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Genome-wide association study for reproduction traits in maternal pig breeds

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ABSTRACT

The number of piglets born alive (NBA) is one of the most important reproduction traits due to its influence on pig production efficiency. It was shown in several studies that NBA has an antagonistic relationship with later fattening performance of the pig. To clarify the genetic background of NBA and to detect possible pleiotropic effects with the production traits growth (ADG), lean meat percentage (LMP) and backfat (BF), Genome-Wide Association Studies (GWAS) using estimated breeding values (EBVs) as phenotypes were performed. Therefore, 4,012 Large White (LW) and Landrace (LR) pigs from herdbook and commercial breeding companies in Germany, Austria and Switzerland were genotyped with the Illumina PorcineSNP60 BeadChip.

The aims of the first study were a) to reveal genetic similarities and differences between LW and LR populations, b) to identify significant associated SNPs with NBA, and c) to clarify the biological relevance of these markers. Because of genetic distances between and within the two breeds, GWAS were performed within each breed and five further sub-clusters for each breed. In total, 17 significant markers affecting NBA were found in regions with known effects on female fertility. No overlapping significant chromosome areas or QTLs for both breeds were detected.

In the second step, GWAS was performed for NBA and production traits (LMP, ADG, BF) to identify possible pleiotropic effects. In a first approach univariate GWAS was performed and resulting SNP positions of all traits were compared. The second approach was based on a principal component analyses (PCA). All EBVs were condensed into representative, uncorrelated principal components (PCs) and used as new phenotype in multivariate GWAS. The relevance of each EBV within a PC was quantified by their corresponding loading. Using univariate method 79 SNPs were identified and only one SNP with potential pleiotropic effects were found. Using the multivariate approach, 98 significant SNPs with partly antagonistic relationships between reproduction and production traits were identified.

In conclusion, population specific SNPs with a significant influence on analyzed traits were identified. Only some of the SNPs were confirmed in direct sub-clusters. Multivariate approach resulted in a higher number of detected pleiotropic effects compared to univariate method. Due to genetic distances between the different populations and the lower number of significant SNPs when GWAS was performed in breeding organization overlapping data sets, a combination of different data sets would not be beneficial.

ZUSAMMENFASSUNG

Die Anzahl lebend geborenen Ferkel (LGF) ist aufgrund der ökonomischen Bedeutung eines der wichtigsten Reproduktionsmerkmale. Frühere Studien zeigten antagonistische Beziehungen zwischen LGF und späteren Mastleistungen der Schweine. Um den genetischen Hintergrund der LGF und pleiotrope Effekte mit den Produktionsmerkmalen tägl. Zunahme (TGZ), Muskelfleischanteil (MFA) und Rückenspeckdicke (RSD) zu klären, wurden genomweite Assoziationsstudien (GWAS) mit dem Zuchtwert als Phänotyp durchgeführt. Dafür wurden 4.012 Edelschwein (LW) und Landrasse (LR) Schweine von Herdbuch und kommerziellen Zuchtorganisationen aus Deutschland, Österreich und der Schweiz mit dem Illumina PorcineSNP60 BeadChip genotypisiert.

Das Ziel der ersten Studie war a) genetische Ähnlichkeiten und Unterschiede zwischen LW und LR Populationen aufzudecken, b) die Identifizierung von SNPs mit signifikanten Einfluss auf LGF, und c) die Klärung der biologischen Relevanz dieser Marker. Aufgrund genetischer Distanzen zwischen und innerhalb beider Rassen wurden die GWAS innerhalb jeder Rasse und in fünf Sub-Clustern je Rasse durchgeführt. Insgesamt wurden 17 signifikante SNPs identifiziert, die innerhalb bekannter Regionen mit Einfluss auf Reproduktion lagen. Gemeinsame signifikante Chromosomen Regionen oder QTLs für beide Rassen wurden nicht identifiziert.

Im zweiten Schritt wurden GWAS für LGF und MFA, TGZ und RSD durchgeführt, um mögliche pleiotrope Effekte zu finden. Im ersten Schritt wurden univariate GWAS durchgeführt und die Ergebnisse aller Merkmale miteinander verglichen. Der zweite Schritt basierte auf einer principal component Analyse (PCA). Alle Zuchtwerte wurden dafür in unkorrelierte principal components (PCs) kondensiert und als neuer Phänotyp für die GWAS genutzt. Die Bedeutung jedes Zuchtwertes innerhalb der PCs wurde anhand des entsprechenden loadings quantifiziert. Mittels des univariaten Ansatzes wurden 79 SNPs gefunden und nur ein SNP zeigte pleiotrope Effekte. Die multivariaten Analysen ergaben 98 SNPs mit zum Teil antagonistischen Beziehungen zwischen den beiden Merkmalskomplexen.

Es lässt sich zusammenfassen, dass signifikante populationsspezifische SNPs für alle untersuchten Merkmale identifiziert wurden. Diese Marker konnten teilweise in direkten Sub-Clustern bestätigt werden. Der multivariate Ansatz ergab eine höhere Anzahl an pleiotropen SNPs im Vergleich zu univariaten Analysen. Aufgrund Poulationsstratifikationen und der niedrigeren Anzahl an signifikanten Markern in Analysen mit überlappenden Datensätzen, kann gefolgert werden, dass eine Kombination der Datensätze nicht vorteilhaft ist.

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LIST OF ABBREVIATIONS

ADG	Average daily gain
AHCTF1	AT hook containing transcription factor 1
AI	Artificial insemination
AKR1C2	Aldo-keto reductase family 1
BF	Backfat
BFT	Backfat thickness
BLUP	Best linear unbiased prediction
BMP5	Bone morphogenetic protein 5
BMP7	Bone morphogenetic protein 7
BW	Birth weight
CAPN1	Calpain 1
CFB	Complement factor B / properdin
CHCHD3	Coiled-coil-helix-coiled-coil-helix domain containing 3
CLN	Corpus luteum number
CRH	Corticotropin releasing hormone
CTSB	Cathepsin B
CTSF	Cathepsin F;
CTSL	Cathepson L
CTSL1	Cathepsin L1
d	Day
DGAT1	Diacylglycerol acyltransferase 1
DIO3	Deiodinase, iodothyronine type III
DU	Duroc
DYD	Daughter yield deviation
EBV	Estimated breeding value

ENO3	Enolase 3
EPBH2	Eph receptor tyrosine kinases B2
EPOR	Erythropoietin receptor
ESR	Estrogen receptor
ESR1	Estrogen receptor 1
ESR2	Estrogen receptor 2
Fbln1	Fibulin -1
Flrt2	Fibronectin leucine-rich repeat transmembrane protein 2
Flt1	FMS-like tyrosine kinase 1
FSH	Follicle stimulating hormone
FSHB	Follicle stimulating hormone beta
FUT1	Fucosyltransferase 1
gBLUP	Genomic Best Linear Unbiased Prediction
GC	Genomic control
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GNRHR	Gonadotropin releasing hormone receptor
GRAMMAR	Genome-wide rapid analysis using mixed models and regression
GS	Genomic Selection
GWAS	Genome-Wide Association Study
h^2	Heritability
HA	Hampshire
HBW	High birth weight
HD	High-density chip
HNF1A	HNF1 homeobox A
HSD17B1	Hydroxysteroid (17-beta) dehydrogenase 1

HWG	Hardy-Weinberg-Equilibrium
IB	Iberian
IBD	Identity-by-descent
IBS	Identity-by-state
IBW	Individual birth weight
IGF2	Insulin like growth factor 2
IGFBP3	Insulin-like growth factor binding protein 3
INPP5F	Inositol polyphosphate-5-phosphatase F
ITGβ1	Integrin β 1
LBW	Low birth weight
LC	Leicoma
LCK	Lymphocyte protein tyrosine kinase
LD	Linkage disequilibrium
LEP	Leptin
LEPR	Leptin receptor
LGF	Lebend geborene Ferkel
LIF	Leukemia inhibitory factor
LMC	Lean meat content
LMP	Lean meat percentage
LR	Landrace
LS	Litter size
LS1-5	Litter size from the first to fifth party
LS5	TNB 5 days after farrowing
LW	Large White
MAF	Minor allele frequency
MBW	Middle birth weight

MC3R	Melanocortin 3 receptor
MC4R	Melanocortin 4 receptor
MDS	Multidimensional scaling
ME1	Malic enzyme 1
MFA	Muskelfleischanteil
MIR27A	MicroRNA 27a
MS	Chinese Meishan
MSTN	Myostatin
MUMM	Mummified piglets
MYF5	Myogenic regulatory factor 5
MYOD1	Myogenic differentiation 1
MYOG	Myogenin
N	Number
NAMPT	Nicotinamide phosphoribosyltransferase
NAT9	N-acetyltransferase 9
NBA	Number of piglets born alive per litter
NBA1	Number of piglets born alive in the first litter
NBA2	Number of piglets born alive in the second litter
NBA2+	Number of piglets born alive in the second and following litters
NBA1-6	Number of piglets born alive in the first to sixth litter
N_e	Effective population size
NPW	Number of piglets weaned
NS	Natural service
NSB	Number of stillborn piglets
OPN	Osteopontin
PC	Principal component

PCA	Principal component analysis
PCR-RFLP	Polymerase chain reaction - restriction fragment length polymorphism
PI	Pietrain
PLA_2G4A	Phospholipase A_2 group 4A
POU1F1	POU class 1 homeobox 1
PPARα	Peroxisome proliferator activated receptor α
PPARγ	*P*eroxisome proliferator-activated receptor gamma
PPARD	Peroxisome proliferator-activated receptor delta
PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
PRLR	Prolactin receptor
PRKAG3	Protein kinase
PTGS2	Prostaglandin-endoperixode synthase 2
PYGM	Phosphorylase, glycogen, muscle
Q-Q Plots	Quantile-Quantile Plots
QTL	Quantitative trait loci
RBP4	Retinol binding protein 4
RNF4	Ring finger protein 4
ROPN1	Rhophilin associated tail protein 1
RSD	Rückenspeckdicke
RYR1	Ryanodine receptor 1
SNP	Single nucleotide polymorphism
SOD1	Superoxide dismutase 1
SPATA19	Spermatogenesis associated 19
SPP1	Secreted phosphoproteine 1
SSC	Sus scrofa chromosome
TBC1D1	TBC1 domain family member

TBG	Thyroxine-binding globulin
TF	Transferrin
TGZ	Tägliche Zunahme
TNB	Total number born piglets
UNC45B	Unc-45 homolog B
VEGF-A	Vascular endothelial growth factor A
VEZT	Vezatin
VRTN	Vertebrae development homolog
WNT10	Wingless-type *MMTV* integration site family
YO	Yorkshire

1 CHAPTER 1: GENERAL INTRODUCTION

1.1 General Background

Reproduction performance of the sow has a major impact on the economic efficiency of pig production. Traits like mothering ability, rearing rate and longevity next to number of piglets born alive (NBA) are of particular interest when reproduction performances of sows are evaluated. A genetic improvement is necessary especially against the background that about 30 % of sows are removed from the herd because of problems in reproduction (Stalder et al., 2005). Especially poor performance in NBA increased the risk of culling for the sow (Hoge and Bates, 2011). Additionally, piglet producers earn 34 € per piglet (25 kg) in North Rhine-Westphalia (Quelle: http://www.agrarmarkt-nrw.de/schweinemarkt.shtm), which is the lowest piglet price in the last two years. In order to generate higher profits in piglet production, selection goals of pig breeding organization are focused on the breeding of sows with high number of NBA (de Koning et al., 2001; Geisert and Schmitt, 2002; Hanenberg et al., 2001; Lewis et al., 2005).

In general, complex genetic basis of reproduction traits is characterized by low heritability (h^2). Mean h^2 of NBA estimated in literature is 0.1 and ranged from 0.0 to 0.6 (Bidanel, 2011; Rothschild and Bidanel, 1998). Severe antagonistic relationships within the trait complex fertility can be found between litter size and birth weight of the piglet and piglet survival (Roehe and Kalm, 2000). Moreover, indirect negative correlation between litter size and later growth performance and carcass traits has been reported (Brien, 1986; Haley et al., 1988). These antagonistic relationships have to be investigated in detail because reproduction and production trait complexes are responsible for the economic profit in swine production (Rothschild et al., 1996).

Improvements in female reproduction and production traits have been achieved with selection based on quantitative genetic theory and the best linear unbiased prediction (BLUP) method. However, low h^2 and sex-limited expression of female reproduction traits represent a challenge for animal breeders. During the last years, genetic maps in livestock species were developed. This is a prerequisite of a better understanding of the genetic architecture of these traits which allows selection on genetic variants affecting these traits known as quantitative trait loci (QTL) (Bidanel et al., 2008; Lande and Thompson, 1990). Moreover, the new tool of high-density (HD) single nucleotide polymorphism (SNP) chips and novel technologies of sequencing enable breeders to benefit from the application of these powerful new methods to understand and investigate the biological basis of genetic variations (Bidanel, 2011). Consequently, the use of molecular marker information may be very useful to increase rates

of genetic improvement and for identification of possible candidate genes for both trait complexes. Moreover, SNPs, quantitative trait locis (QTLs) or even genes could be identified influencing more than one trait. Those pleiotropic effects must be taken into consideration when genetic markers are used for selection methods via modern breeding tool genomic selection (GS). Within this procedure, genetic markers normally get weighed in a statistical optimal way using procedures like genomic BLUP (gBLUP) or Bayesian methods (Goddard and Hayes, 2007; Meuwissen, 2007). However, in order to optimize the overall genetic gain and to avoid negative side effects, it could be useful to modify these marker weights depending on their biologically importance for the trait of interest. The genetic background has to be deciphered in order to improve the biological understanding and to achieve an effective increase in litter size (Hernandez et al., 2014).

In the first section of this study, these main maternal influencing factors on NBA and litter size will be described in brief. In the following chapters, clarifications of the biological and genetic architecture of NBA using Genome-Wide Association Study (GWAS) were performed. Moreover, genetic similarities and differences between Large White (LW) and Landrace (LR) populations used for GWAS will be described. Furthermore, possible pleiotropic effects between NBA and production traits (average daily gain (ADG), backfat (BF) and lean meat percentage (LMP)) were investigated.

1.2 Genetical and biological aspects of reproduction traits

1.2.1 Phenotypic and genetic trends in litter size

Selection on reproduction traits was focused on increasing litter size and especially NBA. Based on this, different selection experiments towards an increase in litter size, number of total born piglets per litter (TNB) and NBA were performed by several studies. Wang et al. (1994) used BLUP breeding values to improve TNB and reached a genetic response of about 1.6 % per year. Direct genetic selection response of 0.43 TNB piglets was achieved using average breeding values of the parents of the litter as selection criteria (Sorensen et al., 2000). Noguera et al. (2002b) concluded, that the highest increase of litter size was achieved, when selection was based on a family selection index combined with intense selection in a large population. With this selection strategy, an increase in NBA up to 6.3 % was achieved in the selection line for litter size in comparison to control line, in which no selection was performed (Noguera et al., 2002b).

In Germany, NBA increased from 10.55 in 1996 to 11.92 in 2009 (ZDS, 2010). In the same period, piglet mortality increased from 16.4 % to 17.6 % (Figure 1).

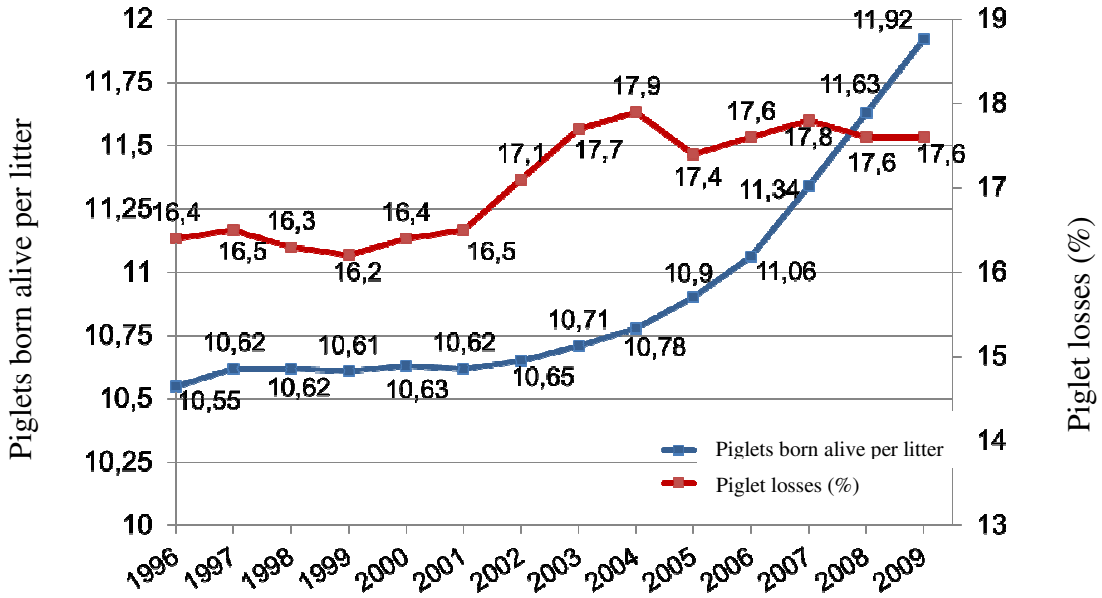

Figure 1: Development of number of piglets born alive per litter and piglet mortality from 1996 till 2009 in Germany, adapted from ZDS (2010)

In comparison to Germany, similar developments have been described in other countries. From 1998 to 2008 NBA increased from 10.2 to 11.35 in USA and Canada (PigCHAMP, 1998, 2008).

1.2.2 Biological aspects of litter size traits

The main limiting factor which determines litter size is the ovulation rate (Bennett and Leymaster, 1989; Buske et al., 2006a; Caárdenas and Pope, 2002; King and Williams, 1984; Lamberson et al., 1991; Tummaruk et al., 2001). Other key factors are uterine capacity, which is described as the maximum number of conceptuses the dam can nourish during gestation (Bennett and Leymaster, 1989) and the embryonic survival (Bennett and Leymaster, 1989; Holm et al., 2005; Rathje et al., 1997; Tummaruk et al., 2001).

Ovulation rate

The ovulation rate is defined as the total number of ovulated ova during one oestrus (Rohrer et al., 1999). Already during early fetal life oogenesis begins. During every oestrus period the number of ovulated follicles is about 10-25 and increases with oestrus and parity number until the fourth or fifth parity (Bidanel, 2011). As a consequence, litter sizes from primiparous and multiparous sows differ significantly.

Positive correlation between ovulation rate and litter size at birth (LS) were detected in a study performed by Benett and Leymaster et al. (1989). Additionally, they detected the largest increase in litter size when selection was focused on ovulation rate and uterine capacity (Bennett and Leymaster, 1989). The hypothesis was supported by more recent studies which also suggested that an increase in ovulation rate could be the main reason for the observed response to selection for litter size (Lamberson et al., 1991; Noguera et al., 2002b; Rathje et al., 1997). Johnson et al. (1999) performed an index selection for ovulation rate leading to a significant increase in litter size after 14 generations of selection. It can be concluded that ovulation rate is the limiting factor of TNB (Bennett and Leymaster, 1989; Buske et al., 2006a; Caárdenas and Pope, 2002; King and Williams, 1984; Lamberson et al., 1991; Tummaruk et al., 2001).

Bidanel et al. (2008) analysed influencing factors on ovulation rate and number of viable embryos in a LW and Chinese Meishan (MS) cross population. They found significant positive correlations between these traits and weight at first mating of the sow. In general,

maternal nutrition before and during gestation has an impact on NBA and litter size because ovarian function is optimal when maternal diet is on a normal level (Caárdenas and Pope, 2002). It is well known, that the maternal diet influences embryonic and fetal growth by releasing glucose and further essential nutrients (Robinson et al., 1999). When nutrition restriction was performed during luteal and follicular phases in post pubertal gilts, ovulation rate decreased (Prunier and Quesnel, 2000). This alteration of ovulation rate might be induced by changes in levels of segregated growth factors, gonadotropin and metabolic hormones (Flowers et al., 1989).

Embryonic and fetal survival

Beside ovulation rate, embryonic survival also has a major impact on NBA (Blasco et al., 1995; Spotter and Distl, 2006). This influence has been shown in an experiment by Johnson et al. (1999) where selection for embryonic survival resulted in increased litter sizes. However, selection for larger litter sizes performed in the last years resulted in an increase of piglet mortality which leads to ethical and economic problems (Cecchinato et al., 2010; Damgaard et al., 2003; Kerr and Cameron, 1995; Knol et al., 2002; Leenhouwers et al., 1999; Lund et al., 2002; Su et al., 2007; Varona and Sorensen, 2014).

Survival rate is a product of embryonic and fetal survival and successful uterus implantation (Blasco et al., 1995). Bennet and Leymaster (1989) defined embryonic survival as litter size divided by ovulation rate which is highly influenced by embryonic viability. They suggested that embryonic survival is equal or less than embryonic viability.

The fertilization rate in sows is almost 100 % but prenatal survival is only about 60 % which means that 40 % of embryos and fetuses die during pregnancy (Christenson et al., 1987; Geisert and Schmitt, 2002). In general, the first four weeks of gestation constitute the most critical phase because embryonic mortality is about 20-30 % during this time period (Geisert and Schmitt, 2002; Pope, 1994). Bennett and Leymaster (1989) suggested that due to limited uterine capacity some embryos will be lost which have an impact in embryonic viability. Furthermore, embryonic implantation at day (d) 13 to 18 is another very critical phase. Most of the embryos die during these phases of implantation and initiation of placental attachment to the uterine surface because of abnormalities in development processes during embryogenesis (Pope and First, 1985; Spotter and Distl, 2006). Fetal death which occurred between d 31-70 and onwards of pregnancy has an average of 10-20 % (Geisert and Schmitt, 2002; Pope, 1994).

A multitude of closely linked signals between the uterus, ovary and conceptus influence the maintenance and establishment of pregnancy (Bazer et al., 1982; Roberts et al., 1993). Early embryonic losses can be induced by inappropriate timing of uterine and conceptuses development. This development is influenced by the nutrients synthesis and factors of attachment, failing in conceptus signaling, competition between embryos (uterus crowding), and genetic impacts affecting the embryonic mortality (Geisert and Schmitt, 2002).

Ford (1995) pointed out, that the passage of nutrients to the placenta maintained by capillary blood flow in the endometrium is the key factor for the survival of the embryo. Therefore, trophoblastic elongation is an important factor for embryonic survival. The trophoblast expansion regulates and limits the final size of the placental surface area of each embryo during gestation. Embryonic mortality or even failure in pregnancy establishment during early gestation can also be caused by asynchronous development of the uterine environment and the individual fetus during preimplantation (Distl, 2007; Geisert and Yelich, 1997). Therefore, embryonic development has an impact on maintaining of the pregnancy because pregnancy is only sustained if a substantial portion of each uterus horn is occupied by conceptus (Geisert et al., 1990).

It can be concluded that the selection for increased litter size led to a reduction of 2-3 % in survival rate for every additionally born piglet (Pettigrew, 1981).

Uterine capacity

Uterine capacity is described as the maximum number of fetuses which can implant in the uterus, assumed that their number is not limited by ovulation rate (Christenson et al., 1987). Vallet et al. (2006) defined uterine capacity as the number of fully formed fetuses which can be held by the uterus till birth. This is a result of interaction between uterine, placental and fetal factors, which contributes to embryonic survival.

Bidanel et al. (2008) found significant negative correlation between embryo survival and ovulation rate (-0.13) in a LW–MS cross. This is an agreement with Sorensen et al. (2000) who found higher proportion of stillborn piglets in increased litter sizes. These findings suggest that increasing litter size goes along with uterine competition between embryos. Additionally, it was indicated that the uterus can only support a limited number of embryos sufficiently. Moreover, the size of the embryo at an early stage of gestation was an influencing factor for embryonic survival and as a consequence for NBA. Embryos which were smaller than their littermates (7-9 mm vs. 10 mm at d 11-12) during first weeks of

gestation exhibited developmental lapse. These less-developed embryos gained reduced uterine space which induces disadvantages in survival if the uterus was crowded (Geisert et al., 1982). It was suggested that a 7 week old embryo needs at least 20 cm of uterine horn for a high survival probability (Wu et al., 1988). Wu et al. (1987) concluded that uterine length is limiting factor determining litter size with increasing ovulation rate.

