the Leydig cells in the testis, and skatole (SKA), which is a product of degradation processes of the amino acid tryptophan in the colon (Zamaratskaia and Squires 2009). Although the origin of these compounds is quite different, there is a moderate positive relationship between androstenone and skatole concentration in backfat. Doran et al. (2002) explained this by decreased degradation of skatole in the liver during the presence of a high androstenone concentration.

For a sustainable prevention of boar taint, breeding against the boar taint compounds is one suitable alternative. Before implementing AND and SKA in selection strategies, possible interactions to other trait complexes like fertility have to be investigated to avoid loss of breeding progress. Previous studies (Moe et al. 2009; Mathur et al. 2013; Hidalgo et al. 2014b), showed contrary results regarding the genetic interaction between boar taint and fertility. Though, some studies are based on routinely collected traits like litter size from commercial breeding organizations, which might not allow an intensive insight into the linked hormonal regulation of boar taint, fertility and robustness.

In order to uncover this complex regulation, we have recorded the hormone profiles of sows and boars which are originating from maternal breeding lines of commercial breeding organizations. Due to the shared synthesis pathway of androstenone and steroid hormones linked to reproduction (Gower 1972; Brooks and Pearson 1986), we focused on the relationship between boar taint compounds and the synthesis of steroid hormones. Therefore, AND and SKA as well as testosterone, estradiol, luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone and cortisol were analyzed due to their relatedness to boar taint or male / female fertility and robustness. The objective of this study was to investigate possible unfavorable genetic relationships between boar taint and the six steroid hormones by variance component estimation (VCE). Additionally, QTL for these traits should be identified in male and female Landrace (LR) and Large White (LW) populations from commercial breeding and herd book organizations to clarify possible pleiotropic genetic backgrounds.

4.4. Material and Methods

Phenotypes

All phenotypes related to boar taint compounds or hormone profiles were recorded within the LR and LW nucleus populations of a commercial breeding organization (C) and a consortium of four herd book organizations (H), respectively. In the next sections these data sets were designated as LR_C, LW_C (commercial breeding organization) and LR_H, LW_H (herd book organizations). For all animals, pedigree information was available up to 15 generations.

Boar taint

A total of 3,775 boars were selected within the LR and LW nucleus population of both breeding organizations (C, H). These boars should cover the genetic variability of the LR and LW populations and were on-station performance tested. LR_C / LW_C boars (n = 1,392 / 1,377) were slaughtered at a constant age (~160 days), LR_H / LW_H boars (n = 744 / 262) at a constant weight (LR_H: 93.4 kg, LW_H: 92.6 kg) in different commercial, EU-certificated abattoirs which were connected to the participating breeding organizations. Adipose tissue samples from these boars were collected from the neck area of the carcasses 24 h after slaughtering and were stored at -20°C until analysis. AND and SKA concentration in adipose tissue was analyzed in all samples by using a standardized stable isotope dilution analysis-headspace solid-phase microextraction-gas chromatography/mass spectrometry (SIDA-HSPM-GC/MS) (Fischer et al. 2011). Because of the skewness of AND and SKA, concentrations were log-transformed for further analyses.

Hormone profiling

Blood collection was performed in accordance with an approval of the German animal protection law and the regulations for the use of animals (reference number: 84-02.04.2016.A541). Samples were taken from a subset of 500 full-sib pairs (male and female) almost equally distributed across C (n = 252) and H (n = 248). Full-sib pairs consisted of one stationary tested boar out of the boar dataset mentioned above and one sister. Blood samples in female pigs were collected at farms at a live weight of 65 to 75 kg (126.37 days on average ± 13.39). This weight range was chosen in order to characterize the hormone status of the sows before puberty. With the same motivation, male pigs were blood sampled at station on week before slaughter (164.81 days on average ± 10.7).

For blood collection, animals were fixed (< 1.5 minutes) and approximately 10 ml blood was taken from the jugular vein. After clotting at room temperature, serum was separated from each blood sample by centrifugation at 1500g for 10 minutes. Serum was stored at -80°C until assay.

Concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured using competitive ELISA Kits (Pig Follicle Stimulating Hormone (FSH) ELISA Kit; Luteinizing Hormone (LH) ELISA Kit, Abbexa Ltd). Minimum detectable concentrations using these assays were 0.469 ng/ml for LH and 14.1 ng/ml for FSH. According to the results of preliminary tests, blood samples from male animals were diluted to 1:5 for measurements of LH. All blood samples (from male and female animals) were diluted to 1:10 for measurements of FSH. Cortisol (CORT), progesterone (PROG), 17-β-estradiol (EST) and testosterone (TEST) concentrations were measured using a Multi-Species Hormone Magnetic Bead Panel (MSHMAG-21K-04 Multi-Species Mag Panel, Merck Chemicals GmbH).

Minimum detectable concentrations using this assay were 0.17 ng/ml for CORT, 0.14 ng/ml for PROG, 0.01 ng/ml for EST and 0.08 ng/ml for TEST. All values under the sensitivity threshold (censored data) were replaced by half of the minimum detectable value as described by Hornung and Reed (Hornung and Reed 1990). Due to the high number of animals with censored data, measurements of PROG for both sexes were removed for further analyses. All analyses were analyzed twice. Samples with an intra-variation coefficient (intra-CV) >30 % were excluded from further analyses due to the quality standards of the corresponding analysis protocols.

Details on the number of investigated animals with hormone profiles per breed and sex can be found in table 10. Resulting concentrations were log-transformed due to their skewed distribution. A control sample was run on every plate to control the measurement quality. It consists of pooled blood serum samples from seven full-sib boars of one litter. The inter-assay coefficients of variation for the control sample varied between 16 and 20%, depending on the analyzed hormone.

Statistical analyses – variance component estimation

Variance components were estimated with a multivariate approach using WOMBAT (Meyer 2007) across and within the breeds LR and LW. Analyzed traits were AND, SKA, CORT, EST, TEST, LH and FSH. In step 1, hormone profiles of male and female animals were treated as different traits. This can be justified by the different ages at blood sampling and the resulting different stage of maturation of both sexes. In case of genetic correlation between corresponding hormones of sows and boars close to one we were motivated to see hormones of both sexes as identical traits (step 2). Step 1 was only possible for animals of the LR population, as the sample

size of LW population was too small to analyze the data by means of a sex specific multi-trait model.

For each hormone in LR, the analysis was performed in a four-trait model, that combined pedigree information with the boar taint compounds AND and SKA and one hormone concentration, measured in males and females, respectively. Variance components for AND and SKA were estimated by using following polygenetic animal model:

$$y = X\beta + Z\alpha + Zu + e \qquad \text{Model 1}$$

where y contains the observed traits. The generalized linear mixed model was fitted to AND and SKA and comprised the fixed environmental effect organization-year-month of slaughter (82 levels in LR, 59 levels in LW) and animal and litter as random effects. Weight and age at slaughter were handled as covariates (model 1). Model 2 for the hormone profiles considered the fixed effects of organization-year season of blood sampling (21 levels in LR, 9 levels in LW) and plate. Animal and litter were implemented as random effects.

$$y = X\beta + Z\alpha + Zu + e \qquad \text{Model 2}$$

Age at blood sampling was used as covariate. X and Z were handled as the incidence matrices.

Taking into account the size of the dataset and the high standard errors, these correlations are only a first rough indicator that a large proportion of overlapping genes are involved in the expression of corresponding hormones in both sexes. Accepting these limitations, further analyses for both breeds were performed in a full multiple seven-trait trait model, including AND and SKA (following Model 1) as well as all hormone concentrations following Model 2 including sex as a fixed effect.

Genotype data

All phenotyped sows and boars (LR: 2,276 LR, LW: 1,694) were genotyped using the Illumina PorcineSNP60 BeadChip (Illumina, San Diego, CA, USA). This data was used to perform a univariate genome-wide association analysis (GWAS) for analyzed hormones AND, SKA, CORT, EST, TEST, LH and FSH.

SNPs and individuals with a call-rate of less than 95% and SNPs with a minor allele frequency (MAF) of less than 5% were excluded from further analysis. The quality control was conducted within the GenABEL package (Aulchenko et al. 2007b). Due to the removal measured hormone phenotypes with a high intra-CV (> 30%), number of genotyped animals varied per trait and sex, as shown in table 9.

GWAS

Within the GWAS, log-transformed concentrations of the analyzed hormones, AND and SKA were regarded as phenotypes. Based on visualized genetic distances, GWAS was performed within clusters, separately. A small amount of animals (n= 20) from the boar taint dataset could not be clearly classified into a cluster and were excluded from further analyses.

Based on the results of the VCE, male and female hormones were analyzed as identical traits. Association test was performed within the R-package GenABEL (Aulchenko et al. 2007b). The hormone data was corrected for sex and organization-year-season of blood sampling as fixed effects and age of blood sampling as covariate. The boar taint data was analyzed under consideration of the fixed effects described by model 1.

Population stratification ranged from 1.009 to 1.72 depending on trait and cluster, so that applying genomic control (GC) as described by Devlin and Roeder (1999) was sufficient to correct for possible population stratification using the following formula:

$$T_{corrected} = \frac{T^2}{\lambda} \qquad \text{Formula 1}$$

whereas T^2 is the empirical test statistic for each locus by a fast score test or t-test and λ is the value of population stratification. Resulting p-values were transformed by Bonferroni correction to avoid error accumulation by multiple testing. Markers with an adjusted p-value < 0.05 were handled as genome-wide / chromosome-wide significant. Additionally, the variance explained by the single SNP was calculated according to the transformation of the student's t-distribution into a z-distribution (Kendall et al. 1977) using following formula:

$$\text{Var } [\%] = \frac{\chi^2_{1df}}{N-2+\chi^2_{1df}} \qquad \text{Formula 2}$$

whereas χ^2_{1df} is the test statistic of each SNP from GWAS and N the number of animals.

Locations of SNPs for all analyzed traits are in accordance with the recent pig genome sequence SusScrofa 11.1, variants are identified according to Ensembl release 95 (Zerbino et al. 2018).

Table 9: Number of animals and markers per trait and cluster

Trait	Cluster	Number of animals	Number of marker
AND	$BT_{LR}C$*	1,293	38,411
	$BT_{LW}C$*	1,317	39,302
	$BT_{LR}H$	735	43,644
SKA	$BT_{LR}C$*	1,293	38,411
	$BT_{LW}C$*	1,317	39,302
	$BT_{LR}H$	735	43,644
CORT	$Ho_{LR}C$	254	40,176
	Ho_{LW}	271	40,972
	$Ho_{LR}H$	434	44,095
TEST	$Ho_{LR}C$	252	40,176
	Ho_{LW}	267	40,972
	$Ho_{LR}H$	423	44,095
EST	$Ho_{LR}C$	251	40,176
	Ho_{LW}	265	40,972
	$Ho_{LR}H$	415	44,095
LH	$Ho_{LR}C$	254	40,176
	Ho_{LW}	272	40,972
	$Ho_{LR}H$	417	44,095
FSH	$Ho_{LR}C$	254	40,176
	Ho_{LW}	272	40,972
	$Ho_{LR}H$	417	44,095

* = results are shown in previous study (Brinke et al. 2020), $HO_{LR}C$ = hormone cluster Landrace from a commercial breeding organization, HO_{LW} = hormone cluster Large White from a commercial breeding organization, $HO_{LR}H$ = hormone cluster Landrace from a herd book organization, AND = log-transformed androstenone, SKA = log-transformed skatole, CORT = log-transformed cortisol, TEST = log-transformed testosterone, EST = log-transformed estradiol, LH = log-transformed luteinizing hormone, FSH = log-transformed follicle stimulating hormone

4.5. Results

Descriptive summary

The number of animals, overall means and standard deviations of raw phenotypes are shown in table 10 for LR and LW, separately. Boars of the datasets LR_C and LW_C were slaughtered at an average age of 163.5±5.0 days in LR (165.2±5.7 days in LW) and an average slaughter weight of 95.1±9.5 kg in LR (89.0±8.8 kg in LW). Boars of the datasets LR_H and LW_H were slaughtered at an age of 175.1 ± 13.3 days in LR (174.1±13.1 days in LW) and weight of 93.4±6.5 kg in LR (92.6±5.8 kg in LW) on average.

Blood samples for hormone profiling from gilts and boars were taken at an average age of 126.37 days (±13.39) from gilts and at an average age of 164.81 days (±10.70) from boars, respectively.

The average CORT concentrations in LW animals were higher than in LR animals and higher in males than in females. Average TEST concentrations of male animals were clearly higher than in female animals. In general, the LR dataset showed higher TEST concentrations than the LW dataset. Concentrations of EST in female animals did not really differ between the breeds, which is in contrast to the male animals were LR animals showed higher concentrations than LW animals. Results of LH and FSH did not show any clear difference between the breeds. Whereas concentrations of LH were up to 2.5 times higher in female animals, FSH concentrations were lower in female animals than in male animals.

Regarding the boar taint compounds boars from the LR population showed higher concentration of AND and SKA, compared to the boars from the LW population.

Table 10: Descriptive statistics of the analyzed traits

Trait	Sex	Landrace			Large White		
		N	Mean	SD	N	Mean	SD
Age	female	353	128.68 ±	31.41	138	124.82 ±	7.74
	male	357	165.81 ±	12.14	148	162.31 ±	4.85
CORT	female	353	28.31 ±	13.32	137	32.71 ±	15.05
	male	357	34.03 ±	17.06	148	38.47 ±	20.74
	male+female	710	31.19 ±	15.58	285	35.70 ±	18.42
TEST	female	344	0.49 ±	1.66	133	0.15 ±	0.12
	male	353	11.53 ±	9.45	148	8.03 ±	7.56
	male+female	697	6.08 ±	8.78	281	4.30 ±	6.75
EST	female	340	0.26 ±	0.26	132	0.21 ±	0.15
	male	346	1.58 ±	1.50	147	0.97 ±	1.15
	male+female	686	0.93 ±	1.27	279	0.61 ±	0.92
LH	female	343	7.03 ±	4.27	138	7.20 ±	2.44
	male	357	2.81 ±	1.54	148	2.59 ±	1.03
	male+female	700	4.88 ±	3.82	286	4.86 ±	2.99
FSH	female	348	1,335.54 ±	767.15	138	1,355.22 ±	461.25
	male	357	1,402.29 ±	1010.81	148	1,440.00 ±	768.22
	male+female	705	1,369.34 ±	898.81	286	1,399.09 ±	639.11
AND	male	2,136	1,653.92 ±	1,509.69	1,639	1,223.45 ±	1,110.99
SKA	male	2,136	219.48 ±	252.99	1,639	93.26 ±	131.26

Age = age at sampling in days, CORT = log-transformed cortisol, TEST = log-transformed testosterone, EST = log-transformed estradiol, LH = log-transformed luteinizing hormone, FSH = log-transformed follicle stimulating hormone, AND = log-transformed androstenone, SKA = log-transformed skatole. AND, SKA concentrations are measured in ng/g fat, CORT, EST; TEST; LH, FSH concentrations are measured in ng/ml serum; SD = standard deviation.

