Breeding for robust sows: Association between leg conformation, fertility and sow stayability

Thu Hong Le
Faculty of Veterinary Medicine and Animal Science
Department of Animal Breeding and Genetics
Uppsala

and

Faculty of Science and Technology
Department of Molecular Biology and Genetics
Aarhus University

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Abstract

The overall aim of this thesis was to investigate the possibility to breed for robust sows by improving the leg quality and conformation traits. The specific goals were to estimate the genetic correlations between leg conformation, reproduction and longevity in Swedish and Danish sows; and to identify quantitative trait loci (QTLs) as well as possible causal genes underlying leg conformation traits in pigs.

Data on leg conformation traits recorded on young pigs in Sweden and Denmark was used in this thesis. In study 1 and 2, the heritabilities of leg conformation traits and their correlations with reproduction and longevity traits in Swedish Yorkshire pigs were estimated. Heritability estimates for leg conformation traits were relatively low, and higher if estimated with a linear-threshold model compared with a linear model. Good leg conformation was favourably correlated with reproduction in study 1 and with sow stayability in study 2. These findings indicate that breeding for better legs would increase the sow reproductive performance and reduce the risk of being early culled.

The genetic correlation between leg conformation, reproduction and longevity were examined in Danish Landrace and Yorkshire pigs (study 3). Heritability estimates were low for leg conformation and low to moderate for longevity traits. Both leg conformation and reproduction traits were favourable correlated with longevity traits, implying the potential of improving longevity by selecting for leg conformation and early reproduction traits.

A genome-wide association study was performed (study 4) to identify possible genes affecting conformation traits in three Danish pig breeds (Landrace, Yorkshire and Duroc). A number of associated QTL regions and diverse candidate genes indicate the complex genetic background of conformation. These traits might be controlled by several genes involved in different biological processes including bone and skeleton development, muscle and fat metabolism and body growth. Meta-analyses can improve the power to detect QTLs compared with single trait association analysis.

Keywords: heritability, genetic correlation, gait, locomotion, reproduction, survival, swine, gene mapping

Author's address: Thu Hong Le, SLU, Department of Animal Breeding and Genetics, P.O. Box 7023, 750 07 Uppsala, Sweden or Thu Hong Le, AU, Department of Molecular Biology and Genetics, Blischers allé 20, 8800 Tjele, Denmark
E-mail: thu.le.hong@slu.se or le.hongthu@mbg.au.dk

Dedication

To my family back in Vietnam …

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List of Publications

This thesis is based on the work contained in the following studies, referred to by Roman numerals in the text:

I Le, T. H., Nilsson, K., Norberg, E., Lundeheim, N. (2015). Genetic association between leg conformation in young pigs and sow reproduction. *Livestock Science* 178, 9-17.

II Le, T. H., Madsen, P., Lundeheim, N., Nilsson, K., Norberg, E. (2016). Genetic association between leg conformation in young pigs and sow longevity. *J. Anim. Breed. Genet.*133, 283-290.

III Le, T. H., Norberg, E., Nielsen, B., Madsen, P. , Nilsson, K., Lundeheim, N. (2015). Genetic correlation between leg conformation in young pigs, sow reproduction and longevity in Danish pig populations. *Acta Agriculturae Scandinavica, Section A — Animal Science* 65, 132-138.

IV Le, T.H., Christensen, O. F., Nielsen, B., Sahana, G. (2016) Genome-wide association study for conformation traits in three Danish pig breeds (manuscript).

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Abbreviations

BACK	Back quality
BW	Body weight
CONF/Oall/o_all/n_all	Overall conformation
CT	Computed Tomography
DanAvl	Danish Pig Research Centre
DD	Duroc
EBV	Estimated breeding value
FRONT/Front	Front legs
GCTA	Genome-wide complex trait analysis
GS	Genomic selection
GWAS	Genome-wide association study
HIND/Hind	Hind legs
LBA	Lifetime number of liveborn piglets
LPL	Length of productive life
LR	Landrace
LS5	Litter size at day 5
LTB	Lifetime number of total born piglets
LW	Large White
MA-GS	Marker-assisted genomic selection
MAS	Marker-assisted selection
n_toes	Toes quality
n_under	Standing-under-position syndrome
NoL	Lifetime number of litters
NTB	Number of total born
o_move/n_move	Movement
OC	Osteochondrosis
QTL	Quantitative trait loci
SNP	Single nucleotide polymorphism

SSC	*Sus scrofa* chromosome
STAY12	Stayability until 2nd parity
WSI	Weaning to service interval
YY	Yorkshire

1 Introduction

The central concept of sustainable breeding is to increase the food production to satisfy the demand of an increasing population and at the same time to reduce the negative effect on the environment. This implies the role of animal breeding in a sustainable production system: improving the productivity without increasing the number of animals and/or reducing their health and welfare. However, simultaneously improving production and functional traits is in general challenging in animal breeding due to their unfavourable genetic correlations. A population under selection for high production often shows more problems in fitness, reproduction, physiology and immune system (Rauw et al., 1998). This is unacceptable from an animal welfare aspect and implies a key area in farm animal breeding, which is breeding for robustness.

In pig breeding, robust pigs were defined by Knap (2005) as “pigs that combine high production potential with resilience to external stressors, allowing for unproblematic expression of high production potential in a wide variety of environmental conditions”. In short, pigs are robust if they are healthy, fertile and profitable. Breeding programs focusing on production traits in the pig industry has been quite successful. Pigs are becoming leaner, grow faster and have larger litter size. However, apart from a great improvement in productivity levels, a deterioration in functional traits has also been observed (Rauw et al., 1998). The number of sows diagnosed to be lame or have poor legs has been increasing (Grandin, 2010). Lameness is a serious welfare issue as affected animals are believed to suffer from the pain and distress. Lameness and leg weakness also represent the second most common reason for premature culling of sows, becoming a major source of economic loss in pig breeding (Engblom et al., 2007). Lame sows are often culled at early parities before reaching the most productive period of their life when they are able to give a positive return to the producers. Therefore, breeding for a robust sow with improved leg quality and improved stayability is one of the goals in many pig breeding programs.

Genetic improvement in a multi-trait breeding program depends on many factors, including the association between component traits and the accuracy of the selection process. The development of high-density genotyping and whole genome sequencing technology allows many quantitative trait loci (QTL) affecting the traits of interest, leg conformation traits included, to be detected. The incorporation of identified QTLs into a genomic prediction model would increase the accuracy of the selection, eventually improving the genetic gain for leg conformation traits (Brøndum et al., 2015).

The overall aim of this thesis was to investigate the possibility to breed for robust sows by improving the leg conformation. The specific goals were to estimate the genetic correlations between leg conformation, reproduction and longevity in Swedish and Danish pigs; and to identify genetic factors affecting leg conformation.

2 General background

2.1 Lameness and leg weakness in pigs

2.1.1 Importance of lameness and leg weakness in pig industry

Breeding programs for production traits has been quite successful with a considerable improvement in productivity by genetic selection. However, apart from a substantial increase in production traits, a decrease in functional traits has also been observed. Populations that have been selected for productivity tent to have more problems in fitness, physiology and immune system (Rauw et al., 1998). The prevalence of lameness and leg weakness among slaughter pigs selected for rapid growth and leanness has been increasing (Grandin, 2010). In the USA, 21% of sows in commercial herds were reported to be lame (Vansickle, 2008) and 15.2% of culled gilts were identified to have problem with feet and legs (USDA-APHIS, 2007). In England, the prevalence of abnormal gaits ranged from 11.8% to 19.7% for pigs at different stages of life (KilBride et al., 2009). In the Nordic countries, lameness and leg weakness are also significant issues in pig breeding. The incidence of lameness in sows was 15% in Denmark (Bonde et al., 2004), 13.1% in Norway, 8.8% in Finland and 8.6% in Sweden (Heinonen et al., 2013). Sows removed from herds due to lameness had in most cases not reached the 4th parity, when the sow is most productive with the highest number of born and weaned piglets (Kraeling and Webel, 2015; Pluym et al., 2013). In other words, lame sows are often culled at early parities before reaching the peak productivity when the profit is returned to the producers. Also, pigs with lameness or leg weakness which have not been culled yet often need treatments, and the cost for that might be substantial. Willgert (2011) estimated the cost of an initial case of lameness in sows in England to be from £19 including the cost for the drugs, bandage, 45 minutes of additional attendance by the stockman and five minutes by a veterinarian. If a lame sow requires repeated treatments, or as in more severe cases where the productivity is affected, the cost can be above £266. Indeed, lameness treatment had a significant negative effect on the growth rate. Jensen et al. (2007) reported that boars that got treatment for lameness had significantly reduced the daily weight gain compared with boars that got treatment for other diseases rather than lameness. Also, the level of weight gain reduction seemed to increase with the number of lameness treatment. Boars with the maximum number of treatments (5 or more) showed the largest reduction in the weight gain. Previously, Lundeheim (1987) also found an unfavorable genetic correlation between leg weakness and growth rate in Swedish pigs. Lameness and leg weakness have become a major source of economic loss in pig production and need to be improved.

Lameness and leg weakness in pigs are also related to animal welfare issue. These disorders are not only causes of the premature culling of gilts and sows, but also can restrict an animal from performing natural behaviours as it prevents animals from normal standing or walking. Lame sows tent to spend more time lying rather than walking which might explain the association between lameness and the risk to develop limb lesion and shoulder ulcers in sows (Bonde et al., 2004; KilBride et al., 2009). Lameness is not only a welfare issue for affected sows, but also for their piglets. Lame sows show a higher frequency of uncontrolled lying down, increasing the risk for the piglets to be crushed (Bonde et al., 2004). The unwillingness of a sow to respond to the sound of a screaming crushed piglet could be a result of the pain during the posture changes between standing and lying. In addition, lame pigs may also have reduced ability to access the feeder and water supplier, as well as to compete for the food, leading to a reduced growth rate compared with the healthy pigs (Jensen et al., 2007; Madec et al., 1986). This negative impact is undesirable from both economic and animal welfare aspects.

Therefore, it is necessary and important to improve lameness and leg weakness in pig production due to its negative impacts on farm economy and animal welfare.

2.1.2 Factors contributing to lameness and leg weakness

According to Heinonen et al. (2013), the causes of lameness and leg weakness are normally difficult to detect by clinical examination at herd level. The most common diagnoses of lameness among breeding sows are claw lesion, trauma, osteochondrosis (OC) and arthritis. These conditions may have a heredity predisposition and/or are caused by inappropriate rearing environment or management. Several structural disorders with a genetic background can cause lameness in pigs. The extremes of muscle development and skeletal proportions can impair the free movement and cause discomfort for the pigs on concrete floor, causing abnormal stance and deteriorating the soundness of leg structures (Wood, 2001). Osteochondrosis is a joint disorder which occurs in young animals whose bones are growing fast. Rapidly growing pigs are likely to fail in the endochondral ossification of the articular cartilage and the growth plate, leading to the deformation of the articular surface. Lundeheim (1987) found a significant genetic correlation between the severity of OC in joints and the incidence of clinical signs of leg weakness. Osteochondrosis is reported as one of the major contributors in lameness and leg weakness, probably as a consequence of the intensive selection for fast growth in pigs (Engblom et al., 2008; Jørgensen, 2003).

Risk factors related to management can be nutrition and housing condition including feed diets, floor type, pen size and amount of bedding. A balanced diet is suggested to prevent some serious structural soundness problems caused by a diet with deficient nutritional factors (Wood, 2001). Regarding the floor type, non-slippery floors are believed to be better for feet and legs (Wood, 2001). It is also known that fully slatted floors or floors with narrow and aluminium slats can cause more leg problems than partly slatted floors or floors with wide, plastic or concrete coated slats. Floors with round edges and smooth concrete is also better for the legs than floors with sharp edges and rough concrete (Wood, 2001; Zoric et al., 2009). It is also reported that the incidence of lameness in piglets was reduced when the amount of straw was doubled (Zoric et al., 2009). Pen size can affect lameness and leg weakness through the impact on animal mobility and activity. Studies indicate that long and narrow pens can give more room for pigs to walk and exercise, improving the muscle strength and coordination (Wood, 2001).

2.1.3 Lameness and leg weakness assessment

Lameness and leg weakness are normally difficult to detect early at the clinical examination at herd level. This syndrome can often be diagnosed when the animals become serious or by post-mortem examination after dead. However, the combination of early clinical and post-mortem examination showed that there is usually an early evidence for lameness in affected animals (Dewey et al., 1993). This suggests that clinical examination to some extent could predict the risk of an animal to become lame. Evaluating the leg conformation and locomotion, thus, becomes the first step in clinical examination for lameness and leg weakness. The evaluation can be objective or subjective. Objective assessment using technologies to evaluate leg conformation seems accurate because the length and diameter of bone can be measured and the joint lesion can be radiography and macroscopically diagnosed (Kirk et al., 2008, 2005; Ytrehus et al., 2004). However, the cost and difficulty in data collecting on live animals using these methods restrict their application in practice. Subjective approach uses linear scales with numbers to score the conformation and locomotion of an animal, normally including pastern postures, claw and hook, gait and movement (Knauer et al., 2010; Nikkilä et al., 2013). According to the selection guideline for gilt replacement from Iowa University, the description of leg and feet of the “ideal” gilt are big feet, good flex to her pastern on both her front and rear legs, and adequate base width both at front and at rear (Stalder et al., 2005). Several scoring systems have been developed to assess the soundness of leg and feet conformation traits. Different scales in which the scores can range from worst to best or between the extreme phenotypes with the optimal score in the middle have been used. The definition of the traits does also vary between studies and systems. Some include the front legs and the rear legs from front view, rear view or side view (Jørgensen and Andersen, 2000; Nikkilä et al., 2013). Other studies defined a leg conformation trait as a phenotypic combination of several single conformation traits (López-Serrano et al., 2000; Serenius and Stalder, 2004; Webb et al., 1983). Locomotion is commonly scored to describe the way an animal moves if it is stiff, winding, wadding or flexible. The age at which animals are evaluated also differs between studies and breeding organizations. The scoring can be performed on young females and males or on gilts and sows during their reproductive life. Normally, animals are scored by trained technicians, but the evaluation can also be done by the breeders or herd staff (Anil et al., 2009). Table 1 presents information about leg conformation scoring in the USA and in the Nordic countries.