It has been demonstrated by several authors that more developed embryos had competitive advantages in embryonic survival within the uterus (Pope and First, 1985; Pope et al., 1986; Wilde et al., 1988). Geisert and Schmitt (2002) mentioned in case of uterus crowding, that embryonic mortality was induced by individual embryo asynchrony with its uterine environment instead of competitive advantages between d 5 to 10 of gestation. These embryonic losses normalize the uterine space which was now available for the surviving embryos. Theoretically, embryo uniformity would be desirable for a high embryonic survival rate (Geisert and Schmitt, 2002). To reach this, uterus crowding which was the exceedance of the uterus capacity due to a too high number of ovulations should be avoided. Additionally, an uniform maturity and viability of ovulated oocytes, synchronously fertilization next to the same genetic potential for rate of development, and equally spacing in uterus were required for high survival rate. Therefore, Geisert and Schmitt (2002) concluded that uterine crowding induced by exceeded uterus capacity by high ovulation rate should be avoided. As a consequence, uterine capacity is another important component contributing to litter size which was supported by the findings of several authors (Buske et al., 2006a; Christenson et al., 1987). When uterine crowding was avoided, the difficulty of gaining enough uterine space for placental development was less important for embryos even when they show some variability in development. For female pigs where uterine capacity was not exceeded, litter size was determined by the number of available embryos at d 12 (Geisert and Schmitt, 2002).

1.2.3 Relationship between litter size, birth weight and pre- and postnatal piglet survival

In the context of our study, the unfavourable relationship between NBA, individual birth weight (IBW) and pre- and postnatal piglet survival is of particular importance. Therefore, the impact of increased litter size on the other two traits is briefly described in the following section.

Knol et al. (2001) suggested that with increasing litter size piglets pre-weaning survival tends to decrease, because of limited maternal ability of the sow to rear the extra piglet. Intense selection for litter size implicates lower IBW, resulting in greater piglet mortality pre- and postnatal and more pigs discounted at market (Fix, 2010; Nielsen et al., 2013). Pre-weaning mortality was in a range of 13 to 25 % (Alonso-Spilsbury et al., 2007; Grandinson et al., 2002). This high piglet mortality raised animal welfare (Jarvis et al., 2005) and economic concerns (Crooks et al., 1992; Serenius et al., 2007) and maked this issue to one of the major problems in pig industry.

The genetic of pre- and postnatal piglet survival is very complex. This trait is mainly influenced by the dam (maternal effect) as well as by the piglet genotype (direct effect) and to a lesser extent by the service sire (paternal effect) (Blasco et al., 1995; Lund et al., 2002; Roehe and Kalm, 2000; van Arendonk et al., 1996). Maternal genetics effects consist of amount of milk, process of birth and mothering ability and illustrate the ability of the dam to create optimal conditions for their piglets to survive. Prenatal survival is mainly influenced by sow's genotype. In this stadium, embryonic or fetal genotype is not important (Blasco et al., 1995; van Arendonk et al., 1996).

IBW and relative birth weight defined as the difference between IBW and the mean birth weight of the litter, were considered to be the most important impact factors influencing the survival of the piglet from birth to weaning (Canario et al., 2006; Knol et al., 2002; Leenhouwers et al., 2003; Roehe and Kalm, 2000). Piglets with low IBW showed reduced postnatal survivability caused by a low level of body energy store, which resulted in a higher sensitivity to hypothermia. Additionally, they had a delayed first suckle and presented a lower ability to get the best teat. The resulting lower amount of colostrum and milk intake was associated with a poorer acquisition of passive immunity and a low nutritional status and, subsequently, with increased postnatal mortality or deteriorated growth performance and subnormal tissue differentiation (Hartsock et al., 1977; Klemcke et al., 1993; Quiniou et al., 2002).

Piglets IBW were mainly influenced by maternal effects, the influence of the dam on intrauterine growth of the embryo. Direct effects like the genetic potential of the piglet for intrauterine growth and the genotype of the sire were less important (Kaufmann et al., 2000; Roehe, 1999b). Dam's genotype contributed to the main part of genetic variation of piglet's birth weight (Arango et al., 2006; Knol et al., 2002). Estimated maternal h^2 for IBW ranged from 0.03 to 0.39 and direct h^2 from 0.02 to 0.36 (Arango et al., 2006; Chimonyo et al., 2006;

Damgaard et al., 2003; Kapell et al., 2010; Kaufmann et al., 2000; Knol et al., 2002; Roehe, 1999b; Roehe et al., 2010; van Arendonk et al., 1996).

Breeding success in increasing litter size raised problems because low IBW was highly negative correlated with postnatal survival, carcass quality and growth performance (Fix et al., 2010b; Kerr and Cameron, 1995; Knol et al., 2001; Leenhouwers et al., 1999; Quiniou et al., 2002; Rehfeldt and Kuhn, 2006). With each additional piglet within a litter, IBW was reduced by 30 to 44 g (Beaulieu et al., 2010; Kerr and Cameron, 1995; Quiniou et al., 2002; Roehe, 1999a; Smit et al., 2013). Estimated correlations between birth weight and litter size/NBA were all negative and ranged from -0.18 to -0.86 (Bidanel, 2011; Hermesch et al., 2000b; Kaufmann et al., 2000; Rosendo et al., 2007b; Rydhmer et al., 2008).

One of the main physiological reasons for decreased postnatal survival was an insufficient fetal nutrition due to poor uterus position and the competition for nutrition between litter mates in uterus (Perry and Rowell, 1969; Rehfeldt and Kuhn, 2006; Wigmore and Stickland, 1983). The effect of uterine crowding due to large litter sizes resulting in low birth weight was discussed in Johnson et al. (1999). Similar findings were reported by two large studies (n > 10,000 pigs): reduced birth weight was associated with increased litter size (Quiniou et al., 2002; Roehe, 1999b).

Due to the negative correlations between IBW and piglet survival as well as IBW and NBA, negative correlations between NBA/litter size and piglet survival can be expected. This antagonistic relationships were found in several analysis (Canario et al., 2006; Johnson et al., 1999; Kerr and Cameron, 1995; Lund et al., 2002; Roehe et al., 2010; Rosendo et al., 2007b). Nielsen et al. (2013) estimated genetic correlation between mortality and litter size between 0.20-0.28. Maternal and direct genetic correlations between birth weight and pre-weaning piglet mortality ranged from -0.16 to -0.43 (Arango et al., 2006). This illustrates that low IBW was associated with higher mortality probability in comparison to high IBW piglets. Mean phenotypic (genetic) correlation between NBA and prenatal survival rate was $r_p = 0.40$ ($r_g = 0.55$) estimated in literature and listed by Bidanel (2011).

Breeding progress for NBA or TNB might have also a negative impact on number of stillborn piglets (NSB). It was reported that the proportion of stillborn piglets was undesirable increased at very small or high litter size values (Canario et al., 2006; Hanenberg et al., 2001; Sorensen et al., 2000) which was the main reason for postnatal piglet mortality (Strange et al., 2013). Selection for increased litter size led to uterus crowding and as a consequence to reduced weight of the embryos. It was suggested by several authors that piglets with low IBW

were more prone to asphyxia or hypoxia during parturition and therefore the risk of mortality increased for those piglets (Alonso-Spilsbury et al., 2007; Herpin et al., 2001; Leenhouwers et al., 1999; Quiniou et al., 2002). Piglets born in small litters might cause problems for the sow during farrowing due to their oversize (Dziuk, 1979). Schneider et al. (2012a) estimated genetic correlations between NBA and number of stillborn piglets, number of mummified piglets and average birth weight of -0.16, -0.04 and -0.31, respectively. Nielsen et al. (2013) found unfavourable phenotypic and genetic correlations between TNB and mortality of the piglet in Landrace (LR) and Yorkshire population. This was an agreement with Su et al. (2007) who detected negative genetic correlation between TNB with piglet survival at birth and survival during suckling. Other studies reported that an intense selection based on embryonic survival and ovulation rate had an unfavourable effect on number of stillborn piglets (Johnson et al., 1999; Petry and Johnson, 2004).

Based on the unfavourable correlation between increased litter size and IBW, and embryonic and piglet mortality, breeding goals have to be adjusted for these relationships. Selection within dam lines should be modified to include an indirect selection for improved survival by selecting for increased IBW (Kapell et al., 2010). Simultaneously, improvement of NBA, IBW and piglet survival might be possible, but there is a limit in how far both, litter size and IBW, can be increased likewise due to their negative correlation (Kapell et al., 2010). In Danish pig breeding programme selection from TNB was changed to TNB at d 5 after farrowing (LS5) (Su et al., 2007). This selection strategy was not focused on the problem of mortality directly, but it seems that this selection strategy had a beneficial effect on litter size as well as on mortality at farrowing and during early suckling period (Nielsen et al., 2013).

1.2.4 Genetic effects on litter size traits

Line and breed differences

Differences in ovulation rate and as a consequence in litter size between breeds, or lines within breeds, have been demonstrated. As a result of selection for ovulation rate, high prolific lines of pigs have been developed (Johnson et al., 1999). Advantages in reproduction of these prolific lines were demonstrated by several authors (Tummaruk et al., 2001, 2000b, c). They found an increase in gilts own reproduction performance between 0.07 to 0.1 more piglets per litter ($p < 0.001$) when this gilt was born in large litters in turn. They concluded that gilts which were born by sows with higher embryonic survival, higher ovulation rate

and/or larger uterus capacity may inherit favorable genes from their mothers affecting these traits. Although, litter size in swine was highly influenced by environmental factors, the favorable genes of their mothers would resulted in an increase of the gilts own reproduction performance, especially of litter size (Tummaruk et al., 2000b, c).

Considerable differences were found in reproduction traits between breeds. The most prolific pig breed was the MS breed. The MS sows had larger litter sizes between three to five more piglets born per litter in comparison to European commercial breeds (Hernandez et al., 2014). However, a commercially breeding of MS was not performed in Europe because of poor growth performance and higher fat content of the carcass of MS pigs (Bidanel et al., 1990; Haley et al., 1992; Serra et al., 1992). Numerous studies have been performed to analyse the superiority of MS regarding litter size. Haley and Lee (1993) found higher prenatal survival at a particular level of ovulation rate and as a consequence larger litters in MS breed. Bidanel et al. (2008) reported that these differences in litter size between breeds like LW and MS were already present at an early stage of pregnancy (d 30). When gilts of MS and LW breeds were compared at the same amount of cycles after puberty, no significant differences in ovulation rate have been found. Differences between the breeds arose and appeared to increase as the sows get older (Bennett and Leymaster, 1989; Haley and Lee, 1993). Additionally, uterine sizes were similar when comparing LW and MS, but uterine capacity was higher in the MS breed. This advantage was reached by a better level of uterus organisation (Haley and Lee, 1993) as well as an increased placental efficiency (defined by the placental/foetal weight ratio) in comparison to European as well as to U.S. breeds (Biensen et al., 1998; Wilson et al., 1999). In comparison to the missing differences in ovulation rate reported by several authors (Bennett and Leymaster, 1989; Haley and Lee, 1993), Miller et al. (1998) found higher number of follicles and subsequently higher ovulation rate in MS sows in comparison to Large White (LW) sows.

In Europe, the breeds LW and LR were mainly used as maternal lines. Between these two maternal lines, differences in litter size have been found. Bidanel et al. (1996) reported higher number of corpora lutea (+1.3 ± 0.3) in LW gilts in comparison to LR gilts but similar number of embryos because of higher embryonic mortality in LW gilts. Other authors found higher number of piglets born per farrowing (approximately 0.5 piglets) of LW sows (Meszaros et al., 2010) in comparison to LR sows (Serenius and Stalder, 2004). Moreover, in comparison to sows from other breeds like Pietrain, LW showed significant higher reproduction performance in lifetime (Hoy, 2014).

Crossbreeding schemes were mostly used to produce commercial dam lines in order to use the heterosis effects. Especially for litter traits, which were influenced by maternal and piglet genotype, performance improvements come from both crossbred piglets (i.e. litter, direct or individual heterosis) and crossbred sows (maternal heterosis). But the largest heterosis effects were associated with the sow (Bidanel, 2011).

Differences in survival rate between purebreds and corssbreds have been reported by several authors. Bidanel et al. (2011) pointed out, that compared to purebred, crossbred dam line had higher conception rates, slightly larger ovulation rate and better prenatal survival rates resulting in larger litters and showed better mothering abilities. As a consequence, crossbred sows had higher postnatal survival rates. These findings were an agreement with other studies who reported higher NBA in crossbred litters in comparison to purebred litters (Holm et al., 2005) and higher embryo survival (5.2 ± 2.2 %) resulting in more living embryos in crossbred sows than purebred animals (+0.9 ± 0.3 embryos) (Bidanel et al., 1996). Blaso et al. (1995) and Cecchinato et al. (2010) found higher survival rates for crossbred pigs than for purebred pigs. Additionally, Knol et al. (2001) reported that the amount of relative heterosis for litter survival was 1.55 %. Differences in survival between lines can be expected as a consequence of genetic and environmental differences between populations (Cecchinato et al., 2010; Kapell et al., 2010). Because of this, selection effects on survival within one line/breed/population cannot be transmitted onto another line/breed/population (Kapell et al., 2010). Cecchinato et al. (2010) suggested that selection success depended on whether purebred performance measured in a nucleus herd can predict performance outcomes in commercial crossbred sows accurately. Moreover, differences in results can also be induced by variations in trait definitions (stillborn piglets, piglets dying in the early hours after birth etc.) (Cecchinato et al., 2010).

Genetic variation within breed

Estimated average h^2 for NBA is low (mean $h^2 = 0.1$) and showed high variation (h^2 range = 0-0.66) (Bidanel, 2011; Rothschild and Bidanel, 1998). Some studies differed between first and later litters and found different h^2 and genetic correlation which differed from unity. Noguera et al. (2002a) estimated h^2 for parities and detected increasing heritability with increasing parity. They concluded that genetic basis for NBA differed across reproductive lifespan of the sow. Furthermore, they suggested that different genes or combination of genes were involved in each parity because of hormonal and physiological maturation. Markedly differences in h^2 between and even within breeds indicated different genetic basis for each

line. An overview over estimated h^2 in different studies in the period from 1995 to 2012 is given in Table 1.

Table 1: Estimated heritability (h^2) for TNB, NBA and litter size

Trait	**h^2**	**Breed**	**Reference**
NBA (AI)	0.09 ± 0.009	LW	Lewis et al. (2005)
NBA (NS)	0.12 ± 0.028	LW	Lewis et al. (2005)
NBA	0.10 – 0-12	LW	Coster et al. (2012)
NBA (AI)	0.056 ± 0.011	LR	Lewis et al. (2005)
NBA (NS)	0.054 ± 0.018	LR	Lewis et al. (2005)
NBA	0.06	LR	Noguera et al. (2002b)
NBA	0.004 ± 0.002*	LR	Noguera et al. (2002a)
NBA	0.07	LR	Chen et al. (2002)
NBA1-6	0.064-0.146 ± 0.019-0.004	LR	Noguera et al. (2002a)
NBA1	0.12	Norwegian LR	Holm et al. (2005)
NBA2	0.14	Norwegian LR	Holm et al. (2005)
NBA1	0.10 ± 0.01	Norwegian LR	Holm et al. (2004)
NBA	0.10 ± 0.01	Norwegian LR	Holm et al. (2004)
NBA1	0.084 ± 0.008	Dutch LR	Hanenberg et al. (2001)
NBA2-6	0.089 ± 0.005	Dutch LR	Hanenberg et al. (2001)
NBA1	0,15	Iberian	Fernandez et al. (2008)
NBA2+	0,12	Iberian	Fernandez et al. (2008)
NBA	0.1	Yorkshire	Chen et al. (2002)
NBA	0.08	Du	Chen et al. (2002)
NBA	0.08	Hampshire	Chen et al. (2002)
NBA	0.19 ± 0.05	LR x Du x Yorkshire	Rempel et al. (2010)
NBA	0.09 ± 0.05	LR -Duroc-LW	Schneider et al. (2012a)
LS	0.06 ± 0.04	LW	Kerr and Cameron (1995)
LS	0.06	LW	Kerr and Cameron (1995)
LS[1]	0.10	LW	Kerr and Cameron (1996b)

Table 1 continued: Estimated heritability (h^2) for TNB, NBA and litter size

Trait	h^2	Breed	Reference
LS[2]	0.21	LW	Kerr and Cameron (1996b)
LS1-5	0.13 – 0.41	Chinese-European composite dam line	Munoz et al. (2010)
TNB1	0,03 ± 0.02	LR	Imboonta et al. (2007)
TNB2+	0.07 ± 0.01	LR	Imboonta et al. (2007)
TNB1	0.093 ± 0.009	Dutch LR	Hanenberg et al. (2001)
TNB2-6	0.101 ± 0.006	Dutch LR	Hanenberg et al. (2001)
TNB	0.11 – 0.16 ± 0.01-0.26	Dam lines PIC	Kapell et al. (2010)
TNB	0.11 – 0.13 ± 0.01-0.27	Sire lines PIC	Kapell et al. (2010)
TNB	0.19 ± 0.06	LR x Du x Yorkshire	Rempel et al. (2010)

NS = natural service; AI = artificial insemination; NBA1 = NBA in the first litter; NBA2 = NBA in the second litter; NBA2+ = NBA in the second and following litters; 1-6 = NBA in the first to sixth litter; [1] = ad-libitum feeding during performance test; [2] = restricted feeding during performance test; * = maternal h^2; LW = Large White; LR = Landrace; LS = litter size; LS1-5 = litter size from the first to fifth party; NBA = number of piglets born alive; TNB = total number born piglets; Du = Duroc

Candidate Genes and detected QTLs

Developments in molecular technologies provide the possibility of selecting for NBA based on genetic marker information (Spotter and Distl, 2006) like SNPs. Mentioned biological constraints can be eliminated by using SNP information because genomic data of every animal is available early in life and the generation interval is shortened. Additionally, accuracy of selection and as a consequence selection success increases by direct selection on beneficial gene variants positively affecting its expression (Spotter and Distl, 2006). Moreover, it can be distinguished between NBA and its component traits like ovulation rate and embryonic survival. Distl (2007) postulated that "using SNP information promises more progress and advantages in optimum balancing of the different physiological mechanisms influencing litter size". Knowledge about beneficial alleles was useful especially for the novel method GS. Here, SNP information was summed up to estimate genomic breeding value for each individual. Normally, anonymous SNP were weighed without knowledge of effects. Information about beneficial alleles on particularly traits increased selection success and

improvement of reproduction traits in swine industry (Rothschild, 1998). Moreover, important SNPs can be weighed differentially in genomic selection method and increase allele frequency of important alleles and therefore improve reproduction traits. It is known that breeding success of traits with low heritability and polygenic character benefit from genomic selection (Lillehammer et al., 2011).

Candidate genes for litter size traits

Two different approaches can be pursued to identify genes with an influence on NBA. The first one was based on investigation of functional candidate genes. Candidate genes were identified because of their physiological role in reproduction in pigs which called physiological candidate gene (Rothschild and Bidanel, 1998). Positional candidate genes were candidate genes which were located close to a genomic region associated with a possible QTL (Haley, 1999). Moreover, candidate genes can be identified by investigating of differentially expressed genes in tissue of investigation or during key processes in reproduction (Distl, 2007; Wilson et al., 2002). Known candidate genes for NBA are listed in Table 2 and Figure 2.

Table 2: Identified candidate genes associated with litter size or components traits, modified by Buske et al. (2006a), Onteru et al. (2009), Distl (2007) and Spotter and Distl (2006)

Gen	**SSC**	**Polymorphism (location)**	**Trait**	**Population**	**N**	**Reference**
ESR1	1	PvuII site (intron)	TNB, NBA	MS x SL;	161;	Rothschild et al. (1996)
				LW	1079	
				LW	4262	Short et al. (1997)
				LW x MS	275	van Rens et al. (2002)
				LW	1030	Goliasova and Wolf (2004)
				LW	226	Horogh et al. (2005)
		C/T (exon 5)		Chinese-European line	408	Munoz et al. (2007)
ESR2	1	A/G (exon)	NBA	Commercial F1 sows	129	Buske et al. (2006b)
FSHB	2	FSHBMS microsatellite (5' flanking region)	TNB, NBA	YO x ER	289	Li et al. (1998)
EPOR	2	Intron 4	UC	4-way cross	402	Vallet et al. (2005)
MIR27A	2	T/C (Hpall site)	LS	LW; synthetic line	142; 140	Lei et al. (2011)
EPBH2	6	Exon 4	LS	LR;YO;DU	485	Fu et al. (2012a)
LEPR	6	Intron 2, exon 2, exon 18	LS	YO; DU	62; 246	Chen et al. (2004a)

Table 2 continued: Identified candidate genes associated with litter size or components traits, modified by Buske et al. (2006a), Onteru et al. (2009), Distl (2007) and Spotter and Distl (2006)

Gen	**SSC**	**Polymorphism (location)**	**Trait**	**Population**	**N**	**Reference**
FUT1	6	Exon 2	TNB, NBA	PBP	104	Horak et al. (2005)
				(LW x LR) LE	123	Buske et al. (2006c)
LCK	6	A/G (1127bp)	LS	LW; LR	100;100	Liu and Xia (2012)
CFB	7	Intron 1	TNB, NBA	(LW x LR) LE	123	Buske et al. (2005)
DIO3	7	-	NBA; LS	LW	1739	Coster et al. (2012)
RNF4	8	C/T (intron 5)	TNB, NBA	QP	159	Niu et al. (2009)
GNRHR	8	3' UTR	OR	MS x LW	200	Jiang et al. (2001)
OPN	8	Intron	TNB, NBA	SL	519	Korwin-Kossakowska et al. (2002)
LIF	8	Exon 3	NBA	LR, LW	850; 604	Spotter et al. (2009)
SPATA19	9	T/C	LS	LW; LR	100;100	Feng et al. (2013)
AKR1C2	10	Ile16Phe (Nt179 in coding region)	OR	¼ MS	191	Nonneman et al. (2006)
HSD17B1	12	A/T (Intron 4)	LS	LW; synthetic line	252; 128	Liu et al. (2009)
NAT9	12	A/G (699bp)	LS	LW; LR	100;100	Zhao et al. (2012)
SOD1	13	Intron	LS	LR	357	Bjerre et al. (2013)
ROPN1	13	T/C (536 bp)	LS	LW; LR	100;100	Lan et al. (2012)

Table 2 continued: Identified candidate genes associated with litter size or components traits, modified by Buske et al. (2006a), Onteru et al. (2009), Distl (2007) and Spotter and Distl (2006)

Gen	**SSC**	**Polymorphism (location)**	**Trait**	**Population**	**N**	**Reference**
PPARγ	13	A/G	LS	LW; LR	564	Wang et al. (2011)
RBP4	14	(Intron)	TNB, NBA	SL	1300	Rothschild et al. (2000)
			NBA	LR; LW	850;604	Spotter et al. (2009)
PRLR	16	Alu site	TNB, NBA	LW;MS;LR	400;261;416	Vincent et al. (1998)
PRLR	16	Alu site	NBA	SL	273	Drogemuller et al. (2001)
BMP7	17	T/C (intron 2)	NBA	LR; LW; DU	25;148;85	Feng et al. (2013)
LEP	18	(Exon 3)	OR, TNB, NBA	MS x LW/LR	55-77	(van Rens et al., 2003; van Rens and van der Lende, 2002)
			TNB	SL	519	Korwin-Kossakowska et al. (2002)
			LS	YO;LR	62; 170	Chen et al. (2004b)
		Intron 1	LS	DU	246	Chen et al. (2004b)

SSC = sus scrofa chromosome*; ESR1* = estrogen receptor 1; *ESR2* = estrogen receptor 2; *FSHB* = follicle stimulating hormone beta; *EPOR* = erythropoietin receptor; *MIR27A* = microRNA 27a; *EPBH2* = Eph receptor tyrosine kinases B2; *LEPR* = leptin receptor; *FUT1* = fucosyltransferase 1; *LCK* = lymphocyte protein tyrosine kinase; *CFB* = complement factor B; *DIO3* = deiodinase, iodothyronine type III; *RNF4* = ring finger protein 4; *GNRHR* = gonadotropin-releasing hormone receptor; *OPN* = osteopontin; *LIF* = leukemia inhibitory factor; *SPATA19* = spermatogenesis associated 19; *AKR1C2* = aldo-keto reductase family 1; *HSD17B1* = hydroxysteroid (17-beta) dehydrogenase 1; *NAT9* = N-acetyltransferase 9; *SOD1* = superoxide dismutase 1; *ROPN1* = rhophilin associated tail protein 1; *PPARγ* = peroxisome proliferator-activated receptor gamma; *RBP4* = retinol binding protein 4; *PRLR* = prolactin receptor; *BMP7* = bone morphogenetic protein 7; *LEP* = leptin

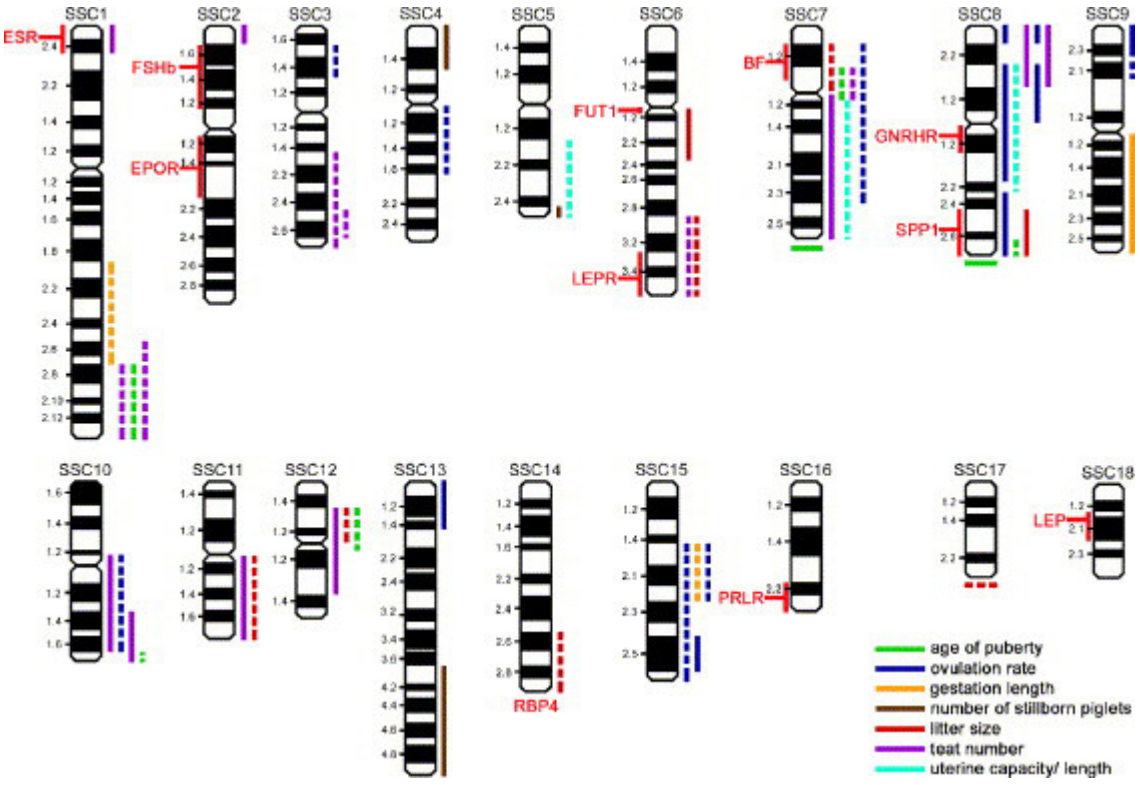

Figure 2: Cytogenetic map of the pig with all QTL and candidate genes influencing fecundity, modified by Buske et al. (2006a).