Variance component estimation

In general, estimated heritabilities (h^2), phenotypic (r_p) and genetic (r_g) correlations are based on the log-transformed values of all parameters and were not transformed in its original scale. An overview about all results of the VCE can be found in table 12. Step 1 of the VCE showed moderate to high r_g between male and female hormone concentrations in LR in a range from 0.40 to 0.91 (see table 11).

Table 11: Genetic correlation (±SE) between male and female hormone concentrations

	CORT	TEST	EST	FSH	LH
Genetic correlation	0.80 (n.E.)	0.91 (n.E.)	0.42 (n.E.)	0.75(±0.59)	0.88(±0.43)

CORT = log-transformed cortisol, TEST = log-transformed testosterone, EST = log-transformed estradiol, FSH = log-transformed follicle-stimulating hormone, LH = log-transformed luteinizing hormone.

Therefore, further analyses for both breeds were performed in a full multiple seven-trait trait model, including AND and SKA (following Model 1) as well as all hormone concentrations following Model 2 including sex as a fixed effect as described in the material and methods section.

Variance component estimation showed moderate to high h^2 of 0.52 for AND in LR (h^2 = 0.44 in LW) and 0.40 for SKA in LR (h^2 = 0.34 in LW) (table 12). Correlations between AND and SKA were higher on the genetic scale in LW (r_g =0.57) than in LR (r_g = 0.42), whereas on the phenotypic scale, it was vice versa (r_p = 0.34 in LR, r_p = 0.27 in LW). The h^2 for CORT was 0.11 in LR and 0.35 in LW. Genetic correlation between CORT and other sexual steroid hormones was mostly different within the LR and LW breed. For example, the r_g between CORT and TEST was -0.35 in LR, but only -0.03 in LW.

Genetic correlations between CORT and EST and CORT and FSH in LR were close to zero, whereas in LW these relations were moderate to high negative between -0.26 and -0.57. As a consequence, a lower concentration of CORT would possibly lead to a higher EST and a higher FSH concentration in LW but not in LR.

Regarding the r_g between CORT and LH similar estimates were found in both breeds (r_g = -0.27 in LR, r_g = -0.42 in LW). The negative sign indicates that breeding against CORT concentrations would lead to increasing LH concentrations in both breeds.

Heritability estimates for steroid hormones were found in a range of 0.03 and 0.42 and were mostly different within the breeds LR and LW. Among the LR and LW breed, estimated h^2 for TEST, EST and LH were clearly distinguishable. In LR, h^2 for TEST and EST was small (h^2 = 0.03 and h^2 = 0.09) but in LW these estimates were moderate to high (h^2 = 0.23 and h^2 = 0.42). A converse breed difference was found for LH, where the h^2 in LR (h^2 = 0.28) was seven times higher than in LW (h^2 = 0.04). For FSH, h^2 was on a moderate level in both breeds (LR: h^2 = 0.28, LW: h^2 = 0.37).

Genetic correlations between the steroid hormones TEST, EST, LH and FSH were in a range of -0.22 and +0.89. Regarding the sign of the estimates, results are mostly consistent within LR and LW. High r_g were found between TEST and EST as well as between LH and FSH in both breeds (r_g = 0.89 / 0.91 in LR, r_g = 0.58 / 0.66 in LW). In contrast, r_g between TEST and LH and TEST and FSH were low in LR (r_g = 0.11 for both) or were close to zero in the LW breed (r_g = 0.01 and r_g = -0.06, respectively).

Possible relevant breed differences were found in the r_g between LH and EST. Genetically induced reduction of EST leads to a higher concentration of LH in LR (r_g= -0.22) but there seems to be no effect on LW (r_g = -0.04). Moreover, r_g between EST and FSH within LR and LW were different in sign (r_g = -0.13 in LR, r_g = 0.17 in LW) but did not significantly differ from zero.

Regarding the objective of our study, r_g between boar taint compounds and CORT as well as steroid hormones are of major interest.

As shown in table 12, r_g between AND and CORT was low to moderate negative in LR (r_g = -0.18) and low in LW (r_g = 0.08). As a consequence, breeding against AND would result in a small increase in CORT concentration in LR, whereas the effect in LW would be zero. A more distinct breed difference was observed for the r_g between SKA and CORT, which was negative (r_g = -0.21) in LR but moderate positive in LW (r_g = 0.38).

AND and TEST as well as SKA and TEST were highly positive correlated to each other in both breeds. The r_g between AND and TEST was 0.62 in LR and 0.83 in LW. Similar high positive r_g (=0.93) was estimated between SKA and TEST in LR, whereas this relationship was on a comparably lower level in the LW breed (r_g = 0.27). A decrease in AND and / or SKA concentration would hence result in a lower concentration of TEST in both breeds.

The estimated r_g between AND and EST slightly differed between both breeds (r_g = 0.49 in LR, r_g = 0.46 in LW). The r_g between SKA and EST was very high in LR (r_g = 0.95), but in contrast to that, the estimate of this relationship in LW did only differ slightly from zero (r_g = 0.03).

Regarding the r_g between AND and LH, similar trends were observed for LR and LW with low to moderate positive relations (r_g = 0.11 in LR, r_g = 0.32 in LW). Genetic relationships between SKA and LH are different for both breeds. In LR, a r_g of -0.16 was estimated, in LW it was the opposite with an estimated r_g of 0.45.

For AND and FSH a moderate r_g of 0.30 was estimated for both breeds. In contrast to that, r_g between SKA and FSH were on a low level ranging between 0.01 (LW) and - 0.14 (LR).

Table 12: h^2, r_g and r_p of boar taint compounds and hormone concentrations (LR and LW)

Trait	Breed	AND	SKA	CORT	TEST	EST	LH	FSH
AND	LR	**0.52 (0.07)**	0.42 (0.11)	-0.18 (0.28)	0.62 (0.91)	0.49 (0.33)	0.11 (0.20)	0.30 (0.19)
	LW	**0.44 (0.07)**	0.57 (0.12)	0.08 (0.26)	0.83 (0.34)	0.46 (0.27)	0.32 (n.E.)	0.30 (0.25)
SKA	LR	0.34	**0.40 (0.06)**	-0.21 (0.30)	0.93 (n.E.)	0.95 (0.40)	-0.16 (0.21)	-0.14 (0.20)
	LW	0.27	**0.34 (0.07)**	0.38 (0.30)	0.27 (0.36)	0.03 (0.30)	0.45 (n.E.)	0.01 (0.29)
CORT	LR	-0.01	-0.01	**0.11 (0.08)**	-0.35 (n.E.)	0.01 (0.60)	-0.27 (0.42)	0.03 (0.37)
	LW	0.02	0.02	**0.35 (0.17)**	-0.03 (0.48)	-0.26 (0.41)	-0.42 (n.E.)	-0.58 (0.38)
TEST	LR	0.32	0.28	-0.02	**0.03 (0.08)**	0.89 (n.E.)	0.11 (0.86)	0.11 (0.72)
	LW	0.52	0.29	0.12	**0.23 (0.18)**	0.58 (0.34)	0.01 (n.E.)	-0.06 (0.45)
EST	LR	0.36	0.41	0.06	0.65	**0.09 (0.08)**	-0.22 (0.42)	-0.13 (0.42)
	LW	0.47	0.27	0.04	0.76	**0.42 (0.25)**	-0.04 (n.E.)	0.17 (0.39)
LH	LR	0.10	-0.03	0.07	-0.32	-0.16	**0.28 (0.10)**	0.91 (0.17)
	LW	0.15	0.12	0.07	0.24	0.15	**0.04 (0.19)**	0.66 (n.E.)
FSH	LR	0.14	0.07	-0.08	0.14	0.17	0.42	**0.28 (0.09)**
	LW	0.20	0.03	-0.04	0.15	0.13	0.52	**0.37 (0.17)**

h^2 (± standard error) on the diagonal, r_p = phenotypic correlation under the diagonal, r_g =genetic correlation above the diagonal, AND = log-transformed androstenone, SKA = log-transformed skatole, CORT = log-transformed cortisol, TEST = log-transformed testosterone, EST = log-transformed estradiol, LH = log-transformed luteinizing hormone, FSH = log-transformed follicle-stimulating hormone

GWAS – genetic structure

Visualization of the genetic relationships of all animals showed three different clusters for the hormone dataset (figure 11). Clusters are containing LR animals from the commercial breeding organization ($Ho_{LR}C$), LR animals from the herd book organizations ($Ho_{LR}H$) and LW animals from all organizations (Ho_{LW}), whereas the latter one also contains obviously misclassified animals. Numbers of animals per cluster were 254 animals in $Ho_{LR}C$, 447 animals in $Ho_{LR}H$ and 272 animals in Ho_{LW}.

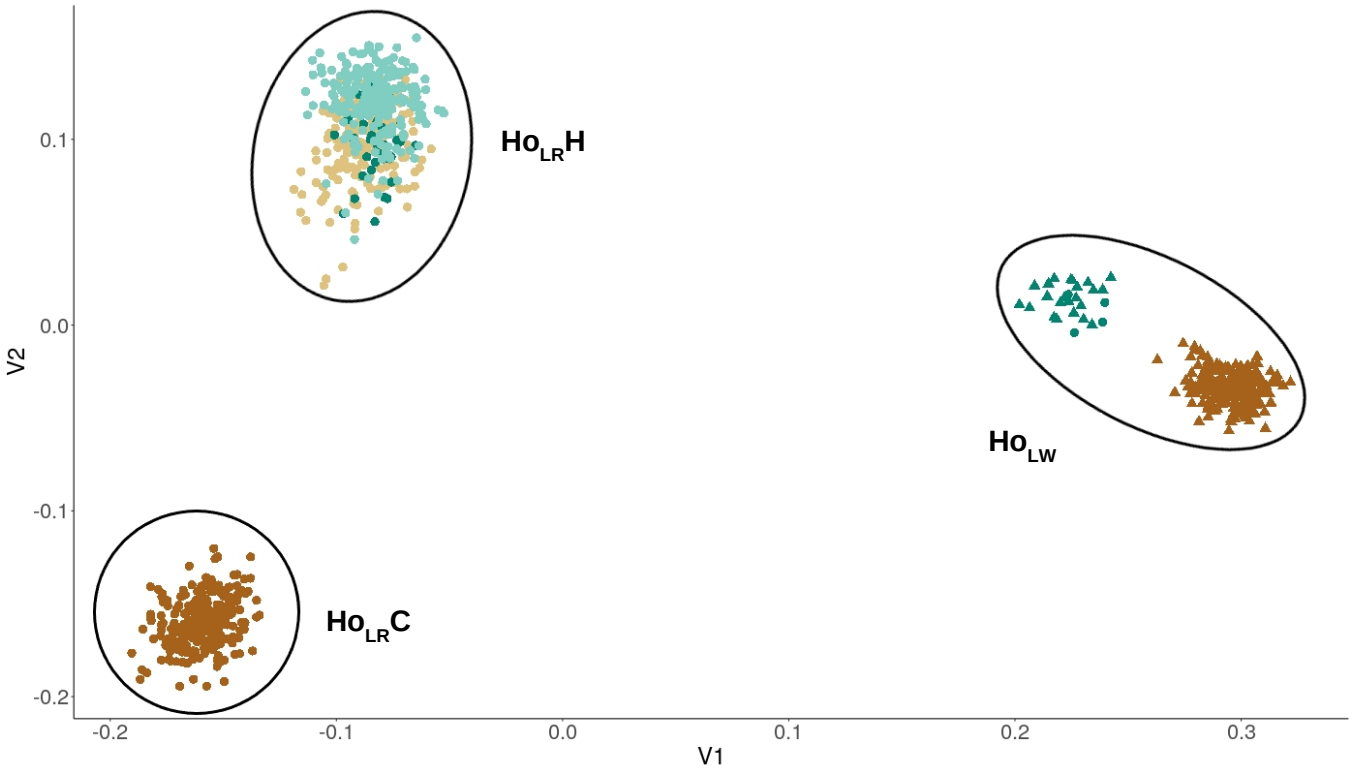

Figure 11: Distribution of animals from hormone dataset in clusters based on genetic relationship matrix, $Ho_{LR}C$ n = 254, Ho_{LW} n = 272, $Ho_{LR}H$ n = 447. Colors are representing the different organizations. Filled-in circles are representing Landrace animals; filled-in triangles are representing Large White animals.

For the boar taint dataset, visualization of the genetic relationships of all animals showed four different clusters (figure 12). 20 animals were removed from further analyses as they cannot be clearly classified into a cluster. Clusters are containing LR animals from the commercial breeding organization ($BT_{LR}C$), LW animals from the commercial breeding organization ($BT_{LW}C$), LR animals from the herd book organizations ($BT_{LR}H$) and LW animals from the

herd book organizations ($BT_{LW}H$). Numbers of animals per cluster were 1,293 animals in $BT_{LR}C$, 1,317 animals in $BT_{LW}C$, 256 in $BT_{LW}H$ and 735 in $BT_{LR}H$.