Despite of being less accurate compared with an objective scoring method, the subjective method is cheap, simple and handy, making this method widely used in practice.

2.1.4 Breeding against lameness in the Nordic countries

Being aware of the impact of lameness and leg weakness on production economy and animal health and welfare, leg conformation has been included in genetic evaluation in most of the Nordic countries (Rydhmer, 2005). However, lameness and leg weakness still seems to be a serious problem in pig production in these countries. In Denmark, Kirk et al. (2005) reported that 72% of the euthanized sows were due to problems in the locomotion system. Leg conformation for all the three Danish breeds (Landrace, Yorkshire and Duroc) has been improved during 2011-2013 but at very low speed, as reported by Danish Pig Research Centre (DanAvl, 2014). Similarly, although both leg conformation and OC had been included in the Swedish genetic evaluation since the 1980’s, arthritis and OC still accounted for the highest proportions of primary diagnosis of the euthanized and dead animals (Engblom et al., 2008). These findings indicate that lameness and leg weakness still need further improvement in the Nordic countries.

Table 1. *Scoring scales used for leg conformation evaluation in different countries*

Country	Trait	Scale	Animal	Age	Place	Reference
USA	11 traits Front leg, hind leg, overall leg action	9-point scale 1=outward from knee or hock, soft pastern, large feet, even hooves 9=inward from knee or hock, upright pastern, small feet, uneven hooves 5=preferred	Gilts	190 days		(Nikkilä et al., 2013)
Finland	13 traits Front leg, hind leg, overall leg action	4- or 5-point scale 1= worst 4 or 5=free of problem, preferred	Gilts and sows	After every farrowing from before 1st mating until culled		(Serenius and Mantysaari, 2001)
Sweden	2 traits Leg weakness OC score	3-point scale 1= worst and 3= best, preferred 0= best, preferred and 5= worst	Female and castrated male pigs	150 days	Testing stations	(Lundeheim, 1987)
Denmark	11 traits Front leg, hind leg, swaying hind quarter	5-point scale 1=normal, preferred 5=severe	Gilts	Before 1st mating	Nucleus herds	(Kirk et al., 2008)
Norway	8 traits Front leg, hind leg, motorics, standing under	4- or 7-point scale 1=sickled knees /crooked hocks, bowed legs, soft pasterns 7=bucked knees/steep hocks, knocked legs, upright or twisted pasterns; stiff movement of wadding hindquarter; severe standing under condition 4=preferred	Gilts	150 days	Nucleus herds	(Aasmundstad et al., 2014)

2.2 Genetics of leg conformation traits

2.2.1 Heritability of leg conformation

The inclusion of leg conformation traits in breeding evaluation requires genetic parameters such as heritabilities and their genetic correlations with other traits to be known. The heritability estimates of leg conformation traits vary between studies, and are generally from low to moderate. The wide range observed can be caused by many factors, including breed, population size, the definition of the studied traits, the scoring scale used as well as on which animals (males or females, gilts or sows) the evaluation was performed. Generally, heritability estimates for locomotion also differ a lot between studies, from close to zero (0.07 - Aasmundstad et al., 2014) to low (0.14 - Luther et al., 2007) to moderate (0.36 - Knauer et al., 2011). A summary of heritability estimates for leg conformation traits in pigs from the literature is shown in Table 2.

2.2.2 Genetic correlations between leg conformation and reproduction and longevity traits

Genetic correlation between leg conformation and reproduction traits

The association between leg conformation and reproduction traits are rarely reported in the literature. Leg score has been showed to have a favourable genetic correlation with age at first farrowing, but not with number of weaned piglets or with first farrowing interval (Serenius and Stalder, 2004). Low to moderate, favourable genetic correlations between front leg front view, rear leg rear view, locomotion and age at first farrowing were also reported by Knauer et al. (2011). Nikkilä et al. (2013) found a moderate genetic correlation between slightly outward turned front leg, less upright rear leg posture, intermediate rear leg foot size in the sows and higher lifetime reproduction performance. However, the authors suggested a careful interpretation of their findings as those characteristics for front legs could be an indicator of OC or other joint lesions.

Along with what has been published on leg conformation, many studies have investigated the association between lameness and reproduction traits. Several studies did not find any significant association (Heinonen et al., 2006; Willgert, 2011), whereas other studies found unfavourable association between lameness and sow reproduction performance. Fewer litters before culling and higher piglets loss were reported for lame sows compared with healthy sows in the study of Grandjot (2007). Similarly, Serenius and Stalder (2007) found a favourable genetic association between leg conformation and litter size, indicating that the better the leg score is, the larger the litter will be at the first parity. Anil et al. (2009) also showed a negative effect of lameness on number of liveborn piglets. This is in agreement with the study of Pluym et al. (2013) in which unfavourable correlation between lameness and number of mummified foetuses was reported.

Genetic correlation between leg conformation traits and sow longevity

Longevity is important in pig production with respect to both economic and ethical aspects. Lameness has been reported to be the second most common reason for early culling of sows (Serenius and Stalder, 2004). Therefore, the association between longevity and lameness or leg weakness have been investigated in a number of studies. Longevity can be defined in different ways. “Stayability” is used to describe the ability of a sow to remain in the herd until a specific time in her life. Longevity can also be defined in terms of reproductive performance, for example as length of productive life (LPL), lifetime number of total piglet born (LTB), lifetime number of piglets born alive (LBA) or lifetime number of litters (NoL).

Generally, the majority of studies report a favourable association between good leg conformation and longevity, but estimated genetic correlation between them seems to vary greatly among the studies and breeds. Serenius and Stalder (2004) found a moderate genetic correlation (0.32) between overall leg action score and LPL in Landrace while the corresponding estimate in Large White was much lower (0.17). López-Serrano et al. (2000) reported no significant genetic association between leg score and stayability in Large White but a favourable genetic correlation (from 0.19 to 0.36) in Landrace, meaning that sows with better leg quality had higher chance to remain in the herd. These findings are consistent with what was reported by de Sevilla et al. (2008) and Anil et al. (2009) in which lame sows exposed a higher risk of culling compared with the healthy sows.

Surprisingly, several studies have also found an insignificant or even unfavourable genetic correlation between leg conformation and longevity (Engblom et al., 2009; Sobczyńska et al., 2013). In the study of Aasmundstad et al. (2014), the genetic correlation between front leg score and longevity only became significant when the breeding value at removal was included in the model for longevity traits. Including breeding value at removal in the statistical model probably accounted for the effect of genetic merit on culling decision, helping to clarify the association between leg conformation traits and sow longevity.

2.3 Quantitative trait loci (QTL) mapping for leg conformation

2.3.1 Quantitative trait loci mapping

Quantitative trait loci (QTL) mapping is the first step to identify the causal genetic variants affecting the traits of interest. The first QTL detected in pigs was for fat deposition using a linkage mapping analysis (Andersson et al., 1994), and since then many other QTL have been identified in pigs using this approach (Rothschild et al., (2007). However, large confidence intervals of the detected QTLs using the linkage approach have restricted their application in breeding programs. Another method for QTL mapping is the candidate gene approach. In this method, the candidate genes are selected based on the known biological functions, and then examined for their association with the studied traits in a target population (Rothschild and Soller, 1997). The disadvantage of this method is the limited knowledge about the biological functions of possible genes. Despite this limitation of the candidate gene approach, several QTL have been detected in pigs, including gene *MC4R* for fatness, growth and feed intake traits and gene *TBC1D21* for number of teats (Jin et al., 2013; Kim et al., 2000). The limitation on resolution in the linkage method and the selection of putative genes in the candidate gene approach can be overcome by using high density markers in the Genome-wide association study (GWAS) approach. In GWAS, markers spread throughout the whole genome are used to evaluate their association with a trait of interest. The development of sequencing technology enabled the availability of high density marker chips and allowed a large number of individuals to be genotyped, facilitating the performance of GWAS (Groenen et al., 2012; Ramos et al., 2009). The single polymorphism nucleotides (SNP) chip with approximately 60,000 markers has become common use in GWAS in pigs (Illumina Inc., San Diego, USA). The assumption behind GWAS is that there are associations between genotyped markers and phenotype of interest because of the linkage disequilibrium between those markers and the causal genes of a given trait. This method is a powerful tool for gene mapping for quantitative traits and complex diseases and have detected a number of QTL affecting traits in pigs, mainly for economically important traits such as growth rate, feed efficiency or litter size (Do et al., 2014; Guo et al., 2016; Kogelman et al., 2014; Sahana et al., 2013).

2.3.2 Quantitative trait loci mapping for leg weakness and leg conformation traits

In comparison with production traits, the number of QTL mapping studies for robustness traits, leg conformation traits included, is quite limited (Andersson-Eklund et al., 2000; Fan et al., 2011, 2009; Laenoi et al., 2011; Lee et al., 2003). The differences in trait definition, scoring scales and the markers used in different mapping studies make the comparison between studies difficult. Generally the genomic regions and genes associated with leg conformation traits are diverse and do not overlap much between studies. However, common genomic regions across studies for similar traits are also reported. For instance, Lee et al. (2003) found several significant QTLs for legs and gait, OC and physis score traits in a Large White x Meishan pig population. Among those, significant QTLs on *Sus scrofa* chromosome (SSC) 13 for front leg score, on SSC 2, 6, 7 for rear leg score, on SSC 4 for gait score were confirmed by Guo et al. (2009). Using 183 microsatellites markers spread over the whole genome Guo et al. (2009) detected 42 QTLs significantly associated with leg weakness related traits. The detected QTLs, however, had relatively large confidence intervals which would need to be narrowed down by fine mapping studies using higher density markers in order to be utilized in practical breeding. Uemoto et al. (2010) also used microsatellites to identify QTL affecting front, rear and feet quality. They did not find significant QTLs but reported nine suggestive QTL regions on SSC1, 2, 3, 10 and 11. They also suggested that QTL on SSC 3 and on SSC 10 might be associated with front leg and rear leg score, respectively.

Studies in human and other species suggested that conformations traits are complex and can be affected by many factors, such as bone strength, muscle growth, fat accumulation and body weight gain (Soranzo et al., 2009; Wu et al., 2013; Yang et al., 2010). This suggests that the candidate gene approach might be a good strategy for QTL detection of leg conformation traits in pigs. Fan et al. (2009) examined 121 SNPs from 95 genes for structure soundness and leg locomotion traits in commercial pigs. Several genes were found to be significantly associated with feet and leg structure (*ALOX5, BMP8, CALCR, OPG*, *OXTR*, *WNT16*), and with overall leg action (*APOE, CALCR, COL1A2, GNRHR, IHH*, *MTHFR*, *WNT16*). A GWAS using high density SNP chips (51,385 SNPs) were later carried out by the same authors (Fan et al., 2011). They reported the association between 14 candidate regions and overall leg score. Among them, the detection of Antp homobox cluster A gene family (HOXA) was noteworthy as this gene family is well known for the evolution of the vertebrates and plays a role on the morphogenesis of skeleton structure along the antero-posterior axis (Favier and Dollé, 1997). Other candidate genes for leg conformation traits reported were *FHL3, TWIST1* and *SP4*. Gene *FHL3* seems to have a regulatory function on the myotube formation and the expression of muscle specific genes (Cottle et al., 2007). Grindflek et al. (2014) reported 10 regions from seven chromosomes to be associated with OC and leg conformation traits in Landrace pigs. The candidate genes on SSC 13 found in their study were in agreement with the previous findings for limb bone lengths and areal bone mineral density of the distal femur (Lee et al., 2003; Mao et al., 2008).

Table 2. *Heritability estimates for leg conformation from different studies in pigs*

Trait	Heritability (± s.e.[1])	Breed[2]	Scoring scale	Animals	Population size	References
Front soundness	0.27 – 0.28[3]	DD + YY	0-10*	Gilts at 91 kg	278 DD 276 YY	(Bereskin, 1979)
Rear soundness	0.15 – 0.38					
Leg action	0.08 ± 0.03	LW	1*-5	Tested boars at 91 kg	14,365 LW	(Webb et al., 1983)
	0.18 ± 0.04	LR			9610 LR	
Aggregate leg score (sum of 19 component traits)	0.17 ± 0.03	LW	0*-2 (for component traits)			
	0.19 ± 0.04	LR				
Conformation score	0.17 ± 0.03	LW	1*-10			
	0.22 ± 0.04	LR				
Leg weakness score	0.14 ± 0.03	LR	1-3*	Castrated males and females at 100 kg	5568 LR	(Lundeheim, 1987)
	0.11 ± 0.03	YY			4318 YY	
Front leg (5 traits: knee, leg turn, pastern, claws)	0.09 – 0.28	LR	1*-5	Boars at 93 kg	1758 LR	(Jørgensen and Andersen, 2000)
	0.01 – 0.35	YY			1888 YY	
Hind legs (5 traits: Standing under, leg turn-out, pastern, claws)	0.02 – 0.29	LR				
	0.10 – 0.32	YY				
Locomotion (4 traits: stiffness, swaying hindquarter, trait sum)	0.10 – 0.30	LR				
	0.11 – 0.33	YY				

Leg status	0.13 ± 0.01 0.13 ± 0.01	LW LR	1-9*	Gilts and sows	21,870 LW 14,944 LR	(López-Serrano et al., 2000)
Overall leg action	0.06 ± 0.02 0.06 ± 0.02	LW LR	1-4* (progeny testing) Binary (performance testing)	Males and females from 25 or 30 kg to 100 kg	6115 LW 7043 LR	(Serenius and Mantysaari, 2001)
Front leg (3 traits: knee, claw, leg turn-out)	0.00 – 0.14 0.02 – 0.19	LW LR				
Hind leg (2 traits: claw, pasterns)	0.02 – 0.18 0.06 – 0.07	LW LR				
Overall leg action	0.07 ± 0.01 0.06 ± 0.01	LW LR	1-5*	Sows	24,007 LW 26,744 LR	(Serenius and Stalder, 2004)
Front leg (angle)	0.14	LW + LR	1-4*-7	Females and castrated males at around 100 kg	7747 LW 1764 LR	(Luther et al., 2007)
Rear leg (4 traits: X-O shape, pastern, angle, claw)	0.10 – 0.26					
Locomotion (stiffness)	0.14		4*-7			
Front leg (2 traits: front and side view)	0.25 – 0.37	LW + LR	1-4*-7	Gilts at 136 kg	1225	(Knauer et al., 2011)
Rear legs (2 traits: front and side view)	0.11 – 0.18					
Locomotion	0.36 ± 0.09		1*-7			

Leg structure (10 traits for front and hind legs)	0.07 – 0.31	LW, LR, YY genetic-originated lines	1-5*-9	Gilts at 124 kg and 190 days	14,471	(Nikkilä et al., 2013)
Overall leg action	0.12		1*-9			
Front leg (knee, pastern)	0.19 ± 0.01	LR	1-4*-7	Gilts on average 150 days and 97 kg	48,210	(Aasmundstad et al., 2014)
Hind leg (stance, hock, pastern)	0.09 ± 0.01					
Locomotion (stiffness, wadding hindquarter)	0.07 ± 0.01					
Standing under	0.05 ± 0.01					

*indicate the optimal score on the scoring scale

[1] standard error [2] LW=Large White, LR=Landrace, YY=Yorkshire, DD=Duroc [3] range of heritability estimates

3 Aims of the thesis

The overall aim of this thesis was to investigate the possibility to breed for robust sows by improving leg conformation. Our hypothesis was that inferior leg strength may cause pain and distress to the sows, resulting in a reduced reproduction and stayability. Thus, selection for better leg conformation in young pigs would improve the reproductive performance and longevity of the sows. For this purpose, genetic parameters including heritabilities of and genetic correlations between related traits need to be known.