Figure legend: bold solid lines = level of significance $p < 0.05$; dashed lines = level of significance $p > 0.05$; cytogenetic positions of the lines at the end of the chromosomes and for *RBP4* were not evaluable; *CFB* = complement factor B; *ESR* = estrogen receptor; *EPOR* = erythropoietin receptor; *FSHB* = follicle stimulating hormone beta; *FUT1* = fucosyltransferase 1; *GNRHR* = gonadotropin releasing hormone receptor; *LEP* = leptin; *LEPR* = leptin receptor; *PRLR* = prolactin receptor; *RBP4* = retinol-binding protein 4; *SPP1* (*OPN*) = secreted phosphoproteine 1

The leptin receptor gene (*LEPR*) was mentioned as candidate gene by Chen et al. (2004a) who have shown that *LEPR* was associated with variation in litter size. This gene is located on sus scrofa chromosome (SSC) 6. Within this candidate gene confidence intervals of reported QTLs were found by several studies (Figure 2) (Bidanel et al., 2008; Li et al., 2009; Wilkie et al., 1999).

Association between properdin (*CFB*) and litter size was first reported by Buske et al. (2005). *CFB* gene plays an important role in the uterine epithelium growth in humans (Hasty et al., 1993). This gene was mapped on SSC7. Several authors found QTLs located in the region of *CFB* (Figure 2) (de Koning et al., 2001; Li et al., 2009; Tribout et al., 2008).

Tribout et al. (2008) detected a QTL affecting NBA close to the prolactin receptor locus (*PRLR*) which was reported as candidate gene for litter size before (Drogemuller et al., 2001; Vincent et al., 1998). The function and effect of *PRLR* is well documented. *PRLR* which was mapped on SSC16 plays an important role in the maintenance of pregnancy (Drogemuller et al., 2001). Mice with null mutations in *PRLR* were steril due to a failure of embryonic implantation and also demonstrate irregular cycles, reduced fertilization rates and defective embryonic development (Ormandy et al., 1997).

Rothschild et al. (1996) were the first who reported an association between estrogen receptor 1 (*ESR1*) on SSC1 and litter size. They detected a PvuII-restriction fragment length polymorphism (*RFLP*) of *ESR1* in both a MS x LW and an European breed synthetic population. *ESR1* is involved in pregnancy establishment by signalling to the uterus and preservation of gestation by spreading the life-span of corpora lutea (Spotter and Distl, 2006). The relationship between *ESR1* and litter size was confirmed by other studies which used different breeds (Chen et al., 2000; Goliasova and Wolf, 2004; Munoz et al., 2007; Short et al., 1997; van Rens et al., 2002). In contrast, several other studies cannot find any association between *ESR1* polymorphisms and litter size (Drogemuller et al., 2001; Gibson et al., 2002; Horak et al., 2005; Isler et al., 2002; Linville et al., 2001; Noguera et al., 2003; Rempel et al., 2010). Additionally, no QTL was detected in the *ESR1* gene region so far (Bidanel, 2011). The inconsistency in results illustrated that the effect of *ESR1* alleles is population specific or maybe not the causative mutation (Bidanel, 2011; Buske et al., 2006a). Other reasons for differences in results might be small and varying sample sizes, differences in environmental influences and genetical background (Buske et al., 2006a).

Candidate genes for litter size with known pleiotropic effects

Moreover, information about possible pleiotropic effects for reproduction and production traits is limited. For genes like retinol binding protein 4 *(RBP4),* peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PPARGC1A), ESR* and insulin like growth factor 2 (*IGF2*) influence on reproduction as well as on production traits is known (Cheng et al., 2013; Jacobs et al., 2006; Munoz et al., 2010; Rempel et al., 2010; Short et al., 1997; Stinckens et al., 2009; Wang et al., 2006a).

Short et al. (1997) detected a favourable pleiotropic effect for BF of the favourable litter size allele in a LW population. No effects of different genotypes were found for ADG in this study. It has been shown that *PPARGC1A* was associated with BF, leaf fat weight and belly

weight in MS cross population (Jacobs et al., 2006). Additionally, this gene was associated with TNB and NBA (Rempel et al., 2010).

A polymorphism located within *IGF2* gene was detected to affect muscle development and fat deposition in swine (Nezer et al., 1999). The paternal allele "A" leads to lower BF thickness and higher lean growth. It can be expected that this allele is present in a higher frequency in swine population because of its favorable effect on lean content (Munoz et al., 2010). Some authors found an effect of *IGF2* on reproduction traits in mouse and farm animals (Badinga et al., 1999; Schams et al., 1999). An influence of *IGF2* on NBA and litter size in pigs was reported in several studies (Buys et al., 2006; Munoz et al., 2010).

QTL detection for litter size traits

The second approach was based on linkage and association analyses. Genomic regions harbouring genes with an influence on the trait of interest should be identified which are called QTL (Distl, 2007; Spotter and Distl, 2006). Above mentioned new genotyping technologies using large-scale SNP chips enable new strategies for genetic mapping (Abecasis et al., 2005; Hirschhorn and Daly, 2005). The new technology of large SNP panels covering the whole genome allows the discovery of loci underlying the genetic variance of QTLs (Bidanel, 2011). It is now practically and financially affordable to genotype a large number of animals with thousands of markers.

Up to the present, 11.610 QTLs have been found in pig genome of which 1.035 QTLs were associated with reproduction traits and 515 QTLs affecting litter size (status 20.11.2014) (Hu et al., 2013). It has to be mentioned, that most of these studies used a low number of microsatellites or SNPs to detect QTLs and recently developed high-density SNP maps (Ramos et al., 2009). An overview of all QTLs affecting NBA is presented in Table 3 and visualized in Figure 3.

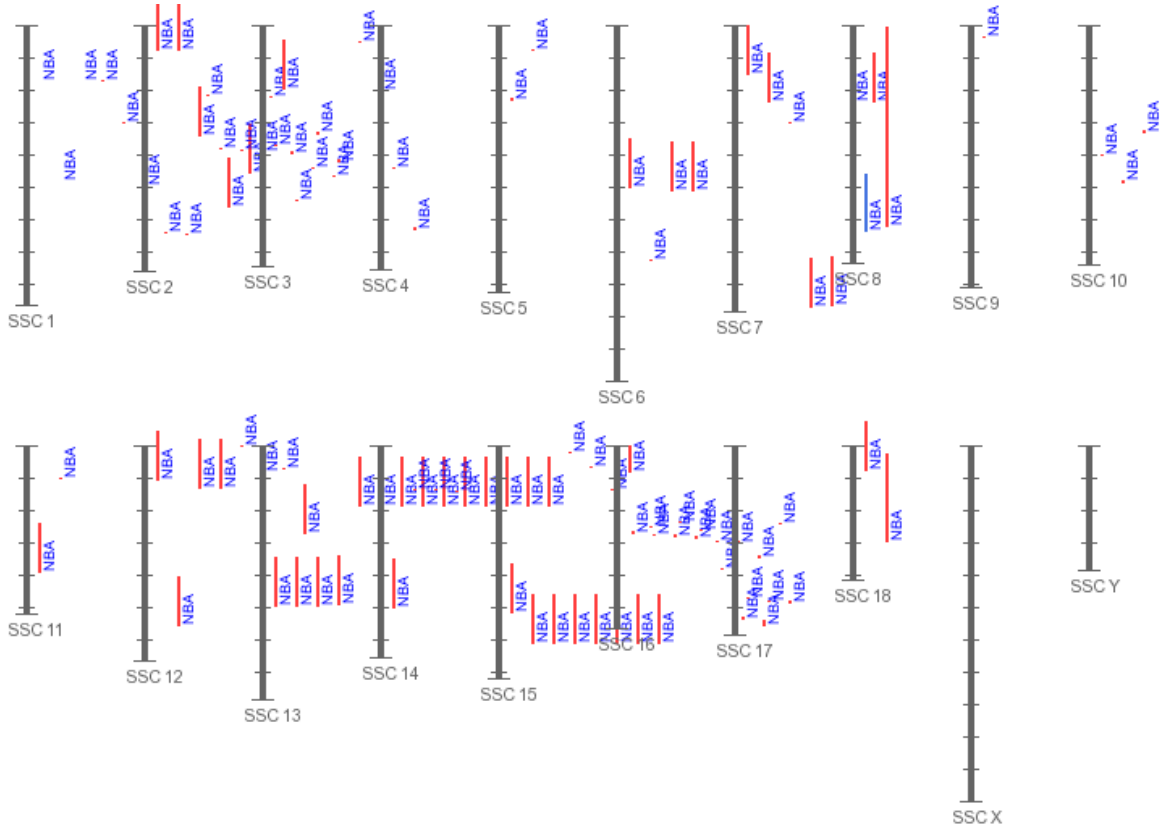

Figure 3: Previously detected QTLs for NBA adapted from Hu et al. (2013)

Table 3: Detected QTLs for NBA

SSC	Population	N animals	Marker	N marker	Reference
1*	Hungarian LW	122	-	-	Horogh et al. (2005)
1*	Commercial F_1 sows	129	SNP	-	Buske et al. (2006b)
1*	MS synthetic line	238	Microsatellite	5	Rothschild et al. (1996)
1*	PI; PI x HA	203; 100	SNP	1	Gunawan et al. (2012)
2*	LW;LR	-	SNP	-	Stinckens et al. (2010)
2*	LW; Chinese breed	182	SNP	1	Lei et al. (2011)
6*	LW; LR	100; 100	SNP	1	Liu and Xia (2012)
7*	(LW x LR) x LC	123	PCR-RFLP	-	Buske et al. (2005)
7*	Commercial line	85	PCR-RFLP	-	Marantidis et al. (2013)
8	MS x LW	220	microsatellites	21	King et al. (2003)

Table 3 continued: Detected QTLs for NBA

SSC	Population	N animals	Marker	N marker	Reference
11	F_2 population	428	microsatellites	151	Cassady et al. (2001)
12*	LW;LR	100;100	SNP	1	Zhao et al. (2012)
12*	LW; LR	100;100	SNP	1	Shifei and Yonggang (2012)
12*	Iberian x MS	-	SNP	-	Fernandez-Rodriguez et al. (2010)
13*	LW; LR	100; 100	SNP	1	Lan et al. (2012)
13*	IB;MS;LW;Vietnamese; LR;PI	11	SNP	16	Balcells et al. (2011b)
13*	IB x MS F_2	347	SNP	2	Balcells et al. (2011a)
15*	LR; YO, DU	765	SNP	2	Fu et al. (2012a)
7, 16, 18	LW;LR	111;84	microsatellites	144	Tribout et al. (2008)

Table 3 continued: Detected QTLs for NBA

SSC	Population	N animals	Marker	N marker	Reference
6, 15	White Duroc x Chinese Erhualian	299	microsatellites	183	Li et al. (2009)
2*, 14*	Chinese-European dam line	408	SNP	6	Munoz et al. (2010)
1, 3,5 ,7, 9, 14	Commercial dam line	818	PorcineSNP60 BeadChip	60k	Onteru et al. (2011)
1,2,3,4,5,6,10, 12,13,14,15,16	Finnish LR	328	SNP	60k	Onteru et al. (2012)
1*,2*,7*	LW	1739	SNP	309	Coster et al. (2012)
1,4,10,13,15,17	crosses	1152	SNP	60k	Schneider et al. (2012b)
8, 18	LW x MS F_2	307	Microsatellites	174	Hernandez et al. (2014)
13, 17	IB x MS	881		109 microsatellites; 6 SNPs	Noguera et al. (2009)

*=Association; N = number; LW = Large White; LR = Landrace; MS = Meishan; IB = Iberian; PI = Pietrain LC = Leicoma; HA = Hampshire; YO = Yorkshire, DU = Duroc; SSC = sus scrofa chromosome SNP = Single nucleotide polymorphism; PCR-RFLP =Polymerase chain reaction - restriction fragment length polymorphism

Most QTLs and associations for NBA and components traits were identified on SSC13 (N = 58) and on SSC1 (N = 40). The lowest amount of QTLs and associations were identified on SSC5 and 11 (N = 8 and 6, respectively). Overall, when results of studies were compared, relatively inconsistent location were reported (Spotter and Distl, 2006). Reasons might be: a) polygenetic control of reproduction traits, b) small sample size, c) breed differences, d) different phenotyping and statistical methodology and e) marker density.

a) Under the assumption of polygenic control of reproduction traits, most loci had only small effects and might interact with other genes and with the environment (Dekkers and Hospital, 2002).

b) Partly low sample sizes used in association studies limit the power to detect QTLs of modest effect (Kirkpatrick, 2002).

c) Genetical heterogeneity of the observed lines and breeds could explain differences in results (Spotter and Distl, 2006). Furthermore, QTLs having an effect in one population do not necessarily have an effect in another population due to varying frequencies of the QTLs (Spotter and Distl, 2006).

d) Differences in phenotyping used as dependent variable in GWAS can also lead to dissimilarities in results between studies. In some studies raw phenotypes were used for association analyses. In other studies estimated breeding values (EBVs) or deregressed proofs which reflect the true EBV of the animal because the EBVs of the parents are removed, were used to detect QTLs. Advantages and disadvantages of using EBVs are discussed by Garrick et al. (2009).

e) Only three studies used high density SNP panels for genotyping animals for GWAS (Onteru et al., 2012; Onteru et al., 2011; Schneider et al., 2012b). All other studies used only few SNPs or microsatellites to genotype pigs for GWAS.

For TNB 55 associations and 83 QTLs located on all pig chromosomes except SSC10 and 11 were identified (Figure 4) (Balcells et al., 2011a; Bjerre et al., 2013; Buske et al., 2005; Coster et al., 2012; Fernandez-Rodriguez et al., 2010; Fu et al., 2012b; Hernandez et al., 2014; Horogh et al., 2005; King et al., 2003; Lan et al., 2012; Lei et al., 2011; Li et al., 2009; Liu and Xia, 2012; Marantidis et al., 2013; Onteru et al., 2012; Onteru et al., 2011; Rothschild et al., 1996; Schneider et al., 2012b; Shifei and Yonggang, 2012; Sironen et al., 2012; Stinckens et al., 2010; Uimari et al., 2011; Wilkie et al., 1999; Zhao et al., 2012).

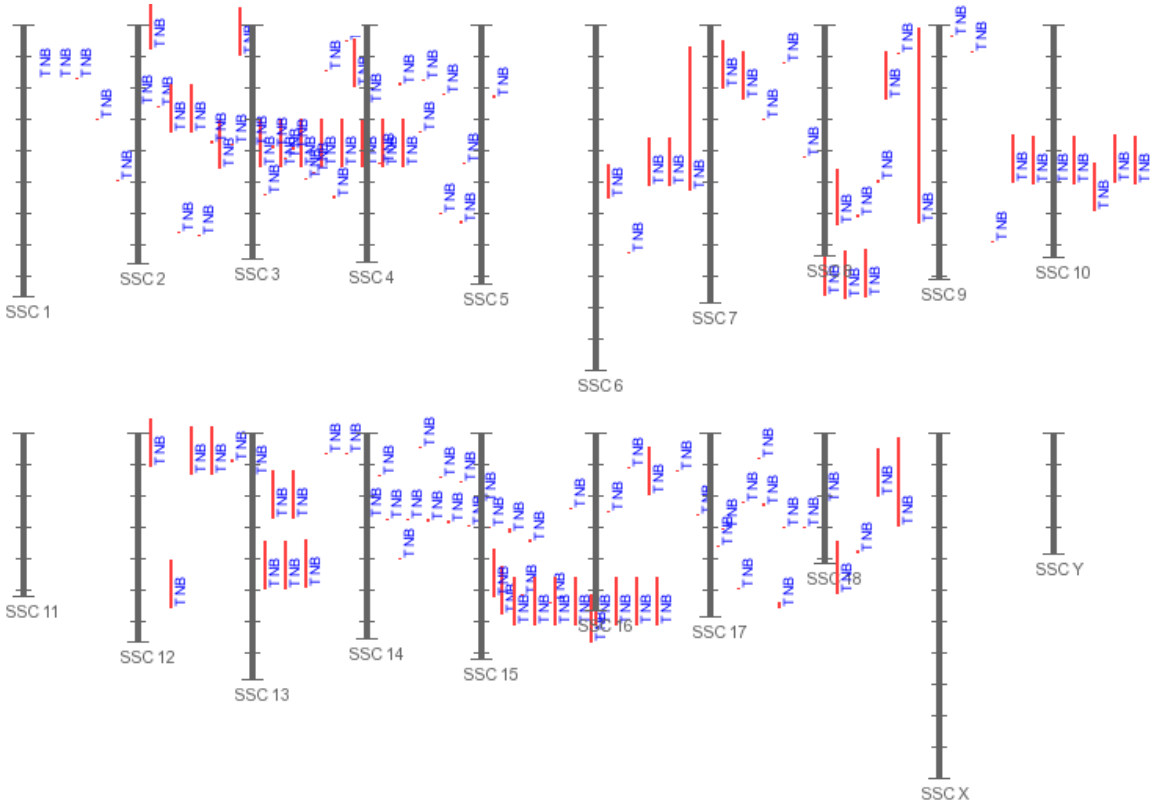

Figure 4: QTLs for Total Number Born (TNB), adapted from Hu et al. (2013)

In total 58 QTLs for number of stillborn piglets (Figure 5) (Cassady et al., 2001; Holl et al., 2004; Li et al., 2009; Onteru et al., 2012; Schneider et al., 2012b; Tribout et al., 2008; Uimari et al., 2011; Wilkie et al., 1999) and 79 QTLs for number of mummified piglets (Figure 6) (Holl et al., 2004; Onteru et al., 2012) located on almost every chromosome were found.

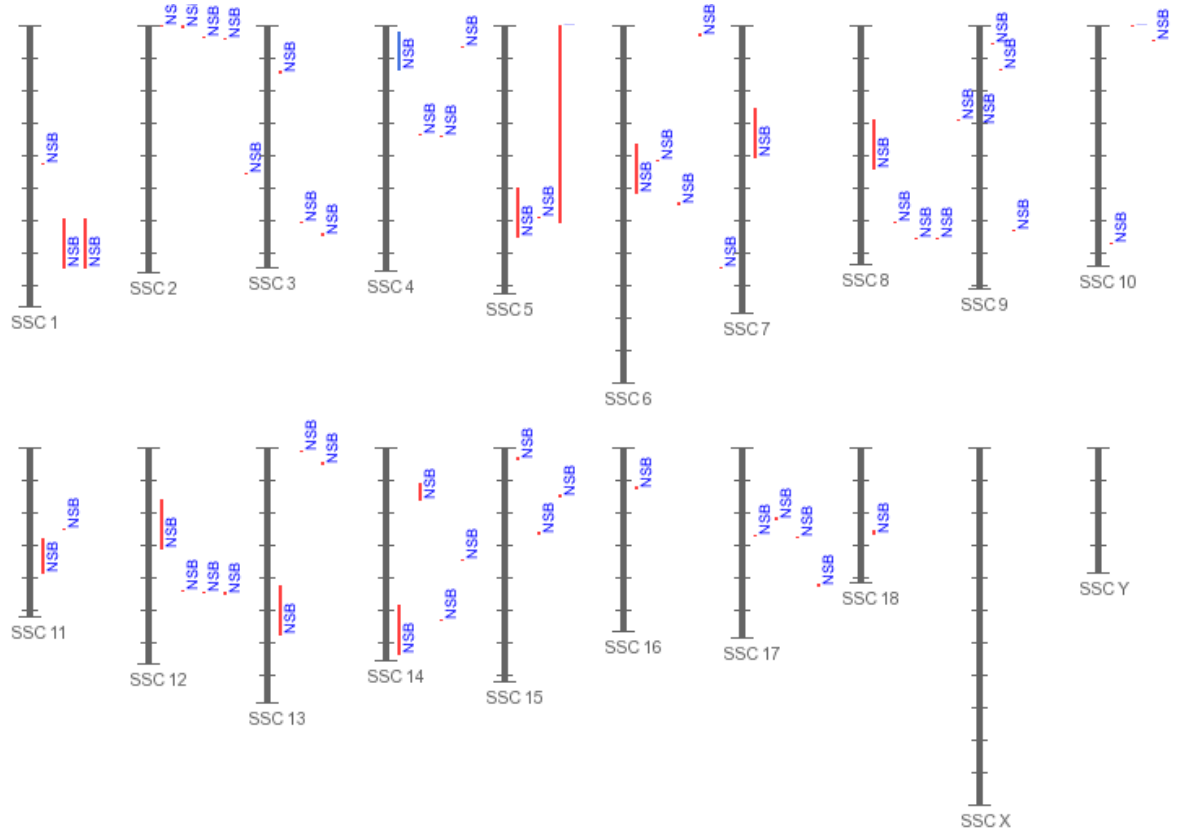

Figure 5: QTLs for number of stillborn piglets (NSB), adapted from Hu et al. (2013).