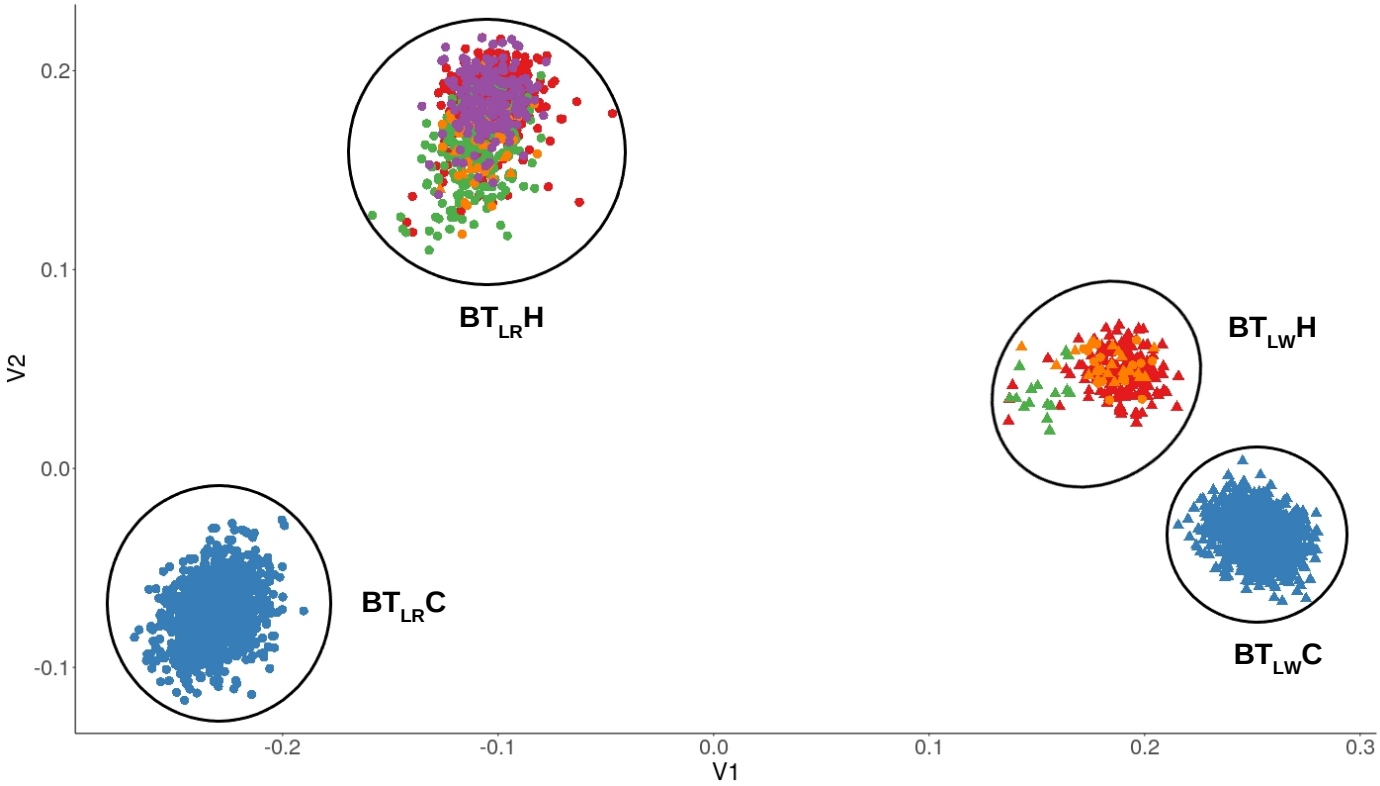

Figure 12: Distribution of animals in clusters based on genetic relationship matrix, $BT_{LR}C$ n = 1,293, $BT_{LW}C$ n = 1,317, $BT_{LW}H$ n = 256, $BT_{LR}H$ n = 735. Colors are representing the different organizations. Filled-in circles are representing Landrace animals, filled-in triangles are representing Large White animals.

Cluster $BT_{LR}C$ and $BT_{LW}C$ are representing LR and LW populations of a commercial breeding organization. GWAS of AND and SKA for these two clusters has already been performed in a previous study (Brinke et al. 2020). A short summary of the results is presented below. Sample size of cluster $BT_{LW}H$ was not sufficient enough for a GWAS (n = 256). Therefore, this study contains an association study of AND and SKA for cluster $BT_{LR}H$ which represents a LR population containing 735 boars of three herd book organizations.

GWAS for hormone profiles

A summary of the genome-wide and chromosome-wide significant associated markers per trait along with their positions for all analyzed traits in this study is presented in table 13. The full table can be found in the appendix (table S3). In total, 48 markers were found to be significantly associated with SKA, CORT, TEST, EST, LH and FSH in the different clusters whereas no QTL was found for AND. Moreover, there were no overlapping QTL regions across traits or cluster regarding an interval of 2,321,253 base pairs.

CORT was found to be significantly associated with two markers in Cluster $Ho_{LR}C$, nine markers in cluster Ho_{LW} and one marker in cluster $Ho_{LR}H$. In Cluster $Ho_{LR}C$, both markers are intron variants and located on *Sus scrofa* chromosome (SSC) 2 at 144.8 Mb. In cluster Ho_{LW}, all markers were located on SSC 7 in a region from 115.2 to 115.6 Mb, four of them were also genome-wide significant. Five variants in this region are from a customized chip, therefore the kind of variant is unknown. The remaining four variants are intron variants. The significantly associated variant in cluster $Ho_{LR}H$ is located at 36.1 Mb on SSC 18 but not mapped until now.

For FSH, one intergenic variant at 58.1 Mb on SSC 10 was found to be chromosome-wide significant associated in cluster $Ho_{LR}C$. Additionally, on SSC 7 one intron variant was significantly associated with at 117.9 Mb in cluster Ho_{LW}.

Table 13: Chromosome wide significant marker in clusters after Bonferroni correction ($p < 0.05$)

Trait	Cluster	SNP name	SSC	Position (Mb)	MAF	Variant	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol
CORT	HoLRC	ALGA0106239	2	144,8	0.15	Intron	0.088	-0.314 (±0.06)	A	NR3C1
		DRGA0017574	2	144,8	0.15	Intron	0.088	-0.314 (±0.06)	T	NR3C1
	HoLW	FBF0920*	7	115,2	0.24	-	0.087	0.274 (±0.05)		-
		CASI0004483*	7	115,3	0.17	Intron	0.115	0.374 (±0.06)	A	DDX24
		ALGA0045097	7	115,6	0.51	Intron	0.071	0.202 (±0.04)	A	alpha-1-antiproteinase-like
		FBF0965	7	115,6	0.51	-	0.069	0.199 (±0.04)		-
		FBF0971*	7	115,6	0.18	-	0.123	0.375 (±0.06)		-
		H3GA0023283	7	115,6	0.72	Intron	0.077	-0.233 (±0.05)	G	SERPINA11
		FBF0974	7	115,6	0.72	-	0.077	-0.233 (±0.05)		-
		MARC0043760*	7	115,6	0.78	Intron	0.109	-0.326 (±0.06)	G	SERPINA11
		FBF0973	7	-	0.71	-	0.076	-0.231 (±0.05)		-
	HoLRH	DIAS0003615	18	33,3	0.35	n.m.	0.038	-0.137 (±0.03)	G	-
TEST	HoLRC	ASGA0001286	1	14,9	0.57	Intron	0.081	0.183 (±0.04)	A	AKAP12
		DRGA0000172	1	15,0	0.57	Intron	0.081	0.183 (±0.04)	T	AKAP12
		ALGA0001286	1	15,0	0.43	intergenic	0.082	-0.184 (±0.04)	C	-
		ASGA0001297	1	150,6	0.43	intergenic	0.082	-0.184 (±0.04)	C	-
		ALGA0044414	7	106,5	0.04	intergenic	0.084	0.487 (±0.10)	T	-
		INRA0028035	7	113,1	0.04	n.m.	0.084	0.487 (±0.10)	C	-

Trait	Cluster	SNP name	SSC	Position (Mb)	MAF	Variant	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol
	HoLW	ASGA0073034	16	33,9	0.40	intergenic	0.074	0.250 (±0.05)	G	-
		ASGA0073036	16	34,0	0.40	intergenic	0.074	0.250 (±0.05)	A	-
		MARC0056521	16	34,2	0.39	UGV	0.079	0.263 (±0.06)	G	Granzyme K
		ALGA0116942	16	34,7	0.37	Intron	0.069	0.242 (±0.05)	C	PLPP1
		ASGA0073065	16	35,0	0.37	Intron	0.068	0.241 (±0.05)	G	IL31RA
		ALGA0090291	16	35,1	0.37	Intron	0.068	0.238 (±0.05)	T	IL6ST
		ASGA0096589	16	35,3	0.37	Intron	0.068	0.241 (±0.05)	C	ANKRD55
		SIRI0000852	16	37,0	0.41	n.m.	0.078	0.249 (±0.05)	A	-
EST	HoLRH	MARC0051573	6	69,5	0.39	Intron	0.044	-0.274 (±0.06)	T	SLC2A5
		ASGA0075694	17	19,6	0.10	NCTEV	0.048	0.445 (±0.10)	T	ENSSSCG00000048560
LH	HoLRH	ALGA0038510	7	9,5	0.21	Intron	0.043	-0.159 (±0.04)	A	PHACTR1
FSH	HoLRC	MARC0079871	10	58,1	0.01	intergenic	0.069	0.628 (±0.14)	C	-
	HoLW	DBNP0002208	7	117,9	0.19	Intron	0.065	0.146 (±0.03)	C	VRK1
SKA	BTLRH	M1GA0020074	14	140,5	0.69	UGV	0.035	-0.233 (±0.04)	A	LRRC27
		MARC0028756	14	140,6	0.69	Intron	0.035	-0.233 (±0.04)	A	LRRC27
		M1GA0020080	14	140,6	0.27	UGV	0.035	0.244 (±0.05)	C	LRRC27, PWWP2B
		M1GA0020121	14	140,9	0.27	3'PUTR	0.035	0.242 (±0.05)	T	CFAP46
		M1GA0020138	14	141,0	0.27	DGV	0.033	0.238 (±0.05)	G	ENSSSCG00000047411
		ALGA0083389	14	141,1	0.27	DGV	0.034	0.239 (±0.05)	T	ADGRA1

Trait	Cluster	SNP name	SSC	Position (Mb)	MAF	Variant	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol
		INRA0048622	14	141,1	0.27	Intron	0.034	0.239 (±0.05)	T	KNDC1
		ASGA0068302	14	141,2	0.27	UGV	0.034	0.239 (±0.05)	G	ADAM8, TUBGCP2
		H3GA0043620	14	141,2	0.27	intergenic	0.034	0.239 (±0.05)	G	-
		ASGA0068308	14	141,3	0.27	Intron	0.034	0.239 (±0.05)	G	CALY
		H3GA0043634	14	141,3	0.27	3'PUTR	0.034	0.239 (±0.05)	T	ECHS1
		H3GA0043632	14	141,3	0.27	Intron	0.034	0.239 (±0.05)	A	MTG1
		INRA0048614	14	152,9	0.27	n.m.	0.034	0.239 (±0.05)	G	-

$Ho_{LR}C$ = hormone dataset Landrace from a commercial breeding organization, Ho_{LW} = hormone dataset Large White from commercial and herd book organizations, $Ho_{LR}H$ = hormone dataset Landrace from herd book organizations, $BT_{LR}H$ = boar taint dataset Landrace from herd book organizations, CORT = log-transformed cortisol, TEST = log-transformed testosterone, EST = log-transformed estradiol, LH = log-transformed luteinizing hormone, FSH = log-transformed follicle stimulating hormone, SKA = log-transformed skatole, SSC = *Sus scrofa* chromosome, MAF = Minor allele frequency, * = also genome-wide significant, n.m. = not mapped, UGV = upstream gene variant, NCTEV = non coding transcript exon variant, 3'PUTR = 3' prime untranslated region, DGV = downstream gene variant

GWAS for TEST revealed six chromosome-wide significant markers for Cluster $Ho_{LR}C$ and eight chromosome-wide significant markers for Cluster Ho_{LW}. Two markers for cluster $Ho_{LR}C$ are located on SSC 7 at 106.5 Mb and 113.1 Mb and were an intergenic and a not mapped variant. Furthermore, four markers were located around 150 Mb on SSC 1 containing two introns and two intergenic variants.

For Cluster Ho_{LW}, seven markers were located in a region from 33.9 Mb to 35.3 Mb on SSC 16 and contained four intron variants, two intergenic variants and one upstream gene variant (UGV). Last variant was located at 37.0 Mb and was not mapped.

For EST and LH, significant markers were only found in cluster $Ho_{LR}H$. Whereas GWAS for LH showed an intron variant as chromosome-wide significant marker on SSC 7 at 9.5 Mb, GWAS for EST revealed one intron variant on SSC 6 at 69.5 Mb and one non coding transcript exon variant at 19.6 Mb on SSC 17.

GWAS for AND and SKA

For clusters $BT_{LR}C$ and $BT_{LW}C$, which were analyzed in a previous study (Brinke et al. 2020), 25 markers in LR and 18 markers in LW were found to be significantly associated with both boar taint compounds. The most important region for AND in LR was ranging from 20.9 Mb to 22.9 Mb on SSC 5 and contained 12 significant SNPs. In LW, one marker was found to be chromosome-wide significant associated with AND at 48.1 Mb on SSC 17. Associations with SKA were found in a shared region for LR and LW on SSC 14 in a region from 140.5 Mb to 141.6 Mb. Additionally, nine markers were found to be chromosome-wide significant associated with SKA in LR on SSC 6. More details about the described regions above can be found in the preceding study by Brinke et al. (2020).

For Cluster $BT_{LR}H$, analyzed in this study, no significant markers were found to be associated with AND. For SKA, 12 markers were found to be chromosome-wide significant associated on SSC 14 in a region from 140.5 to 141.3 Mb (figure 13). It contains four introns, three upstream gene variants, two downstream gene variants, two 3’ prime untranslated region variants and one intergenic variant. Ten of these markers were also found to be significantly associated with SKA in Cluster $BT_{LW}C$ in the previous study (Brinke et al. 2020). Phenotypic variance explained by a significant SNP in this region varied between 3.3% and 3.5%. Additionally, one significant marker was located at 152.9 Mb which is not mapped until now.