The specific goals of individual studies included in this thesis, therefore, were:

- To estimate the genetic parameters of leg conformation traits recorded in young animals and their genetic correlations with reproduction traits in Swedish Yorkshire sows (Study 1).

- To estimate genetic parameters of leg conformation traits recorded in young animals and their genetic correlations with longevity/stayability in Swedish Yorkshire sows (Study 2).

- To estimate the genetic parameters of leg conformation traits recorded in young pigs and their genetic correlations with litter size and stayability in Danish Landrace and Yorkshire sows (Study 3).

- To perform a GWAS and meta-analyses to identify QTLs and possible causal genes affecting conformation traits in three Danish pig breeds (Study 4).

4 Summary of investigations

In study 1, 2 and 3 the genetic parameters, including heritabilities and genetic correlations, between leg conformation, reproduction and longevity traits in Swedish and Danish pigs were estimated. In study 4, a GWAS was performed for conformation traits in three Danish breeds (Landrace, Yorkshire and Duroc). A brief summary of the four studies is presented in Table 3.

Table 3. *Brief summary of the four studies included in this thesis*

Population	Study	Studied traits			Results
		Conformation	Reproduction	Longevity	
Swedish Yorkshire	1	8 traits for leg conformation and locomotion	4 traits in 2 first parities		- Low to moderate heritabilities - Favourable genetic correlations between conformation and reproduction traits
	2	2 traits for leg conformation and locomotion		3 traits for longevity	- Low to moderate heritabilities - Favourable genetic correlations between conformation and longevity traits
Danish Landrace, Yorkshire, Duroc	3	3 traits for leg conformation and overall score	2 traits for litter size in first parity	3 traits for longevity	- Low to moderate heritabilities - Favourable genetic correlations between conformation and litter size with longevity - Unfavourable correlations between conformation and litter size
	4	4 traits for legs, back and overall conformation			- A number of QTLs and genes showed association with conformation traits - QTL detection power can be improved in meta-analyses

4.1 Material and methods

4.1.1 Study 1 and study 2

Study 1 and 2 aimed to estimate genetic correlations between leg conformation and reproductive performance traits (study 1) and between leg conformation and longevity/stayability traits (study 2) in Swedish Yorkshire pigs.

Data and data editing

Study 1 and 2 were based on data on purebred Yorkshire pigs from the breeding company Nordic Genetics (www.nordicgenetics.se). Both male and female pigs born around between 2005 and 2012 were performance tested for leg conformation and locomotion at around five months of age and approximately 100 kg weight. Selected purebred Yorkshire gilts and sows were inseminated to produce purebred (Yorkshire x Yorkshire) or crossbred (Yorkshire x Landrace) litters. The reproduction performance of sows (litter size and weaning to service interval) and their longevity (stayability and lifetime reproductive performance related traits) were analysed. Data on the studied traits were edited to exclude the possible recording errors in body weight at testing or age at 1st farrowing and to include only herds with reasonable number of animals and proportion of crossbred or purebred litters during the studied period.

In study 1, records on 123,307 pigs for eight conformation traits (toes, front leg, rear leg, standing under syndrome, 2 movement traits and 2 overall conformation traits scored using different scales according to old and new scoring systems) and four reproduction traits recorded at the first two litters from 14,329 sows (number of total born, liveborn and stillborn piglets and weaning to service interval) were analysed. A normal scoring method was performed by the procedure RANK in SAS to transform conformation traits and number of stillborn piglets, in order that they more closely follow the normal distribution.

In study 2, two conformation traits (movement and overall conformation) from 97,575 pigs and three longevity traits (stayability to 2nd parity, lifetime number of litters and lifetime number of liveborn piglets) from 26,962 sows were analysed.

Statistical analysis

The statistical models for traits analyzed in study 1 and study 2 were:

$$y_{conformation} = hy_{bir} + sex + a + litter + hysp + e$$

$$y_{reproduction} = hy_{farrow} + boar + a + hys_{farrow} + e$$

$$y_{longevity} = herd + year_{1farrow} + m_{group} + a + hys_{1farrow} + e$$

$y_{conformation}$ is the transformed score for conformation traits; $y_{reproduction}$ is the number of total born/born alive/transformed number of stillborn piglets and transformed weaning to service interval (study 1); $y_{longevity}$ is longevity traits (study 2). Fixed effects were herd-year at birth (hy_{bir}) and sex (sex) for conformation traits; herd-year of farrowing (hy_{farrow}) and boar $(boar)$ for reproduction traits; herd $(herd)$, year at 1st farrowing $(year_{1farrow})$ and mating group $(m_{group}$- purebred or crossbred litters producing group) for longevity traits. Random effects were genetic effect of animal (a); birth litter $(litter)$; herd-year-season-pen at testing $(hysp)$ for conformation traits; herd-year-season at farrowing hys_{farrow} for reproduction; herd-year-season at 1st farrowing $hys_{1farrow}$ for longevity traits.

Genetic (co)variance estimation of studied traits were performed using the DMU package. In study 1, linear mixed model using the Restricted Maximum Likelihood (REML) method was applied for both conformation and reproduction traits. In study 2, linear models were applied for all traits if using REML method; linear models were used for continuous traits (number of litters and liveborn piglets) and threshold models for categorical (conformation traits) and binary traits (stayability to 2nd parity) if using Gibb sampling method. Heritability estimates were estimated in univariate analysis (study 1) and in multivariate analysis (study 2), while the genetic correlations between traits were estimated in multivariate analyses.

4.1.2 Study 3

Study 3 aimed to estimate the genetic parameters of leg conformation, reproduction and longevity trait in Danish Landrace and Yorkshire pigs.

Data and data editing

This study was based on records on Landrace and Yorkshire pigs provided by the Danish breeding company DanAvl (http://www.danavl.dk/). Similar to study 1 and 2, conformation traits were recorded at performance testing for purebred male and female pigs born between 2005 and 2007 when they were five months of age and 100 kg weight. The final data used for statistical analyses consisted of 116,733 Landrace and 89,963 Yorkshire pigs having records on three leg conformation traits: front leg (Front), hind leg (Hind) and overall conformation (Oall).

Data on reproduction performance and longevity of sows were edited for age at 1st farrowing, farrowing interval between litters and the size of herds as well as the percentage of crossbred litters produced in each herd. This editing was done to exclude unrealistic records and to account for the censoring of longevity traits. The final data on reproduction and longevity used for statistical analysis was from 27,070 Landrace and 11,895 Yorkshire sows from nucleus and multiplier herds. Two reproduction traits at 1st parity and three longevity traits analysed were litter size a birth (NTB), litter size at day 5 (LS5), stayability to the second parity (STAY12), lifetime number of litter (NoL) and lifetime number of piglet born alive (LBA).

Statistical analysis

The linear animal mixed models used in study 3 are presented as below:

$$y_{conformation} = hy_{bir} + sex + tech + a + litter + bpt + e + BW$$

$$y_{reproduction} = hy_{1farrow} + a + hyss_{1farrow} + e$$

$$y_{longevity} = hy_{1farrow} + a + hyss_{1farrow} + e$$

$y_{conformation}$ is conformation; $y_{reproduction}$ is litter size; $y_{longevity}$ is longevity. Fixed effects were the combination of herd-year at birth (hy_{bir}), sex (sex) and technician ($tech$) for conformation traits; the combination of herd-year at 1st farrowing ($hy_{1farrow}$) for reproduction and longevity traits. Random effects were birth litter ($litter$), the combination of batch-pen-technician at testing (bpt) for conformation traits, and the combination of herd-year-2month-stable at 1st farrowing ($hyss_{1farrow}$) for reproduction and longevity traits. Body weight at performance testing (BW) was treated as covariate for conformation traits.

4.1.3 Study 4

Study 4 aimed to identify genetic variants affecting conformation traits by performing a GWAS followed by meta-analyses in three Danish breeds.

Data

SNP data on 23,989 Landrace, 24,130 Yorkshire and 16,524 Duroc pigs genotyped by either 8.5K, 60K or 70K SNP chips was available. After quality control and imputation 37,080 SNPs, 36,080 SNPs and 32,376 SNPs on 18 autosomes for Landrace, Yorkshire and Duroc, respectively were retained for single trait association analyses. In total, there were 44,390 different SNPs for the three breeds which were later used in the meta-analysis across breed.

Corrected phenotypes on four conformation traits: front leg (FRONT), back (BACK), hind leg (HIND) and overall conformation (CONF) recorded on young pigs (five months of age and 100 kg weight) were used for association analyses.

Single trait association analysis

Phenotypes were corrected for effects of sex, herd-year-month and regressed on body weight at performance testing was used as the dependent variable in the association analysis rather than the raw phenotype. Single trait association analyses were performed using the software Genome-wide complex trait analysis (GCTA) (Yang et al., 2011). The top ten eigenvectors with the largest eigenvalues from Principal Component Analysis were subsequently included as covariates in the statistical model to account for the confounding effect of population structure. Association analysis for each SNP was carried out using a mixed linear model with leaving-one-chromosome-out approach (Yang et al., 2014). Due to multiple testing the Bonferroni correction was applied.

Meta-analysis

SNP effects estimated from single trait association analyses were used to perform two types of meta-analyses: 1) within breed multi-trait meta-analysis to examine if a genomic region was associated with multiple conformation traits; and 2) meta-analysis for single trait by combining association results from multiple breeds to increase power of QTL detection. Multi-trait within breed meta-analysis was carried out using approximate multi-trait test statistic (Bolormaa et al., 2014). A meta-analysis across three breeds was performed using software METAL to test if the same QTLs for a trait were segregating across the breeds (Willer et al., 2010). The contribution of the most significant SNPs within the detected QTL region was calculated as the proportion of phenotypic or genetic variance explained by that SNP. Candidate genes closest to the most significant SNP within detected QTLs were searched via the pig genome assembly, Sscrofa 10.2 (http://www.ensembl.org/Sus scrofa/Info/Index).

4.2 Results

4.2.1 Study 1 and study 2

Heritabilities

In study 1, heritability estimates ranged from low to moderate (from 0.02 to 0.20) for conformation traits and relatively low (from 0.06 to 0.10) for reproduction traits. Among conformation traits, front leg, rear leg and overall conformation score had the highest heritabilities with an estimate of 0.19, 0.17 and 0.20 respectively.

In study 2, both conformation and longevity traits were heritable, but at relatively low to moderate levels. Heritabilities estimated with linear-threshold model were higher than those estimated with linear model, ranging from 0.10 to 0.24 and 0.07 to 0.20 respectively. Among longevity traits, the heritability estimates for STAY12 (from 0.08 to 0.18) was lower than those for NoL and LBA (from 0.17 to 0.24).

Genetic correlations

Significant genetic correlations between conformation and reproduction traits in study 1 are summarized in Table 4. They are the genetic correlations between movement and litter size traits, between toes, overall conformation trait and number of born alive piglets. Front leg and number of stillborn were weakly genetically correlated. The highest genetic correlation estimates were found between standing under syndrome and litter size traits.

Table 4. *Significant genetic correlations between conformation and reproduction traits in study 1*

Conformation	Reproduction	Genetic correlation estimates	SE
o_move	NTB1, NTB2, NBA1, NBA2	0.20 – 0.36	0.09 – 0.10
	WSI2	-0.35	0.11
n_toes	NBA1	-0.35	0.13
n_front	NSB1	0.26	0.10
n_under	NTB1, NTB2, NBA1, NBA2	-0.35 – (-0.54)	0.16 – 0.18
n_all	NBA1	-0.31	0.13

o_move=movement scored from 1 (bad) to 3 (good); n_toes=toes quality scored from 4 (good) to 7 (bad); n_front=front quality scored from 1 to 7 and 4 is optimal; n_under=standing under scored from 4 (good) to 7 (bad); n_all=overall conformation scored from 1 (good) to 6 (bad); NTB1,2= number of total born in parity 1,2; NBA1,2=number of born alive in parity 1,2; NSB1=number of stillborn in parity 1; WSI1=weaning to service interval in parity 1; SE=standard errors

Table 5. *Genetic correlations between conformation and longevity traits in study 2*

Longevity trait	Movement		Overall conformation score	
	Linear model	Linear-threshold model	Linear model	Linear-threshold model
STAY12	$0.42_{0.07}$	$0.36_{0.07}$	$0.24_{0.07}$	$0.17_{0.06}$
NoL	$0.39_{0.05}$	$0.36_{0.06}$	$0.20_{0.05}$	$0.18_{0.06}$
LBA	$0.34_{0.05}$	$0.32_{0.06}$	$0.17_{0.05}$	$0.15_{0.05}$

STAY12=stayability until 2nd parity; NoL=number of litters; LBA=lifetime number of born alive piglets; Standard errors are given as subscripts

Genetic correlations between conformation and longevity traits in study 2 are presented in Table 5. They were moderately correlated to each other. Longevity showed higher genetic correlation estimates with movement than with overall score. Regardless of the model used, the genetic correlation estimates ranged from 0.32 to 0.42 and 0.15 to 0.24 respectively.