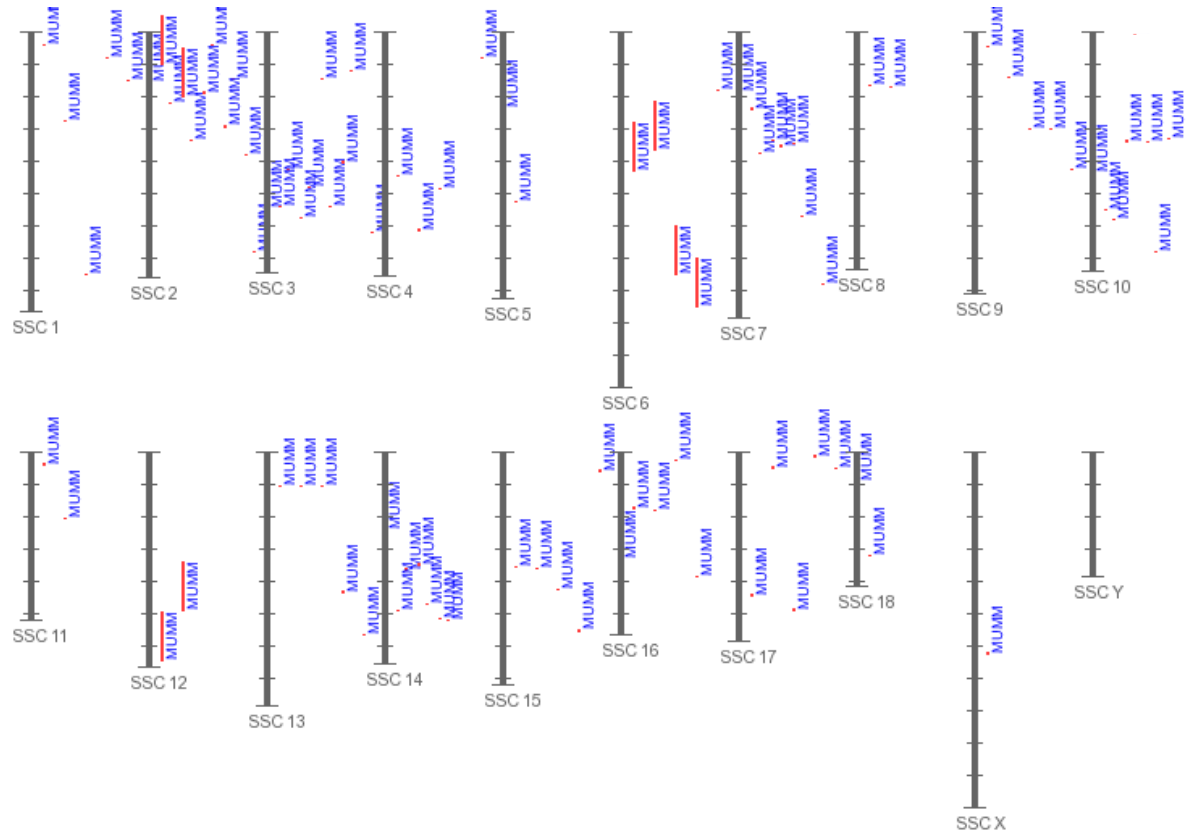

Figure 6: QTLs for mummified piglets (MUMM), adapted from Hu et al. (2013).

Up to now, in total 34 QTLs located on SSC3, 4, 5, 7, 8, 9, 10, 13, 15, 18 were detected for corpus luteum number (CLN) (Figure 7) (Bidanel et al., 2008; Braunschweig et al., 2001; Campbell et al., 2008; Campbell et al., 2003; Cassady et al., 2001; Hernandez et al., 2014; Jiang et al., 2001; Rathje et al., 1997; Rohrer et al., 1999; Sato et al., 2006; Sato et al., 2011; Wilkie et al., 1999), three QTLs affecting embryonic survival located on SSC9, 12 and 18 and six QTLs on SSC6, 9, 12 and 18 affecting number of viable embryos were detected (Bidanel et al., 2008).

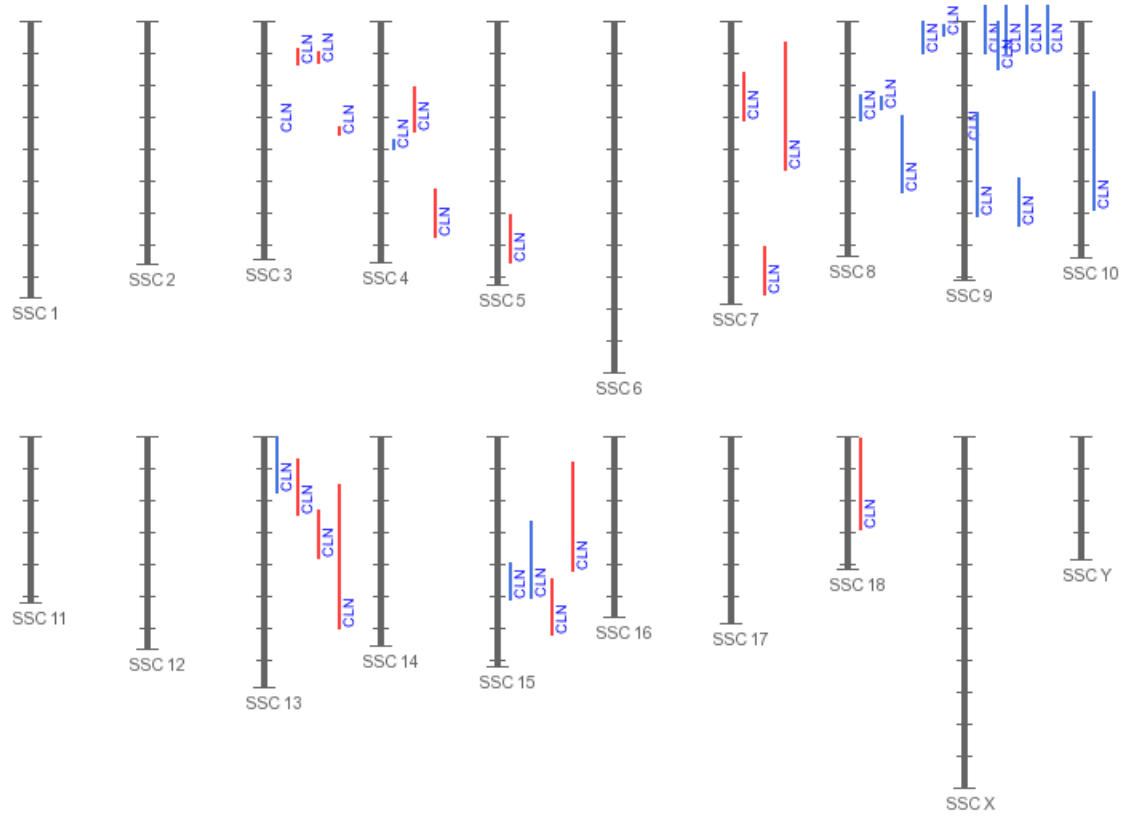

Figure 7: QTLs for Corpus Luteum Number (CLN) adapted from Hu et al. (Hu et al., 2013)

The only overlap of QTLs reported for litter size were found on SSC6 (Bidanel, 2001; Li et al., 2009; Wilkie et al., 1999), on SSC7 (de Koning et al., 2001; Li et al., 2009; Tribout et al., 2008), on SSC13 (Bidanel, 2001; Noguera et al., 2009) and on SSC17 (de Koning et al., 2001; Noguera et al., 2009). When comparing confidence intervals and locations of QTLs for NBA and CLN, overlapping regions can be found, for example on SSC7, 13 and 18 (Figure 3 and Figure 7). On SSC7 one QTL affecting NBA was detected by Tribout et al. (2008) at 0.0-11.6 Mb. Within this QTL span Hernandez et al. (2014) detected one chromosome-wide significant QTL affecting CLN. The same authors found a QTL for CLN on SSC13 (18.3-206.7 MB) (Hernandez et al., 2014). Within the QTL span, a large amount of QTLs for NBA and TNB and one for mummified piglets were identified by Onteru et al. (2012). Hernandez et al. (2014) identified QTLs affecting NBA as well as CLN at the p-arm of SSC 18. These QTLs have a small overlap confidence interval. Moreover, Bidanel et al. (2008) detected a QTL for number of viable embryos at this position on SSC18. CLN and therefore OR is one of the most important influencing factor for litter size (Bennett and Leymaster, 1989). Because of this relationship, it can be expected that QTLs for both traits can be found in the same

genomic region. Overlapping QTLs for ovulation rate and NBA or TNB indicated that there could be one or more gene having an effect on litter size in this region.

Overlaps with candidate gene studies have been reported by some studies. Buske et al. (2006a) gave an overview of previously found QTLs and known candidate genes. Till know, more QTLs and candidate genes are known. Figure 2 illustrates the state of research up to year 2006.

1.3 Genetical and biological aspects of production traits

Fourtythree (43) % of the world´s red meat is produced by pigs. Therefore, of particular interest for pig producers has been selection for high lean growth rates to enlarge market weights of around 115 kg (Bunger et al., 2005). During the last years improvements in economical traits like ADG, BF and LMP of the pig carcass were achieved using traditional selection practice (Imboonta et al., 2007). This selection was mainly focused on sire lines. But also dam lines undergone considerable selection for production traits next to selection for increased NBA and piglet survival (Bunger et al., 2005). In the framework of our study we are particular interested in the correlated genetic response when selecting on reproduction and production traits. From the animal breeding point of view, genetic correlation between production traits and genetically linked or pleiotropic acting genes or genome regions are of particular importance.

In literature, weak correlations between litter size and growth and carcass traits (BF and LMP) have been reported (Brien, 1986; Haley et al., 1988). Several authors found a relationship between selection for production traits and a decrease in reproductive performance (Hermesch et al., 2000b; Holm et al., 2004; Knol, 2001; Serenius et al., 2004; Zhang et al., 2000), whereas other authors have not found any correlation between production traits and litter size (Kerr and Cameron, 1996a; Kuhlers and Jungst, 1991; Noguera et al., 2002a, b). Holm et al. (2004) hypothized that selection for lean growth might have a negative genetic relationship with NBA in high prolific animals.

These differences may in some cases be related to the limited precision of estimates, but also reflect genetic differences in average performance levels and metabolic efficiency, as well as variation in management practices (Rosendo et al., 2007a).

1.3.1 Relationship between carcass composition and litter size traits

Genetic foundation of carcass composition traits

Heritability of most carcass traits is medium to high, what makes genetic improvement easier in comparison to reproduction traits. In the recent reviews of Ciobanu et al. (2011) and Clutter (2011) average h^2 for BF and LMP in a range of 0.43 and 0.49 were reported. In some of the reviewed studies, the statistical models comprised maternal and direct genetic effects. It can

be concluded that maternal effects have a higher influence on BF during suckling, whereas during performance test, direct effects are more important for pig's growth and BF (Crump et al., 1997; Rosendo et al., 2007a; Solanes et al., 2004; Zhang et al., 2000).

Carcass composition and NBA

The genetic improvement of both, NBA and lean growth/BF, is essential to increase the efficiency of pork production (Chen et al., 2003). A genetic antagonism between reproduction traits and carcass confirmation was reported by Kerr and Cameron (1996b). Moreover, Young et al. (1991) concluded that selection focused on production traits, especially on an increase in lean meat content (LMC), resulted in a decrease in litter size. Consequences of high LMP of sows and their litter sizes was studied by Beckova et al. (2005). They described sows with high LMP at mating that showed significantly reduced TNB and NBA. This is an agreement with Kerr and Cameron (1996b), who reported a decrease in reproduction efficiency in LR gilts with a high proportion of lean meat. This can be explained by deterioration in the body's ability for lipid mobilization during gestation and / or suckling period (Johansson and Kennedy, 1983a). The influence of BF of the sow and its reproduction performance was analysed by several authors. A significant influence of BF at first insemination on TNB, NBA and number of weaned piglets was found for sows with high BF having the largest litters (Beckova et al., 2005; Wahner et al., 2001).

Inconsistent correlations were described between carcass traits and litter size in literature. Slightly positive genetic and phenotypic correlations were estimated between TNB and BF in the first parity by Imboonta et a. (2007). Phenotypic correlation between TNB and BF in later parities was negative (-0.44). Positive (unfavorable) genetic correlations between TNB and carcass fat percentage were estimated by Serenius et al. (2004) (0.17 ± 0.11 in Finnish LR and 0.19 ± 0.11 in Finnish LW) and Chen et al. (2003) who reported genetic correlation between BF (cm) and NBA in Yorkshire, Duroc, Hampshire and LR pigs (r_g = 0.176 ± 0.032-0.201 ± 0.042). The signs of the correlations indicate that selection for BF could slightly decrease litter size but increase litter weight (Chen et al., 2003; Chen et al., 2002). In an early study performed by Short et al. (1994) negative and one positive genetic correlation between BF and TNB of -0.12, -0.03, -0.08 and 0.06 in two maternal dam lines were found.

In recent studies genetic relationships between litter traits and carcass lean content were slightly positive, e.g. unfavorable (Chen et al., 2003; Imboonta et al., 2007; Serenius et al., 2004; Zhang et al., 2000). In comparison, other authors estimated negative (unfavorable)

genetic correlations between TNB and LMP in a range of -0.02 and -0.35 (Holm et al., 2004; Serenius et al., 2004).

Birth weight and carcass composition

No differences in lean content and BF due to differences in birth weight were found (Berard et al., 2008; Gondret et al., 2005; Nissen et al., 2004; Powell and Aberle, 1980; Rehfeldt et al., 2008). Other authors reported increased BF in low birth weight pigs (Gondret et al., 2006; Poore and Fowden, 2004; Schinckel et al., 2010). Gondret et al. (2005) compared the carcass quality of pigs of low and heavy birth weight and observed reduced muscle fiber number (-19 %), lower LMC, and a higher proportion of subcutaneous fat.

Several authors reported reduced LMC in piglets with low IBW compared to high IBW (Gondret et al., 2006; Rehfeldt et al., 2008; Schinckel et al., 2010). In comparison to this, Fix et al. (2010) reported lowest lean percentage in pigs having intermediate IBW. Light weighted and heavier piglets showed higher lean percentage. They concluded that these findings were based on a combined effect of birth weight on BF and longissimus muscle area. Piglets which were heavy at birth were fatter later in live, but stronger muscled. In addition, Fix et al. (2010) reported that light weighted piglets at birth were less muscled and leaner.

Berard et al. (2008) analyzed fat content in barrows from large litters compared to barrows from small litters. Carcasses of pigs which were born in large litters had numerically greater amount of subcutaneous fat percentage. The authors suggested that the carcass compositions of all barrows born in large litters were similar to those of pigs with low IBW reported in previous studies. Thus, they hypothesized that intrauterine crowding reduced prenatal development which influenced the composition of carcass in all, heavier and lighter, barrows of larger litters (Berard et al., 2008).

Candidate genes and detected QTLs

For average BF thickness 225 QTLs were found located on each chromosome except SSCY. Again, phenotypes used for QTL studies or GWAS differed markedly (BF 13 weeks of age, BF 17 weeks of age, BF 22 weeks of age, BF 40 kg live weight, BF 60 kg live weight, average BF-by Fat-O-Meter, average BF-ultrasound, BF above muscle dorsi, BF at first rib, BF at first rib (14 weeks of age), BF at first rib (26 weeks of age), BF at last lumbar, BF at last lumbar (14 weeks of age), BF at last lumbar (26 weeks of age), BF ats last rib, BF ats last rib (10 weeks), BF ats last rib (13 weeks), BF ats last rib (14 weeks), BF ats last rib (16

weeks), BF ats last rib (19 weeks), BF ats last rib (22 weeks), BF ats last rib (26 weeks), BF at mid-back, BF at P2 position, BF at rump, BF at tenth rib, BF at tenth rib (10 weeks), BF at tenth rib (13 weeks), BF at tenth rib (16 weeks), BF at tenth rib (19 weeks), BF at tenth rib (22 weeks), BF between 3rd and 4th last ribs, BF between 6th and 7th ribs, BF between the last 3rd and 4th lumbar, BF linear at last rib, BF at tenth rib, BF percentage, BF thickness (EBV), BF thickness between 3rd and 4th rib). An overview over all detected QTLs is given in Figure 8.

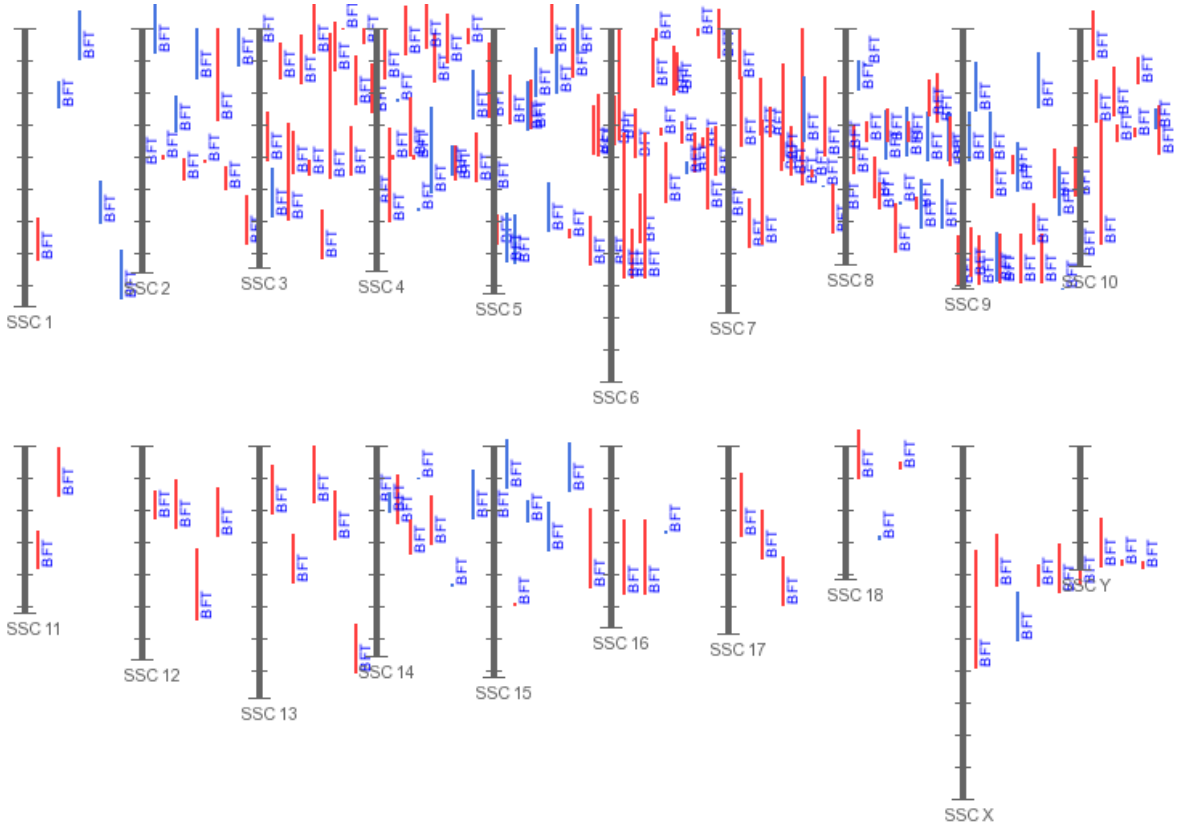

Figure 8: Detected quantitative trait locis for average backfat thickness (BFT), adapted from Hu et al. (2013)

27 QTLs affecting LMP on all chromsomoes except SSC10 and Y were found up to the present. These QTLs are given in Figure 9.

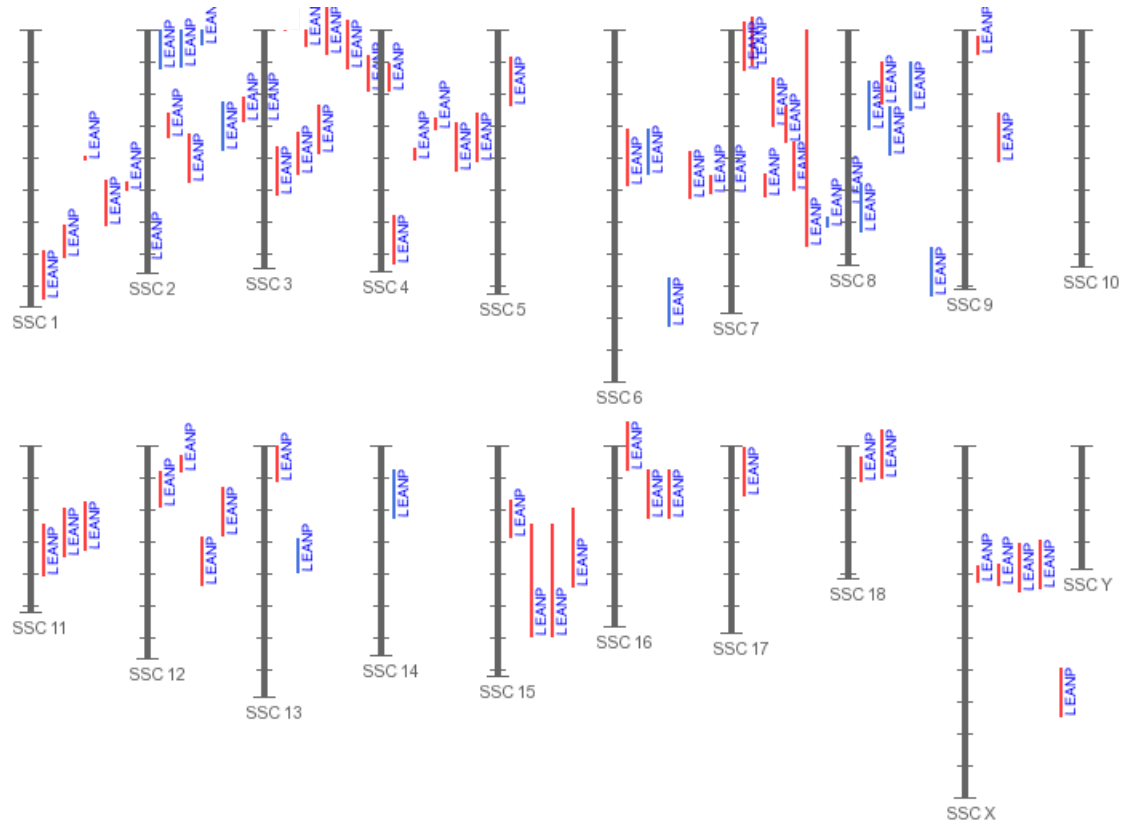

Figure 9: Detected quantitative trait locis for lean meat percentage (LMP), adapted from Hu et al. (2013)

1.3.2 Growth traits and litter size traits

Prenatal and postnatal growth of pigs is complex and influenced by a large amount of factors. Complexity of growth is illustrated in Figure 10. It can be seen that genetic as well as exogenous factors like litter size has an impact on growth performance of the pig.

The influence of litter size on later growth performance has been well studied. It is known that selection for improvements of efficiency and productivity affects litter size of gilts and sows, because litter size and growth showed highly antagonistic genetic associations (Holm et al., 2004). Other authors have found increased growth rate when ovulation rate, prenatal survival or piglet survival were increased (Knol et al., 2001; Rosendo et al., 2007a). Thus, it might be that pigs with genetically potential for high growth are more likely to survive during suckling period in comparison to pigs having low growth potential (Serenius et al., 2004).