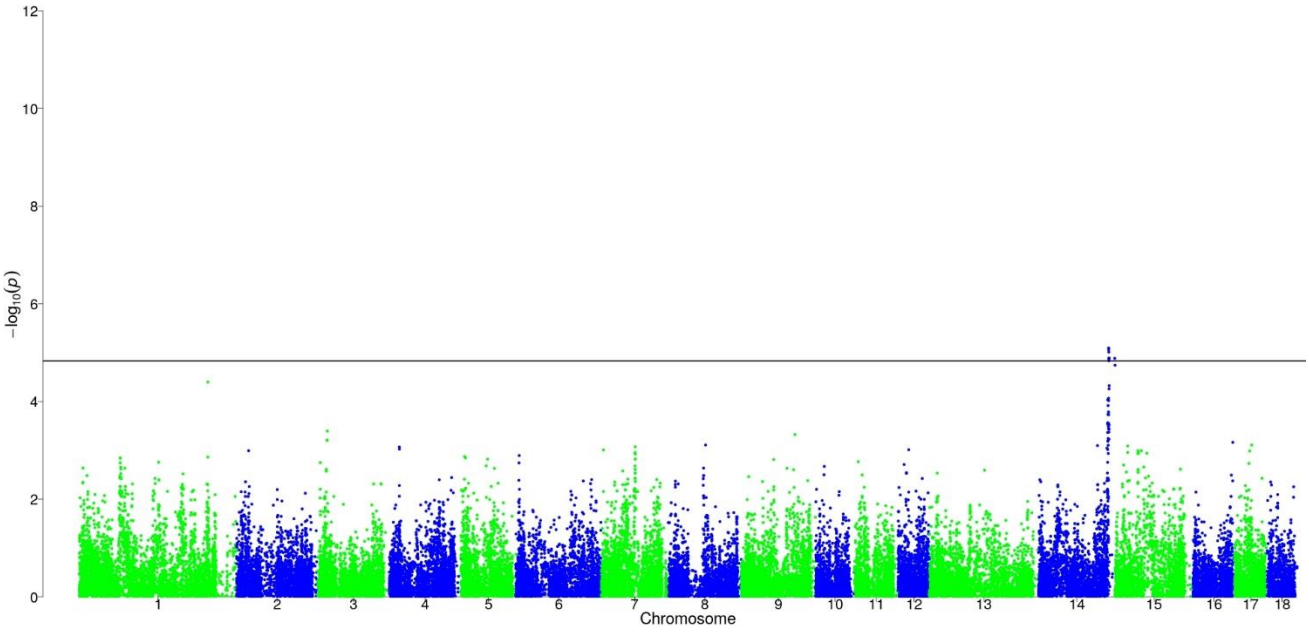

Figure 13: Distribution of SNPs for log-transformed skatole in $BT_{LR}H$. Black line corresponds to the threshold of chromosome-wide significance.

4.6. Discussion

Breeding against boar taint is a long-term, sustainable and animal welfare-friendly alternative to surgical castration of young male piglets during their first week of life. As unfavorable relationships to the fertility complex can be expected due to a shared synthesis pathway (Gower 1972; Brooks and Pearson 1986), it is important to clarify this relationships as well as to reveal possible common genetic backgrounds to avoid loss of breeding progress in fertility traits which was accomplished during the past decades. Therefore, this study aims to reveal antagonistic relationships as well as to identify genes or QTL with possible pleiotropic effects on boar taint and the analyzed endocrine parameters.

Descriptive summary for boar taint and endocrine parameters

The descriptive data showed that the concentrations of AND and SKA in fat were on average greater in LR (1653.92 ng/g for AND, 219.48 ng/g for SKA) than in LW (1223.45 ng/g for AND, 93.26 ng/g for SKA). A reason for that could be the leaner carcass composition of LW pigs in comparison to LR pigs (Bunter et al. 2008).

For the hormone concentrations, the number of analyzed LW animals was lower (n= 286) than LR animals (n= 710) (table 10) which resulted from the limited availability of purebred LW

animals. As a consequence, results, especially for LW animals, estimated parameters have to be interpreted with caution, as can be also seen from the high standard errors of the estimators.

Mean CORT concentrations in this study are in a range between 28.31 ng/ml and 38.47 ng/ml. Comparing CORT concentrations to references is not recommendable as CORT is a hormone which presence is highly influenced by many factors e.g. the individual animal, breed, or the circadian rhythm, which cannot be expressed by a single measurement (Kress et al. 2019)

Mean TEST concentration was 11.82 ± 9.53 ng/ml for LR boars and 8.17 ± 7.53 ng/ml for LW boars. This is higher than reported values of Colenbrander et al. (1978), who measured about 4 ng/ml for a Dutch LR × Yorkshire cross at similar age. Nevertheless, the increasing TEST concentration after this age has been described by Colenbrander et al. (1978) as a consequence of increasing activity in the testis. Newer studies have shown TEST concentrations in a range of of 0.73 to 3.06 ng/ml in Duroc boars depending on the season (Cheon et al. 2002) or concentrations of 6.6 ng/ml in a Pietrain × LR cross (Zoels et al. 2020). Average TEST concentration of females was 0.49 ± 1.66 in LR and 0.15 ± 0.12 in LW. The high amount of females with a TEST concentration under the sensitivity threshold of 0.08 ng/ml underlines the hypothesis that the female animals in this study were not in estrus yet.

Average EST concentrations of 1.58 ± 1.50 ng/ml in LR and 0.97 ± 1.15 in LW were greater in the male animals than in the female animals (0.26 ± 0.26 ng/ml in LR, 0.21 ± 0.15 ng/ml in LW). The comparative high concentration in male animals can be explained due to the fact, that boars produce more estrogens in the testis than other animals do (Booth 1980a). Additionally, female animals were younger and due to their PROG profile it can be assumed that these gilts were not in estrus yet.

Mean LH concentrations in this study were higher in females (7.03 ng/ml ± 4.27 in LR, 7.20 ng/ml ± 2.44 ng/ml in LW) than in males (2.81 ng/ml ± 1.54 in LR, 2.59 ng/ml ±1.03 in LW). For FSH, concentrations of male and female animals did only slightly differ. Whereas female animals showed an average FSH concentration of 1,335.54 ng/ml in LR and 1,355.22 ng/ml in LW, male animals showed FSH concentrations of 1,402.29 ng/ml in LR and 1,440.00 ng/ml in LW.

PROG was excluded from further analysis as there were too many values in the assay under the sensitivity threshold of 0.14 ng/ml. An explanation for the high amount of animals under this threshold could be their age. As PROG was earlier described as an endocrine indicator for the number of ovulations in prepubertal gilts (Wettemann et al. 1980), animals in our study may

not have reached this hormonal status of puberty as they were too young with an average age of 126 days for females and 164 days for males. Additionally, studies dealing with physiological background of PROG are often performed as a challenge study, during the estrous cycle or in pregnant or sows or gilts (Henricks et al. 1972; Hoving et al. 2017; van Wettere et al. 2018). As PROG during pregnancy plays another role than in prepubertal pigs, these studies cannot be used as references.

Variance component estimation

The results for the h^2 for boar taint compounds AND (h^2 = 0.52 in LR, h^2 = 0.44 in LW) and SKA (h^2 = 0.40 in LR, h^2 =0.34 in LW) are in accordance to previous reported ranges of 0.25 to 0.88 for AND and 0.19 to 0.54 for SKA (Sellier et al. 2000; Engelsma et al.; Robic et al. 2008; Strathe et al. 2013a). The genetic correlation between AND and SKA was r_g = 0.42 in LR and r_g = 0.57 in LW. In LR, this result is close to previously reported r_g of 0.35 and 0.36 (Tajet et al. 2006; Strathe et al. 2013a) and is comparable to results from our previous study which represents a subset of this dataset (r_g = 0.29 in LR, r_g = 0.41 in LW) (Brinke et al. 2020). The physiological point of view for this relationship is described by Doran et al. (2002) who described that SKA metabolism is inhibited by high AND concentrations in hepatocytes. Therefore, increasing AND concentrations leads to increasing SKA concentration which is reflected by the genetic correlations estimated in this study.

As the common synthesis pathway for boar taint compounds and sex steroid hormones is well known for a long time, a few studies were already conducted on the genetic relationship between boar taint and TEST and / or EST (Zamaratskaia et al. 2004; Moe et al. 2009; Grindflek et al. 2011b). But as endocrine parameters are underlying a regulatory network, it is important to not only focus on TEST or EST but also reveal the relationships of boar taint to regulatory hormones like LH, or FSH.

Regarding the results in table 12, it should be taken into account, that individual variation of these hormone concentrations are very high and the standard error of both, h^2 and r_g limits the significance of our study.

Moderate h^2 of 0.35 for CORT in LW is in accordance with what has been reported in the literature by Larzul et al. (2015) who have reported a h^2 of 0.36 for a baseline of CORT in purebred LW. A study on Swiss LR showed h^2 between 0.40 and 0.70 depending on the used model (Kadarmideen and Janss 2007), which is higher than the h^2 of 0.11 that was estimated for LR animals in this study. Genetic relationship between boar taint compounds and CORT was moderately negative in LR with a r_g = -0.18 for AND and SKA and -0.21 for SKA and

CORT. Breeding against AND or SKA could therefore result in a higher CORT concentration in LR animals.

The h^2 for TEST differed between the breeds. The moderate h^2 of 0.23 in LW is in more comparable to reported values for purebred Duroc (DU) ($h^2 = 0.19$) and a DU × LW cross ($h^2 = 0.29$) (Parois et al. 2015). This study has also reported a high r_g between AND and TEST of 0.75 in the purebred boars (Parois et al. 2015) which we can generally confirm in LR ($r_g = 0.62$), whereas the r_g in LW was higher in this study ($r_g = 0.93$). These findings are in accordance with our expectations, because the synthesis pathway of AND is along the sex steroid synthesis, where testosterone shows up as a precursor of AND (Bonneau 1982; Gower 1972). Due to the moderate to high r_g between AND and SKA, similar values were expected for r_g between SKA and TEST. In LR, r_g between AND and SKA was very high ($r_g = 0.83$) and comparable with the results of the study of Parois et al. (2015) ($r_g = 0.71$), whereas the r_g in LW was lower ($r_g = 0.27$). As TEST has been reported to be essential for spermatogenesis and male fertility in general (Walker et al. 2004; Walker 2010), breeding against AND and / or SKA would have clear unfavorable consequences on the concentration of TEST in purebred LR and LW animals.

EST is not only one of the most important estrogens for female fertility as reflected by Grindflek et al. (2011b), it is also very important for sexual behavior in boars. Genetic correlations between AND and EST have been reported in several studies in a range between 0.42 to 0.93 as summarized by Moe et al. (2009), where r_g in this study was 0.49 for LR and 0.46 for LW. In contrast to that, r_g between SKA and EST in LR was very high in this study ($r_g = 0.95$) and low in LW ($r_g = 0.03$) compared to literature references of 0.29 to 0.53 as reviewed by Moe et al. (2009). Nevertheless, it should be noted that sample size for LW animals in this study was comparably low to LR sample size which has an impact on the estimates and the standard errors.

Genetic correlation between FSH and AND was 0.30 in LR and LW, respectively, which indicates that breeding against AND would result in lower FSH concentrations. This could have relevant undesirable consequences, as has been shown by Wise et al. (1996) in Meishan pigs which were used to improve the fertility of European pig populations. Within this breed, the authors have shown that there is a negative relationship between FSH concentration in boars and increased litter size in sows. Regarding the genetic relationship between FSH and SKA, the risk of reduced fertility by breeding against SKA is small because the r_g is close to zero in LW ($r_g = 0.01$) and even favorable expressed in LR ($r_g = -0.14$).

GWAS

In this study, univariate GWAS for the analyzed hormones TEST, CORT, EST, LH and FSH was performed for three different clusters: $Ho_{LR}C$ (commercial LR population), Ho_{LW} (LW population from herd book and commercial breeding organization) and $Ho_{LR}H$ (herd book LR population). This association analysis revealed nine markers in cluster $Ho_{LR}C$, 19 (4) markers in cluster Ho_{LW} and four markers in cluster $Ho_{LR}H$ which were found to be chromosome-wide (genome-wide) significantly associated with one of the analyzed hormones.

GWAS hormones

For CORT in $Ho_{LR}C$, two intron variants were identified around 144.8 Mb on SSC 2 belonging to the gene Nuclear Receptor Subfamily 3 Group C Member 1 (*NR3C1*). This gene is known as a candidate gene for affecting the regulation of the HPA axis in pigs (Muráni et al. 2010; Muráni et al. 2012). Additionally, a polymorphism of this gene was later identified with variations in plasma cortisol levels in purebred LR and LW populations and in a Pietrain × (LW × LR) crossbred (Muráni et al. 2012).

For Ho_{LW}, a region around 115.6 Mb on SSC 7 contained significantly associated variants with CORT. One of them is an intron variant of the gene DEAD-Box Helicase 24 (*DDX24*). Although this gene is not further investigated in pig, members of this gene family are potentially involved in e.g. spermatogenesis in humans (Gashaw et al. 2005). Based on this information, *DDX24* can be regarded as a possible candidate gene for TEST concentration in pigs.

Previously reported associations with CORT and variations in CORT concentrations in a LW × Meishan cross by Désautés et al. (2002) are located on SSC 7 at 149 Mb and 156 Mb. Additionally, Ponsuksili et al. (2012) identified two regions on SSC 7 at 123.2 Mb and 85.9 Mb to be significantly associated with plasma CORT concentration in a Pietrain × (LW × LR) crossbred. A marker on SSC 18 at 33.3 Mb was identified in this study for $Ho_{LR}H$, but the position is not in accordance with results described by Désautés et al. (2002).

Within $Ho_{LR}C$ and Ho_{LW}, GWAS for TEST revealed six and eight chromosome-wide associations, respectively. For $Ho_{LR}C$, two regions were identified on SSC 1 around 15 Mb and 150.6 Mb. The first region contains two intergenic variants and two intron variants of the gene A-Kinase Anchoring Protein 12 (*AKAP12*). Until now, no specific function of this gene is known in the porcine organism. Two additional markers were identified on SSC 7 at 106.5 Mb and 113.1 Mb in $Ho_{LR}C$ affecting TEST. As the first marker is an intergenic variant and the second one is an unknown variant, no statement can be made due to their relevance for TEST.

The region on SSC 7 at 71 Mb described by Ren et al. (2009) in a F_2 LW × Erhualian intercross was not confirmed.

For the Ho_{LW} cluster, a region between 33.9 Mb to 37.0 Mb on SSC 16 was identified to be significantly associated with TEST. This region contains an upstream gene variant of Granzyme K (*GZMK*). Additionally, this region comprises four introns, two intergenic variants and one unknown variant. None of these variants are in genes that seems to play a role in TEST synthesis or regulation. Nevertheless, one intron variant is located in interleukin-6 receptor subunit beta (*IL6ST*). Variations in this gene or members of his family could be possibly involved in alterations in the expression of these receptors in porcine follicular fluid during atresia (Maeda et al. 2007).