4.2.2 Study 3

Generally, heritability estimates were higher for Hind and Oall (from 0.08 to 0.15) than for Front (from 0.04 to 0.05). Heritability estimates were low for reproduction traits with the range from 0.02 to 0.06. Higher heritability estimates were found for longevity traits, ranged from 0.10 to 0.30 for Landrace and 0.22 to 0.41 for Yorkshire. Longevity traits in Landrace pigs had slightly lower heritability estimates than those in Yorkshire pigs. Genetic correlation estimates between the studied traits in Landrace pigs (lower diagonal) and in Yorkshire pigs (upper diagonal) are presented in Table 6.

Table 6. *Genetic correlation estimates between studied traits in Landrace pigs (lower diagonal) and in Yorkshire pigs (upper diagonal) in study 3*

Trait	Front	Hind	Oall	NTB	LS5	STAY12	NoL	LPL
Front	-	$0.69_{0.04}$	$0.84_{0.03}$	$-0.35_{0.13}$	$-0.17_{0.16}$	$0.13_{0.10}$	$0.14_{0.08}$	$0.12_{0.08}$
Hind	$0.82_{0.03}$	-	$0.93_{0.01}$	$-0.35_{0.12}$	$-0.28_{0.15}$	$0.12_{0.09}$	$0.15_{0.07}$	$0.14_{0.07}$
Oall	$0.85_{0.02}$	$0.92_{0.01}$	-	$-0.39_{0.11}$	$-0.32_{0.14}$	$0.20_{0.08}$	$0.20_{0.06}$	$0.19_{0.06}$
NTB	$-0.24_{0.10}$	$-0.46_{0.08}$	$-0.38_{0.08}$	-	$0.74_{0.09}$	$0.22_{0.11}$	$0.24_{0.10}$	$0.26_{0.10}$
LS5	$-0.24_{0.14}$	$-0.45_{0.12}$	$-0.37_{0.11}$	$0.79_{0.05}$	-	$0.35_{0.13}$	$0.51_{0.10}$	$0.53_{0.10}$
STAY12	$0.26_{0.09}$	$0.28_{0.07}$	$0.39_{0.07}$	$0.00_{0.09}$	$0.28_{0.12}$	-	$0.77_{0.03}$	$0.80_{0.03}$
NoL	$0.10_{0.07}$	$0.13_{0.06}$	$0.20_{0.05}$	$0.23_{0.07}$	$0.55_{0.09}$	$0.80_{0.03}$	-	$0.99_{0.00}$
LPL	$0.07_{0.07}$	$0.08_{0.06}$	$0.16_{0.05}$	$0.26_{0.07}$	$0.58_{0.09}$	$0.79_{0.03}$	$0.99_{0.00}$	-

Front=front leg quality scored from 1 (bad) to 3 (good); Hind=hind leg quality scored from 1 (bad) to 3 (good); Oall=overall conformation score scored from 1 (bad) to 5 (good); NTB=number of total born at 1st parity; LS5= Litter size at day 5 after 1st farrowing; STAY12=Survival to the 2nd parity; NoL= Number of litters; LPL= length of productive life. Standard errors are as subscripts

4.2.3 Study 4

Single trait association analyses

Single trait association analyses detected 14, 13 and 12 QTLs associated with conformation traits in Landrace, Yorkshire and Duroc, respectively. Manhattan plots in Figure 1 gives an overview of their distribution on the genome in Duroc pigs. Generally, more association regions were found for CONF than for other traits. A number of candidate genes underlying the significant QTLs were also identified. Genes *LRPPRC, WRAP73, VRTN* and *PPARD* are likely to control the conformation traits through the regulation of the bone and muscle development, while *IGF2BP2, GH1, CCND2* and *MSH2* might influence through growth-related processes. Table 7 presents the most significantly associated QTL regions along with the top SNP in those regions for each breed. In Landrace the most significant SNP (SSC7: 36.2 Mb) located within gene *PPARD* was associated with BACK. This SNP explained 0.19% of the phenotypic variance, equivalent to 2.04% of the genetic variance of this trait. In Yorkshire, the most significant SNP was located on SSC1:199.4 Mb and explained 0.22% of the phenotypic variance (2.27% of the genetic variance) of CONF. In Duroc, the most significant SNP contributed 0.85% of the phenotypic variance (equal to 11.36% of the genetic variance) of BACK. This SNP was located at SSC3: 100.2 Mb between gene *EPAS1* and gene *PRKCE*.

Multi-trait within breed meta-analyses

The number of significant SNPs identified in multi-trait meta-analysis for Landrace, Yorkshire and Duroc (206, 257 and 306 SNPs) were higher than in single trait analysis. Many SNPs associated with more than one trait in single trait analysis were confirmed in the multi-trait analysis, suggesting the possible pleiotropic effects of the QTLs containing these SNPs. Figure 2 shows that associated SNPs on SSC6 and SSC3 in single trait analysis in Duroc were also significant in multi-trait meta-analysis. This indicates the potential QTL regions affecting multiple traits on these chromosomes. Moreover, significant SNPs in multi-trait meta-analysis generally showed lower P-value than in single trait analyses.

Across breeds meta-analysis for single trait

In total, 36 regions were significantly associated with studied traits in meta-analysis across breeds. Meta-analysis across breed not only confirmed many QTLs found in single trait analysis, but also detected several additional QTLs and novel candidate genes. A QTL at SSC6:52.6 Mb associated with CONF containing two coding genes *ENSSCG00000003243* and *ZNF614* can be cited as an example.

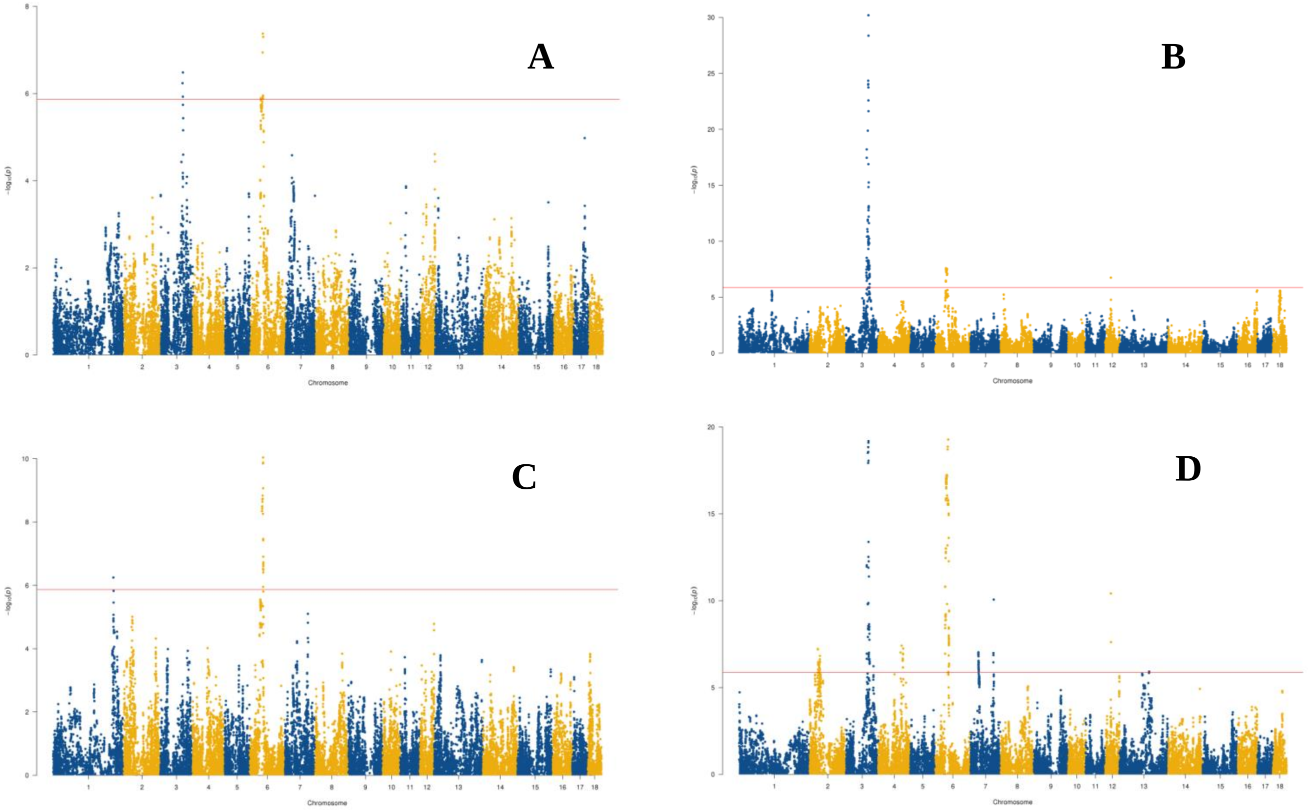

Figure 1. Manhattan plot of GWAS for conformation traits in Duroc pigs (A) FRONT, (B) BACK, (C) HIND and (D) CONF; x-axis represents chromosome and y-axis represents $-log_{10}(P - value)$. The red line indicates genome-wide significance threshold at P-value < 1.38×10^{-6}.

Table 7. *The most significant association QTL in Landrace, Yorkshire and Duroc along with the top SNP in that region*

Breed	Trait[1]	CHR[2]	Right position (bp)	Left position (bp)	Most significant SNP[3]						Candidate genes[4]
					SNP[2]	Position (bp)	P_value	B_value	SE`	% σ_P^2	
Landrace	BACK	7	36202231	37157566	rs80828473	36202231	$7.41x10^{-12}$	-0.0233	0.0034	0.19	**PPARD**
Yorkshire	CONF	1	198725106	208707283	rs80783847	199414449	$2.42x10^{-13}$	-0.0389	0.0053	0.22	ENSSSCT00000005518
Duroc	BACK	3	100232086	100448894	rs81373717	100232086	$6.41x10^{-31}$	-0.0465	0.0040	0.85	EPAS1, PRKCE

[1] *FRONT* - front leg quality, *BACK* - back quality, *HIND* - hind leg quality, *CONF* - overall conformation [2]*CHR* Chromosome [3]*SNP* rsID [4]Candidate gene in which the most significant SNPs located are in bold

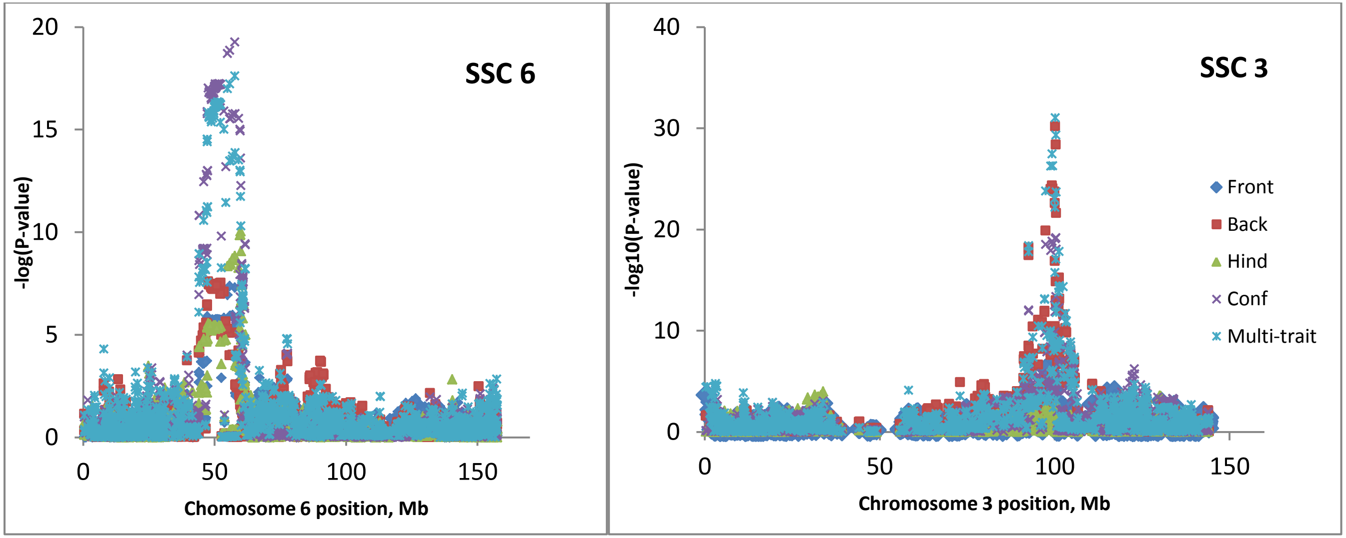

Figure 2. The $-log(P - value)$ of SNPs on SSC6 and SSC3 from single trait analysis for four traits and from multi-trait within breed analysis in Duroc

5 General discussion

5.1 Leg conformation and locomotion evaluation

Lameness is a serious problem in pig production with respect to both economic and ethical aspects. Lameness represents the second most important reason for premature culling of sows in many pig production systems (Engblom et al., 2007; Heinonen et al., 2013; Pluym et al., 2013). In Sweden, arthritis and OC were still diagnosed as the main reason for the deadth and euthanasia of sows in spite of leg conformation and OC being part of the genetic evaluation for three decades (Engblom et al., 2008). This indicates that selection for conformation traits over the past years has not been as efficient as expected. One of the possible reasons for this limited improvement are the negative genetic correlations between leg conformation and other economically production traits, especially growth rate and lean meat percentage (Rauw et al., 1998). The low to moderate heritability estimates for leg conformation traits can be another possible reason for the slow improvement of these traits. The lack of a proper scoring system can limit a proper evaluation and use of genetic variability which can be the reason for low heritability estimates of conformation traits. Normally, pigs are evaluated for conformation and locomotion by technicians using a subjective scale. Conformation traits usually include front leg, hind leg, back, standing under, claws and toe quality. Compared with conformation, locomotion is more difficult and time consuming to measure as it requires an animal walking on a flat surface calmly for a certain amount of time. This might not be feasible since the assessment period is usually short and the movement of a pig can be influenced if the pig is nervous or stressed. An automatic recording system could possibly collect information on leg quality and locomotion in the field without requiring the presence of evaluators. In pigs, the use of accelerometer technology during the last years has offered an opportunity to automatically record sow postures and stepping behaviors (Conte et al., 2014; Ringgenberg et al., 2010). Different postures (standing, sitting, lying ventrally or lying laterally) of the sows can be detected using the data from an accelerometer attached to their backs or legs. The ability to accurately detect the sow postures using this method suggested the potential application of accelerometer on farm for a large number of pigs (Ringgenberg et al., 2010). Indeed, by analyzing the number of steps of rear legs and the postures of sows during morning feeding this method has shown to be efficient in assessing lameness of sows at farm level (Conte et al., 2014). In a Swedish study from 1970 where the pigs walked on a moving mat, the swaying of the back and the step length was filmed and measured to analyze the quality of legs and conformation (Bring-Larsson and Sundgren, 1977). In Norway, male selection candidates have been subjected to Computed Tomography (CT) scan to assess OC since 2008 (Norsvin, 2013). However, the expenses, time consumption and complex algorithms to analyze the big data created from CT appear to be the main drawbacks of this method.