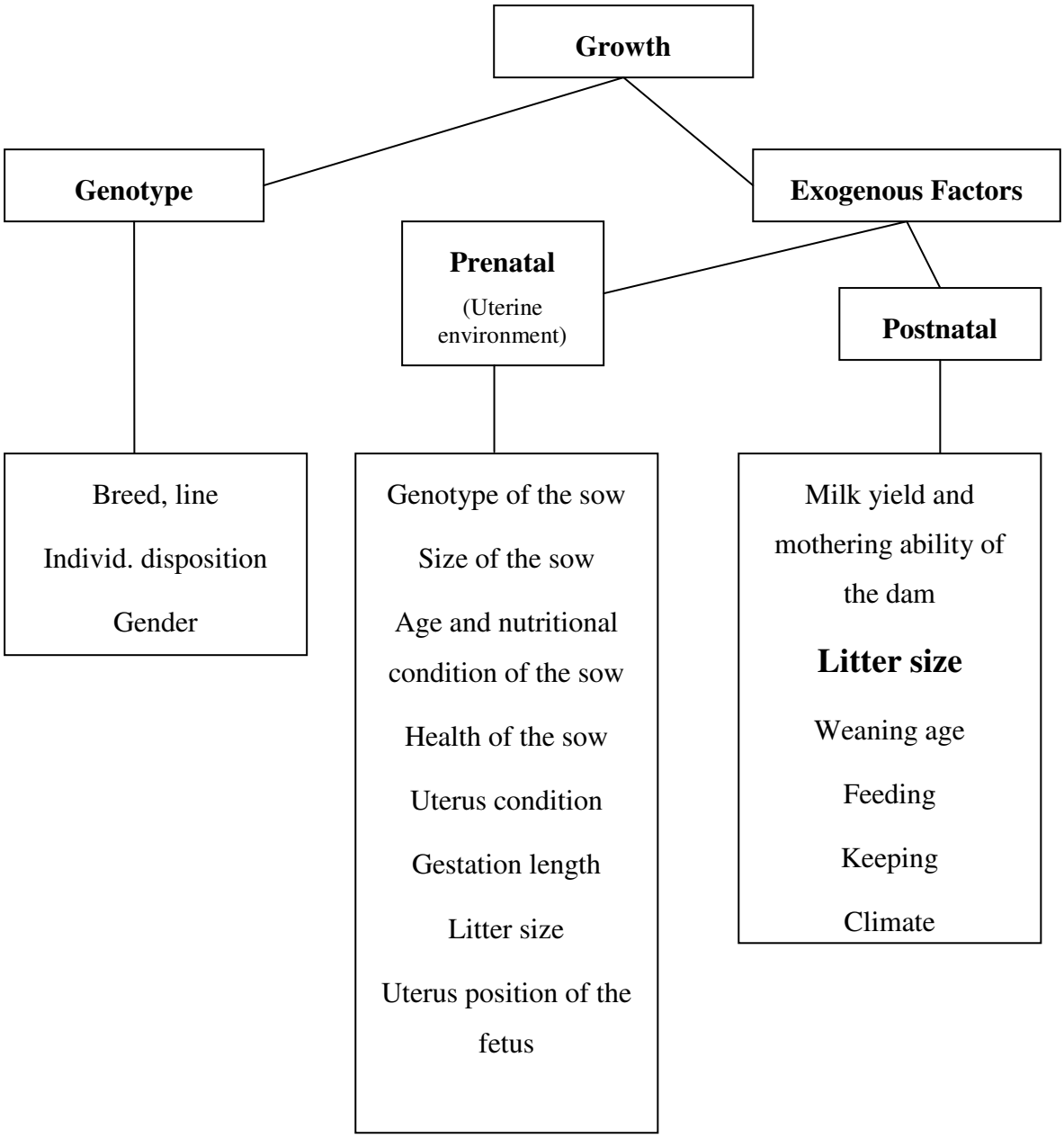

Figure 10: Influencing factors on growth, adapted from Biedermann (1999)

Growth rate and NBA of the sow

Positive relationship of growth rate with the subsequent reproductive performance of the gilt was reported by several authors (Filha et al., 2010; Kummer et al., 2006; Tummaruk et al., 2001). Filha et al. (2010) found a tendency but not significant higher NBA in gilts having higher growth rates before farrowing compared to gilts with a lower growth rate from birth to first farrowing. The disadvantage was that the piglets which were born by gilts with high growth rates showed lower IBW with higher variation. The greater litter size in gilts with high growth rate has also reported by other authors (Beckova et al., 2005; Kummer et al., 2006; Tummaruk et al., 2001, 2000a; Young et al., 2008). Tummuruk et al. (2001) reported, that an increase in growth rate by 100 g/d resulted in an increase of 0.3 additional piglets per litter. Beckova et al. (2005) found a significant association between higher ADG of the sow and larger litter sizes and a tendency of increased NBA in LR population. A larger litter size in fast growing gilts may be explained by a higher ovulation rate of these gilts. This was indicated by a probably increased *IGF-I* and insulin concentrations which was related to ovulation rate (Cox, 1997). Moreover, gilts with higher growth rates consumed higher amount of feed during rearing, had higher body weights and advanced nutrient status resulting in larger litters than gilts with reduced growth rates (Kummer et al., 2006). Lower daily food intake during pregnancy could impair the prenatal development of the piglet (Kerr and Cameron, 1995).

From a biological point of view, high growth rates place high demands on the sow that required resources for growth. In turn, this has a undesirable genetic influence on sow´s ability to give birth to large litter (Holm et al., 2004). Brien (1986) pointed out, that gilts with high growth rates might exhibit a reduced sexually maturity at the same weight than gilts having a lower growth rates, again affecting the gilt's uterine capacity. The age of the gilt was the most important factor in reaching puberty (Hughes, 1982), so that gilts with high ADG tended to be heavier at puberty (Imboonta et al., 2007). In an early study, it was demonstrated that the weight at an age of 165 d, rather than BF, influenced gilts ovulation rate (King, 1989). Imboonta et al. (2007) concluded that selection for increased ADG may increase TNB but at the same time piglet mortality in the first parity will increase.

Litter size, birth weight and later growth performance of the piglet

As already mentioned, selection for increased litters during the last decades led to reduced IBWs (Quiniou et al., 2002). This is mainly induced to a greater competition between the

fetuses in utero which is also reflected by an inverse correlation of IBW and litter size (Milligan et al., 2002; Quiniou et al., 2002). Previous studies showed that piglets with low IBW established a lower number of muscle fibers during prenatal development in comparison to their heavier littermates (Gondret et al., 2006; Gondret et al., 2005; Handel and Stickland, 1984; Nissen et al., 2004; Rehfeldt and Kuhn, 2006; Wigmore and Stickland, 1983) and had a reduced ADG during postnatal period (Milligan et al., 2002; Powell and Aberle, 1980; Quiniou et al., 2002; Rehfeldt and Kuhn, 2006). Dwyer et al. (1994) found a positive correlation between postnatal growth and number of muscle fibers.

As a result of large litter sizes, within-litter variation of IBW increases (Bee, 2007). Generally, both variation of IBW within one litter and low IBW cause problems for future performances like greater pre-weaning piglet mortality, decreased pork quality and slower growth rates (Herpin et al., 2002; Quiniou et al., 2002). Competition for dam´s teats and colostrum which provides energy and maternal antibodies to newborn piglets (Le Dividich et al., 2005) between litter mates occured during the first days after farrowing. Light weighted piglets were disadvantaged in comparison to their heavier litter mates and were not able to prevail. As a result, light weight piglets were replaced to the posterior teats which exhibit a decreased galactorhoea and piglets got insufficient nutrition (milk) supply (Devillers et al., 2007; Gondret et al., 2005; Schinckel et al., 2004). Heavier littermates had a better milk intake due to their ability for better teat stimulation which induced development and distribution of milk secreting hormones Prolactin and Oxytocin. Moreover, ingredients in milk increased (Algers et al., 1991). Piglets with a lower IBW showed reduced teat stimulation and had a significantly reduced ADG. This led to a reduced weaning weight and in fattening period to a reduced slaughter weight. Haley et al. (1995) estimated positive correlation between birth weight and weaning weight and ADG from birth to weaning. In their analyses weaning weight increased for 3 kg per 1 kg higher birth weight.

It was shown that light weight piglets had reduced growth rate during fattening period and reached slaughter weight at a later time point (Berard et al., 2008; Gondret et al., 2006; Gondret et al., 2005; Nissen et al., 2004; Poore and Fowden, 2004; Powell and Aberle, 1980; Quiniou et al., 2002; Rehfeldt and Kuhn, 2006; Rehfeldt et al., 2008; Smith et al., 2007; Wolter et al., 2002). Additionally, it was reported that with increased IBW, body weight in later life increased at a decreasing rate (Quiniou et al., 2002; Schinckel et al., 2007; Schinckel et al., 2010). Berard et al. (2008) compared light vs. medium vs. high birth weight piglets. In their study, light weight piglets at birth showed significant reduced growth from weaning to slaughter in comparison to high and medium birth weight piglets. When piglets with high

IBW (1.75-2.05 kg) were compared with light IBW piglets (0.8-1.1 kg), light weighted pigs needed 12 additional days to reach a slaughter weight of 102 kg which was a result of a reduced ADG of 31 % (Berard et al., 2008). This is an agreement with Rehfeldt et al. (2008) who found significant differences in ADG in light weight compared to heavy weight IBW piglets during the entire life. Fix et al. (2010a) detected a significant influence of birth weight on ADG during all periods of rearing and fattening in pigs of an U.S. LW x LR sows bred to Duroc sires. Piglets with higher birth weight showed higher ADG. They concluded that birth weight affect later body weight, mainly because of differences in ADG. The increase in ADG prior to weaning leads to heavier body weight at weaning which has been shown to resulted in increased post-weaning gain (Klindt, 2003). Moreover, Fix (2010) suggested that an indirect improvement of birth weight through selection for increased body weight measured later in life should be possible. With an indirect or direct selection for increased IBW improvements in piglet survival could be achieved, as well. The relationship between birth weight of the piglet and later growth performance measured by Fix (2010) is illustrated in Figure 11.

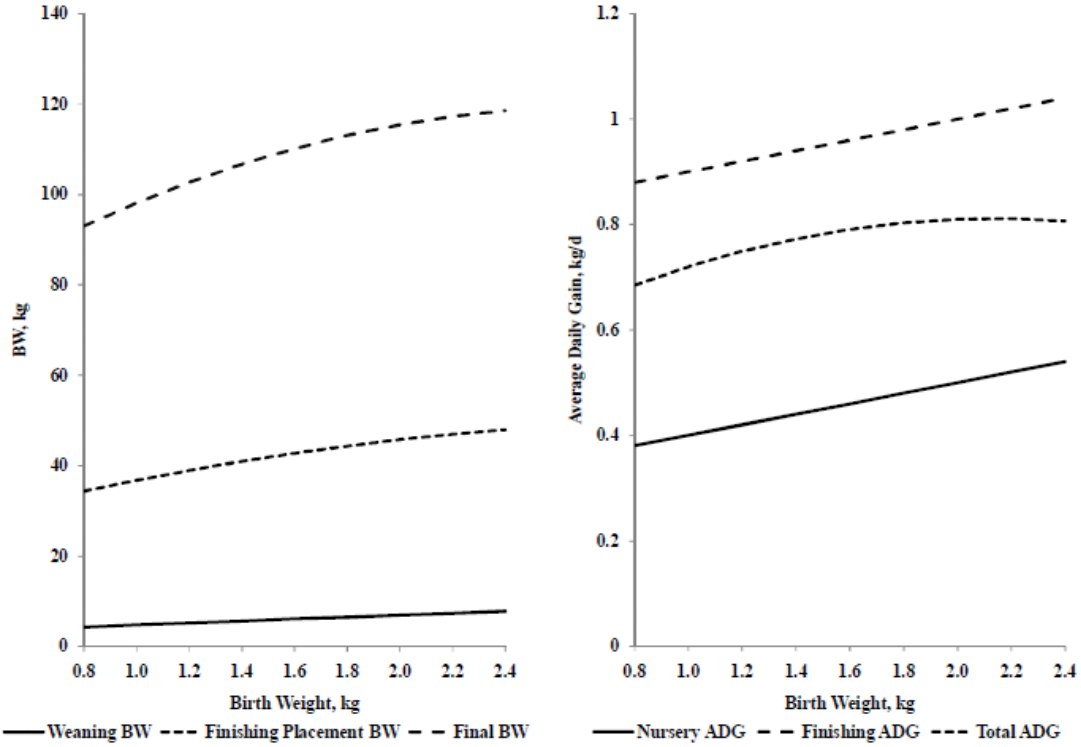

Figure 11: Effect of birth weight on body weights and ADG, adapted from Fix (2010)

Rehfeldt and Kuhn (2006) reviewed previously papers which analyzed the influence of fetal growth retardation and the influence of low IBW and as a result low number of muscle fibers

on carcass compositions and postnatal growth (Kuhn et al., 2002; Rehfeldt, 2005). These studies used piglets of German LR sows and formed three different birth weight groups. The first group consisted of piglets with a birth weight lower 1.2 kg (LBW). Middle weight piglets formed group number two (MBW) and piglets with a larger birth weight than 1.6 kg group number three (HBW). Piglets of low birth weight grew slower compared to the two other groups (ADG LBW: 582 g/d, MBW: 619 g/d, 641 g/d). The ranking in weight was the same at birth and at slaughter, although the differences between MBW and HBW were no longer significant (Figure 12).

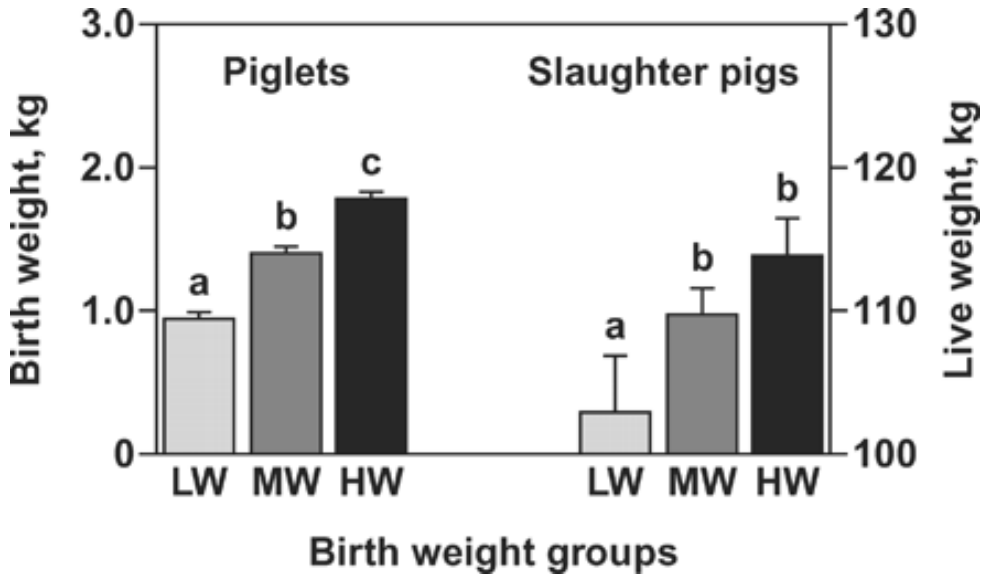

Figure 12: Birth weight (N = 180) and live weights 1 week before slaughter (n = 58; d 175) of pigs divided by birth weight groups (LBW = low, MBW = middle, HBW = heavy). Within age group, least squares means without a common superscript differ between the birth weight groups ($P < 0.05$), adapted from Rehfeldt and Kuhn (2006)

Reduction in ADG due to low birth weight has also been reported by Quiniou et al. (2002) who analysed the effect of the piglet´s birth weight of LW x LR crosses on pre- and post-weaning performance. After weaning, differences between low and high IBW piglets increased so that piglets with an IBW of 1 kg needed 14 additional days to reach finishing weight of 105 kg in comparison to piglets with an IBW of 2 kg (Quiniou et al., 2002). This is an agreement with Gondret et al. (2005). They reported an increase of 12 days of fattening period for light weighted piglets (IBW 0.8-1.1 kg) in comparison to heavy piglets (IBW 1.75-

2.05). This was a result of a significantly reduced ADG during lifetime (birth-slaughter) of light weighted piglets in comparsion to piglets with high birth weight (605 ± 8 vs. 658 ± 10 g/d). Additionally, Gondret et al. (2005) found a strong positive correlation (r=0.65, P<0.01) between birth weight and ADG up to 67 days or 27.5 kg. According to this, influence of birth weights on ADG decreased. The authors concluded that differences in ADG between birth weight classes was the result of reduced food intake of light weightes piglets. Another reason for reduced ADG could be poorer ability of light weight pigs to compete against heavier pigs for nutrition during fattening period (Gondret et al., 2005).

Generally, it can be concluded, that low birth weights in piglets is highly correlated with postnatal growth rates (Knol et al., 2001; Milligan et al., 2002; Quiniou et al., 2002; Smit et al., 2013) (Fix, 2010; Fix et al., 2010a; Gondret et al., 2005; Rehfeldt and Kuhn, 2006).

Genetic foundation of growth traits

Mean h^2 for ADG was 0.4 and ranged from 0 to 0.6 (Bidanel et al., 1996; Bryner et al., 1992; Hermesch et al., 2000a; Imboonta et al., 2007; Johnson et al., 2002; Kerr and Cameron, 1996a; Knol et al., 2001; Rosendo et al., 2007a; Stern et al., 1994; Suzuki et al., 2005). Direct comparisons of h^2 between studies are difficult, because estimations were performed in different breeds, where phenotyping differed markedly. Beside different phenotyping, statistical model to analyze growth traits contained different factors. In some studies the genetic model comprised common litter, maternal and direct effects.

Negative genetic correlations between later growth performance and litter size have been reported in literature (Ducos and Bidanel, 1996; Hermesch et al., 2000a; Holm et al., 2004; Peskovicova et al., 2002; Zhang et al., 2000). Chen et al. (2003) estimated slightly negative genetic correlation between lean growth rate, kg/d and NBA in Yorkshire, Duroc, Hampshire and LR pigs (r_g = -0.082 ± 0.033 – -0.113 ± 0.062). Holm et al. (2004) estimated a positive (unfavorable) genetic correlation between NBA and age at 100 kg. In comparison to that, slightly positive and favorable genetic and phenotypic correlations were estimated between TNB and ADG in the first and later parities by Imboonta et a. (2007) which was an agreement with previous studies (Kerr and Cameron, 1996a; Serenius et al., 2004). Moreover, Rosendo et al. (2007a) calculated slightly positive genetic and phenotypic correlation between ovulation rate and ADG. Crump et al. (1997) estimated a genetic correlation between ADG and NBA of 0.084 in British LR pigs and Short et al. (1994) observed positive and negative genetic correlations of 0.04, 0.05, 0.23 and -0.15 between ADG and TNB in two dam lines. Serenius et al. (2004) estimated slightly positive (favorable) genetic correlations between

TNB and ADG in Finnish LR pigs. The correlation between ADG and TNB was negative (-0.16 ± 0.13) (unfavorable) when a Finnish LW population was investigated. Chen et al. (2003) estimated slightly negative genetic correlation between growth rate (days to 113.5 kg) and NBA in Yorkshire, Hampshire and LR pigs (r_g = -0.041 ± 0.025 to -0.072 ± 0.036). Only in Duroc breed relationship was slightly positive (r_g = 0.051 ± 0.031). It was reported that gilts with higher growth rates had larger litter sizes compared with gilts having low growth rates (Tummaruk et al., 2001). One explanation for this subsequent better reproductive performance could be a possible healthier and better nutrient supply of gilts with high growth rates.

Candidate genes and detected QTLs

Threehundred-twelve (312) QTLs were found for ADG on every chromosome except SSC Y (Hu et al., 2013) (Figure 13). The interpretation of these QTLs is difficult, because of different phenotyping of growth traits. As can be seen in literature, ADG is recorded in different fattening or rearing periods.

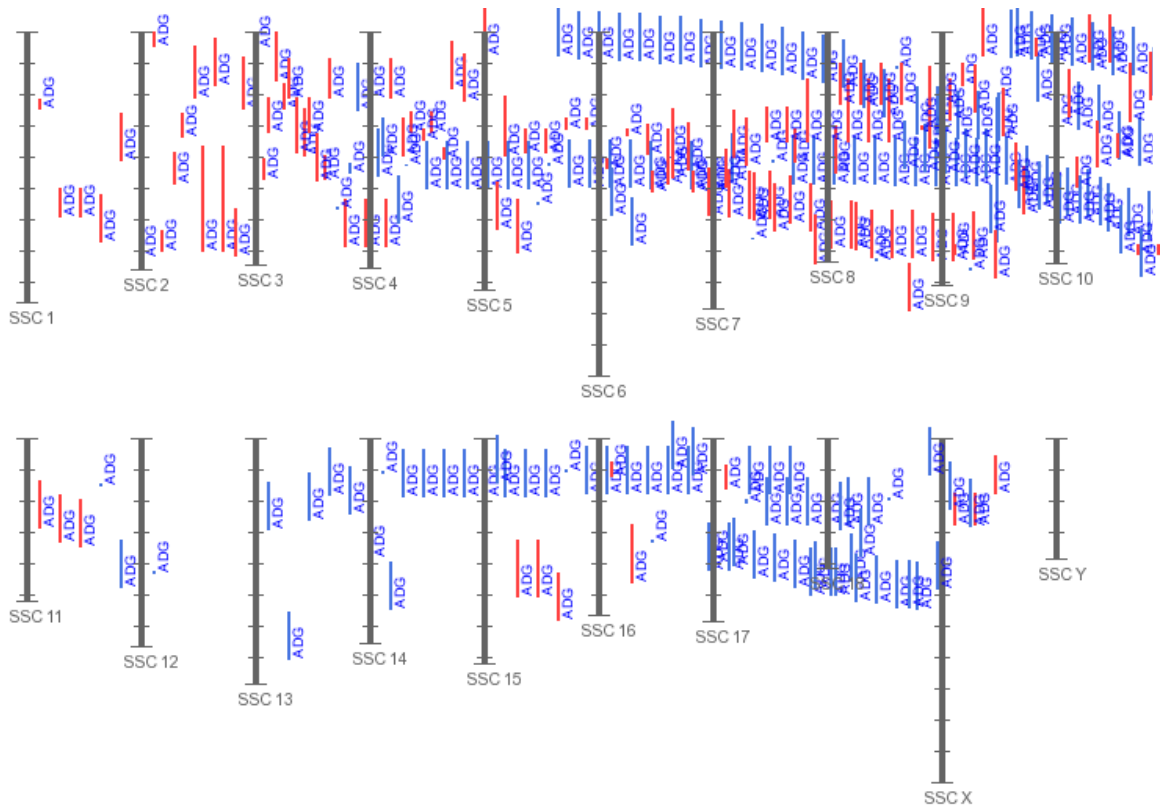

Figure 13: Detected QTLs for ADG, adapted from Hu et al. (2013)

Candidate genes for carcass and growth traits

Several candidate genes affecting ADG, BF and LMP were identified. For some candidate genes, associations for more than one of the analyzed traits were identified. That is the reason why these genes are presented in one section. An overview over all detected candidate genes is given in Table 4.

Table 4: Potential candidate genes affecting analyzed production traits

Gene Name	SSC	Trait	Reference
MC4R	1	BF, ADG	Kim et al. (2000)
		LMC	Weisz et al. (2011)
		ADG	Meidtner et al. (2006)
ME1	1	BF	Vidal et al. (2006)
IGF2	2	ADG	Van den Maagdenberg et al. (2008)
		ADG, BF	Han et al. (2014)
		LMP, BF	Nezer et al. (1999)
CAPN1	2	LMP	Yang et al. (2008)
PYGM	2	LMP	Xu et al. (2012)
MYOD1	2	ADG, LMP	te Pas and Visscher (1994)
CTSF	2	ADG, LMC, BF	Russo et al. (2004)
CRH	4	BF, ADG	Murani et al. (2006)
DGAT1	4	LMC	Weisz et al. (2011)
CTSK	4	BF, ADG	Fontanesi et al. (2010a)
WNT10B	5	BF	He et al. (2011)
MYF5	5	LMC	Verner et al. (2007)
RYR1	6	BF, LMC, ADG	Krenkova et al. (1999)
LEPR	6	BF	Munoz et al. (2009)
PPARD	7	BF	Meidtner et al. (2009)
VRTN	7	BF, ADG	Hirose et al. (2014)
BMP5	7	BF, LMP	Shao et al. (2011)
TBC1D1	8	BF, LMP	Fontanesi et al. (2011a)
PPARGC1A	8	BF	Stachowiak et al. (2007)
		LMP	Kim et al. (2012)
MYOG	9	BF	Xue and Zhou (2006)
NAMPT	9	BF	Wang et al. (2007)
GH	12	ADG	Krenkova et al. (1999)
UNC45B	12	BF	Xu et al. (2008)

Table 4 continued: Potential candidate genes affecting analyzed production traits

Gene Name	SSC	Trait	Reference
ENO3	12	BF	Wu et al. (2008)
TF	13	BF	Krenkova et al. (1999)
CSTB	13	ADG	Russo et al. (2003)
POU1F1	13	BF	De Smet et al. (2003)
CTSB	14	BF	Russo et al. (2003)
HNF1A	14	LMP, BF	Kayan et al. (2013)
MSTN	14	ADG	Liu et al. (2011)
PRKAG3	14	BF	Kocwin-Podsiadla et al. (2006)
INPP5F	14	ADG	Zhou et al. (2009)
MC3R	17	ADG	Weisz et al. (2011)
GHRH	17	BF, LMC, ADG	(Franco et al., 2005; Pierzchała et al., 2003)
CHCHD3	18	BF	Fan et al. (2011)
LEP	18	ADG	Krenkova et al. (1999)
IGFBP3	18	BF	Wang et al. (2009)
TBG	X	BF	Kuehn et al. (2007)

MC4R = melanocortin 4 receptor; *ME1* = malic enzyme 1; *IGF2* = insulin-like growth factor 2; *CAPN1* = calpain 1; *PYGM* = phosphorylase, glycogen, muscle; *MYOD1* = myogenic differentiation 1; *CTSF* = cathepsin F; *CRH* = corticotropin releasing hormone; *DGAT1* = diacylglycerol acyltransferase 1; *WNT10* = wingless-type *MMTV* integration site family; *MYF5* = Myogenic regulatory factor 5; *RYR1* = ryanodine receptor 1; *LEPR* = leptin receptor; *PPARD* = peroxisome proliferator-activated receptor delta; *VRTN* = vertebrae development homolog; *BMP5* = bone morphogenetic protein 5; *TBC1D1* = TBC1 domain family member; *PPARGC1A*= peroxisome proliferator-activated receptor gamma-coactivator 1A; *MYOG* = myogenin; *NAMPT* = nicotinamide phosphoribosyltransferase; *GH* = Growth hormone; *UNC45B* = unc-45 homolog B; *ENO3* = enolase 3; *TF* = transferrin; *CSTB* = cystatin B; *POU1F1* = POU class 1 homeobox 1; *CTSB* = cathepsin B; *HNF1A* = HNF1 homeobox A; *MSTN* = myostatin; *PRKAG3* = protein kinase, *INPP5F* = inositol polyphosphate-5-phosphatase F; *MC3R* = melanocortin 3 receptor; *GHRH* = growth hormone releasing hormone; *CHCHD3* = coiled-coil-helix-coiled-coil-helix domain containing 3; *LEP* = leptin; *IGFBP3* = insulin-like growth factor binding protein 3; *TBG* = thyroxine-binding globulin; BF = backfat; ADG = average daily gain; LMP = lean meat percentage; LMC = lean meat content; SSC = sus scrofa chromosome

One of the most discussed candidate gene for growth and carcass traits is *IGF2*. The first time, Nezer et al. (1999) reported an effect of *IGF2* on production traits. Progenies which inhered the paternal *IGF2* A allele showed reduced BF and higher lean growth. Previous studies confirmed these findings in different experimental crosses and commercial populations (Estelle et al., 2005; Fontanesi et al., 2012a; Fontanesi et al., 2011b; Fontanesi et al., 2010b; Han et al., 2014; Jungerius et al., 2004; Van Laere et al., 2003; Vykoukalova et al., 2006). *IGF2* is part of insulin-like growth-factor system. This is important for promotion of cell proliferation and for inhibition of apoptosis (Oksbjerg et al., 2004). Moreover, *IGF* system has a major impact on normal fetal and postnatal growth and development and on myogenesis (Florini et al., 1996).