On the same chromosome at 21 to 24 Mb, Grindflek et al. (2011a) identified QTL for TEST in Norwegian LR and Duroc. Previously identified regions on SSC 3 (Grindflek et al. 2011a), SSC 7 or SSC 13 (Ren et al. 2009) for TEST could not be confirmed in this study.

GWAS for EST showed only chromosome-wide significant associations with two markers in cluster $Ho_{LR}H$. One marker was located on SSC 6 at 69.5 Mb and was identified as an intron variant of the Solute Carrier Family 2 Member 5 (*SLC2A5*). This gene is involved in transporting fructose during pregnancy from uterus to conceptus (Steinhauser et al. 2016). Additionally it is hypothesized by Steinhauser et al. (2016) that an increasing concentration of PROG is resulting in a higher expression of *SLC2A5*. In conclusion, it cannot be hypothesized that *SLC2A5* is a specific candidate gene for EST, but for fertility in general. Associations for EST on SSC 1, 13 or 15 as reported by Grindflek et al. (2011a) could not be confirmed in our study.

For LH, one marker was found to be chromosome-wide significant associated in $Ho_{LR}H$. This marker an intron variant of Phosphatase And Actin Regulator 1 (*PHACTR1*) was located on SSC 7 at 9.5 Mb. The specific function of this gene in pigs is still unknown. In human, a homologue of this gene is associated with the risk for coronary artery diseases (Chen et al. 2019a). In a broad sense, this gene function can be linked to robustness, but not to maternal or paternal fertility characteristics.

FSH was associated with at least one marker when analyzing the clusters $Ho_{LR}C$ and Ho_{LW}, respectively. For $Ho_{LR}C$, the marker was an intergenic variant on SSC 10. For Ho_{LW}, an intron variant on SSC 7 at 117.9 Mb was identified, lying in the gene VRK Serine / Threonine Kinase 1 (*VRK1*). This gene is involved in the phosphate metabolism at least in Berkshire and Korean

native breeds (Edea and Kim 2014) but until now, it is not possible to relate this gene function to any kind of fertility influencing metabolism.

In general, GWAS of hormone data showed significant regions that differed per trait and cluster. No overlapping regions were found in the analysis of endocrine parameters, although a region on SSC 7 contained significant associations for CORT in Ho_{LW}, TEST in $Ho_{LR}C$ and FSH in Ho_{LW} in adjacent regions between 113.1 Mb to 117.9 Mb.

GWAS boar taint cluster $BT_{LR}H$

Association studies for a LR and LW population from a commercial breeding organization were already performed in a previous study (Brinke et al. 2020). This study showed genome-wide associations with AND in LR on SSC 5 and in LW on SSC 17. For SKA, GWAS showed significant associations in both breeds for the region around 141 Mb on SSC 14. Furthermore, a region on SSC 6 at 0.3 Mb to 0.4 Mb was significantly associated with SKA in LR (Brinke et al. 2020).

The results of this analysis could be partly confirmed by the GWAS in this study which was performed in the LR herd book dataset $BT_{LR}H$. Because of the small sample size, GWAS was not performed within the LW herd book dataset ($BT_{LW}H$).

GWAS for cluster $BT_{LR}H$ revealed 13 markers, which were found to be chromosome-wide significant associated with SKA. For AND, no significant associated markers were found. For SKA, 12 of the 13 identified markers are located in a region on SSC 14, ranging from 140.5 Mb to 141.3 Mb. Although none of these markers are located in genes, that are further investigated in pigs, the identified region was identified as the promotor region of the Cytochrome P450 Family 2 Subfamily E Member 1 (*CYP2E1*) gene, which is well known to be involved in SKA metabolism from several previous studies (Squires and Lundström 1997; Skinner et al. 2005; Moe et al. 2009; Mörlein et al. 2012; Wiercinska et al. 2012). Nine of these 12 markers were also found to be significant associated with SKA in the $BT_{LW}C$ cluster in the previous study (Brinke et al. 2020) which enhances the importance of this region for a genetic background for SKA.

Regarding the key question whether selection against boar taint has negative consequences on sexual hormones, knowledge about overlapping gene regions is of particular interest. However, none of the identified regions for endocrine parameters in this study are located in regions that were identified for AND or SKA or reproduction traits in our previous study (Brinke et al.

2020). Consequently, clear genomic indicators which provide evidence of an antagonistic relationship between boar taint and fertility were not found in this study.

4.7. Conclusion

In conclusion, the results showed contrary directions regarding possible unfavorable relationships between boar taint compounds and reproduction hormones in both breeds. However, in the hormones EST and TEST, which are well-known for their importance for female and male fertility, r_g are showing consistent unfavorable relationships among both breeds regarding breeding against AND. These results confirm the physiologically expected relationship also on the genetically level.

GWAS could not identify regions with pleiotropic effects on boar taint and EFP but enhances the importance of the identified region on SSC 14 for SKA. The performed GWAS for endocrine parameters revealed possible candidate genes for fertility. A region on SSC 7 between 113.1 Mb to 117.9 Mb showed pleiotropic potential for CORT, TEST, and FSH, which should be further analyzed using multivariate approaches. Although high h^2 of AND and SKA seems to be promising regarding breeding against boar taint, generally, selection should be handled with care as a deterioration of breeding progress in reproduction traits should be avoided.

Chapter 5. General discussion

In recent years many pig breeding companies have changed their breeding objective in order to focus more on the balance between animal welfare, consumers' acceptance, ecological performance and economic success. Particular ethical concerns against painful invasive methods like the surgical castration of young piglets during their first week of life constitutes new challenges for all participants of the pig production chain. As a consequence of these discussions, German legislators have changed the animal protection law in 2013 to ban surgical castration from 2019. Although this deadline has already been extended until 2021, generally accepted methods to avoid surgical castration of piglets without anesthesia are still not found and a controversial debate about alternatives is still ongoing. In order to reflect these discussions, section 5.1 summarizes the pros and cons of the most common available alternatives for surgical castration.

5.1. Alternatives for surgical castration of young male piglets without anesthetics

In the past two years following alternatives have been prepared to control the risk of tainted meat:

- Castration under general anesthetic or under local anesthetic
- Immunization against boar taint
- Fattening of entire males.

5.1.1. Castration under anesthetics

Surgical castration can be performed under the application of anesthetics. Beside a castration under general anesthetic (intramuscular injection or inhalation), a castration under local anesthetic is possible. Generally, these approaches aim to control and to eliminate occurring pain during castration. Although general anesthetics eliminate the pain during castration due to unconsciousness, it is mandatory to use analgesia after castration to prevent post-operative pain (see review Prunier et al. (2006)). Castration with intramuscularly anesthetic is usually performed with ketamine and azaperone (Bonneau and Weiler 2019). These anesthetics lead to a long recovery time of the piglet, which could increase pre-weaning losses as the piglet is e.g. not able to drink or regulate body temperature.

Castration under inhalation anesthetic is mostly conducted by using isoflurane or CO_2 / O_2. Whereas CO_2 / O_2 is discussed to be a non-efficient stress eliminator because animals showed

restlessness during anesthesia and higher cortisol levels after waking up (Kohler et al. 1998), isoflurane has been approved for castration of piglets in November 2018 by the Federal Office of Consumer Protection and Food Safety (BVL 2018).

Castration under local anesthetics implies the injection of a local anesthetic in the spermatic cord of the piglet (Bonneau and Weiler 2019). Undoubtedly, as described before, the use of a post-operative analgesia is mandatory. The used substance is often a mixture of lidocaine or procaine and adrenaline (Bonneau and Weiler 2019) to lower the toxicity of lidocaine (Prunier et al. 2006). The injection itself is suggested to cause pain and stress reaction as it has been reported by Saller et al. (2020) and Hofmann et al. (2019). The feasibility of castration of male piglets under anesthetics depends on the regulation by the Federal Ministry of Food and Agriculture (BMEL) and allows farmers with an appropriate certificate of competence to conduct the castration. The occurring costs and the workload varies strongly between the methodologies.

5.1.2. Immunization against boar taint

Another alternative to prevent boar taint, that is commonly practiced in some countries e.g. Australia, New Zealand, Brazil and Belgium is the vaccination against boar taint by an active immunization (immunocastration) which results in the synthesis of a biological production of antibodies against the natural GnRH (Brunius 2011). In Europe this alternative is only applied in Belgium (Bonneau and Weiler 2019). For a successful immunocastration, animals were vaccinated against GnRH twice in their life (first vaccination: age of 8-12 weeks, second vaccination: 4-6 weeks before slaughter).

As described in in chapter 2.2.1 (page 21), a reduction of the GnRH level leads to a decreased synthesis of steroid hormones (*inter alia* testosterone) and to a decreasing testis size. Improvac® (Zoetis Deutschland GmbH, Berlin, Germany) is an GnRH vaccine that is mostly used for immunocastration. It leads to the production of antibodies against the natural GnRH and therefore blocks the synthesis of steroid hormones. As has been shown by the predominant part of the studies, Improvac® combines the benefits from surgical castration (prevention of ranking fights and sexual aggressive behavior) and fattening of entire males (increased feed conversion and growth rate). Nevertheless, studies by Vanhonacker and Verbeke (2011) and Fredriksen et al. (2011) indicate that a relevant proportion of consumers have serious reservations against immunocastration. From a scientific point of view, the reasons for these reservations are incomprehensible and have led to an adverse position of retailers and

commercial abattoirs in Germany against immunocastration (Bundesministerium für Ernährung und Landwirtschaft (BMEL) 2016). As a consequence, immunocastrated boars are of minor importance for the pig supply chain in Germany.

5.1.3. Fattening of entire males

Entire boars have a better feed conversion, a higher growth rate due to their metabolic state, a higher proportion of lean meat (Bonneau and Desmoulin 1982; Walstra et al. 1999; Lundström et al. 2009) and a lower environmental food print (Bonneau and Weiler 2019; Dugué et al. 2020).

These advantages speak in favor of fattening of boars as the method of choice. Strategies to detect the off-odor by means of human nose scores (HNS), electronic noses or automated detection systems have been developed in recent years. Although there are remaining tasks like to improve the detection accuracy or the detection speed, these systems are able to protect the consumer from tainted pork. In order to lower the number of rejected – or as inferior classified carcasses, the potential of breeding against boar taint is high and can be used.

Thus, several projects are currently running to evaluate the potential of including breeding against boar taint in their breeding programs. Progress in breeding lines has already been made in sire breeds (BHZP GmbH; Sauter 2012; Schrade 2013) but until now, no information can be found about the implementation of breeding against boar taint in dam breeds. This is a consequence of the feared unfavorable effects as presented in chapter 2.3 where the physiological relationship of the boar taint compounds AND and SKA and the reproduction traits is emphasized due to their common synthesis background.

The high content of unsaturated fatty acids within the backfat of entire males remains an unsolved problem. From consumers' perspective the fat composition of meat from entire males is healthier (Bonneau and Weiler 2019). On the other hand, the high amount of unsaturated fatty acids leads to a softer backfat consistency which requires processing adaptations (Candek-Potokar et al. 2015) and limits the further expansion of meat from entire males in Germany.

Reduced animal welfare is another criticized problem regarding fattening of entire males. In this context, studies often report ranking fights (Rydhmer et al. 2006; Rydhmer et al. 2013) and penile injuries (Zoels et al. 2020). In order to solve this problem, housing systems has to be adapted. Reduction of stocking density, enrichment of pen facilities and the avoidance of

mixing groups when moving units (Rydhmer et al. 2013) are presumable effective to ensure acceptable animal welfare conditions.

Another frequently mentioned opportunity to avoid the risk of boar taint as well as harm- and painful consequences of ranking fights and penile injuries is to slaughter boars before the beginning of puberty. This is a common practice e.g. in Spain or Great Britain where males are slaughtered with a slaughter weight of approximately 70 kg. From a short-to-medium term perspective, this strategy is not an acceptable alternative for commercial slaughterhouses in Germany because of unpredictable economic consequences for the national and international pig supply chain.

In conclusion, some issues of the fattening of entire males have good chances for being solved by technical innovations (like the improvement of boar taint detection methods) or by enriched housing or feeding systems of entire boars. However, the adaption of pork processing systems is mandatory for successful fattening of entire males. The restricted access of pork to important export markets (Bee et al. 2020) as well as the effective reduction of tainted carcasses without adversely affecting animal health and fertility are causing further problems which are currently limiting a successful expansion of fattening of entire males in Germany.

This thesis was part of the G-I-FER project, that aims to genetically improve boar taint in maternal breeds (LR, LW) without impairing achieved and further planned progress in fertility and robustness traits. Therefore, the main target of this work was to analyze the complex genomic interactions between the trait complexes boar taint and fertility within both sexes. Furthermore, QTL with possible pleiotropic effects should be identified in order to efficiently support balanced selection strategies in pig breeding.

5.2. Relationship between boar taint compounds, fertility and robustness

Animal welfare is not only determined by the physical integrity of an animal. As described in 5.1, fattening of entire males might lead to higher rate of aggressive or sexual behavior, e.g. mounting behavior or ranking fights. These behaviors are causing stress in the animals, which is harmful with respect to animal welfare, meat quality (Foury et al. 2005; Dokmanovic et al. 2015) as well as reproduction and fertility traits (Einarsson et al. 2008).

Relationships between boar taint, fertility and stress can also be explained by linked biological pathways of involved hormones as displayed in figure 2 (page 7).

The stress potential of a pig is commonly evaluated by the baseline and fluctuation of the glucocorticoid CORT. CORT is built in the *zona fasciculata* of the adrenal cortex in the adrenal gland (Taves et al. 2011). Glucocorticoids are involved in the regulation of metabolic activity, immune function and behavior.