The difference in the number of classes of the scoring scale between recording systems can influence the heritability estimates. A three-point scale is normally used to judge an animal from bad, normal to good; or to describe a trait from one extreme phenotype to normal to the opposite extreme phenotype (e.g. X-shaped knee, normal knee, O-shape knee). On the one hand, few categories in a scale has an advantage of easy scoring as there is a clear difference between healthy and diseased or between two opposite extreme phenotypes. However, it does not allow the differentiation between the different severity levels of the traits, and is therefore less suitable for estimating genetic variation and heritabilities. Data from two different scoring systems were used in study 1 in this thesis. Lower heritabilities were estimated for traits scored by a 3-point scale compared with traits scored using a 4- or 7-point scale. On the other hand, using a scale with large number of categories makes it harder to clarify the differences between adjacent classes. This might make the evaluators end up using only a few of the categories available when scoring the animals. For instance, among 17 possible classes in a study of Steenbergen et al. (1990), the rare use of the intermediate points resulted in the most use of the main 9 points. We found in study 1 that for the traits scored by a 7-point scale, the number of observations scored in the extreme categories 1, 2 and 6, 7 were relatively low. This raises a question of how many classes would be suitable for conformation scoring. A national system for recording conformation traits in Canada has suggested that a 5-point scale might be the best option since it not only allows the differentiation between adjacent classes but also enables the identification of the genetic variation of the traits.

Heritability estimate for leg conformation also depends on the statistical model used. Generally they are higher when estimated with threshold models compared with linear model (Kadarmideen et al., 2000; Veselá et al., 2011; Weigel and Rekaya, 2000). This phenomenon was also observed in our study (study 2) where the heritability estimates range from 0.10-0.14 when using threshold model and from 0.07-0.12 when using linear model. The ability to proper account for the non-normal distribution of conformation traits of threshold model can explain this difference. However, it is also shown in our study that different models did not significantly change either the genetic correlations between leg traits and reproductive and longevity traits, or the ranking of animals for selection. Thus, linear model was applied for conformation traits in our next study (study 3) as commonly used in routine genetic evaluation in practice.

5.2 Genetic association between leg conformation, reproduction and sow longevity

5.2.1 Genetic correlations between leg conformation, reproduction and longevity

Results from study 1 indicates that sows with better leg conformation tend to have better reproductive performance (even toes, good overall leg score, better movement and not suffering from standing under - higher number of total and liveborn piglets and shorter weaning to service interval). This favourable correlation between leg conformation and reproduction is in agreement with what has been reported in the literature. However, in study 3 we found unfavourable genetic correlation between these traits for Danish Landrace and Yorkshire pigs. Pigs with inferior leg conformation at performance testing seem to have larger litter size at their 1st parity. There are possible explanations for this unexpected finding. Bad leg conformation might not yet negatively affect reproduction performance of first-litter sows since it is reported that sows in later parities (2-5) had the highest proportion of leg disorders (Engblom et al., 2007; Mote et al., 2009; Tummaruk et al., 2008). The relatively strong selection on litter size compared with the selection on leg conformation in Danish pig breeding might also be a reason for the unfavourable correlation between these two traits. Another possible explanation is the pre-selection for leg conformation that occurred before or after performance testing. Lame gilts or gilts with bad legs were probably already excluded before they had a chance to enter the farrowing units to express their reproductive capacity. Only sows which had at least one litter were included and the gilts which failed to produce any litter might be the ones with inferior legs. Information about culling reasons before the 1st farrowing is necessary to be able to answer this hypothesis.

Favourable genetic correlation between leg conformation and longevity found in study 2 and 3 in this thesis are consistent with the findings previously reported in the literature. Study 2 on Swedish Yorkshire pigs showed that sows with better movement and overall leg score had a higher chance to stay until 2nd parity and thereby had a potential to produce more piglets before being culled. Similar results were found for Danish Landrace and Yorkshire pigs in study 3 where sows with better legs and overall conformation had a lower risk to be culled, stayed longer in the herds and produced a larger number of litters.

In study 3, we also observed a favourable genetic correlation between litter size at 1st parity and longevity. This is expected because poor reproduction performance represents the most common reason for culling in commercial herds (Engblom et al., 2007; Mote et al., 2009). The favourable correlation between litter size and longevity is partly due to the selection bias, since the litter size affects the decision of farmers when selecting sows for the next insemination. Sows with very small litter size are likely to be culled earlier, creating the automatic positive correlation between litter size and longevity.

The association between leg conformation, litter size and longevity found in our studies and in the literature suggest a relationship between them which is graphically presented in Figure 3. On the one hand, leg conformation and reproduction have a direct impact on longevity in which good leg and outstanding reproductive capacity tend to give the sows higher stayability. On the other hand, leg conformation can also indirectly affect longevity through its impact on reproduction. Sows with good legs are likely to have better reproduction performance which would increase their chances to remain longer in the herds.

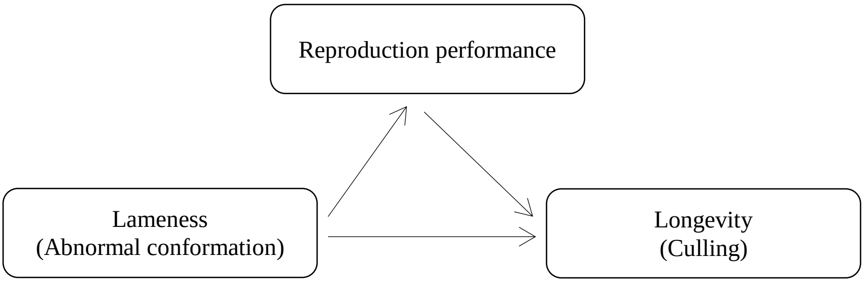

Figure 3. Causal relationship between leg conformation, reproduction performance and longevity

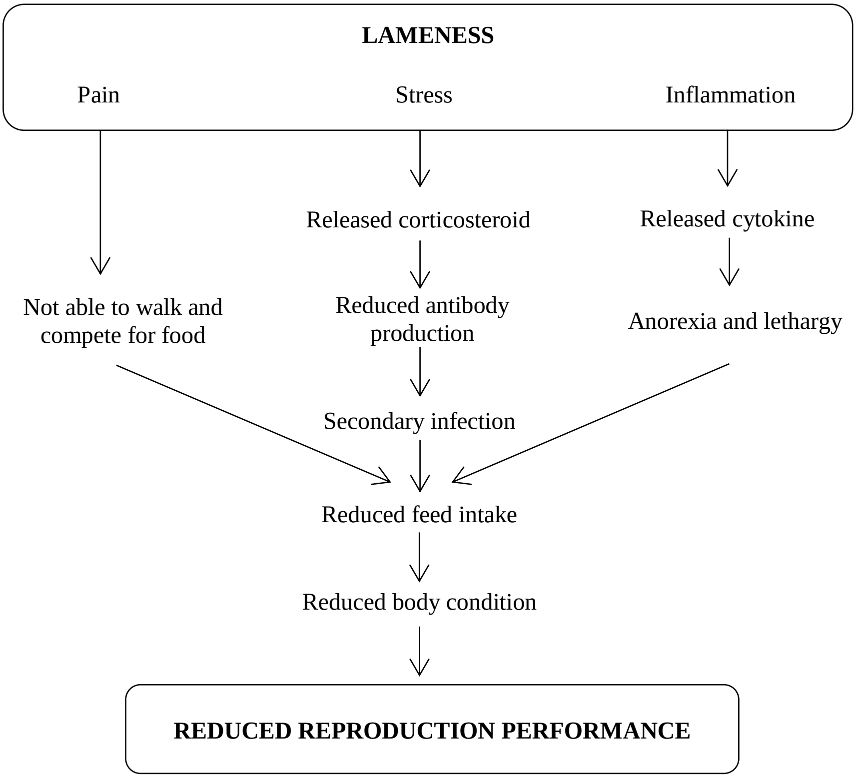

Figure 4. Biological background underlying association between leg conformation and reproductive performance

5.2.2 Biological background underlying the association between leg conformation and reproductive performance

The biological background underlying the favourable correlation between lameness and reproduction performance found in our study 1 and the literature is summarized in Figure 4 (Anil et al., 2009; Einarsson et al., 2008; Heinonen et al., 2013). Lame sows are believed to suffer from pain and stress which can negatively affect the reproduction (Einarsson et al., 2008). High level of corticosteroids released under stressful situations can decrease the production of antibodies, resulting in a reduction in the ability resist to the infection. Similarly, cytokines released during the inflammatory processes in lame sows can cause anorexia and lethargy. Also, lame sows normally have difficulties to walk to the feeder and compete with the pen mates for food. Sows in these situations are likely to reduce feed intake and consequently might lose their body condition. Reduced body condition during lactation has been reported to have negative influence on the subsequent reproduction cycle (Anil et al., 2009).

5.2.3 Leg conformation can be an early indicator for sow longevity

True longevity of an individual sow can only be recorded when she has been culled or is dead, which makes direct selection on longevity inefficient due to prolonged generation interval. In contrast to the late recording of longevity, leg conformation traits are usually recorded early at performance testing when pigs are approximately 5 months of age and 100 kg weight. This fact together with the reported significant correlations between leg conformation and longevity suggest that leg conformation can be an early indicator for sow longevity. However, the selection decision based on this early recorded information needs to be done with caution since very little is known about how well leg quality recorded on young animals can predict the leg soundness in the adult animals. In the study of Stock et al. (2016b), joint angles for feet and leg conformation traits between gilts at selection, sows at 2nd gestation and at parity 5 and above were compared. The authors found significant differences in joint angles at knee, pasterns, hock and rear stance between different groups of gilts/sows. This indicates that feet and leg structure change with age during the reproductive life of a sow. The authors also investigated the effect of gestation stage on the difference in joint angles for feet and leg conformation traits in 2nd gestation sows (Stock et al., 2016a). Results showed changes in knee, front pastern and hock join angle as the gestation progressed. Taken together, it is possible to use leg conformation as an early indicator for longevity due to their significant correlation but it is also challenging because of the possible change in leg conformation as the pigs get older.

The question now is which traits, among several conformation traits recorded, would be the best indicators for sow longevity. The best indicators should be the ones easy to record, highly heritable and correlated with longevity. It is suggested that locomotion would be the best candidate due to its significant impact on longevity (Steenbergen et al., 1990). In our study (study 2) on Swedish Yorkshire pigs, locomotion (movement) also showed higher genetic correlation with longevity compared with overall leg score. However, locomotion generally had lower heritability estimates than other conformation traits, probably due to the inaccuracy in recording (Jørgensen and Andersen, 2000; Aasmundstad et al., 2014). In our studies (study 1 and 2) on Swedish pigs, heritability estimates for locomotion were 0.05 whereas those for other conformation traits ranged from 0.07 to 0.20. For the Danish pig population analysed in study 3, our results suggested that the best indicator for longevity might be overall conformation trait. Compared with front and hind leg, overall score showed the highest heritability estimates and highest genetic correlations with longevity traits.

5.3 Breeding for productive and robust sows

5.3.1 Improving robustness in breeding programs

Generally, breeding goals have put more emphasis on production traits because of their direct benefit for farm profit. However, the negative impact of unsound pigs on economic profitability and animal welfare implies the need to improve the robustness. The first question here is if it is worth to improve robustness traits in pig production in terms of economic gain. Knap (2005) calculated the marginal economic value (MEV) for the component traits in the profit equation either with or without including the robustness traits. According to him, a classical profit equation for slaughter pigs includes five economically important traits: carcass weight, lean percentage, number of days to slaughter weight, the cost for daily feed, and the cost to produce litters. An extended profit equation had included three additional robustness traits: the growing pig survival, number of litter produced during lifetime and pre-weaning survival. Results showed that MEV standardized to money/trait σ_G for robustness traits were at similar magnitude as for conventional production traits (1-3 money/σ_G vs. 2-4 money/σ_G, respectively). This indicates that if the improvement rate used for production traits is applied for the robustness traits, an enhanced profitability can be expected. In other words, it seems economically worthwhile to improve robustness. The next question now will be if it is possible to improve robustness traits by breeding. Generally, several indicators are used to evaluate robustness traits. For instance, in the study of Knap (2005), parity number at culling, culling rate, disease resistance, heat tolerance, leg quality and udder quality are suggested to be used for sow longevity prediction. In this thesis, robustness traits are restricted to conformation traits and sow longevity. As reported previously, both conformation and longevity traits are heritable which indicates that it is feasible to improve these robustness traits by breeding. However, the speed of improvement may be slow due to their low to moderate heritability estimates. Only the genetic improvement for leg conformation traits will be discussed here. Several experiments on selection for lameness or leg conformation traits have been reported in the literature. In the USA, a 5-generation divergent selection experiment on front leg weakness was carried out to create three lines: high line with least weakness pigs, control line with intermediate weakness pigs and low line with severe weakness pigs (Rothschild and Christian, 1988). Results in generation 5 showed that high line pigs had better mean scores for front leg and lower OC lesion score for the proximal radius and ulna than the control and low line. These findings indicate that leg conformation can be changed and the leg soundness traits can be improved by breeding.