The second intensive discussed candidate gene is melanocortin-4 receptor (*MC4R*). This gene is part of G protein-coupled receptors family. This gene is mainly expressed in nervous system. *MC4R* plays a major role in leptin-regulated melanocortin feedback system controlling energy homeostasis and in turn, food intake with effects on body weight and obesity (reviewed by Tao (2010)). A missense mutation within the *MC4R* gene has been associated with BF, ADG, feed intake (reported by Kim et al. (2000)) and was confirmed by several other studies (Bruun et al., 2006; Davoli et al., 2012; Fan et al., 2010; Fontanesi et al., 2012a; Galve et al., 2012; Hernandez-Sanchez et al., 2003; Houston et al., 2004; Jokubka et al., 2006; Kim et al., 2000; Kim et al., 2006; Kim et al., 2004; Meidtner et al., 2006; Munoz et al., 2011; Ovilo et al., 2006; Piorkowska et al., 2010; Stachowiak et al., 2006; Szyndler-Nedza et al., 2010; Tao, 2010; Van den Maagdenberg et al., 2007).

1.4 Statistical Analyses

1.4.1 Methology of Genome-Wide Association Study

Since the very beginning of QTL mapping (Andersson et al., 1994), thousands of QTLs have been detected for several traits in swine. An overview is given in the pigQTL database (Hu et al., 2013). Most of these QTLs have small effects on traits and only a small number of QTLs have large effects on quantitative traits (Goddard and Hayes, 2009; Hayes et al., 2010; Visscher and Haley, 1996). Additionally, these effects are often breed specific and can altered over generations due to selection (Flori et al., 2009; Signorelli et al., 2009; Thaller et al., 2003). Moreover, proportion of variation explained by an average QTL is normally very small (Pausch et al., 2011). Besides that, major determinant of the mapping power is the heritability of the trait (Goddard and Hayes, 2009). That could be one explanation why most QTLs have been detected for production traits, which normally exhibit high heritability.

One tool for QTL detection is GWAS with high number of markers and a sufficient number of animals. GWAS is defined as analyses using a dense array of markers, which capture significant proportion of common genomic variation, and which are typed in DNA samples that were informative for the trait of interest. The aim of GWA studies are the mapping of effects for the particularly trait of investigation through the identification of association between genotype and trait (McCarthy et al., 2008) which forms the basis of GWAS. To date, GWAS mainly relied heavily upon microsatellites to identify regions of interest. However, the implementation of the PorcineSNP60 BeadChip (Ramos et al., 2009) have offered advances for determining QTL. It is now practical and feasible to genotype a large number of animals. The most common used model found in literature to detect QTL is a simple linear model (univariate analyses). With this univariate approach, the association between one trait and the markers is tested at the same time.

Several conditions should be considered and be fulfilled when performing a GWAS. First of all, number of animals used in GWA study should be determined. The required number of animals depends on the effect to be detected. It has been shown that the number of genotyped animals can increase the power of GWAS. It has been concluded that a sufficiently sized samples is crucial for successful GWAS analyses of complex traits (Pausch and Fries, 2014). For the genotyped animals a precise recorded phenotype has to be available. Often, phenotypes are recorded in progeny. The mean of the progeny can be used as phenotype

instead of the own phenotype of studied animal (Goddard and Hayes, 2009). Often, EBVs are used as phenotypes for GWAS and should be preferred over raw phenotypes (Pausch and Fries, 2014). Using EBVs as phenotypes for GWAS it is possible to detect QTLs for traits with low heritability and low population sizes (Pausch et al., 2011).

Secondly, the number of SNPs should be determined. Increasing the number of SNPs will increase the power to detect QTLs. QTLs are only detected, when the marker is in allocated linkage disequilibrium (LD) with the QTL (Goddard and Hayes, 2009). A large number of SNPs increases the probability that the marker was in LD with the QTL which will be detected. While enlarging the reference population substantially increases the accuracy of genomic breeding values, applying denser SNP panels results in moderate gain only (Lund et al., 2011; VanRaden et al., 2011). Effective population size (N_e) is the major determinant for the number of existing independent chromosome segments (Daetwyler et al., 2010), implying that denser SNP panels are necessary to capture the genetic variation for population with large N_e. The findings in the studies by Pausch et al. (2013) provide evidence that the increased density of the 777k-panel allows to identify QTL in cattle populations much more precisely than the 54k-panel. The very dense SNP map enable to capture genetic effects at a better resolution and might result in substantially higher accuracies of genomic breeding values at least in the cattle (Fleckvieh) population.

Thirdly, the structure of the population should be considered. An important prerequisite is homogeneity of studied population because mapping is based on LD (Devlin and Roeder, 1999). Potential existing population stratification due to random mating or different breeds used within data set can result in an increase of false-positive associations. A data set consisting of different breeds cause the biggest problems. When population stratification is not taken into consideration, possibility to identify false-positive associations increases which has been shown in literature (Erbe et al., 2010; Pausch et al., 2011). The relationship between the animals is another form of admixture. In swine, bred usually took place in full-sib families, whereas in cattle half-sib families have been used for breeding. Therefore, the relationships among studied individuals will influence LD between loci even if they are not linked (Goddard and Hayes, 2009).

Quality control of genotype data is necessary to ensure high quality of the data. Often, standard quality criteria for minor allele frequency (MAF), Hardy-Weinberg-Equilibrium (HWG), Call Rate and identy-by-state (IBS) are chosen.

The approach which is applied for GWAS is another very important factor that had to be considered before detection is performed. Several approaches can be used for GWA studies (case-control, cohort, trio, family-based association and DNA pooling) (McCarthy et al., 2008; Pearson and Manolio, 2008). In case-control studies, allele frequencies of animals with specific phenotypes are compared with allele frequencies of animals which do not exhibit this specific phenotype. Most case-control studies have been performed for diseases.

Moreover, different methods exist which can be used depending on population stratification and of the degree of kinship. Performing association studies using stratified samples may lead to false-positive results, "i.e. detected associations can be due to the underlying structure of the population instead of a biologically meaningful association with one or several genes" (Becker et al., 2013). In Figure 14, different methods which can be used depending on population structure and degree of relationship are illustrated. In the following section the methods "genomic control", "structured association", "mixed model approaches" and "eigenstrat method" will be presented.

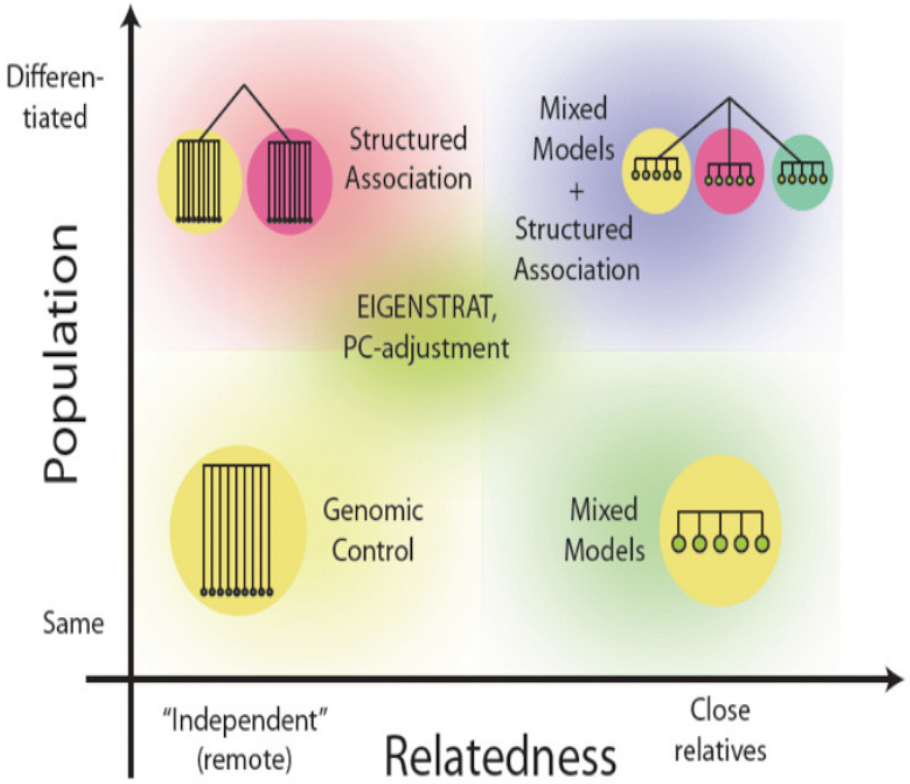

Figure 14: Applied methods for GWAS depends on population structure and degree of kinship, adapted from Aulchenko et al. (2007b)

„Genomic Control“

The method „genomic control“ (GC) is based on a correction of possible population stratification by an adjustment of the significance of the test statistic. GC can only be applied when limited pronounced population stratification exists. To correct the test statistic for existing population stratification, value of the test statistic ($T^2_{original}$) is divided by the inflation factor λ.

$$T^2_{corrected} = \frac{T^2_{original}}{\lambda}$$

This inflation factor λ is calculated as follows (Devlin and Roeder, 1999):

$$\hat{\lambda} = \frac{Median\ (T_i^2)}{0{,}4549}$$

λ is an indicator for how good population stratification was corrected. When λ = 1 no stratification exists, whereas λ > 1 indicates stratification or other confounders (family structure, cryptic relatedness) still exist (Clayton et al., 2005). Quantile-Quantile plots (Q-Q plots) are standard tool for visualization of test statistics. Values of λ < 1.05 are considered as sufficient, although inflation in λ is proportional to sample size (Price et al., 2010).

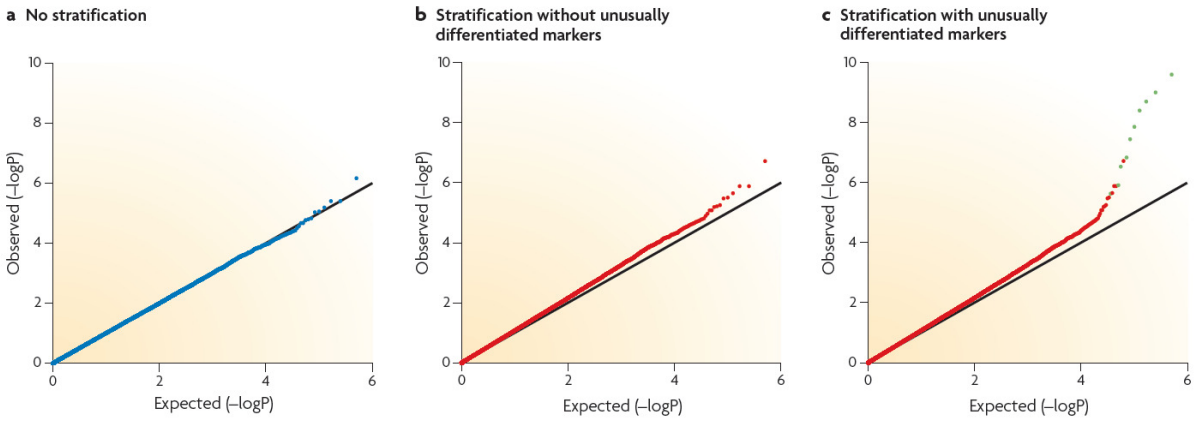

Figure 15: Q-Q plots for the visualization of stratification or other confounders, adapted from Price et al. (Price et al., 2010)

In Figure 15 Q-Q plots of three different scenarios are illustrated. The first one shows the "perfect" Q-Q plot when no stratification exists and p-values fit the expected distribution. In part b stratification without unusually differentiated markers is illustrated. The p-values exhibit modest genome-wide inflation. Part C illustrates stratification with unusually differentiated marker P-values exhibit modest genome-wide inflation and severe inflation at a small number of markers (Price et al., 2010).

„Structured Association"

This method is based on the fact that analyzed animals originate from genetically independent populations (Figure 14). Within these sub-populations animals have low degree of kinship. SNP effects and variances are estimated within each sub-population. Then, these effects and variances are pooled over all sub-populations whereby a general test statistic is produced.

„Mixed Model Based Approaches"

Conditions for „mixed model based approaches" are that the degree of kinship between analyzed animals must be relatively high. This method can further be divided into family-based method and a general regression approach. The family-based association is based on family structure which can be included in the model as identity-by-descent (IBD) information (Chen and Abecasis, 2007). The regression approach is called „Genome-wide rapid analysis using mixed models and regression" (GRAMMAR) (Amin et al., 2007; Aulchenko et al., 2007a). With this method the phenotype was corrected for a polygenic effect in a first step. Resulting residuals are used as a new phenotype in GWAS. Within this method the genomic kinship matrix is used.

Eigenstrat

The method eigenstrat was implemented in several GWAS programmes like GenABEL (Aulchenko et al., 2007b), EIGENSOFT and Plink. This method was introduced by Price et al. (2006) to correct for existing population stratifications and is a combination of structured association and genomic kinship matrix. Differences between animals and populations should not be too large but a genetic linkage must exist. In the first step based on genomic kinship matrix principal components (PC) are calculated, which illustrate genetic variation between animals in compressed form.

The PCs are used to correct the genotype and the phenotype for existing population stratification. For this, PCs are implemented as covariates in the model for GWAS. With the software GenABEL, a varying number of PC can be included in the model, depending on population stratification. The software PLINK uses the first 10 PC to correct for population stratification.

1.5 Multivariate Analyses

In multivariate analyses associations of more than one trait are identified at the same time. Using complex multivariate models are useful to detect pleiotropic QTL effects. Additionally, multivariate models increase the precision of the estimated QTL position in the genome (Knott and Haley, 2000).

The influence of one QTL/SNP/gene on more than one trait is termed pleiotropy (Bolormaa et al., 2014; David et al., 2013; Solovieff et al., 2013). Solovieff et al. (2013) distinguished pleiotropy further in biological pleiotropy, mediated pleiotropy and spurios pleiotropy. Biological pleiotropy occurs when one gene has a direct effect on at least two different traits. Spurious pleiotropy is defined as a genetic variant falsely identified to be associated with more than one phenotype, whereas mediated pleiotropy exists if one phenotype is causally related to another phenotype (Figure 16).

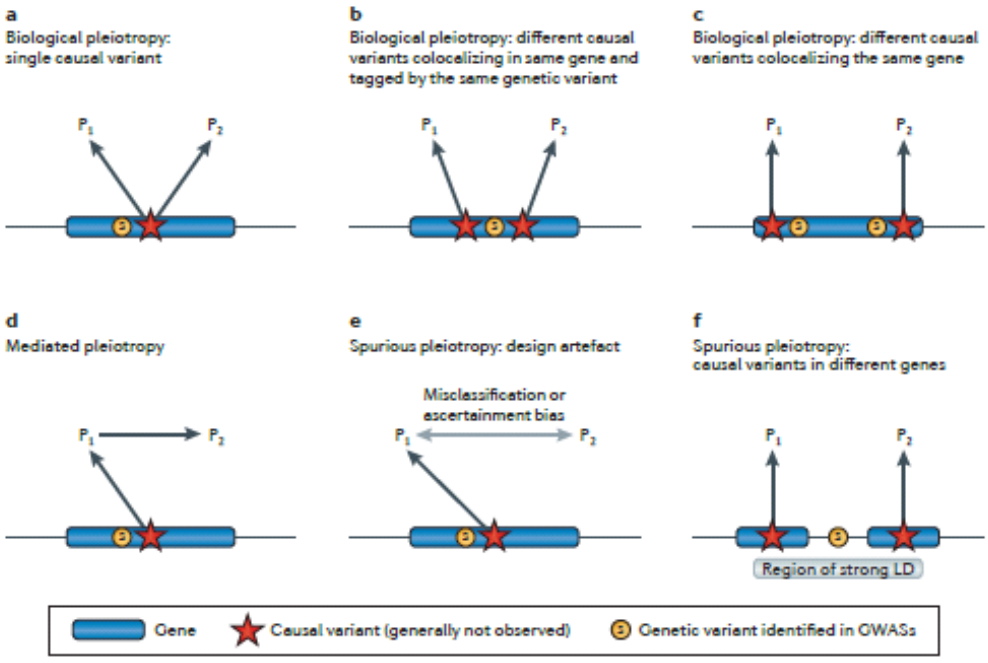

Figure 16: Types of pleiotropy, adapted from Solovieff et al. (2013)

Figure legend: In each scenario, the observed genetic variant (S) is associated with phenotypes 1 and 2 (P1 and P2). We assume that the observed genetic variant is in linkage disequilibrium (LD) with a causal variant (red star) that affects one or more phenotypes. In some cases, the causal variant may be identified directly and the figures can be simplified accordingly. The various figures correspond to the unobserved underlying pleiotropic structure. a | Biological pleiotropy at the allelic level: the causal variant affects both phenotypes. b | Colocalizing association (biological pleiotropy): the observed genetic variant is in strong LD with two causal variants in the same gene that affect different phenotypes. c | Biological pleiotropy at the genic level: two independent causal variants in the same gene affect different phenotypes. d | Mediated pleiotropy: the causal variant affects P1, which lies on the causal path to P2, and thus an association occurs between the observed variant and both phenotypes. e | Spurious pleiotropy: the causal variant affects only P1, but P2 is enriched for P1 owing to misclassification or ascertainment bias, and a spurious association occurs between the observed variant and the phenotype 2. f | Spurious pleiotropy: the observed variant is in LD with two causal variants in different genes that affect different phenotypes. GWAS, Genome-Wide Association Study.

Pleiotropic effects are the main cause of genetic correlations between two or more traits. However, another possible reason for genetic correlations is linkage disequilibrium (LD) between the QTL for more than one trait (Bolormaa et al., 2014). Thereby, QTLs can be affected in the same or in opposite directions. Identifying QTLs, SNPs or genes with pleiotropic effects might help to understand genetic architecture and interaction of multiple traits.

The principal component analysis (PCA) is one variant of multivariate analyses. PCA condensates a large number of variables to a small number that still contained most of the information of the large set. The PCA is accomplished from the phenotypic covariance matrix of the data set, results as estimations of the residual covariance matrix. Analyzing a *p* number of traits results in *p* number of phenotypically uncorrelated combinations resulted from the components of the eigenvectors of the phenotypic covariance matrix. Each eigenvalue stands for the part of phenotypic variability explained by the corresponding PC variable (Gilbert and Le Roy, 2003). Such a multitrait analysis might be particular beneficial in a situation, where the effect of a pleiotropic locus is too small to be detected by single-trait analyses only (Mangin et al., 1998). It has been proposed that PCA should be used for multitrait detection of pleiotropic QTL (Weller et al., 1996). Moreover, it has been reported that the PCA was generally more powerful and accurate than single trait analyses (Gilbert and Le Roy, 2007; Klei et al., 2008; Mangin et al., 1998).

The interpretation on a biological basis of the results of PCA might be difficult especially when a significant locus has an antagonistic effect on more than one trait. In literature it has been discussed how many PC should be analyzed and interpreted. Some others suggested just analyzing the first PC which explained the majority of variation (Liu et al., 1996; Mahler et al., 2002). On the other hand, it has been shown by Olsen et al. (1999) that the first PC not always identified the highest phenotypic proportion explained by a genetic marker, because of large number of influencing factors on the phenotype, the contribution of any individual gene to overall phenotypic variation might be small for a complex trait (Olson et al., 1999). This was supported by Aschard et al. (2014) who investigated the importance of the second and following PC. They concluded that PCs explaining only a low amount of the phenotypic variance might harbor a substantial part of the total genetic association and seemed to be very powerful when QTL effects are opposite to positively correlated traits.

Up to the present, studies analyzing or detected pleiotropic effects on production and reproduction traits in swine are very limited (Gilbert et al., 2007; Guo et al., 2009; Knott and

Haley, 2000; Mercade et al., 2005; Munoz et al., 2013; Nagamine et al., 2009; Revilla et al., 2014; Spotter et al., 2005; Stearns et al., 2005; Uddin et al., 2011).

1.6 Scope of the study

In the recent past, pig breeding organizations have been focused on the breeding of sows with high number of NBA in order to generate higher profits in piglet production. Despite the low heritability (h^2) and the complex genetic basis, a considerable genetic progress has been achieved for NBA. Simultaneously, antagonistic relationships between production, fitness and reproduction trait complexes were reported by several authors.

Against this background, the aims of the present work were to clarify the genetic basis of NBA and to detect possible pleiotropic effects between the two trait complexes reproduction and production using different GWAS models and methods.

For all statistical analysis a large data set consisting of 4,012 LW and LR pigs from herdbook and commercial breeding companies in Germany (3), Austria (1) and Switzerland (1) was analysed. All pigs had EBVs for NBA and production traits (ADG, LMP, BF) and were genotyped with the Illumina PorcineSNP60 BeadChip.

Theoretically, combing data from different breeding organisation will increase the power of the study. However, the risk of false positive results is increased if the populations are stratified. This aspect was analysed in the first part of the project (**Chapter 2**), where the extent of genetic distance between LW and LR populations of different breeding organisations was quantified. Based on these results, GWA studies were performed for NBA within the two maternal dam lines LW and LR and their corresponding sub-clusters.

In the second part (**Chapter 3**) possible pleiotropic effects between NBA and three production traits (ADG, LMP and BF) were investigated with univariate and multivariate approaches. In the univariate GWA studies, overlapping significant SNPs or genomic regions for different traits were identified. In multivariate approach, EBVs of all traits were condensed into a series of uncorrelated PCs. These PCs comprised all EBVs which were differently weighted, so that a rough genetic interpretation was possible. Theoretically, the power of the detection of pleiotropic effects using multivariate statistical methods like PCA was increased, so that efficiency of detection of pleiotropic effects is improved.

2 CHAPTER 2: A GENOME-WIDE ASSOCIATION STUDY IN LARGE WHITE AND LANDRACE PIG POPULATIONS FOR NUMBER PIGLETS BORN ALIVE

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Abstract:

The number of piglets born alive (NBA) per litter is one of the most important traits in pig breeding due to its influence on production efficiency. It is difficult to improve NBA because the heritability of the trait is low and it is governed by a high number of loci with low to moderate effects. To clarify the biological and genetic background of NBA, Genome-Wide Association Studies (GWAS) were performed using 4,012 Large White and Landrace pigs from herdbook and commercial breeding companies in Germany (3), Austria (1) and Switzerland (1). The animals were genotyped with the Illumina PorcineSNP60 BeadChip. Because of population stratifications within and between breeds, clusters were formed using the genetic distances between the populations. Five clusters for each breed were formed and analysed by GWAS approaches. In total, 17 different significant markers affecting NBA were found in regions with known effects on female reproduction. No overlapping significant chromosome areas or QTL between Large White and Landrace breed were detected.