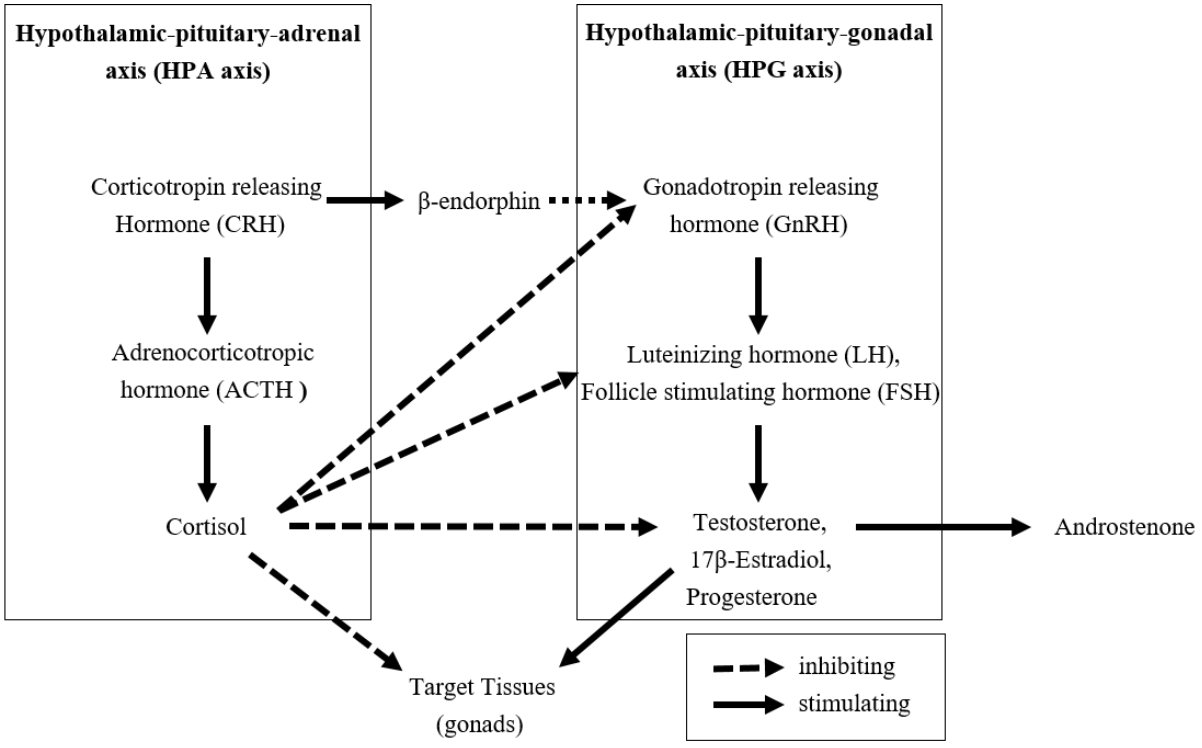

Figure 14: Interaction of the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal axis. Modified according to Rabin et al. (1988).

In general, a low CORT potential is desirable as high stress exposure leads to a lower weight gain (Parois et al. 2017; Prims et al. 2019), but there are also situations which are requiring increasing CORT levels, especially in the immune functions or anoestrous (Einarsson et al. 2008). Regulation of CORT production underlies the hypothalamic pituitary adrenal axis

(HPA-axis). In a stressful situation, more CORT is produced in the adrenal cortex (see figure 14) due to an increased release of the corticotropin-releasing hormone (CRH) and the adrenocorticotropic hormone (ACTH).

Noteworthy, it has to be mentioned, that synthesis of CORT as a glucocorticoid is following the same precursor as the sex steroids – cholesterol. As a consequence, sexual regulation and stress regulation is controlled by connected pathways. This relatedness was partly verified by the GWAS results described in chapter 4 of this thesis. A common genetic foundation was found for at least CORT, TEST and FSH with significant associations in adjacent regions between 113.1 Mb to 117.9 Mb on SSC 7 which indicates a possible pleiotropic relationship between these endocrine fertility parameters. Moreover, cholesterol as precursor in the synthesis pathway is not the only connection between stress management and reproduction. Figure 14 describes the common neuroendocrine interaction between the hypothalamic-pituitary-adrenal axis (HPA-axis) and the hypothalamic-pituitary-gonadal axis (HPG-axis).

Increasing cortisol concentration in the blood leads to the inhibition of GnRH production in the hypothalamus which leads furthermore to a reduced steroid hormone synthesis. Therefore, it is hypothesized that increased stress leads to a decreased fertility potential and (due to the linked synthesis) to a simultaneous decrease of AND production. By transferring this hypothesis to the boar taint issue, it would imply that breeding against boar taint could lead to a higher stress potential of the animal.

Under the condition of a breeding program with focus on fertility and boar taint, it could be beneficial to genetically improve or stabilize the CORT levels, which is a heuristic suggestion as a clear definition of an optimal baseline or optimal fluctuation in stress-responding CORT levels is hardly possible.

However, as has been described in chapter 4, estimated h^2 of CORT (0.11 in LR, 0.35 in LW) are moderate. These results demonstrate that CORT could be used as a measurable biomarker for stress in both breeds which can be changed by selection.

Genetic correlations between CORT and the other endocrine parameters (TEST, EST, LH, FSH) showed consistent negative values in LW in a range between -0.03 and -0.58.

In LR, these negative r_g were confirmed between between CORT and LH or TEST (rg = -0.27 ± 0.42 for LH, r_g = -0.35 ± n.E. for TEST); but were close to zero for the relationship CORT to FSH and EST (r_g = 0.03 ± 0.37 for FSH, r_g = 0.01 ± 0.60 for EST). These distinct negative r_g validate the physiological expectations due to the common neuroendocrine regulation as it is

described above. Moreover, GWAS in chapter 4 showed a region with adjacent associations for TEST in a commercial LR population and CORT and FSH in a combined LW population. This suggests a common genetic foundation which can also be explained by the above-mentioned common regulation and makes it an interesting region for fertility.

Little is known from literature about the r_g between AND and CORT. Wesoly et al. (2015) estimated a Pearson correlation between AND in fat and CORT in urine of 0.17. In this study, r_g between AND and CORT were small, but reflected the antagonistic regulation at least in LR (r_g = -0.18 ± 0.28). In LW, this r_g was close to zero.

The r_g between SKA and CORT drew a similar picture in LR (r_g = -0.21 ± 0.30), whereas the r_g in LW did not fit our expectations (r_g = 0.38 ±0.30). That means, that breeding against AND or SKA could result in a higher CORT concentration in LR animals. It has to be considered, that the significance of these results is limited by the high standard errors. Moreover, it is noteworthy to mention that CORT concentration in chapter 4 was measured once, without determining a CORT baseline for each animal.

CORT concentrations are underlying a high fluctuation due to the individual animal, sex, age, weight, and circadian rhythm (Einarsson et al. 2008; Kress et al. 2019). This results clearly show the necessity of repeated measurements of CORT at different time points or under varying stress conditions.

Despite these concerns, these results indicate, that at least monitoring of the CORT baseline or the stress response level is useful in breeding programs with a strong emphasis on boar taint and fertility. This monitoring enables to identify unexpected negative consequences on robustness at an early stage and allows breeding organizations to counteract in time.

5.3. Genetic relationships between boar taint, reproduction traits and endocrine fertility parameters in pig production

In the current situation, reproduction is a crucial trait complex for efficiency and economic success of pig production (Schneider et al. 2015). Therefore, it is of crucial importance to know whether the implementation of boar taint in selection strategies will adversely affect essential reproduction traits.

The interpretation of r_g between boar taint and fertility traits is only useful if the h^2 of these traits is above zero. Heritabilities of AND and SKA found in this study were in a range between 0.32 and 0.52. These values are in accordance to the range of estimates given by several studies, e.g. by Baes et al. (2013), Rowe et al. (2014) or Parois et al. (2015).

Heritabilities for maternal fertility were low for the traits NBA and NBA in a range between 0.07 and 0.14 for both breeds and moderate for AFI (h^2 = 0.27-0.34). Similar values were estimated by Heuß (2020) for NBA and NBD in a range between 0.08 and 0.12 by using partial overlapping data.

For paternal fertility traits, h^2 in this study were moderate to high (h^2 = 0.39-0.48). These are higher values than previously reported values for purebred LR and LW boars (Wolf 2010; Strathe et al. 2013b).

In comparison to boar taint and classical reproduction traits, little is known about the h^2 of hormones. In this study, h^2 for the hormones ranged between 0.03 and 0.42. These results are partly in accordance with literature (Kadarmideen and Janss 2007; Larzul et al. 2015; Parois et al. 2015).

In general, with a small number of exceptions in hormones, all traits showed an additive polygenetic inheritance with clearly recognizable h^2 in a wide range from 0.03 to 0.52.

Regarding the r_g between boar taint and fertility, earlier studies showed conflicting results. The hypothesis, that breeding against boar taint impairs reproduction was partly neglected (Strathe et al. 2013a; Strathe et al. 2013b; Hidalgo et al. 2014a) or considered as a minor problem (Tajet et al. 2006; Moe et al. 2009) by some studies. On the other hand, some studies (Grindflek et al. 2011b; Grindflek et al. 2011a; Frieden et al. 2014; Dugué et al. 2020) showed alarming indicators up to the extreme position of Mathur et al. (2013) who has postulated, that the problem of boar taint has only arisen by the focused selection on reproduction.

Physiologically expected antagonisms between fertility and boar taint (see chapter 2.3) are genetically induced by genes, whose expression is closely associated with both complexes. An example of this would be ESR1, which is described as a candidate gene for boar taint compounds as well as for reproduction traits (see tables 1-3, p. 17-30). SULT2A1 is also named as a candidate gene for AND (Sinclair et al. 2006; Duijvesteijn et al. 2010), but is simultaneously involved in other steroid synthesis pathways (Sinclair et al. 2006). Furthermore, different expressions of CYB5A and CYP17A1 are associated with diverging boar taint levels (Davis and Squires 1999; Moe et al. 2007; Grindflek et al. 2010; Leung et al. 2010; Gunawan et al. 2013) but also involved in the metabolism of pregnolone, earlier in the steroid synthesis pathway (Squires et al. 2019).

Regarding the results of our quantitative genetic studies, the picture of possible antagonistic relationships between boar taint and fertility is not consistent. Estimated r_g between boar taint and fertility traits are different in sign or extent between the breeds LR and LW. In LR, the r_g between AND and NBA is moderate unfavorable ($r_g = 0.31 \pm 0.15$) which is in accordance with the physiological expectation. In LW, this r_g is favorable ($r_g = -0.15 \pm 0.16$), which could be interpreted as a distinctive genetic feature of the LW breed.

Further breed differences in the r_g can be observed between AND and NBD, as the r_g was zero in LR ($r_g = 0.00 \pm 0.16$) and slightly positive in LW ($r_g = 0.15 \pm 0.19$). These results indicate no genetic risk of increased NBD. A similar result was also found by Mathur et al. (2013) in a YS and LR population ($r_g = 0.04 \pm 0.11$). In the same study, a more worrying result was observed within a purebred PI with a slightly unfavorable r_g of -0.24 (± 0.16) between AND and NBD.

In this study, r_g between AND and paternal reproduction traits were slightly negative (r_g = -0.17- -0.25) for sperm volume (SV) and sperm count (SC) and close to zero for sperm density (SP) in both breeds. From the perspective of an AI station, SP seems to be the most important of these traits. As a consequence, it can be assumed that selection against AND has no economically relevant adverse effects on paternal fertility.

Although SKA is not directly involved in the steroid synthesis or the steroid metabolism pathways, unfavorable relationships between SKA and SC ($r_g = 0.37 \pm 0.14$) / SP ($r_g = 0.32 \pm 0.14$) are of considerable importance in LW. At first glance, this result is unexpected but can be indirectly explained by the genetic influence of AND on the degradation of SKA (see chapter 2.1.3). In LR, these r_g were close to zero and are in accordance to the conclusions of Strathe et al. (2013b) who concluded, that breeding against boar taint would not impair paternal fertility.

Although these results indicate a low risk for paternal fertility, they should be handled with care. Suppliers for paternal fertility records were commercial AI stations which have implemented a quality insurance system. This ensures that all boars fulfill generally accepted sperm quality thresholds. The resulting preselection of boars might have a veiling impact on the size of the estimated genetic parameters.

In order to obtain a deeper insight into the complex common regulation of boar taint and fertility, we have also analyzed the hormone profiles of boars and their female full-sibs. From the physiological point of view, efficiency of maternal and paternal reproduction reflects the endocrine status of the animal. That means, analogical interrelations are expected between AND and the endocrine parameters, like 17β-estradiol (EST) or LH / FSH which have a direct influence on maternal fertility traits.

The results of our quantitative genetic analysis confirmed the expected antagonistic relationships between AND and EST / LH / FSH in both breeds in a range from 0.11 to 0.83.

Especially, r_g between AND and TEST was expected to be very high, as TEST is a precursor of AND. A current study of Dugué et al. (2020) showed a high r_g between AND in fat and TEST in plasma (r_g = 0.80) in a purebred PI population. Results of the recent study confirmed these findings, as r_g between AND and TEST was estimated as 0.62 in LR and 0.83 in LW. As EST is also known to be important for male fertility, r_g between AND and EST in this study showed a clear unfavorable relationship (r_g = 0.49 in LR, r_g = 0.46 in LW). This is in accordance to previously reported values in a range between 0.42 to 0.93 as summarized by Moe et al. (2009).

GWAS identified QTL for all traits (except paternal fertility traits) but in contrast to the variance component estimation, our results did not show any regions with possible pleiotropic effects on boar taint and fertility. This indicates a polygenetic cause for the antagonistic relationships.

In conclusion, r_g between boar taint compounds and endocrine fertility showed a more consistent picture regarding expected unfavorable relationships. These results are not in accordance with the partly less expressed or even favorable r_g between boar taint and the common reproduction traits in LR or LW. With the exception of CORT, the r_g between AND and all EFP were in an unfavorable moderate to high range. This clearly indicates that breeding against AND results in lower EFP concentrations. Especially for paternal fertility, the high r_g between AND and TEST is alarming, as TEST is of major importance for spermatogenesis. As a consequence, the r_g close to zero between AND and SP found in this study can hardly be used

as a valid counter argument of this genetic risk. It is more likely that this relationship found in this analysis is underestimating the true effect because of the preselection, the low number of boars and the sensitivity of sperm quality traits to environmental effects or measurement errors. For maternal fertility, no clear conclusion can be given regarding the consequences of lower EFP for common reproduction traits like NBA or NBD. Although EST, LH and FSH are important hormones for female fertility and will be all lowered by selection against AND (r_g between 0.11 and 0.49), only little is known about the relationship between NBA and the EFP. Regarding our results, selection against AND will lead to lower EST in both breeds, but only to lower NBA in LR. This enhances the complexity of these relationships and makes it difficult to assess the risk of breeding against boar taint for female fertility.