There are several options to compute estimated breeding values (EBV) for conformation traits for genetic evaluation in practice. One option is to create single EBV for each trait and then combine into a single index based on genetic correlations between each trait and longevity or their economic importance. This single index for conformation will work as other production traits in the breeding goal. The advantage of this method is that it allows the adjustment of genetic progress in each component trait according to the progress achieved and the future need in different breeds and populations. An example of the use of this approach is in The Netherlands. The breeding program used in Hendrix Genetics creates a combined conformation index with the weight for each component trait depending on its economic importance or the need to improve that trait in that breed or population (Huisman, 2007). Another approach for EBV computation is to compute a phenotypic combined score by simply summing all conformation scores or summing deviations from optimum values or based on the relationship between component traits. This approach, can on the one hand, prevent the overweight on individual traits, but on the other hand, makes it difficult to predict the response to selection of the component traits. This method is used for EBV calculation for conformation traits in Sweden and Denmark. In Sweden, Hampshire sire line gets relatively high emphasis on the soundness traits in which the economic contribution to the breeding goal is 17% for conformation (9% for traits scored on farm and 8% scored on test station) and 9% for OC (http://www.nordicgenetics.se/en-us/avel/avelsm%C3%A5l.aspx). In Denmark, front leg, back and rear leg are phenotypically combined in an overall conformation trait (i.e. Oall in study 3 and CONF in study 4). This combined trait is the only conformation trait included in the breeding goal. With the economic contribution of 5% of the breeding objective, the average genetic improvement of leg conformation for all the three Danish breeds (Landrace, Yorkshire and Duroc) during 2011-2013 was +0.04 point (the score mean increased 0.04 point), as reported by Danish Pig Research Centre (DanAvl, 2014).

Table 8. *Genetic correlations between leg weakness related traits and production traits*

Conformation trait	Score scale[1]	Growth	Fatness	Lean percentage	Result interpretation	Reference
Front leg	0-10*	0.33	0.35	-0.33	Good legs: fast growth to 91kg, more fat, less lean percentage	(Bereskin, 1979)
Rear leg	0-10*	0.09	0.52	-0.19		
Leg weakness score	1-3*	-0.26-(-0.35)	-	-0.09-(-0.43)	Good legs: slower growth until 100kg, shorter carcass length, less lean percentage	(Lundeheim, 1987)
OC score	0*-5	0.03-0.29	-	0.08-0.28		
Sum of leg weakness	1*-5	-0.24	-	0.20	Good legs: less lean	(Jørgensen and Andersen, 2000)
Sum of OC	1*-5	0.24-0.34	-	0.29	Less OC: slow growth, less lean	
OC score	1*-6	0.31	-	0.21-0.32	Less OC: slow growth, less meat quantity	(Kadarmideen et al., 2004)
OC score	0*-5	-0.40-(-0.74)	-	-	Less OC: slow growth to 100kg, especially to 30kg	(Aasmundstad et al., 2013)

[1] Star (*) indicates the optimal score on the linear scale

5.3.2 Breeding for both production and robustness

Lameness and leg weakness can be caused by abnormal leg conformation, gait and locomotion, and also the occurrence of OC. It is a relatively consistent conclusion in the literature that production traits are unfavourably genetically correlated to conformation traits (Aasmundstad et al., 2013; Bereskin, 1979; Jørgensen and Andersen, 2000; Lundeheim, 1987). The associations between leg weakness or OC lesions and growth rate, back-fat thickness, and lean meat percentage from these studies are presented in Table 8. Generally, pigs that grow faster before reaching 100kg, have less fat, longer carcass and more lean meat percentage at slaughter are at a higher risk of becoming lame. Also, the selection experiment carried out in the USA showed that the line selected for high level of leg quality had more fat, less extensor muscle of forearm and shorter length of the humeral and metacarpal bone compared with the lines selected for more leg weakness (Draper et al., 1992; Rothschild et al., 1988). In conclusion, the unfavourable correlations between lameness and growth rate, fat content, lean meat percentage in the literature indicate that selection for better leg conformation might undermine the effort to improve production traits. Although in the current pig production, almost all breeding systems are willing to accept leg conformation traits in the breeding goal but not at the expense of production traits. An optimal trade-off between these traits is a difficult question and controlled by economics. As discussed previously, the incorporation of robust traits (leg quality included) in the profit equation for pig production showed that the economic value achieved from improvement of leg conformation traits is similar to the value of improving production traits (Knap, 2005). Leenstra et al. (1984) performed a selection experiment to investigate the influence of breeding for leg quality on growth rate and vice versa in broiler chicken. In that experiment, three sublines from the same broiler chicken sire line base were selected for high average daily gain, low twisted leg incidence or a combination of both two traits. Results showed that the line selected for reduced twisted legs had a decreased growth rate and the line selected for faster growth had higher incidence of twisted legs. However, the line selected for the combination of two traits showed a considerable reduction in twisted legs while still remained the growth rate at nearly maximum level. This result suggests that it would be also feasible to breed for improved leg quality in pigs without sacrificing their production performance.

The focus on the only sire line Hampshire in the current Swedish pig production is on growth rate, feed efficiency and meat quality. Along with a total weight of 26% for conformation traits (9% for conformation scored on farm, 8% on test station and 9% for OC), the economic contribution of production traits in the breeding objective is 13% for growth rate from birth to 35 kg, 24% for growth rate from birth to 100kg, 11% for fat thickness and 15% for meat percentage at 100kg. This distribution results in an increase of 22 SEK (approximately USD 2.5) per slaughter pig per year. In Denmark, the conformation trait accounts for 5% of the breeding goal while the contribution of growth rate and lean percentage ranges from 13 to 25% and from 7 to 16%, respectively (DanAvl, 2014). With this goal, the average genetic gains for all these traits during 2011-2014 regardless of the breed were positive (increased 0.04 point for conformation, 16g/day for growth rate from 30kg to 100kg and 0.10% for lean meat percentage). The progress achieved for both conformation and production traits in Sweden and Denmark confirms the possibility to simultaneously improve these traits in pig breeding.

5.3.3 Genome wide association study and the use of GWAS results in breeding for robustness traits

Genome wide association study for conformation

In study 4 we have identified several chromosomal regions on SSC 1, 2, 3, 4, 5, 6, 7, 10, 12, 13 and 18. Many candidate genes within the identified regions in this study were reported to be related to bone and muscle development (*LRPPRC, WRAP73, VRTN, HMGA* and *PPARD*) or fat metabolism (*PRKCE*). Other candidate genes seem to be involved in the growth pathway (*IGF2BP2, GH1, CCND2* and *MSH2*) or play roles in the central nervous system (*SLC14A2*). Findings from our study support the GWAS results from human about the complexity of conformation traits where the interaction between bone and cartilage development, muscle growth, fat metabolism and body weight gain determines the skeleton structure and movement patter of an individual.

Meta-analysis for multiple traits within breed detected a higher number of significant variants as well as achieved higher significance levels of the top variants compared with single trait analysis. This enhanced QTL detection capacity has been earlier reported in beef cattle for stature, fatness and reproduction related traits (Bolormaa et al., 2014). Meta-analyses across breeds not only confirm many associated QTLs with enhanced significance levels of the top SNPs within these QTL regions, but also discovered several new QTL regions and candidate genes associated with conformation. Some of these genes were reported to be linked with bone, skeletal or muscle development such as *SOS2*, *TRIM24* and *ELMO*. A region associated with overall conformation trait on SSC 12: 25 Mb could be interesting as it harbours the Antp homobox (HOX) gene family. Four clusters of *HOX* genes (*HOXA*, *HOXB*, *HOXC* and *HOXD*) are well known to be associated with the formation and development of vertebrae (Kessel and Gruss, 1991).

The use of GWAS results in pig breeding

The selection process in conventional breeding is based on the breeding values estimated from phenotypic records and pedigree information. This selection method has been successfully used in pig breeding with an increase in genetic gain for many production traits. However, conventional selection seems not effective to improve robustness traits because of their low heritabilities (e.g. conformation) and late or difficult to record (e.g. longevity and disease resistance). Instead, selection approaches which integrate QTLs information in breeding value estimation are expected to enhance the selection accuracy and eventually improve the genetic gains (Dekkers, 2007). One such approach is marker-assisted selection (MAS) in which markers associated with traits of interest are identified and animals carrying desirable marker alleles are selected (Dekkers, 2007). Though GWAS have identified a large number of significant associated QTLs, however, the use of these associated variants in pig breeding is still limited. This is because 1) many of identified QTLs have not been validated; 2) causal variants within identified QTLs in one population need to be identified to be able to use in selection in other populations; and 3) the identified QTLs only explained a small proportion of the phenotypic (and/or genetic) variation due to the polygenic nature of most of the traits of interest. Indeed, in our study 4 on Danish pigs, we found 170 significant SNPs associated with conformation traits in Landrace, 283 significant SNPs in Yorkshire and 406 significant SNP in Duroc pigs. Among those the most significant SNPs only explained 0.19%, 0.22% and 0.85% of phenotypic variance (equivalent to 2.04%, 2.27% and 11.36% of genetic variance) of back trait in Landrace, overall conformation trait in Yorkshire and back trait in Duroc, respectively. However, these identified QTLs and the candidate genes need to be validated in an independent sample before they can be used in MAS which could be difficult in practice (Goddard and Hayes, 2009). Despite of these challenges, MAS still has been applied in practice but at limited level. For instance, the use of estrogen receptor gene for the selection of litter size and the use of 15 markers for traits related with reproduction, feed intake, growth, body composition and meat quality in pigs (Dekkers, 2004). Associated markers were also incorporated into a customized SNP panels for genetic evaluation in some pig breeding companies (Ibáñez-Escriche et al., 2014; Van Eenennaam et al., 2014).

Although GWAS results so far had limited use in MAS in practice, it could be advantageous to use them in combination with other methods, such as genomic selection (GS). In GS, a high density marker panel spread over the genome are used to estimate genomic breeding value of an animal. This enables GS to capture "all" the genetic variance caused by the infinitesimal nature of complex traits and thus, overcome the limitation of MAS. The integration of significant markers from GWAS into GS results in "marker-assisted genomic selection (MA-GS)". In this approach, significant markers are fitted into the genomic prediction model as fixed effects; i.e the estimated breeding value of an animal is the sum of estimated breeding value and the estimated effect of the included markers. The prediction accuracies of MA-GS, GS, MAS and traditional selection for androstenone level and gestation length were compared by Hidalgo (2015). He showed that the prediction accuracy in MAS was higher than that in pedigree-based traditional selection; and the highest accuracy was obtained in MA-GS. The explanation for the former result is that animals can be better genetically differentiated by including significant QTLs affecting the traits. For the latter result, even when markers are already accounted for in the genomic relationship matrix in GS, the marker effects are still better captured if they are fitted as fixed effects in MA-GS. The improved prediction accuracy was also reported in dairy cattle by including markers significant in GWASs on whole genome sequence data alongside the 54K SNP set (Brøndum et al., 2015).

Application of GWAS results in selection for robustness traits in pigs

Although it would be advantageous to use associated markers to select for robustness traits, the use of MAS or MA-GS for robustness traits in pigs, so far, is relatively limited (Ibáñez-Escriche et al., 2014). One possible reason for this is the lack of putative QTLs for robustness traits due to limited number of GWASs for these traits as well the lack of validation of the identified QTLs. Among robustness traits, disease resistance or susceptibility would be beneficial from application of MAS because it is challenging to collect data for these traits and they are normally not included in the breeding goal. In fact, MAS has been successfully used to select against the halothane gene that causes an increase in both muscle growth and susceptibility to stress in pigs (Dekkers, 2004; Hanset et al., 1995). Another example is the selection program for resistant animals to *Escherichia Coli* (*E.coli*) which started in 2003 in Denmark. The selection of resistant boars only had increased the frequency of resistant animals from approximately 1% in 2003 to more than 90% in 2009. The prevalence of faeces samples with *E.coli* O149 diarrhoea had also decreased from 20% to 5% after this selective program (Nielsen et al., 2009). Currently in Denmark, genomic selection has been used to estimate breeding values for the traits in the breeding goal, conformation and sow longevity included, for purebred animals in all three pig breeds (DanAvl, 2014). Significant markers from GWAS for leg conformation traits have also been incorporated in genetic evaluation process in a pig company in the USA, Pig Improvement Company (PIC). A “trait-line-specific low-density-panel” containing less than 30 significantly SNPs associated with leg score is used in their routine evaluation system to select for this trait (Van Eenennaam et al., 2014).

Most of the pig companies only reported the implementation of GS in the last few years and the use of GS and MA-GS for robustness traits is relatively new in pig breeding. Although expected, it might be too early to assess the potential of GS or MA-GS to accelerate the genetic gain of robustness traits in pigs.

6 Conclusions

The main conclusions of this thesis are:

- Both leg conformation/locomotion and sow longevity/stayability are heritable in Swedish and Danish pig populations. Thus, it is possible to improve these traits in breeding programs, but the response to selection might be slow because of their low to moderate heritability estimates.

- The genetic correlation between leg conformation/locomotion and traits related to litter size is significant and favourable in Swedish Yorkshire pigs, but unfavourable in Danish Landrace and Yorkshire pigs. Therefore, selection on leg quality traits would affect the reproduction performance and vice versa. Standing-under-position syndrome should be avoided in breeding due to its high genetic correlations with number of total born and liveborn piglets in Swedish Yorkshire pigs.

- Leg conformation and reproduction traits are favourably genetically correlated with sow longevity: the better leg quality and the larger litter size a sow has, the longer she will stay in the herd. This indicates that conformation recorded on young animals and litter size at early parities can be early indicators for sow longevity.

- A large number of QTLs and candidate genes identified for conformation traits show their polygenic architecture and also indicate that they might be controlled by genes involved in bone and skeleton development, muscle growth, fat metabolism and body weight gain processes.

- Meta-analysis in which data on different traits and/or from different breeds are combined is a powerful approach to detect QTLs affecting traits of interest. Possible QTLs with pleiotropic effect and novel candidate genes were identified in meta-analysis for conformation traits in three Danish pig breeds.

7 Future research

Current pig breeding programs worldwide are continuing to focus on a simultaneous improvement of both production and robustness. For the results of this thesis, several future steps should be carried out to facilitate the improvement process of robustness traits:

- Increase the accuracy of genetic evaluation and selection by applying an automatic recording and computational assessment for leg conformation and locomotion. The link between information from accelerometer and visual scoring information in large herds should be analysed to evaluate the potential to apply this method in practice.

- Validate significant QTLs and candidate genes associated with conformation traits identified in this thesis; and examine the possibility of further use in fine mapping to verify the effect of genes on conformation traits in pigs as well as in marker assisted (genomic) selection to improve these traits.