Keywords: NBA; Pig; Fertility; GWAS

Introduction:

Reproduction traits of livestock are important because of the major role they play in the economic success of production [1]. The efficiency of pig production largely depends on the number of piglets born alive (NBA) and the number of piglets weaned (NPW). Up to the present, selection based on traditional breeding programmes using Best Linear Unbiased Prediction (BLUP) has been successful in improving maternal reproductive traits such as NBA. However, genetic improvement of female reproduction traits is difficult and complex because of low heritability and sex limited expression and because phenotyping is only possible late in a sow's life. These conditions constitute a challenge for traditional animal breeding programmes. The exploration of the genetic architecture of reproduction traits is necessary because of the complex genetic and biological processes involved [1,2].

Since the very beginning of quantitative trait loci (QTL) mapping [3], about 10,000 QTL for 653 different traits have been identified in the pig genome (PigQTLdb, http://www.animalgenome.org/cgi-bin/QTLdb/SS/index, [4]). Most of the reported QTL affect production and meat quality traits. For reproduction traits, 137 QTL were identified for total number born, 110 QTL for body weight at birth and 106 QTL for NBA (July 2014).

Several studies have investigated the biological foundation in regard to the high impact of NBA on pig production. Genes such as retinol binding protein 4 (*RBP4*), estrogen receptor 1 and 2 (*ESR1*, *ESR2*) and porcine insulin-like growth factor 2 (*IGF2*) were identified to be positively associated with NBA [1,5-7], but these genes explain only a relatively small proportion of the genetic variation of NBA.

In the past, genome-wide scans using microsatellites were performed to identify regions affecting the potentially interesting traits. The development of the PorcineSNP60 BeadChip [8] allows the detection of QTL and candidate genes in a higher resolution. In a recent study Onteru et al. [9] have detected novel QTL regions for pig reproduction traits which do not overlap with QTL intervals previously reported using microsatellites.

In Europe, the two breeds Large White (LW) and Landrace (LR) are typical dam lines in commercial pig breeding programmes. However, differences between the two breeds were found in several studies which investigated reproduction traits such as NBA. For example, it was shown that LW sows had slightly higher NBA compared to LR sows [10-12]. Moreover,

most breeding companies have their own LW and LR populations with different breeding objectives. Breeding stock is not normally exchanged between organisations. This leads one to expect differences between the breeding companies and their breeding stock.

In order to map QTL affecting NBA, Genome-Wide Association Studies were performed in LW and LR populations of different breeding companies located in Germany, Switzerland, and Austria. The aims of the study were

a) to reveal genetic similarities and differences between LW and LR populations of different breeding organisations,
b) to identify significant associated SNPs for NBA, and
c) to clarify the biological relevance of these significant markers.

Material and Methods:

Animals and phenotype data:

The study included a total of 4,012 LW and LR pigs from herdbook and commercial breeding companies across Germany (3), Austria (1) and Switzerland (1). Data of 2,365 (boars: 1,435, sows: 930) LW and 1,647 (boars: 1,159, sows: 488) LR animals born between 1990 and 2011 were recorded (Table 5).

The frequencies of years of birth of all animals are shown by gender in Figure 17. Breeding values for NBA were routinely estimated by the breeding companies using a standard animal repeatability model and were provided for the study.

SNP Quality control

Tissue samples (semen or hair follicle) of the pigs were genotyped with the Illumina PorcineSNP60 Bead Chip [8] in the laboratory Life & Brain GmbH, Bonn.

SNPs were excluded from further analysis under the following conditions: a) Minor allele frequency (MAF) < 0.5 %, b) Call rate < 95 % and c) strong deviation from the Hardy-Weinberg-Equilibrium ($p < 10^{-3}$). Quality control was performed as implemented in the GenABEL package [13] within defined population clusters.

Population structure

GWAS were performed within breeds (LW or LR) and clusters comprising different sub-populations. In order to visualize possible population stratifications, multidimensional scaling (MDS) plots of an identity-by-state (IBS) matrix were generated containing the two most important principal components of the underlying genetic variation. These two-dimensional MDS plots of the IBS matrix revealed the overall genetic distances between the animals. Based on the visualized genetic distances, animals of the LW and LR populations were analysed separately. In addition, four sub-populations were identified within the LW and LR breeds. Additional GWAS were performed within these clusters, which comprise animals from one to four different breeding organisations.

Genome-wide association study

The GWAS were based on an combined approach developed by Amin et al. [14] and Price et al. [15] and implemented in the R-Package GenABEL [13,14,16]. In order to control population stratification the "Genome-wide Rapid Analysis using Mixed Models and Regression" (GRAMMAR) [14] combined with EIGENSTRAT [15] was used. A similar, combined procedure was suggested by Zhao et al. [17].

In a first step, the phenotypic data (breeding values) were corrected for the fixed effect "breeding organization" and a polygenetic effect *(a)* by means of equation (1):

$$y^{*} = y - (\mu + X\beta + Za) \qquad (1)$$

with y^{*} and y as vectors of pre-corrected and original estimated breeding values (EBVs), respectively, β as solution vector of the fixed effect 'breeding organisation', and a as random additive polygenic $(a_i \sim N (0, G \times \sigma^2_a))$ effect, which estimates the contribution from the polygene (breeding value) with G as the genomic kinship matrix and the additive genetic variance σ^2_a. X and Z are the corresponding design matrices for the fixed and random effects.

The genomic kinship (G_{ij}) was estimated by applying the method suggested by Astle & Balding [18]:

$$G_{ij} = \frac{1}{L} \sum_{l=1}^{L} \frac{(g_{l,i} - p_l)(g_{l,j} - p_l)}{p_l(1 - p_l)} \qquad (1)$$

with L as the number of SNP, p_l as the allelic frequency at l-th locus (major allele) and $g_{l,j}$ / $g_{l,i}$ as the genotype of j-th / i-th individual at the l-th locus, coded as 0, 1/2 and 1, corresponding to the rare homozygous, heterozygous, and common homozygous genotype.

Ignoring the covariance between animals from one family can lead to a high number of false-positive SNPs. The residuals computed with GRAMMAR are corrected for polygenic relationships between the animals and can be used as a new phenotype in association analyses [14,16].

In a second step, these familial correlation-free residuals were included in a simple linear regression as new phenotype for association test (2):

$$y^* = \mu + kg + e \qquad (2)$$

with y^* as the vector pre-corrected EBVs from (1), μ as the mean, g is the vector of genotypes at the marker, k as the marker genotype effect and e as the vector of random residuals.

In order to verify remaining population stratification, the inflation factor λ, which depends on the squared original test statistic of the *i-th* SNP (T_i^2) was calculated as

$$\lambda = \frac{Median(T_i^2)}{0.4549}.$$

Aluchenko et al. [13] and Price et al. [19] showed that an inflation factor λ in the range of 1.0 to 1.05 is an indicator of a sufficiently corrected population stratification which can be analysed with an acceptable risk of false positive results. Preliminary results of our analysis showed that λ deviates considerably from this optimum. This implies that serious population stratifications still exist.

In order to correct for this problem, model 2 was extended by principal components (PC) estimated from the genomic kinship (EIGENSTRAT) [13,15] which were included as fixed covariables. The genomic kinship matrix was used to reveal the PC reflecting the axes of genetic variation and describing the stratification of the populations involved in this study. These PC were used to adjust the phenotype and the genotype for population stratification. The estimation of the PC and the association analysis was performed with the function 'egscore' as implemented in the R-package GenABEL [13].

The number of PC used in this step is variable and depends on the ability to correct different levels of population stratifications. The number of PC was increased stepwise and after each step the level of population stratification was quantified via the inflation factor λ.

The final number of PC was chosen so that the inflation factor λ [20] was nearest to 1.

The inflation factor λ and the observed versus the expected p-values for each SNP are illustrated in quantile-quantile (Q-Q) plots for each cluster. Two regression lines are fitted which represent the optimal ($\lambda = 1$) and the calculated inflation factor λ. In case of unstratified population structures, no visible differences can be observed between the two regression lines.

In order to reduce the risk of false-positive associations, the p-values of the SNP significance tests were corrected using the Bonferroni-adjustment. Thresholds for genome-wide and chromosome-wide significance levels were 5 %.

Variance of the pre-corrected EBVs (σ^2_{y*}) explained by each SNP was calculated approximately using following formula:

$$r^2 = \frac{\chi^2_{1df}}{N - 2 + \chi^2_{1df}} \qquad (3)$$

with χ^2_{1df} as the test statistic for each SNP resulted from association test and N as the number of animals. This formula resulted from the transformation of a student's t-distribution into a z-distribution [21]. In our analysis, r^2 cannot be interpreted as the proportion of explained phenotypic variance of NBA – as is usually the case –, because pre-corrected EBVs were analyzed instead of phenotypes. However, r^2 might be a rough indicator of the explained proportion of the additive genetic variance of NBA and could be used to rank the importance of QTL only.

Pig Sscrofa 10.2 (International Swine Genome Sequencing Consortium) [22] was used to annotate the significant associated SNPs. The search for biologically relevant genes was performed with Ensembl BioMart [23,24]. For that, a 2 Mb window around a significant region was chosen.

Results:

Population structure analysis

MDS plots were used to visualize the genomic distances between the animals (Figure 18-Figure 20). Figure 18 revealed that the breeds LW and LR had a large genetic distance and should be regarded as more or less genetically disconnected. Each breed was analysed separately because of distinct genetic differences between LW and LR.

Additionally performed visual inspections of the breed specific MDS plots of LW and LR populations led to various cluster definitions (Figure 19, Figure 20).

The animals of the breed LW (LW_1) were grouped into four sub-clusters (Figure 19). Cluster LW_3 and LW_2b contained only animals of one breeding organisation, whereas cluster LW_2a covered genetically overlapping pigs of three breeding organisations. In addition, cluster LW_2 combined the clusters LW_2a and LW_2b, which overlap only to a small extent.

In the LR population (cluster LR_1) four sub-clusters were assigned (Figure 20). Cluster LR_2 was formed by excluding the breeding company (cluster LR_3) with the highest deviation from the LR_1 dataset. In addition, two distinct sub-populations were extracted from cluster LR_3 which form cluster LR_3a and LR_3b.

Quality control

SNP quality control was performed within the various clusters. The quantity of remaining genetic markers lay between 39,408 and 45,303 (LW) and 42,205 and 46,066 (LR) clusters. The number of animals ranged between 553 and 2,272 for LW or 206 and 1,598 for LR clusters. More detailed information about each cluster is given in Table 6.

Influence of population stratification

In order to ensure the power and accuracy of GWAS, it is essential to take possible population stratifications [13,25,26] into consideration. Therefore, associations between SNP and NBA were estimated within the genetically more or less overlapping clusters. In addition, PC which condensed the genetic relationships between the animals was used in the statistical model as

covariates to correct for existing population stratification. Depending on the cluster, different numbers of PC were required in order to avoid negative effects of population stratification on the validity of the GWAS analysis. The number of PC used in the analyses of various clusters ranged from 22 (LR_3b) to 372 (LW_1). Genomic inflation factors in all clusters were close to one (Table 6).

Cluster specific Q-Q plots (Figure 22) contain regression lines which were calculated by a linear regression of expected test statistics (independent variable) on observed test statistic (dependent variable). The slopes of these lines correspond to the calculated inflation factor, which is close to 1 in all clusters analysed. This shows that possibly existing stratifications of the populations do not adversely affect the validity of corresponding GWAS analysis.

Genome-wide association analyses

The Manhattan plots show the p-values of the SNP association test for the target trait NBA ordered according to the genomic positions (representative by Figure 21; SI 1-SI 9). 14 different chromosome-wide and three genome-wide significant SNPs were detected in the analysed clusters. Three of these SNPs had a MAF below 1 %.

SNPs which were significant in both breeds or in different clusters containing animals from different breeding organisations would have been of particular interest. However, no significant markers or chromosome regions were found to be shared by the breeds. Moreover, only a small number of SNPs were found to be identical in the different clusters of each breed. These SNPs and cluster specific significant markers will be described in the following sections.

Large White

In LW_1 three chromosome-wide significant markers were found on SSC5 and SSC10. Each of these markers explained less than 1.0 % of σ^2_{y*}. The population LW_1 was subdivided into clusters LW_2 (animals from four breeding organisations) and LW_3 (one breeding organisation). In LW_2 and LW_3 no genome-wide significant SNPs were found. However, within cluster LW_2 five QTL were detected on a chromosome-wide significant level. Each of these QTL explained between 1.1 to 1.3 % of σ^2_{y*} of the target trait NBA (SI 1 and SI 4, Table 6 and Table 8). Because of a smaller degree of genetic overlapping, LW_2 was further subdivided into clusters LW_2a and LW_2b. The analyses of these clusters revealed three

(LW_2a) and two (LW_2b) chromosome-wide significant SNPs for NBA (Table 6 and Table 8), which explained 2.4 to 4.6 % and 1.8 to 2.2 % of σ^2_{y*}. Three of the QTL detected in dataset LW_2a were significant on a genome-wide level (SI 2 and SI 3). One of the significant SNP associations on SSC9, identified in cluster LW_2, was confirmed by the analysis of sub-cluster LW_2a. Additionally, three SNPs which were found on SSC5 and SSC10 in LW_1 were also identified in LW_2. This was to be expected, because LW_2 is a subset of the larger cluster LW_1 and LW_2a is one of LW_2.

Landrace

In the data set LR_1 two SNPs reached the chromosome-wide significance threshold of 5 % (SI 5, Table 6 and Table 9). These associations were located on chromosome 9 and 11, they explained up to 1.3 % of σ^2_{y*}. After visual inspection of the MDS plots, LR_1 was subdivided into clusters LR_2 and LR_3 which contained 4 or 1 breeding organisations, respectively. In the case of LR_2, no SNP reached the genome- or chromosome-wide significance level (SI 6, Table 6 and Table 9). On the other hand, association test performed for cluster LR_3 resulted in two SNPs with chromosome-wide significance, explaining up to 4.8 % of σ^2_{y*}. These significant SNPs were located on SSC 7 and SSC16 (SI 7, Table 6 and Table 9). Although cluster LR_3 contained only animals from one breeding organisation, two genetically disconnected sub-clusters (LR_3a and LR_3b) were identified. Association tests in LR_3a resulted in no significant SNPs. For LR_3b and LR_3, one marker located on SSC16 reached the chromosome-wide significance level and explained up to 8.0 % of σ^2_{y*} (SI 9 and SI 7, Table 6 and Table 9).

Discussion:

Population stratification

In the present study, a combined GWAS-approach was used to identify QTL influencing NBA in two maternal pig breeds. When analysing such large scale heterogeneous data, it is of major importance to correct for potential population stratifications in order to ensure the accuracy of the statistical analysis. Several studies have shown that ignoring population stratification will lead to an inflation of false positive QTL and to a loss of statistical power [13,25,26]. In order to avoid such negative effects, our study analysed several clusters compromising animals from only one or from genetically overlapping breeding organisations.

As a first result, it was found that animals of the LW and LR breed in the present study do not genetically overlap. This can be seen in the corresponding MDS plot (Figure 18). For this reason both breeds were analysed separately. In addition, sub-clusters within the two breeds were identified. These sub-clusters are presumably the result of the different selection strategies used by the different breeding organisations. Sub-populations from a limited number of breeding organisations were investigated to identify common regions affecting the target trait NBA. This is a generally accepted procedure and has been utilised in several GWAS in pigs and cattle [27,28].

The defined clusters were statistically evaluated with an approach that combines the GRAMMAR [14] und EIGENSTRAT [15] methods. Within the GRAMMAR approach estimated breeding values for the trait NBA are pre-corrected for the effects 'breeding organisation' and 'familial correlations', taking into account the genomic "true" relationship between animals. This approach has two advantages: a) the genomic kinship matrix shows the true proportion of shared alleles whereas a pedigree based kinship matrix displays the expected proportion and b) familial correlations are removed from the new phenotype by calculating environmental residuals for association test [14,29]. This is especially important for analysing EBVs as dependent variables because in this case distinct correlations between the EBVs of relatives can be expected. Despite these corrections, the inflation factor, which was calculated according to model 1 (GRAMMAR approach), deviates considerably from the optimum of λ=1 in each cluster. Therefore, in the second part of the combined approach (EIGENSTRAT), the detection of QTL is based on a model which includes a number of genomic PC depending on the cluster as fixed covariates. This method (EIGENSTRAT) has been applied in several other studies [26-28,30]. The PC condenses the genomic covariance structure of the animals into a series of factors with decreasing importance. The PC act as a correction factor for possible population stratification, but on the other hand, they also reduce the genetic variation which can be used to detect QTL. Although this method leads to an efficient elimination of population stratification, it remains unclear if the inclusion of a high number of PC (>10) leads to an unacceptable loss of utilizable genetic variation. This might have a considerable impact on the power of the association tests [25,28]. In order to balance the two conflicting objectives – removal of population stratification and retention of utilizable genetic variation –, we increased the number of PC stepwise until an acceptable solution was found. The effects of increasing the number of PC were monitored by evaluating the level of the inflation factor λ, which is an indicator of the remaining population stratification.

Generally, a value of λ between 1.00 and 1.05 is regarded as tolerable [13,19]. This acceptable range was reached in all analyses after the inclusion of 22 to 372 PC. Aulchenko et al. [13] suggested including 10 PC in the GWAS model in human, which can be regarded as a compromise between correcting for population stratification and retention of utilizable genetic variation. As expected, the number of significant markers increased substantially when 10 PC were used. However, the inflation factors in all analysis were below one, so that the results were not further interpreted.

Minor allele frequency

In GWAS, SNPs with a MAF lower than 1 % are frequently excluded from the data set. In the present study a threshold of 0.5 % was chosen instead, which can be justified by the findings of Tabangin et al. [31] and Stephens & Balding [32]. Tabangin et al. [31] found that rare SNPs did not show significantly higher false-positive results than common SNPs. They concluded that the removal of SNPs with a low MAF would not be necessary to reduce false-positive results. Stephens & Balding [32] pointed out that the consideration of the p-value alone is not sufficient to characterize the association between the SNP and trait. The statistical power in association tests is of high importance in order to quantify the true dimension of the association. This power is influenced by the MAF and is reduced when SNPs with low MAFs are removed [32,33].

Only five out of a total 17 significant SNPs in the present study had a MAF of < 1 %. These SNPs were located in regions where trait specific QTL or genes have been mapped (Table 8-Table 7). Their physiological role could indicate a functional relevance regarding the variation of the trait examined here. Gorlov et al. [33] and Cargill et al. [34] found in their analyses that the proportion of functional SNPs was highest among SNPs with a low MAF. The elimination of rare SNPs could thus decrease the potential for genetic improvement when using genomic selection in animal breeding.

Significant markers for NBA: Across population

In LW, SNPs significant across sub-populations were found in the analysis of clusters LW_1 and LW_2 as well as in LW_2 and LW_2a, which had a certain proportion of animals in common contain shared proportions of identical animals. A remarkably low number of QTL

were found in the genomically homogeneous cluster LW_2b, which consists of animals from only one breeding organisation. The high number of PC (151) with negative impact on the utilizable genetic variation might explain this result. In addition, the year of birth of the pigs from this breeding organisation covers the years 1990 to 2011 (Figure 17). This long period of selection might influence the frequency of important genes and/or the linkage phase between marker and QTL, but not necessarily the genomic population structure displayed by the MDS plots (Figure 19 and Figure 20).

The LR population of one breeding organisation (LR_3) was genetically disconnected, so that two sub-clusters (LR_3a and LR_3b) were formed and analysed separately. The genetic disconnection can be explained by the import of breeding animals into this breeding organization in the past. Within the different LR clusters, only one SNP located on SSC16 was found in two clusters, LR_3 and its subset LR_3b.

Significant markers for NBA: Position and biological relevance

Detailed information about significant SNPs and the results of annotation for all analyses with previously reported candidate genes, QTL or association in SNP regions are given in Table 7.

In the analysis of LW_2a, one SNP significantly associated with NBA on SSC3 at 27.9 Mb was located within a region where QTL have been found for NBA and ovulation rate (OR) in previous studies [9,35] (Table 7). Up to the present, no gene with an influence on these reproductive traits has been located in this chromosome region.

At the distal end of the p-arm of SSC5 two significant markers (ASGA0023685, MARC0103593) were found in LW_1 as well as in LW_2 (Table 7). In the cluster LW_2b, these two markers slightly exceeded the 5 % significance threshold. The gene peroxisome proliferator activated receptor α (*PPARα*), which is part of a nuclear hormone receptor family, was mapped within the 2 Mb window around these marker positions. In Polish LR and Pietrain, it has been shown that the expression of *PPARα* is significantly higher in endometrial tissue at early stage of pregnancy than during the estrous cycle [36]. Gene expression was lower at day 10-12 and 22-30 of pregnancy when the maternal recognition of pregnancy and the end of the implantation of the fetus in the endometrium take place. The study concluded that *PPARα* is involved in these two important events. A second gene (Fibulin-1, *Fbln1*), involved in building blood vessel walls, is located at 1.07-1.16 Mb on SSC5 (Table 7). The importance of this gene was illustrated by a perinatal mortality of mice

with homozygous knock-out phenotype [37]. Vezatin (*VEZT*) was located at 92.2 to 92.3 Mb which was next to the found marker at 91.5 Mb on SSC5 when analyzing cluster LW_2a. The physiological role of *VEZT* has not been established in pigs, but Hyenne et al. [38] reported a function of *VEZT* during preimplantation of mice embryos. They inhibited the expression of this gene and found developmentally arrested embryos with limited cell-cell interactions which failed to form a young blastocyst. This finding underlines the potential importance of *VEZT* for maternal reproduction.

In cluster LR_3, one chromosome-wide significant marker (CASI0006750) was found at 115.5 Mb on SSC7 with a MAF of 2 %. Fibronectin leucine-rich repeat transmembrane protein (*Flrt2*) was mapped close to this marker (114.35-114.36 Mb) (Table 7) which is involved in the embryonic development of the heart. Mice homozygous null embryos were developmentally arrested and died at mid-gestation caused by cardiac insufficiency [39].

At position 14.8 Mb the marker MARC0070952 was found on SSC9 in LR_1 and in LR_2, but in LR_2 the marker exceeds the chromosome-wide 5 % p-value threshold only by a small amount (p = 5,5 %). In pigs, Onteru et al. [9] detected one QTL affecting TNB in this region (Table 7). Up to the present, no genes with an influence on reproduction in pigs have been identified in this chromosome region. A second detected marker on SSC9 was found in the overlapping clusters LW_2 and LW_2a (ALGA0055303, 139.0 Mb) with a genome-wide significance in LW_2a although the MAF was below 1 %. In a previous study, QTL for corpus luteum number have been detected in this chromosome region of SSC9 [35]. Additionally, prostaglandin-endoperixode synthase 2 (*PTGS2*, also known as cyclooxygenase II), was mapped in this area of SSC9 (140.2-140.3 Mb) (Table 7). *PTSG2*-null mice showed defects in the mentioned reproduction traits [40,41], e.g. implantation failure [41]. Ashworth et al. [42] investigated the role of *PTSG2* in the estrous cycle and early pregnancy of pigs. They concluded that this gene has an impact on placental attachment and embryo survival in pigs. An early estrogen exposure at the beginning of the pig's pregnancy leads to an altered *PTSG2* expression. This could be one of the reasons for a total embryonic loss during implantation due to endocrine disruption of pregnancy [42]. Additionally, it has been shown that *PTGS2* is important for the regulation of ovulation and fertilization which determine the number of preimplanted embryos [41,43,44] and therefore influences litter size in pigs. Phospholipase A_2 group 4A (*PLA_2G4A*) is required for a normal *PTGS2* induction [45,46]. *PLA_2G4A* is also mapped in the chromosome region of the significant associated marker, which was found in LW_2 and LW_2a on SSC9 (140.4-140.6 Mb) (Table 7). Knocking out

this gene leads to reduced litter sizes in mice caused by defects during implantation [47-51]. Kurusu et al. [52] also found a significantly reduced number of oocytes and preimplanted embryos in *PLA$_2$G4A*$^{-/-}$ mice in comparison to *PLA$_2$G4A*$^{+/+}$ mice leading to a reduction in litter size.