5.4. Integration of boar taint and fertility parameters in selection strategies – challenges and perspectives

Including the boar taint compounds in breeding programs is necessary to establish the fattening of entire males as a future standard solution for surgical castration. Recording AND and SKA by chemical analysis or via human nose scoring system (HNS) is challenging as both compounds are "age-limited, sex-limited and destructive" (Lukić et al. 2015). As has been realized in this study, AND and SKA can be chemically determined with a high accuracy which leads to high h^2 estimates. In contrast, records of the HNS scoring system have lower estimated h^2 and the accuracy is strongly depending on the scoring panelists (Mörlein et al. 2016). Nevertheless, HNS recording is less cost-intensive per animal than a chemical analysis. Both recording systems are bound to the system of progeny testing, which causes a prolonged generation interval. This generation interval could be shortened, if concentrations of boar taint compounds are directly measured in biopsy backfat samples of the selection candidates (Baes et al. 2013; Haberland et al. 2014). However, biopsy sampling is an invasive method which is painful and violates animal welfare. Therefore, the use of this method can hardly be accepted as a standard performance testing method.

Genomic selection for boar taint

A second promising possibility to reduce the generation interval is to implement boar taint into genomic selection (GS). Besides the reduced generation interval, GS would be appropriate to evaluate not only the sires' but also the dams' potential for boar taint earlier in life. Until now, only a limited number of studies have confirmed the eligibility of GS to solve the boar taint issue (Squires 2006; de Campos et al. 2015; Lukić et al. 2015).

The low number of animals that can be used in the training data set can be seen as a bottleneck of implementing GS in pig breeding. In case of a small sample size, low accuracies of the estimated breeding values can be expected which limits genetic progress (Kang et al. 2017). In this regard, pooling data from different populations might be a possible solution. On the other hand, mixing of different populations is not beneficial in case of distinct genetic distances between the combined populations.

In this study, we verified different scenarios of pooling different breeds and lines (Brinke et al. 2019). GS was performed for AND and SKA based on breeding values. GS accuracies were estimated by cross validation or forward prediction using the latest generation of genotyped animals and corrected for the estimated h^2 according to the method of Daetwyler et al. (2013).

In summary, highest accuracies in a range between 0.55 to 0.83 were found when GS estimation was conducted only within the data of a single population (Brinke et al. 2019). These results state that the genetic distance between animals of different dam lines used in this study is obviously too high to develop a common GS formula. Although reference populations in dam lines are small, resulting accuracies of about 60 % are sufficiently high. Against this background, GS seems to be the method of choice to improve boar taint as it is already implemented for other traits in commercial pig breeding organizations. In this situation, genotype information is used for all kind of traits so that only the costs of phenotyping boar taint have to be considered. As an important challenge, breeding organizations should focus on a rapid development of an informative reference pool. As has been demonstrated in this study, this can be efficiently achieved by geno- and phenotyping (AND / SKA) carcasses of entire males.

Regarding the statistical methodology of implementing GS, Bayesian methods should be favored for both, AND and SKA. This was demonstrated by the studies of Lukić et al. (2015) and de Campos et al. (2015) who showed higher accuracies for these methods in simulated and real data, which is explained by fact that the "exponential distribution reasonably reflect[s] the nature of the QTL effects", particularly of SKA (de Campos et al. 2015).

Consequences for fertility

The most critical and not finally solved challenge in breeding against boar taint in dam lines is the risk of deteriorating maternal and paternal fertility. As has been intensively discussed in section 5.3, r_g between AND / SKA and common reproduction traits were sporadically unfavorable, whereas r_g between AND and hormones are consistently undesirable. In general, these results cannot be used as a conclusive evidence either for or against serious consequences for fertility traits by boar taint selection. Nevertheless, the risk for impairing paternal fertility should not be neglected. As a consequence, selection strategies against boar taint should also include paternal fertility traits. For maternal fertility, a similar conclusion can be drawn, although the r_g between AND / SKA and fertility traits are not as consistent as in paternal fertility. Due to the strong focus on reproduction traits in dam lines, economic and genetic progress in both trait complexes have to be balanced.

Impact of identified QTL

The GS method utilizes each small or large genetic effect of boar taint compounds which can be found in the underlying data. Although it can be assumed that boar taint (especially AND)

has a polygenic inheritance, QTL with large effects are of particular importance. QTL might help to accelerate genetic progress but can also be used as an explanatory indicator for physiological coherences.

In this study, a few QTL for AND were found, but none of them are clearly located in a region, which is known for the synthesis, accumulation or metabolism of AND. Moreover, these QTL are found in LR or LW and have minor effect sizes. This underlines the polygenic inheritance of AND which can be utilized by GS. As no relevant pleiotropic effects were found for AND and fertility traits, there is no need to exclude (penalize) specific QTL regions for AND from GS prediction formula.

In comparison to AND, more QTL were found for SKA, which were located in two regions of moderate size. One region on SSC 14 which contains the promotor region for CYP2E1 was relevant for both breeds. As has been shown in several studies (Squires and Lundström 1997; Doran et al. 2002; Tambyrajah et al. 2004; Skinner et al. 2005; Moe et al. 2009; Mörlein et al. 2012; Wiercinska et al. 2012; Zadinová et al. 2017), CYP2E1 plays a key role in the metabolism of SKA and thus reduces the amount of stored SKA in backfat. Gene information about CYP2E1 is already located on the standard Illumina PorcineSNP60 BeadChip (Illumina, San Diego, CA, USA). Taking these arguments into account, a direct fixation on the desirable alleles would accelerate the genetic progress for SKA. Similar to AND, no regions with pleiotropic effects on fertility have been found for SKA so there is no need for excluding certain QTL SKA from GS in order to avoid negative consequences for fertility traits.

Summarizing the results of this study, the question arises, whether selection against boar taint should focus only on SKA without considering AND. There are some arguments in favor of this option: Firstly, there is at least one major QTL (CYP2E1) with a high impact available for SKA. This QTL has no obviously adverse effects on fertility and reproduction (van Son et al. 2017) and can be efficiently used for selection. Secondly, studies of Weiler et al. (2000) and Bekaert et al. (2011) showed that SKA has a much higher olfactory perception rate compared to AND. Finally, Mörlein et al. (2016) showed that SKA plays a more important role in olfactory perception of boar taint. For breeding companies, who would like to breed against boar taint without taking the risk for impairing fertility, this option could be a recommendable alternative.

Chapter 6. Conclusion

Implementation of the boar taint compounds AND and SKA in breeding programs is an essential part on the way to a future without surgical castration. This study and results from literature showed, that genetic variation and genetic potential of both compounds is sufficient to generate future populations with a lower risk of tainted carcasses of entire males. Simultaneously, studies reported possible antagonistic relationships to fertility which presents another important trait complex in pig production systems. The results presented above showed inconclusive and breed specific results regarding unfavorable relationships between AND / SKA and number of piglets born alive in LR and SKA and age at first insemination in LW. For paternal fertility traits, distinct antagonistic relationships could only be found between SKA and sperm count / sperm density in LW. However, both analyzed populations showed clear antagonistic relationships between AND and all analyzed hormones except for cortisol. SKA was only unfavorably correlated with testosterone in both breeds, 17β-estradiol in LR and LH in LW. Hence, breeding programs against boar taint should include paternal fertility and should balance breeding progress for maternal fertility traits and boar taint by appropriate economic weights. With the present data no unambiguous candidate genes for AND were found. Possible QTL regions and markers for AND were identified on SSC 5 for LR and 17 for LW. In addition to the identified regions from literature, these results underline the polygenic character of AND as it was described before. In contrast to that, GWAS for SKA identified a region in all analyzed populations that was previously identified as containing a candidate gene for SKA. This confirms the assumption that the gene CYP2E1 on SSC 14, whose promotor region lies within this region, is involved in SKA metabolism. Candidate genes or QTL regions with a possible pleiotropic effect on both, boar taint and fertility could not be found but regions with a possible pleiotropic effect within the analyzed endocrine parameters were identified.

In accordance to other production and reproduction traits, GS seems to be the method of choice to improve boar taint efficiently. AND and SKA measured in entire male progenies of nucleus boars can be used to establish informative GS reference data. As has been shown in this study, a reference data set of a size between 500 and 1000 animals results in accuracies between 0.55 to 0.83%. Regarding GS accuracies, pooling data of different dam breeds or lines was not beneficial due to the distinct genetic distances. Because no QTL with pleiotropic effects for AND / SKA and fertility traits were found, the exclusion or penalization of QTL for boar taint in the GS formula is not necessary. As mentioned above, close monitoring of fertility traits is necessary to maintain the chance of intervention in case of relevant decline in fertility traits. In case of an unacceptable regress in fertility, GS only for SKA could be recommendable.

Chapter 7. Summary

To prevent boar taint, fattening of entire males represents a long-term and sustainable alternative to surgical castration regarding several issues like animal welfare, consumers' acceptance and environmental aspects like the carbon footprint. For realizing this alternative in future, it is mandatory to reduce the number of tainted carcasses of entire males at slaughterhouse. Previous studies showed that the genetic potential for breeding against the causing compounds AND and SKA is sufficient. Until now, breeding values for boar taint are already implemented in several breeding programs of sire breeds. Due to possible expected antagonistic relationships to fertility, this breeding progress has not been made in dam breeds so far. AND is produced in the same steroid synthesis pathway as other steroid hormones like 17β-estradiol or testosterone, that are important for both, male and female fertility and reproduction.

The aim of this thesis was to analyze and discuss the interactions between the boar taint compounds AND and SKA and the trait complexes fertility and robustness with a particular emphasis on the genomic background of these traits and their genetic relationships. Additionally, traits representing boar taint, fertility and robustness were genetically analyzed in the two dam lines LR and LW. QTL with possible pleiotropic effects should be identified to assess the opportunity of a permanent integration of boar taint in selection strategies without impairing fertility or reproduction traits. Therefore, datasets from commercial breeding and herd book organizations were provided that included records from at least 4,678 LR and 3,378 LW animals.

In the first study (chapter 3), only data from the commercial breeding organization was considered. The genetic background of the boar taint compounds AND and SKA as well as the genetic foundation for the reproduction traits for both sexes was analyzed using a univariate GWAS. Moreover, a multi-trait VCE was conducted to reveal genetic relationships between the investigated traits. VCE confirmed the potential of breeding against boar taint by resulting in moderate to high h^2 for AND and SKA but showed diverging results regarding antagonistic relationships between boar taint and fertility among both breeds. GWAS results could confirm results from earlier studies regarding CYP2E1 as a candidate gene for SKA metabolism. Regions with possible pleiotropic effects on boar taint and fertility could not be identified. Considering the results from VCE, breeding against AND or SKA could be integrated in the selection process but only with a coincident monitoring of the effect on reproduction traits.

In chapter 4, endocrine fertility parameters and their relationship to AND and SKA were genetically analyzed using data from commercial breeding and herd book organizations. Again,

a multi-trait VCE for EFP, AND and SKA was conducted to revise if physiologically expected relationships are reflected in the genetic relationships. GWAS was performed to analyze possible pleiotropic effects on the genomic level. Results confirmed expected physiological relationships based on shared synthesis pathways, especially between AND and EST / TEST. Similar antagonistic relationships were observed between SKA and TEST and EST in LR and SKA and LH in LW. In general, results from VCE in chapter 4 were not completely consistent with results from chapter 3. More analyses between EFP and reproduction traits are necessary to reveal their interrelation. GWAS for SKA based on the herd book data confirmed the CYP2E1 gene again as it was already detected for both commercial breeding populations in chapter 3. Genomic univariate analyses for the EFP showed a region with possible pleiotropic potential for CORT, TEST and FSH at SSC 7 between 113.1 Mb to 117.9 Mb. Under consideration of the results from VCE, breeding against AND could impair concentrations of EST and TEST which are both important hormones for male and female fertility.

Results from various GS scenarios demonstrated, that a GS formula has to be developed breed-specific to achieve sufficiently accurate results in genomic prediction. Pooling data of different dam lines or breeds did not improve the accuracies of GS because the underlying populations were genetically too far distant.

In conclusion, including the boar taint compounds into selection strategies is possible but challenging. Whereas genetic relationships between boar taint compounds and reproduction traits showed contrary results among the breeds, analyses of relationships between AND and EFP showed the unambiguous potential of adversely affecting fertility in both breeds. As a consequence, a close monitoring of genetic changes of fertility traits is highly recommended. Based on these results, balancing the importance of boar taint and fertility traits by appropriate economic weights is necessary. To avoid any risk of an impaired fertility in dam lines, the development of a selection strategy only against SKA could be a useful alternative since SKA is less likely to affect reproduction as well as it has a higher impact on the olfactory perception of boar taint than AND.