- Perform a simulation study to investigate the possibility to have a better alternative breeding scheme for production and robustness using the genetic parameters (heritability and genetic correlations) estimated in this thesis and the parameters currently use in genetic evaluation system in practice. The total progress and partial progress in different component traits should be predicted when different economic weights are put on leg conformation/locomotion and production traits in breeding goal. The response of conformation traits under marker assisted genomic selection should also be predicted in such a simulation study.

8 Sammanfattning

Avelsarbetet har under senaste 50-årsperioden resulterat i stora förbättringar i grisarnas produktionseffektivitet: snabbare tillväxt, bättre foderutnyttjande, lägre fettansättning och större kullar. Vissa negativa effekter av denna avel har observerats för funktionella egenskaper såsom ben och rörelser. Svaga ben och dåliga rörelser är negativt både för suggan och för de växande grisarna. Svenska studier har visat att dåliga ben och rörelser är den näst vanligaste orsaken till att suggorna slaktas. Den vanligaste utslagsorsaken är dålig reproduktion (suggorna kommer inte i brunst eller blir inte dräktiga). Varje år slaktas hälften av suggorna i Sverige, och byts ut mot gyltor. Detta innebär att ofta slaktas suggorna innan de nått sin mest produktiva kapacitet (störst kullar i kullnummer 3 till 6). Dåliga ben innebär högst sannolikt smärta och nedsatt djurvälfärd hos de drabbade djuren, samt försämrad produktionsekonomi för grisproducenten. Avelsurval för bättre ben och högre livstidsproduktion ingår idag i avelsmålet i flertalet avelsorganisationer.

I ett avelsprogram är det viktigt att ha information om de genetiska sambanden mellan såväl mätegenskaper som egenskaper som ingår i avelsmålet. Med modern molekylär teknik finns nu möjlighet att identifiera gener som har inverkan på enskilda mått/egenskaper. Nyttjande av denna information i avelsvärderingen ger möjlighet till ökad säkerhet i avelsurvalet, alltså en ökad sannolikhet att det verkligen är de bästa djuren vi väljer för att producera nästa generation djur.

Det övergripande målet med denna avhandling var att undersöka möjligheterna att genom avelsurval för bättre ben och rörelser öka suggornas livstidsproduktion. Avhandlingens specifika mål var att skatta genetiska korrelationer mellan benbedömningar, fruktsamhet och risk för tidig utslagning av renrasiga suggor i Sverige och Danmark, samt att identifiera gener som påverka grisarnas ben och rörelser.

Sammanfattning av de enskilda studierna

Information om ben och rörelser hos renrasiga grisar (svensk yorkshire samt dansk lantras och yorkshire) som bedömts vid ca 100 kg vikt (ca 5 månader gamla) analyserades tillsammans med information om suggornas fruktsamhet och utslagning. De skattade arvbarheterna för olika bedömningar av de unga djurens ben och rörelser var låga (<0.2), vilket innebär samma nivå som för fruktsamhet och för olika mått på suggans livstidsproduktion. Bra ben och rörelser var genetiskt korrelerade till god reproduktion (i svensk data, men ej i dansk data) och hög livstidsproduktion (både svensk och dansk data). Detta indikerar möjligheten att genom selektion för bra ben och rörelser hos de unga djuren selektera för en hög livstidsproduktion hos suggorna.

Information om ben och rörelser hos renrasig dansk lantras, yorkshire och duroc (100 kg, 5 månader) kopplades samman med information om gener hos dessa djur (GWAS; Genome-Wide Association Study). Ett antal gener och gen-regioner uppvisade koppling till bedömningarna av ben och rörelser, men dock ingen riktigt starkt kopplad gen som direkt skulle kunna användas i avelsurvalet.

References

Aasmundstad, T., Kongsro, J., Wetten, M., Dolvik, N.I., Vangen, O., 2013. Osteochondrosis in pigs diagnosed with computed tomography: heritabilities and genetic correlations to weight gain in specific age intervals. Animal 7, 1576–82.

Aasmundstad, T., Olsen, D., Sehested, E., Vangen, O., 2014. The genetic relationships between conformation assessment of gilts and sow production and longevity. Livest. Sci. 167, 33–40.

Andersson, L., Haley, C., Ellegren, H., Knott, S., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M., Hansson, I., 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science (80-.). 263, 1771–1774.

Andersson-Eklund, L., Uhlhorn, H., Lundeheim, N., Dalin, G., Andersson, L., 2000. Mapping quantitative trait loci for principal components of bone measurements and osteochondrosis scores in a wild boar x large white intercross. Genet. Res. 75, 223–30.

Anil, S.S., Anil, L., Deen, J., 2009. Effect of lameness on sow longevity. J. Am. Vet. Med. Assoc. 235, 734–8.

Bereskin, B., 1979. Genetic Aspects of Feet and Legs Soundness in Swine. J. Anim. Sci. 48, 1322–1328.

Bolormaa, S., Pryce, J.E., Reverter, A., Zhang, Y., Barendse, W., Kemper, K., Tier, B., Savin, K., Hayes, B.J., Goddard, M.E., 2014. A Multi-Trait, Meta-analysis for Detecting Pleiotropic Polymorphisms for Stature, Fatness and Reproduction in Beef Cattle. PLoS Genet. 10, e1004198.

Bonde, M., Rousing, T., Badsberg, J.H., Sørensen, J.T., 2004. Associations between lying-down behaviour problems and body condition, limb disorders and skin lesions of lactating sows housed in farrowing crates in commercial sow herds. Livest. Prod. Sci. 87, 179–187.

Bring-Lasson, K., Sundgren, P.E., 1977. Reports of the Agricultural College of Sweden. Report no A284

Brøndum, R.F., Su, G., Janss, L., Sahana, G., Guldbrandtsen, B., Boichard, D., Lund, M.S., 2015. Quantitative trait loci markers derived from whole genome sequence data increases the reliability of genomic prediction. J. Dairy Sci. 98, 4107–16.

Conte, S., Bergeron, R., Grégoire, J., Gète, M., D'Allaire, S., Meunier-Salaün, M.-C., Devillers, N., 2014. On-farm evaluation of methods to assess welfare of gestating sows. Animal 8, 1153–1161.

Cottle, D.L., McGrath, M.J., Cowling, B.S., Coghill, I.D., Brown, S., Mitchell, C. a, 2007. FHL3 binds MyoD and negatively regulates myotube formation. J. Cell Sci. 120, 1423–35.

DanAvl, 2014. Annual Report. URL: http://www.danavl.com/~/media/danbred/News/DanAvl_profil_global_UK.pdf

de Sevilla, X.F., Fàbrega, E., Tibau, J., Casellas, J., 2008. Effect of leg conformation on survivability of Duroc, Landrace, and Large White sows. J. Anim. Sci. 86, 2392–400.

Dekkers, J.C., 2004. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J. Anim. Sci. 82, E313–E328.

Dekkers, J.C.M., 2007. Marker-assisted selection for commercial crossbred performance. J. Anim. Sci. 85, 2104–2114.

Dewey, C.E., Friendship, R.M., Wilson, M.R., 1993. Clinical and postmortem examination of sows culled for lameness. Can. Vet. J. 34, 555–556.

Do, D.N., Ostersen, T., Strathe, A.B., Mark, T., Jensen, J., Kadarmideen, H.N., 2014. Genome-wide association and systems genetic analyses of residual feed intake, daily feed consumption, backfat and weight gain in pigs. BMC Genet. 15, 27.

Draper, D.D., Rothschild, M.F., Christian, L.L., 1992. Effects of Divergent Selection for Leg Weakness on Muscle and Bone Characteristics in Duroc Swine. Genet Sel Evol 24, 363–374.

Einarsson, S., Brandt, Y., Lundeheim, N., Madej, A., 2008. Stress and its influence on reproduction in pigs: a review. Acta Vet. Scand. 50, 48.

Engblom, L., Eliasson-Selling, L., Lundeheim, N., Belák, K., Andersson, K., Dalin, A.-M., 2008. Post mortem findings in sows and gilts euthanised or found dead in a large Swedish herd. Acta Vet. Scand. 50, 25.

Engblom, L., Lundeheim, N., Dalin, A.-M., Andersson, K., 2007. Sow removal in Swedish commercial herds. Livest. Sci. 106, 76–86.

Engblom, L., Lundeheim, N., Schneider, M.D.P., Dalin, A.-M., Andersson, K., 2009. Genetics of crossbred sow longevity. Animal 3, 783–90.

Engblom, L., Lundeheim, N., Strandberg, E., Schneider, M.D.P., Dalin, A.-M., Andersson, K., 2008. Factors affecting length of productive life in Swedish commercial sows. J. Anim. Sci. 86, 432–41.

Fan, B., Onteru, S.K., Du, Z.Q., Garrick, D.J., Stalder, K.J., Rothschild, M.F., 2011. Genome-wide association study identifies loci for body composition and structural soundness traits in pigs. PLoS One 6(2), e14726.

Fan, B., Onteru, S.K., Mote, B.E., Serenius, T., Stalder, K.J., Rothschild, M.F., 2009. Large-scale association study for structural soundness and leg locomotion traits in the pig. Genet. Sel. Evol. 41, 14.

Favier, B., Dollé, P., 1997. Developmental functions of mammalian Hox genes. Mol. Hum. Reprod. 3, 115–131.

Goddard, M.E., Hayes, B.J., 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nat. Rev. Genet. 10, 381–391.

Grandin, T., 2010. The importance of measurement to improve the welfare of livestock, poultry and fish. URL: http://www.grandin.com/importance.measurement.improve.welfare.html

Grandjot, G., 2007. Claw problems cost money. SUS - Schewinezucht und Schweinesmast. Munst. Ger. Landwirtschaftsverlag GmbH 5, 28–31.

Grindflek, E., Hamland, H., Aasmundstad, T., 2014. Genome-wide association study for conformation traits and osteochondrosis in pigs, in: Proceedings of the 10th World Congress of Genetics Applied to Livestock Production. pp. 1–3.

Groenen, M.A.M., Archibald, A.L., Uenishi, H., Tuggle, C.K., Takeuchi, Y., Rothschild, M.F., Rogel-gaillard, C., Park, C., Megens, H., Li, S., Larkin, D.M., Kim, H., Laurent, A., Frantz, F., Caccamo, M., Ahn, H., Aken, B.L., Anselmo, A., Berman, D., Blecha, F., Blomberg, J., Bolund, L., Botti, S., Bujie, Z., Bystrom, M., Capitanu, B., Carvalho, D., Chardon, P., Chen, C., Cheng, R., Choi, S., Clark, R.C., Clee, C., Crooijmans, R.P.M.A., Harry, D., Dehais, P., Sapio, F. De, Dibbits, B., Drou, N., Eversole, K., Fadista, J., Fairley, S., Faraut, T., Geoffrey, J., Fowler, K.E., Fredholm, M., Fritz, E., Gilbert, J.G.R., Giuffra, E., Gorodkin, J., Griffin, D.K., Harrow, J.L., Howe, K., Hu, Z., Humphray, S.J., Hunt, T., 2012. Analyses of pig genomes provide insigt into porcine demography and evolution. Nature 491, 393–398.

Guo, X., Su, G., Christensen, O.F., Janss, L., Lund, M.S., 2016. Genome-wide association analyses using a Bayesian approach for litter size and piglet mortality in Danish Landrace and Yorkshire pigs. BMC Genomics 17, 468.

Guo, Y.M., Ai, H.S., Ren, J., Wang, G.J., Wen, Y., Mao, H.R., Lan, L.T., Ma, J.W., Brenig, B., Rothschild, M.F., Haley, C.S., Huang, L.S., 2009. A whole genome scan for quantitative trait loci for leg weakness and its related traits in a large F2 intercross population between White Duroc and Erhualian. J. Anim. Sci. 87, 1569–1575.

Hanset, R., Dasnois, C., Scalais, S., Michaux, C., Grobet, L., 1995. Effets de l’introgression dans le génome Piétrain de l'allèle normal au locus de sensibilité à l'halothane. Genet. Sel. Evol. 27, 77–88.

Heinonen, M., Oravainen, J., Orro, T., Virolainen, J., Tast, A., Peltoniemi, O.A.T., 2006. Lameness and fertility of sows and gilts in randomly selected loose-housed herds in Finland. Vet. Rec. 159, 383–387.

Heinonen, M., Peltoniemi, O., Valros, A., 2013. Impact of lameness and claw lesions in sows on welfare, health and production. Livest. Sci. 156, 2–9.

Hidalgo, A.M., 2015. Exploiting genomic information on purebred and crossbred pigs [dissertation]. [Wageningen]: Wageningen University.

Huisman, A., 2007. More Accurate Breeding for Conformation. URL: http://www.thepigsite.com/articles/2073/more-accurate-breeding-for-conformation/

Ibáñez-Escriche, N., Forni, S., Noguera, J.L., Varona, L., 2014. Genomic information in pig breeding: Science meets industry needs. Livest. Sci. 166, 94–100.

Jensen, T.B., Baadsgaard, N.P., Houe, H., Toft, N., Østergaard, S., 2007. The effect of lameness treatments and treatments for other health disorders on the weight gain and feed conversion in boars at a Danish test station. Livest. Sci. 112, 34–42.

Jin, S., Lee, J.B., Kang, K., Yoo, C.K., Kim, B.M., Park, H.B., Lim, H.T., Cho, I.C., Maharani, D., Lee, J.H., 2013. The possibility of TBC1D21 as a Candidate gene for teat numbers in pigs. Asian-Australasian J. Anim. Sci. 26, 1374–1378.

Jørgensen, B., 2003. Influence of floor type and stocking density on leg weakness, osteochondrosis and claw disorders in slaughter pigs. Anim. Sci. 77, 439–449.

Jørgensen, B., Andersen, S., 2000. Genetic parameters for osteochondrosis in Danish Landrace and Yorkshire boars and correlations with leg weakness and production. Anim. Sci. 71, 427–434.

Kadarmideen, H.N., Schworer, D., Ilahi, H., Malek, M., Hofer, A., 2004. Genetics of osteochondral disease and its relationship with meat quality and quantity, growth, and feed conversion traits in pigs. J. Anim. Sci. 82, 3118–3127.

Kadarmideen, H.N., Thompson, R., Simm, G., Eh, M., 2000. Linear and threshold model genetic parameters for disease, fertility and milk production in dairy cattle. Anim. Sci. 71, 411–419.

Kessel, M., Gruss, P., 1991. Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell 67, 89–104.

KilBride, A.L., Gillman, C.E., Green, L.E., 2009. A cross sectional study of the prevalence, risk factors and population attributable fractions for limb and body lesions in lactating sows on commercial farms in England. BMC Vet. Res. 5, 30.

Kim, K.S., Larsen, N., Short, T., Plastow, G., Rothschild, M.F., 2000. A missense variant of the porcine melanocortin-4 receptor (MC4R) gene is associated with fatness, growth, and feed intake traits. Mamm. Genome 11, 131–135.

Kirk, R.K., Jørgensen, B., Jensen, H.E., 2008. The impact of elbow and knee joint lesions on abnormal gait and posture of sows. Acta Vet. Scand. 50, 5.

Kirk, R.K., Svensmark, B., Ellegaard, L.P., Jensen, H.E., 2005. Locomotive disorders associated with sow mortality in Danish pig herds. J. Vet. Med. Ser. A Physiol. Pathol. Clin. Med. 52, 423–428.

Knap, P.W., 2005. Breeding robust pigs. Aust. J. Exp. Agric. 45, 763–773.

Knauer, M., Stalder, K.J., Serenius, T., Baas, T.J., Berger, P.J., Karriker, L., Goodwin, R.N., Johnson, R.K., Mabry, J.W., Miller, R.K., Robison, O.W., Tokach, M.D., 2010. Factors associated with sow stayability in 6 genotypes. J. Anim. Sci. 88, 3486–92.

Knauer, M.T., Cassady, J.P., Newcom, D.W., See, M.T., 2011. Phenotypic and genetic correlations between gilt estrus, puberty, growth, composition, and structural conformation traits with first-litter reproductive measures. J. Anim. Sci. 89, 935–42.

Kogelman, L.J.A., Pant, S.D., Fredholm, M., Kadarmideen, H.N., 2014. Systems genetics of obesity in an F2 pig model by genome-wide association, genetic network, and pathway analyses. Front. Genet. 5, 1–15.

Kraeling, R.R., Webel, S.K., 2015. Current strategies for reproductive management of gilts and sows in North America. J. Anim. Sci. Biotechnol. 6, 3.

Laenoi, W., Uddin, M.J., Cinar, M.U., Grosse-Brinkhaus, C., Tesfaye, D., Jonas, E., Scholz, A.M., Tholen, E., Looft, C., Wimmers, K., Phatsara, C., Juengst, H., Sauerwein, H., Mielenz, M., Schellander, K., 2011. Quantitative trait loci analysis for leg weakness-related traits in a Duroc x Pietrain crossbred population. Genet. Sel. Evol. 43, e13.

Lee, G.J., Archibald, A.L., Garth, G.B., Law, A.S., Nicholson, D., Barr, A., Haley, C.S., 2003. Detection of quantitative trait loci for locomotion and osteochondrosis-related traits in Large White × Meishan pigs. Anim. Sci. 76, 155–165.

Leenstra, F.R., Voorst, A. van, Haye, U., 1984. Genetic aspects of twisted legs in a broiler sire strain. Ann. Agric. Fenn. 23, 261–270.

López-Serrano, M., Reinsch, N., Looft, H., Kalm, E., 2000. Genetic correlations of growth, backfat thickness and exterior with stayability in large white and landrace sows. Livest. Prod. Sci. 64, 121–131.

Lundeheim, N., 1987. Genetic Analysis of Osteochondrosis and leg weakness in the Swedish Pig Progeny Testing scheme. Acta Agric. Scand. 37, 159–173.

Luther, H., Schwörer, D., Hofer, A., 2007. Heritabilities of osteochondral lesions and genetic correlations with production and exterior traits in station-tested pigs. Animal 1, 1105–11.

Madec, F., Cariolet, R., Dantzer, R., Croix, L., Carreire, D. De, 1986. Relevance of some behavioural criteria concerning the sow (motor activity and water intake) in the intensive pig farming and veterinary practice. Ann. Vet. Res. 17, 177–184.

Mao, H., Guo, Y., Yang, G., Yang, B., Ren, J., Liu, S., Ai, H., Ma, J., Brenig, B., Huang, L., 2008. A genome-wide scan for quantitative trait loci affecting limb bone lengths and areal bone mineral density of the distal femur in a White Duroc x Erhualian F2 population. BMC Genet. 9, 63.

Mote, B.E., Mabry, J.W., Stalder, K.J., Rothschild, M.F., 2009. Evaluation of current reasons for removal of sows from commercial farms. Prof. Anim. Sci. 25, 1–7.

Nielsen, B., Jørgensen, C.B., Vernersen, A., Fredholm, M., 2009. Effect of selection for E . coli F4ab / ac resistance in pigs, in: 60th Annual Meeting of the European Association for Animal Production. p. 208.

Nikkilä, M.T., Karriker, A., Boggess, M. V, Serenius, T. V, 2013. Genetic associations for gilt growth, compositional, and structural soundness traits with sow longevity and lifetime reproductive performance. J. Anim. Sci. 1570–1579.

Norsvin, 2013. Annual Report Norsvin Genetics 2013.

Pluym, L.M., Van Nuffel, A., Van Weyenberg, S., Maes, D., 2013. Prevalence of lameness and claw lesions during different stages in the reproductive cycle of sows and the impact on reproduction results. Animal 7, 1174–81.

Ramos, A.M., Crooijmans, R.P.M.A., Affara, N.A., Amaral, A.J., Archibald, A.L., Beever, J.E., Bendixen, C., Churcher, C., Clark, R., Dehais, P., Hansen, M.S., Hedegaard, J., Hu, Z.L., Kerstens, H.H., Law, A.S., Megens, H.J., Milan, D., Nonneman, D.J., Rohrer, G.A., Rothschild, M.F., Smith, T.P.L., Schnabel, R.D., Van Tassell, C.P., Taylor, J.F., Wiedmann, R.T., Schook, L.B., Groenen, M.A.M., 2009. Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. PLoS One 4, e6524.

Rauw, W.M., Kanis, E., Noordhuizen-Stassen, E.N., Grommers, F.J., 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livest. Prod. Sci. 56, 15–33.

Ringgenberg, N., Bergeron, R., Devillers, N., 2010. Validation of accelerometers to automatically record sow postures and stepping behaviour. Appl. Anim. Behav. Sci. 128, 37–44.

Rothschild, M., Soller, M., 1997. Candidate gene analysis to detect gene controlling traits of economic important in domestic livestock. Probe(Lond) 8, 13–20.

Rothschild, M.F., Christian, L.L., 1988. Genetic control of front-leg weakness in Duroc swine. I. Direct Response to five generations of divergent selection. Livest. Prod. Sci. 19, 459–471.

Rothschild, M.F., Christian, L.L., Jung, Y.C., 1988. Genetic control of front-leg weakness in Duroc swine. II. Correlated responses in growth rate, backfat and reproduction from five generations of divergent selection. Livest. Prod. Sci. 19, 473–485.

Rothschild, M.F., Hu, Z.L., Jiang, Z., 2007. Advances in QTL mapping in pigs. Int. J. Biol. Sci. 3, 192–197.

Rydhmer, L., 2005. Swine breeding programmes in the Nordic countries, National Swine Improvement Federation. URL: http://www.nsif.com/conferences/2005/pdf%5CBreedingNordicCountries.pdf

Sahana, G., Kadlecová, V., Hornshøj, H., Nielsen, B., Ole, F., Christensen, O.F., 2013. A genome-wide association scan in pig identifies novel regions associated with feed efficiency trait. J. Anim. Sci. 1041–1050.

Serenius, T., Mantysaari, E.A., 2001. The genetics of leg weakness in Finnish Large White and Landrace populations. Livest. Prod. Sci. 69, 101–111.

Serenius, T., Stalder, K.J., 2007. Length of productive life of crossbred sows is affected by farm management, leg conformation, sow's own prolificacy, sow's origin parity and genetics. Animal 1, 745–50.

Serenius, T., Stalder, K.J., 2004. Genetics of length of productive life and lifetime prolificacy in the Finnish Landrace and Large White pig populations. J. Anim. Sci. 82, 3111–3117.

Sobczyńska, M., Blicharski, T., Tyra, M., 2013. Relationships between longevity, lifetime productivity, carcass traits and conformation in Polish maternal pig breeds. J. Anim. Breed. Genet. 130, 361–71.

Soranzo, N., Rivadeneira, F., Chinappen-Horsley, U., Malkina, I., Richards, J.B., Hammond, N., Stolk, L., Nica, A., Inouye, M., Hofman, A., Stephens, J., Wheeler, E., Arp, P., Gwilliam, R., Jhamai, P.M., Potter, S., Chaney, A., Ghori, M.J.R., Ravindrarajah, R., Ermakov, S., Estrada, K., Pols, H.A.P., Williams, F.M., McArdle, W.L., Van Meurs, J.B., Loos, R.J.F., Dermitzakis, E.T., Ahmadi, K.R., Hart, D.J., Ouwehand, W.H., Wareham, N.J., Barroso, I., Sandhu, M.S., Strachan, D.P., Livshits, G., Spector, T.D., Uitterlinden, A.G., Deloukas, P., 2009. Meta-analysis of genome-wide scans for human adult stature identifies novel loci and associations with measures of skeletal frame size. PLoS Genet. 5, e1000445.

Stalder, K.J., Johnson, C., Miller, D.P., Baas, T.J., Berry, N., Christian, A.E., Serenius, T. V., 2005. Pocket guide for the evaluation of structural, feet, leg, and reproductive soundness in replacement gilts. Des Moines, Iowa Natl. Pork Board. Pub. no. 04764 1–26.

Steenbergen, E.J. Van, Kanis, E., Steen, H.A.M. Van Der, 1990. Genetic parameters of fattening performance and exterior traits of boars tested in central stations. Livest. Prod. Sci. 24, 65–82.

Stock, J.D., Díaz, J.A.C., Mote, B., Stalder, K.J., 2016a. Joint Angles for Feet and Leg Conformation Traits in Second Gestation Sows. Iowa State University. Faculty Papers and Publications in Animal Science. 892.

Stock, J.D., Díaz, J.A.C., Mote, B.E., Stalder, K.J., 2016b. Comparison of Joint Angles for Feet and Leg Conformation Traits between Gilts at Selection and at their Second Gestation and Sows Parity 5 and Above. Fac. Iowa State University. Faculty Papers and Publications in Animal Science. 890.

Tummaruk, P., Tantasuparak, W., Techakumphu, M., Kunavongkrit, A., 2008. Effect of the Removal Reasons on Longevity and Lifetime Production of Sows in a Commercial Swine Herd in Thailand, in: Federation of Asian Veterinary Association. pp. 2–3.

Uemoto, Y., Sato, S., Ohnishi, C., Hirose, K., Kameyama, K., Fukawa, K., Kudo, O., Kobayashi, E., 2010. Quantitative trait loci for leg weakness traits in a Landrace purebred population. Anim. Sci. J. 81, 28–33.

USDA-APHIS, 2007. Highlights of Swine 2006 Part I: Reference of Swine Health and Health Management Practices in the United States, 2006. URL: https://www.aphis.usda.gov/animal_health/nahms/swine/downloads/swine2006/Swine2006_is_PartI_Highlights.pdf

Van Eenennaam, A.L., Weigel, K. A, Young, A.E., Cleveland, M. A, Dekkers, J.C.M., 2014. Applied

Animal Genomics: Results from the Field. Annu. Rev. Anim. Biosci. 2, 105–139.

Vansickle, J., 2008. Sow Lameness Underrated. Natl. Hog Farmer. URL: http://nationalhogfarmer.com/health-diseases/0615-sow-lameness-underrated

Veselá, Z., Vostrý, L., Šafus, P., 2011. Linear and linear-threshold model for genetic parameters for SEUROP carcass traits in Czech beef cattle. Czech J. Anim. Sci. 9, 414–425.

Webb, A.J., Russell, W.S., Sales, D.I., 1983. Genetics of leg weakness in performance-tested boars. Anim. Prod. 36, 117–130.

Weigel, K. a, Rekaya, R., 2000. Genetic parameters for reproductive traits of Holstein cattle in California and Minnesota. J. Dairy Sci. 83, 1072–1080.

Willer, C.J., Li, Y., Abecasis, G.R., 2010. METAL: Fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26, 2190–2191.

Willgert, K., 2011. The economic and welfare impact of lameness in sows in England 1–16. URL: http://www.fao.org/fileadmin/user_upload/animalwelfare/TheeconomicandwelfareimpactoflamenessinsowsinEngland.pdf

Wood, C.M., 2001. Don't Ignore Feet and Leg Soundness in Pigs. URL: http://www.sites.ext.vt.edu/newsletter-archive/livestock/aps-01_06/aps-0375.html

Wu, X., Fang, M., Liu, L., Wang, S., Liu, J., Ding, X., Zhang, S., Zhang, Q., Zhang, Y., Qiao, L., Lund, M.S., Su, G., Sun, D., 2013. Genome wide association studies for body conformation traits in the Chinese Holstein cattle population. BMC Genomics 14, 897.

Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Others, 2010. Common SNPs explain a large proportion of the heritability for human height. Nat Gen 42, 565–569.

Yang, J., Lee, S.H., Goddard, M.E., Visscher, P.M., 2011. GCTA: A tool for genome-wide complex trait analysis. Am. J. Hum. Genet. 88, 76–82. doi:10.1016/j.ajhg.2010.11.011

Yang, J., Zaitlen, N.A., Goddard, M.E., Visscher, P.M., Price, A.L., 2014. Advantages and pitfalls in the application of mixed-model association methods. Nat. Genet. 46, 100–106.

Ytrehus, B., Andreas Haga, H., Mellum, C.N., Mathisen, L., Carlson, C.S., Ekman, S., Teige, J., Reinholt, F.P., 2004. Experimental ischemia of porcine growth cartilage produces lesions of osteochondrosis. J. Orthop. Res. 22, 1201–9.

Zoric, M., Nilsson, E., Lundeheim, N., Wallgren, P., 2009. Incidence of lameness and abrasions in piglets in identical farrowing pens with four different types of floor. Acta Vet. Scand. 51, 23.

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Lê Hồng Thư