Chapter 8. References

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Chapter 9. Appendix

Table S1: Chromosome wide significant marker in LR after Bonferroni correction ($p < 0.05$)

Trait	SNP name	SSC	Position	MAF	Variant	Empirical p-value	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol	Gene ID
log_AND	ALGA0031240	5	20‘959‘194	0.13	n.m.	2.97E-05	0.013	0.115 (±0.03)	C	-	-
	ASGA0025066	5	21‘181‘048	0.19	UGV	5.30E-06	0.016	0.104 (±0.02)	A	CD63	100155929
	ASGA0025070	5	21‘293'188	0.19	IG	5.25E-06	0.016	0.104 (±0.02)	C	-	-
	ALGA0031253*	5	21‘534‘750	0.24	3'PUTR	1.48E-08	0.024	0.119 (±0.02)	G	ESYT1	100519485
	ASGA0025077*	5	21‘606‘108	0.17	I	3.24E-08	0.023	0.131 (±0.02)	G	RNF41	100157936
	DIAS0001551*	5	21‘635‘545	0.17	I	3.24E-08	0.023	0.131 (±0.02)	C	SLC39A5	100155542
	ASGA0025075*	5	21‘678‘113	0.17	I	3.24E-08	0.023	0.131 (±0.02)	T	CS	397519
	CADI0000251*	5	21‘745‘636	0.18	syn	2.87E-08	0.023	0.131 (±0.02)	T	STAT2	396923
	ALGA0031261*	5	21‘987‘782	0.18	I	2.54E-08	0.023	0.131 (±0.02)	A	ENSSSCG00000000405	100157156
	H3GA0016074*	5	22‘066‘047	0.18	I	2.54E-08	0.023	0.131 (±0.02)	G	ENSSSCG00000026055	100157576
	ASGA0103650*	5	22‘323‘436	0.19	DGV	1.39E-10	0.031	0.147 (±0.02)	A	TAC3	492314
	DIAS0004585*	5	22‘338‘939	0.19	SRV	1.24E-10	0.031	0.147 (±0.02)	G	TAC3, MYO1A	492314, 100739662
	ASGA0025072	5	22‘969‘678	0.20	n.m.	3.29E-06	0.016	0.106 (±0.02)	G	-	-
	ASGA0025136	5	23‘898‘188	0.07	IG	4.71E-06	0.016	0.166 (±0.04)	G	-	-
log_SKA	M1GA0026184	6	378‘156	0.11	UGV	1.66E-05	0.014	0.150 (±0.04)	G	DPEP1	397196
	H3GA0055463	6	489‘077	0.11	I	2.03E-05	0.014	0.149 (±0.04)	T	ANKRD11	100627188
	H3GA0017433*	6	5‘567‘530	0.33	I	1.07E-06	0.018	-0.115 (±0.02)	A	CDH143	100126163
	ASGA0027409	6	6‘215‘657	0.43	I	1.59E-06	0.018	-0.110 (±0.02)	T	ENSSSCG00000049504	-
	M1GA0008302	6	6‘674‘723	0.34	I	1.35E-06	0.018	-0.115 (±0.02)	G	CMIP	100518307
	ALGA0034498	6	6‘854‘501	0.43	I	3.88E-06	0.016	0.106 (±0.02)	T	CMIP	100518307
	M1GA0008318*	6	6‘879‘834	0.29	I	1.67E-09	0.027	0.149 (±0.02)	C	CMIP	100518307
	MARC0006941	6	7‘526‘054	0.40	I	1.16E-05	0.015	-0.098 (±0.02)	C	CDYL2	100627470
	ASGA0094340*	6	7‘528‘105	0.48	I	1.03E-07	0.021	-0.122 (±0.02)	T	CDYL2	100627470
	ASGA0068311*	14	141'410'475	0.32	IG	6.73E-07	0.019	-0.118 (±0.02)	A	-	-

Trait	SNP name	SSC	Position	MAF	Variant	Empirical p-value	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol	Gene ID
	SIRI0000194*	14	141'690'183	0.32	UGV	5.96E-07	0.019	-0.118 (±0.02)	C	CYP2E1	403216
AFI	FBF0282TUM_custom_NGS_12*	1	400‘589	0.28	-	1.26E-10	0.042	3.480 (±0.54)	T	-	-
	FBF0302TUM_custom_NGS_32*	2	11’736’404	0.15	-	2.13E-07	0.028	3.315 (±0.64)	T	-	-
NBD	ASGA0003865	1	92’140’037	0.45	I	4.30E-06	0.029	0.051 (±0.01)	C	CD109	100155478

* = also genome wide significant ($p < 0.05$), SSC = *Sus scrofa* chromosome, n.m. = not mapped, UGV = upstream gene variant, DGV =downstream gene variant, SRV = splice region variant, I = intron variant, SYN = synonymous variant, IG = intergenic variant, 5’PUTR = 5′ prime UTR variant, 3’PUTR = 3′ prime UTR variant; Variance per SNP = explained phenotypic variance per significant SNP, SE = standard error.

Table S2: Chromosome wide significant marker in LW after Bonferroni correction ($p < 0.05$)

Trait	SNP name	SSC	Position	MAF	Variant	Empirical p-value	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol	Gene ID
log_AND	ASGA0077319	17	48‘131‘652	0.17	3‘PUTR	2.78E-05	0.013	0.105 (± 0.03)	A	PCIF1, ZNF335	100153535, 100516768
log_SKA	M1GA0020074*	14	140'598'974	0.48	UGV	9.18E-10	0.028	-0.130 (± 0.02)	A	LRRC27	100737235
	MARC0028756*	14	140'612'004	0.48	I	1.05E-09	0.028	-0.130 (± 0.02)	A	LRRC27	100737235
	M1GA0020121	14	140'916'098	0.36	3‘PUTR	1.53E-05	0.014	0.094 (± 0.02)	T	CFAP46	100158140
	ALGA0083385	14	140'967'546	0.36	I	1.53E-05	0.014	0.094 (± 0.02)	C	CFAP46	100158140
	INRA0048622	14	141'151'934	0.35	I	5.78E-06	0.015	0.099 (± 0.02)	T	KNDC1	100152900
	M1GA0020149	14	141'173'643	0.34	I	8.43E-06	0.015	0.097 (± 0.02)	G	KNDC1	100152900
	MARC0033858	14	141'191'697	0.35	UGV	5.78E-06	0.015	0.099 (± 0.02)	A	ENSSSCG00000010771	100158138
	ASGA0068302	14	141'232'780	0.35	UGV	5.78E-06	0.015	0.099 (± 0.02)	G	ADAM8	100156146
	H3GA0043620	14	141'279'745	0.35	IG	5.78E-06	0.015	0.099 (± 0.02)	G	-	-
	ASGA0068308	14	141'309'602	0.35	I	5.78E-06	0.015	0.099 (± 0.02)	G	CALY	100158118
	H3GA0043634	14	141'338'056	0.35	3’PUTR	5.78E-06	0.015	0.099 (± 0.02)	T	ECHS1	100156927
	H3GA0043632	14	141'374'711	0.35	I	5.78E-06	0.015	0.099 (± 0.02)	A	MTG1	100153322
	M1GA0020167	14	141'395'576	0.35	UGV	5.78E-06	0.015	0.099 (± 0.02)	G	ENSSSCG00000039939	100620717
	ASGA0068311*	14	141'410'475	0.47	IG	1.83E-10	0.030	-0.135 (± 0.02)	A	-	-
	SIRI0000194*	14	141'690'183	0.47	UGV	3.01E-10	0.029	-0.133 (± 0.02)	C	CYP2E1	403216
	INRA0048614	14	152'927'262	0.35	n.m.	6.36E-06	0.015	0.098 (± 0.02)	G	-	-
	UMB10000045	14	153'479'067	0.35	n.m.	6.09E-06	0.015	0.099 (± 0.02)	C	-	-

* = also genome wide significant ($p < 0.05$), SSC = *Sus scrofa* chromosome, n.m. = not mapped, UGV = upstream gene variant, DGV =downstream gene variant, SRV = splice region variant, I = intron variant, SYN = synonymous variant, IG = intergenic variant, 5’PUTR = 5′ prime UTR variant, 3’PUTR = 3′ prime UTR variant; Variance per SNP = explained phenotypic variance per significant SNP, SE = standard error.

Table S3: Chromosome wide significant marker in clusters after Bonferroni correction ($p < 0.05$)

Trait	Cluster	SNP name	SSC	Position	MAF	Variant	Empirical p-value	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol	Gene ID
CORT	HO_LR_C	ALGA0106239	2	144,841,166	0.15	Intron	3.94E-06	0.088	-0.314 (±0.06)	A	NR3C1	396740
		DRGA0017574	2	144,842,462	0.15	Intron	3.94E-06	0.088	-0.314 (±0.06)	T	NR3C1	396740
	HO_LW	FBF0920*	7	115,258,459	0.24	-	6.01E-07	0.087	0.274 (±0.05)		-	-
		CASI0004483*	7	115,347,654	0.17	Intron	5.66E-09	0.115	0.374 (±0.06)	A	DDX24	100157901
		ALGA0045097	7	115,603,615	0.51	Intron	8.57E-06	0.071	0.202 (±0.04)	A	alpha-1-antiproteinase-like	100621494
		FBF0965	7	115,613,969	0.51	-	1.07E-05	0.069	0.199 (±0.04)		-	-
		FBF0971*	7	115,643,210	0.18	-	1.56E-09	0.123	0.375 (±0.06)		-	-
		H3GA0023283	7	115,666,493	0.72	Intron	3.44E-06	0.077	-0.233 (±0.05)	G	SERPINA11	100155953
		FBF0974	7	115,678,076	0.72	-	3.44E-06	0.077	-0.233 (±0.05)		-	-
		MARC0043760*	7	115,679,840	0.78	Intron	1.71E-08	0.109	-0.326 (±0.06)	G	SERPINA11	100155953
		FBF0973	7	-	0.71	-	4.05E-06	0.076	-0.231 (±0.05)		-	-
	HO_LR_H	DIAS0003615	18	33,314,578-	0.35	n.m.	3.91E-05	0.038	-0.137 (±0.03)	G	-	-
TEST	HO_LR_C	ASGA0001286	1	14,988,130	0.57	Intron	6.63E-06	0.081	0.183 (±0.04)	A	AKAP12	100152595
		DRGA0000172	1	15,010,885	0.57	Intron	6.63E-06	0.081	0.183 (±0.04)	T	AKAP12	100152595
		ALGA0001286	1	15,081,855	0.43	intergenic	5.80E-06	0.082	-0.184 (±0.04)	C	-	-
		ASGA0001297	1	150,645,589	0.43	intergenic	5.80E-06	0.082	-0.184 (±0.04)	C	-	-
		ALGA0044414	7	106,555,323	0.04	intergenic	4.02E-06	0.084	0.487 (±0.10)	T	-	-
		INRA0028035	7	113,153,185	0.04	n.m.	4.02E-06	0.084	0.487 (±0.10)	C	-	-
	HO_LW	ASGA0073034	16	33,990,830	0.4	intergenic	1.25E-05	0.074	0.250 (±0.05)	G	-	-
		ASGA0073036	16	34,002,361	0.4	intergenic	1.25E-05	0.074	0.250 (±0.05)	A	-	-
		MARC0056521	16	34,212,785	0.39	UGV	5.22E-06	0.079	0.263 (±0.06)	G	Granzyme K	100233185
		ALGA0116942	16	34,735,906	0.37	Intron	2.65E-05	0.069	0.242 (±0.05)	C	PLPP1	100515451/100521873
		ASGA0073065	16	35,043,618	0.37	Intron	2.73E-05	0.068	0.241 (±0.05)	G	IL31RA	100522044
		ALGA0090291	16	35,169,994	0.37	Intron	2.95E-05	0.068	0.238 (±0.05)	T	IL6ST	100037294
		ASGA0096589	16	35,336,487	0.37	Intron	2.73E-05	0.068	0.241 (±0.05)	C	ANKRD55	100520647
		SIRI0000852	16	37,039,866	0.41	n.m.	7.80E-06	0.078	0.249 (±0.05)	A	-	-

Trait	Cluster	SNP name	SSC	Position	MAF	Variant	Empirical p-value	Variance by SNP	SNP effect (± SE)	Effect allele	Gene symbol	Gene ID
EST	HO_LR_H	MARC0051573	6	69,534,577	0.39	Intron	1.16E-05	0.044	-0.274 (±0.06)	T	SLC2A5	100625876
		ASGA0075694	17	19,679,917	0.10	NCTEV	4.73E-06	0.048	0.445 (±0.10)	T	ENSSSCG00000048560	-
LH	HO_LR_H	ALGA0038510	7	9,593,061	0.21	Intron	1.41E-05	0.043	-0.159 (±0.04)	A	PHACTR1	100153737
FSH	HO_LR_C	MARC0079871	10	58,195,169	0.01	intergenic	1.46E-05	0.069	0.628 (±0.14)	C	-	-
	HO_LW	DBNP0002208	7	117,999,329	0.19	Intron	1.59E-05	0.065	0.146 (±0.03)	C	VRK1	100157930
SKA	$BT_{LR}H$	M1GA0020074	14	140,598,974	0.69	UGV	8.07E-06	0.035	-0.233 (±0.04)	A	LRRC27	100737235
		MARC0028756	14	140,612,004	0.69	Intron	8.07E-06	0.035	-0.233 (±0.04)	A	LRRC27	100737235
		M1GA0020080	14	140,632,401	0.27	UGV	8.64E-06	0.035	0.244 (±0.05)	C	LRRC27, PWWP2B	100737235/100154915
		M1GA0020121	14	140,916,098	0.27	3'PUTR	9.71E-06	0.035	0.242 (±0.05)	T	CFAP46	100158140
		M1GA0020138	14	141,069,021	0.27	DGV	1.46E-05	0.033	0.238 (±0.05)	G	ENSSSCG00000047411	-
		ALGA0083389	14	141,123,029	0.27	DGV	1.31E-05	0.034	0.239 (±0.05)	T	ADGRA1	100623440
		INRA0048622	14	141,151,934	0.27	Intron	1.31E-05	0.034	0.239 (±0.05)	T	KNDC1	100152900
		ASGA0068302	14	141,232,780	0.27	UGV	1.31E-05	0.034	0.239 (±0.05)	G	ADAM8, TUBGCP2	100156146/100154072
		H3GA0043620	14	141,279,745	0.27	intergenic	1.31E-05	0.034	0.239 (±0.05)	G	-	-
		ASGA0068308	14	141,309,602	0.27	Intron	1.31E-05	0.034	0.239 (±0.05)	G	CALY	100158118
		H3GA0043634	14	141,338,056	0.27	3'PUTR	1.31E-05	0.034	0.239 (±0.05)	T	ECHS1	100156927
		H3GA0043632	14	141,374,711	0.27	Intron	1.31E-05	0.034	0.239 (±0.05)	A	MTG1	100153322
		INRA0048614	14	152,927,262	0.27	n.m.	1.31E-05	0.034	0.239 (±0.05)	G	-	-

$Ho_{LR}C$ = hormone dataset Landrace from a commercial breeding organization, Ho_{LW} = hormone dataset Large White from commercial and herd book organizations, $Ho_{LR}H$ = hormone dataset Landrace from herd book organizations, $BT_{LR}H$ = boar taint dataset Landrace from herd book organizations, CORT = log-transformed cortisol, TEST = log-transformed testosterone, EST = log-transformed estradiol, LH = log-transformed luteinizing hormone, FSH = log-transformed follicle stimulating hormone, SKA = log-transformed skatole, SSC = *Sus scrofa* chromosome, MAF = Minor allele frequency, * = also genome-wide significant, n.m. = not mapped, UGV = upstream gene variant, NCTEV = non coding transcript exon variant, 3'PUTR = 3' prime untranslated region, DGV = downstream gene variant, SE = standard error.

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