The SNP ASGA0046811 at position 18.2 Mb on SSC10 was significantly associated with NBA in LW_2a. The gene AT hook containing transcription factor 1 (*AHCTF1* also known as *ELYS*), was mapped close to this marker (17.3-17.4 Mb) (Table 7). The function of this gene in pigs is not clarified yet. Okita et al. [53] showed that *AHCTF1* deficient mice with a homozygous genotype for this mutation died after implantation phase. They observed impaired proliferation of the inner cells of the embryos and concluded that this gene is an important factor for the proliferation and survival of the inner cells and thus for the survival of the mouse embryo [53]. SNP MARC0070030 mapped on SSC10 at 32.5MB was found in LW_1 and LW_2, but had a MAF below 1 % in both sub-populations. This marker is located in a previously described QTL for corpus luteum number [35] which is one of the main factors influencing NBA [54]. In the upstream chromosome region of SSC10, the SNPs DRGA0010601 and MARC0043480 (63.8 Mb) were associated with NBA in LW_2b. QTL affecting ovulation rate and plasma follicle-stimulating hormone (*FSH*) concentration were detected within that chromosome region in previous studies [35,55] (Table 7). In addition, integrin β 1 (*ITGβ1*) was mapped close to these markers (61.4-61.5 Mb). It has been shown that the G allele of *ITGβ1* has an effect on litter size in LW and LR [56]. Cathepsin L1 (*CTSL1,* at 76.9-77.0 Mb) is located close to the significant marker which was identified at position 76.8 Mb and was found to be associated with NBA in LW_2a. In pigs, this gene has the function of regulating the transport of macromolecules between mother and embryo. This is essential for the nutrition and development and thus the survival of the embryo [57].

On the p-arm of SSC11 one marker was found to be associated with NBA in LW_2. This is the first time that a QTL for NBA has been reported in this region. The chromosome-wide significant SNP H3GA0030985 was found at position 3.7 Mb in LR_1. The FMS-like tyrosine kinase 1 (*Flt1*) gene, which is one of the two receptors for vascular endothelial growth factor (*VEGF*)-A [58], was mapped at 5.3-5.5 Mb. It has a major impact on embryonic vascular development and on the cyclic blood vessel proliferation in the female reproduction tract [59]. An adequate vascular development is a key factor for the fetal-maternal exchange of nutrients, gases and wastes [60]. It has been shown that a targeted change of *VEGF-A* in mice leads to embryonic death [61,62]. Fong et al. [63] found that the gene *Flt1* has an

essential function in embryonic vasculature. This was underlined by the fact that mutant mice homozygous in the *Flt1* locus did not survive the embryonic stage. Death was caused by abnormal vascular channels which these mutant embryos had developed. Furthermore, Ferrara [59] suggested that *Flt1* appears as a "decoy" receptor for *VEGF-A* agonist during embryogenesis. In LW_2a, one marker was found on the q-arm of SSC11 in the QTL region which was reported to be responsible for the number of stillborn piglets in LW and French LR populations by Tribout et al. [64].

The SNP ASGA0072103, located on SSC 16, had chromosome-wide significance in LR_3 and LR_3b. Tribout et al. [64] detected a QTL affecting NBA at this position in LW and French LR populations.

In the same study, a QTL for NBA was found on SSC18 [64]. This supports the findings of the present study. We detected a SNP with genome-wide significance at position 47.3 Mb on SSC18 with a MAF of 0.6 %. The results reported by Tribout et al. [6464] and our own findings indicate that this chromosome region may have an impact on NBA in Large White populations.

Conclusion

A distinct genetic stratification between different pig breeds and pig sub-populations was detected in our data set. This might be characteristic for commercial pig populations from competing pig breeding organisation with different breeding goals.

In summary, we found 17 different SNPs in the various sub-clusters. Five of the SNPs had a low MAF (<1 %). Taking into account the long selection history for fertility traits and the low heritability of NBA, this result was to be expected. Most of the significant SNPs were detected in chromosome regions where candidate genes or QTL affecting litter size had been mapped in previous studies. Against this background, the removal of SNPs with a low MAF jeopardises the potential for genetic progress in genomic selection programs. Because of the low MAF of many QTL, the probability of finding many SNPs which act as QTL across breeds or sub-clusters was low. This assumption was supported by the low number of across sub-cluster QTL in our study. It appears that in each sub-population litter size is influenced by different alleles. Because there are no such overlapping QTL regions, it is questionable if the combination of genetically divergent sub-populations is a useful strategy for detecting relevant QTL or improving the accuracy of genomic selection.

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Table 5: Number of genotyped animals

	Landrace			**Large White**		
Country	**N**	**Boar**	**Sow**	**N**	**Boar**	**Sow**
Germany	**1288**	925	363	**1146**	790	356
Austria	**266**	141	125	**191**	148	43
Switzerland	**93**	93	-	**1028**	497	531
Σ	**1647**	1159	488	**2365**	1435	930

Table 6: Dataset and results of association analyses

	Dataset		**Association analyses***				
Data set	**N animal**	**N marker**	**PC**	**λ**	**Chromosome (Genome)-wide significant SNPs**	σ^2_{y*} **(%)**	**MAF**
LW_1	2272	39408	372	1.004	3 (0)	0.7-0.9	0.8-21.1
LW_2	1719	43216	256	1.005	5 (0)	1.1-1.4	0.5-22.5
LW_2a	738	45242	74	1.002	4 (3)	2.4-4.6	0.6-21.2
LW_2b	938	45303	151	1.004	2 (0)	1.8-2.1	16.3-17.6
LW_3	553	43549	109	1.004	0 (0)	-	-
LR_1	1598	42721	293	1.004	2 (0)	1.1-1.3	31.4-39.4
LR_2	1144	46066	185	1.001	0 (0)	0	0
LR_3	454	42205	76	1.009	2 (0)	4.2-4.8	1.2-3.7
LR_3a	206	43416	26	1.015	0 (0)	-	-
LR_3b	248	44013	22	1.009	1 (0)	8.0	2.2

*= Numbers of chromosome-wide and genome-wide significant associated SNPs with NBA (p>0.05 %); PC = number of principal components; λ = inflation factor; MAF = minor allele frequency; σ^2_{y*} = Variance of the pre-corrected EBVs

Table 7: Results of annotation for all analyses with previously reported candidate genes, QTL or association in SNP region

SSC	SNP	Position (Mbp)	Genes in SNP Region*	Previously reported QTL or Associations in	Cluster
3	ALGA0018160	27925965	-	NBA, CLN	LW_2a
5	ASGA0023685	876762	*PPARα, Fbln1*	NSB	LW_1,LW_2
5	MARC0103593	961240	*PPARα, Fbln1*	NSB	LW_1,LW_2
5	MARC0104982	91550413	*VEZT*	-	LW_2a
7	CASI0006750	115511369	*FLRT2*	-	LR_3
9	MARC0070952	14861213	-	TNB	LR_2
9	ALGA0055303	139041276	*PTGS2,PLA2G4A*	CLN	LW_2,LW_2a
10	ASGA0046811	18203672	*AHCTF1*	-	LW_2a
10	MARC0070030	32526661	-	CLN	LW_1,LW_2
10	MARC0043480	63867699	*ITGβ1*	CLN, FSH	LW_2b
10	DRGA0010601	63869377	*ITGβ1*	CLN, FSH	LW_2b
10	ASGA0090608	76815569	*CTSL*	-	LW_2a
11	H3GA0030853	82720	-	-	LW_2
11	H3GA0030985	3733271	*FLT1*	-	LR_1
11	MARC0006510	74240078	-	NSB	LW_2a
16	ASGA0072103	6470509	-	NBA	LR_3,LR_3b
18	ASGA0079878	47312409	-	NBA	LW_2a

SSC= Sus scrofa; TNB = total number born; NBA = number born alive, NSB = number of stillborn piglets; CLN = corpus luteum number, FSH = follicle-stimulating hormone, *AHCTF1* = AT hook containing transcription factor 1; * The declaration of gene symbols can be obtained from Ensembl or http://www.ncbi.nlm.nih.gov/gene; ** The QTL information was obtained using http://www.animalgenome.org/cgi-bin/gbrowse/pig/

Table 8: Statistic of significant SNPs in LW

SNP	**SSC**	**N[A]**	**MAF[B]**	**Var[C]**	**p-value[D]**	**Cluster**
ALGA0018160	3	738	2.0	3.4	0.001**	LW_2a
ASGA0023685	5	2272	20.9	0.8	0.02	LW_1
	5	1719	22.3	1.2	0.01	LW_2
MARC0103593	5	2272	21.1	0.8	0.05	LW_1
	5	1719	22.5	1.1	0.03	LW _2
MARC0104982	5	738	0.6	2.5	0.03	LW _2a
ALGA0055303	9	1719	0.6	1.3	0.004	LW _2
	9	738	0.6	4.6	<0.001**	LW _2a
ASGA0046811	10	738	21.3	2.6	0.02	LW _2a
MARC0070030	10	2272	0.8	0.9	0.007	LW _1
	10	1719	0.8	1.1	0.01	LW _2
MARC0043480	10	938	16.3	2.2	0.01	LW _2b
DRGA0010601	10	938	17.6	1.9	0.04	LW _2b
ASGA0090608	10	738	5.7	2.4	0.05	LW _2a
H3GA0030853	11	1718	1.9	1.1	0.03	LW _2
MARC0006510	11	738	0.6	3.0	<0.001**	LW _2a
ASGA0079878	18	738	0.6	3.5	0.0004**	LW _2a

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] σ^2_{y*} = Variance of the pre-corrected EBVs (Var, %), [D] nominal p-value and corresponding significant thresholds: **genome-wide significant ($p_{gem} < 0.05$)

Table 9: Statistic of significant SNPs in LR

SNP	**SSC**	**N[A]**	**MAF[B]**	**Var[C]**	**p-value[D]**	**Cluster**
CASI0006750	7	454	3.7	4.2	0.04	LR_3
MARC0070952	9	1598	31.3	1.3	0.01	LR _1
H3GA0030985	11	1598	39.4	1.1	0.05	LR _1
ASGA0072103	16	454	1.2	4.8	0.005	LR _3
	16	248	2.2	8.0	0.01	LR _3b

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] σ^2_{y*} = Variance of the pre-corrected EBVs (Var, %), [D] nominal p-value and corresponding significant thresholds

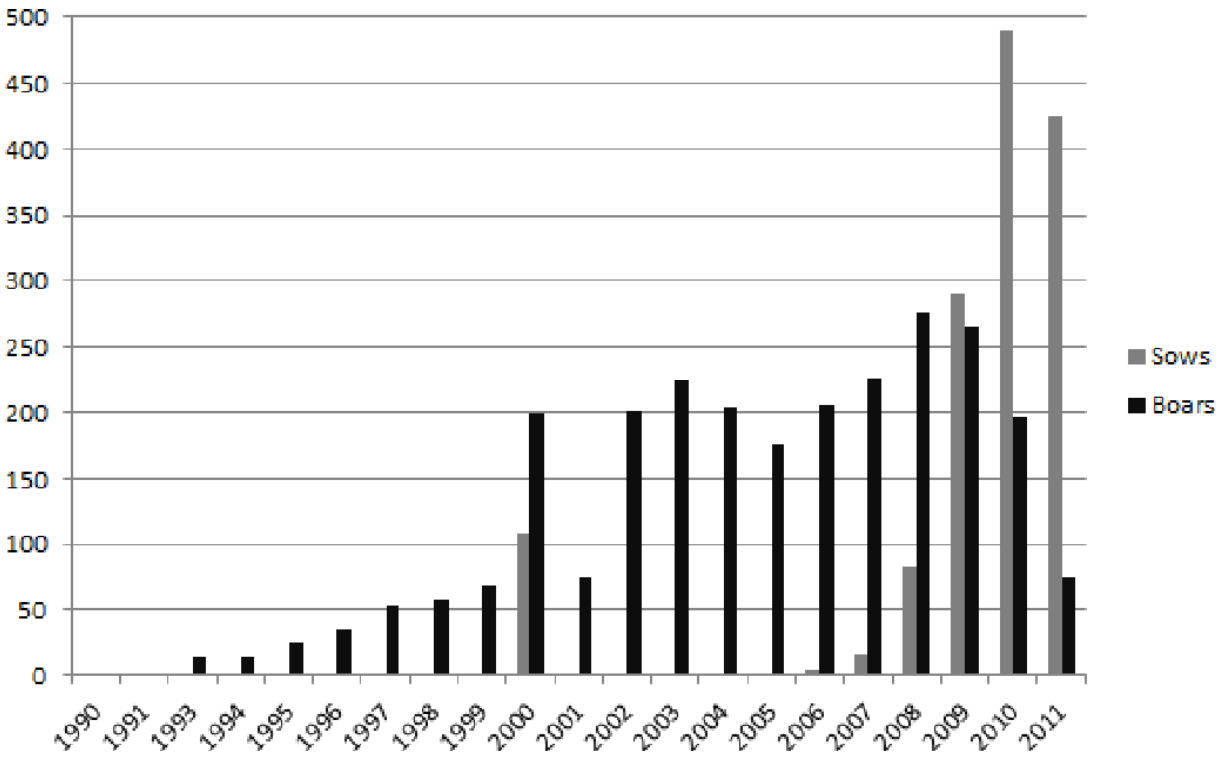

Figure 17: Frequencies of years of birth from all animals by gender

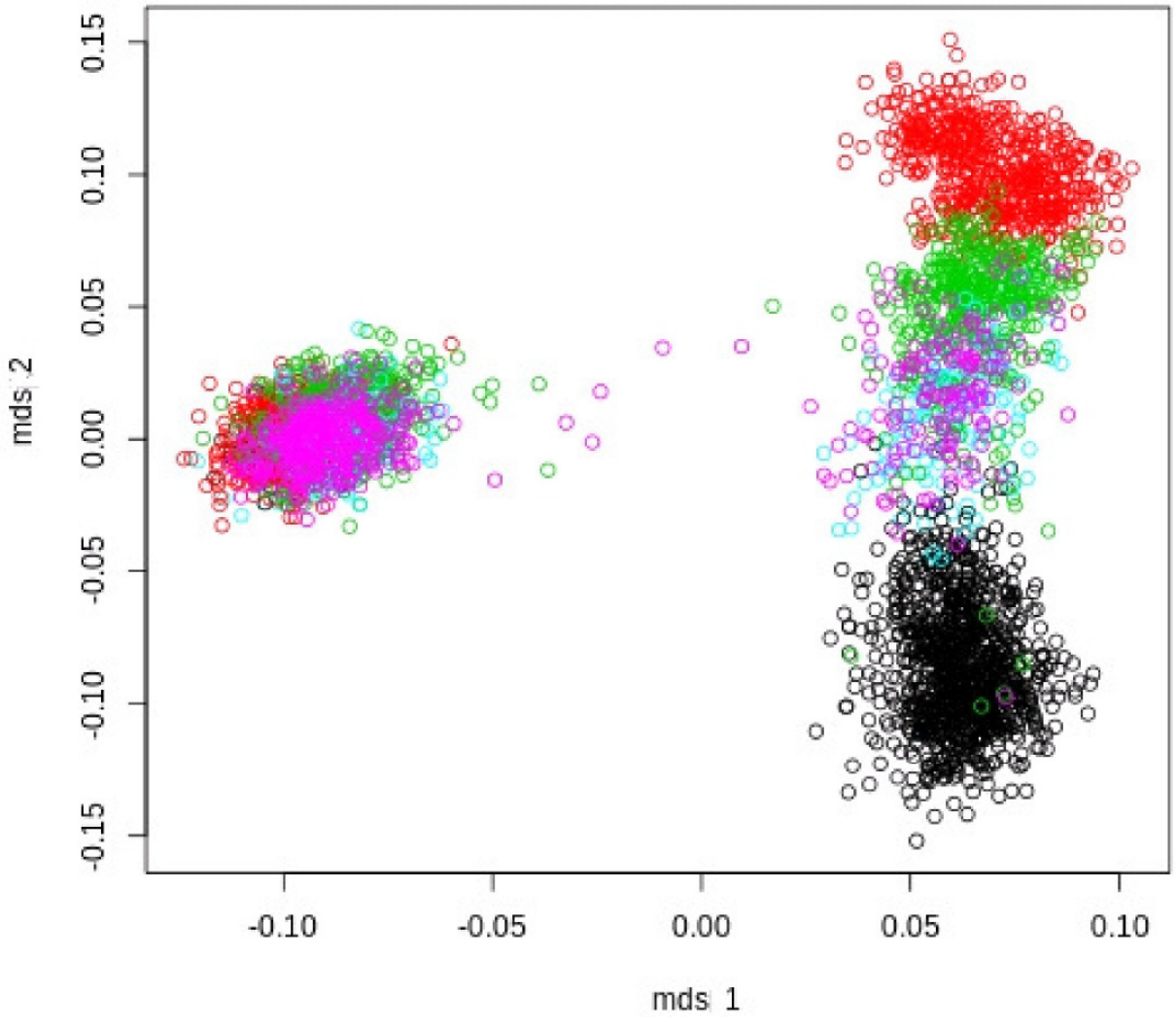

Figure 18: MDS Plot of Landrace (left) and Large White (right) populations of 5 European breeding companies

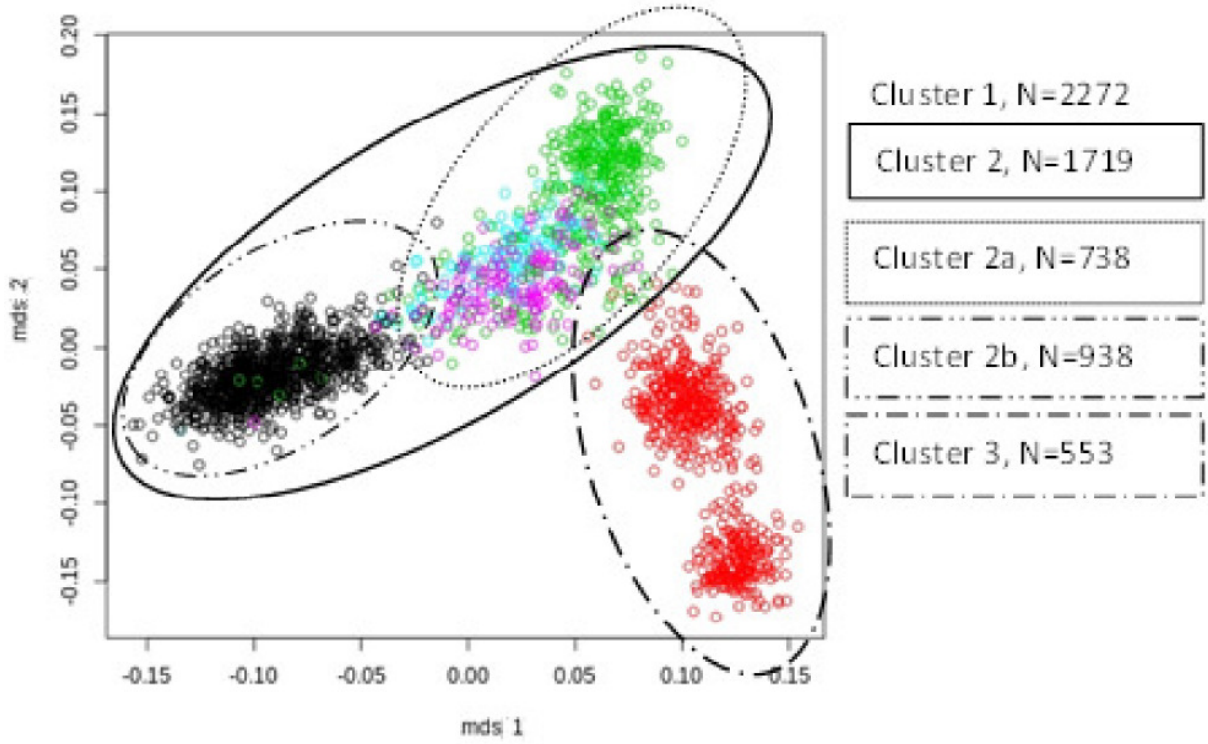

Figure 19: MDS plot of Large White population, each colour represents one breeding company, circles show two different clusters

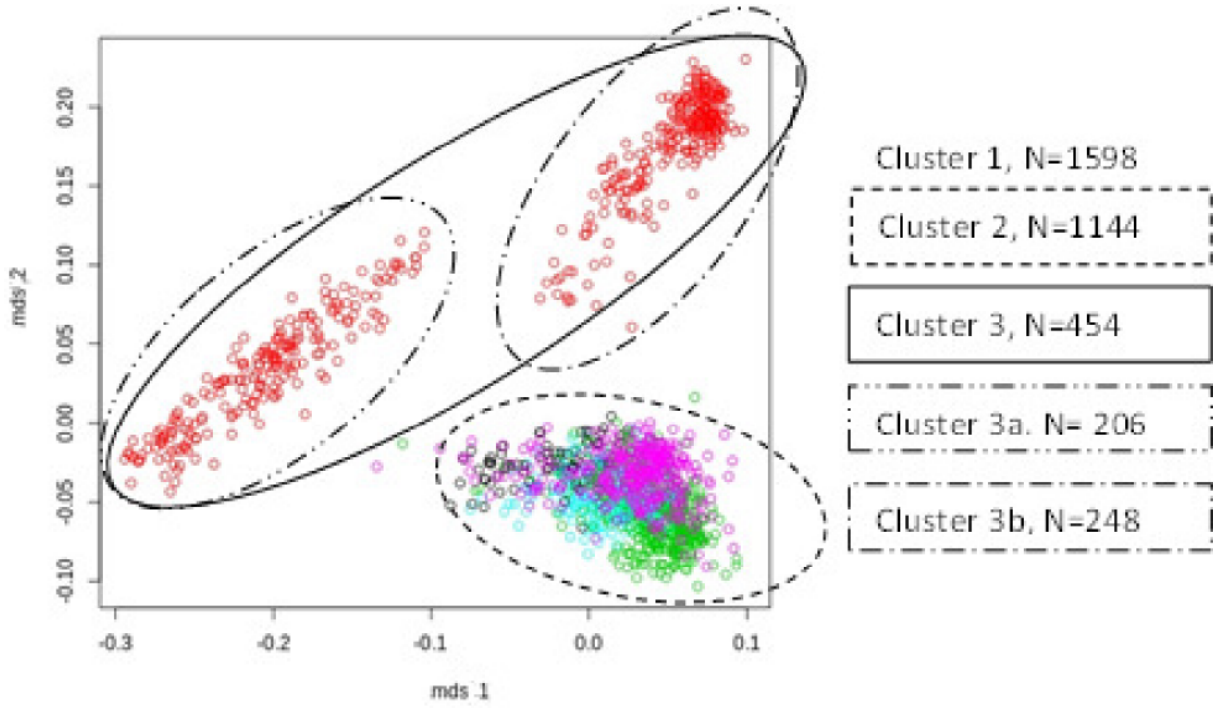

Figure 20: MDS Plot of Landrace population of 5 European breeding companies, circles indicate different clusters

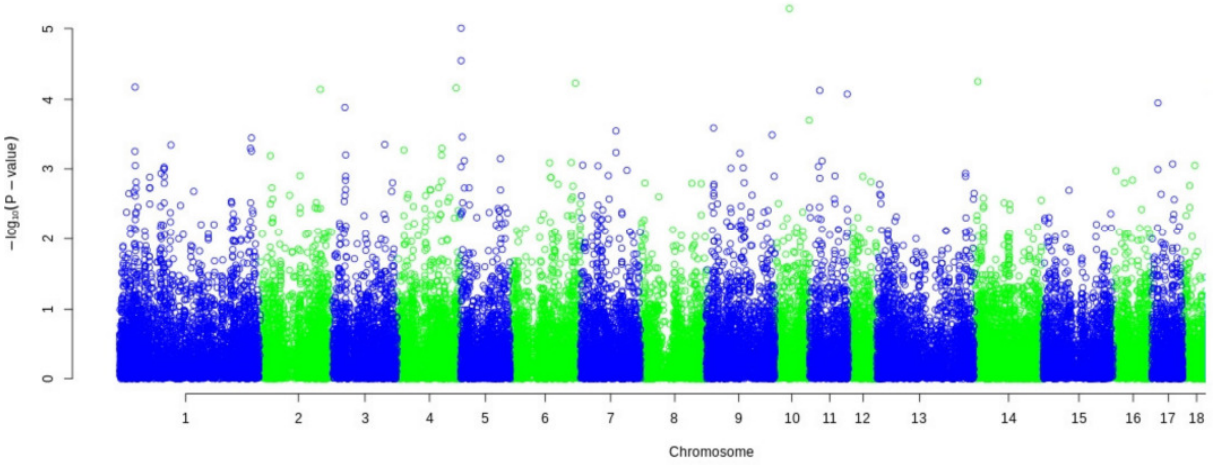

Figure 21: Manhattan plot of Genome-Wide Association Study for NBA in LW_1

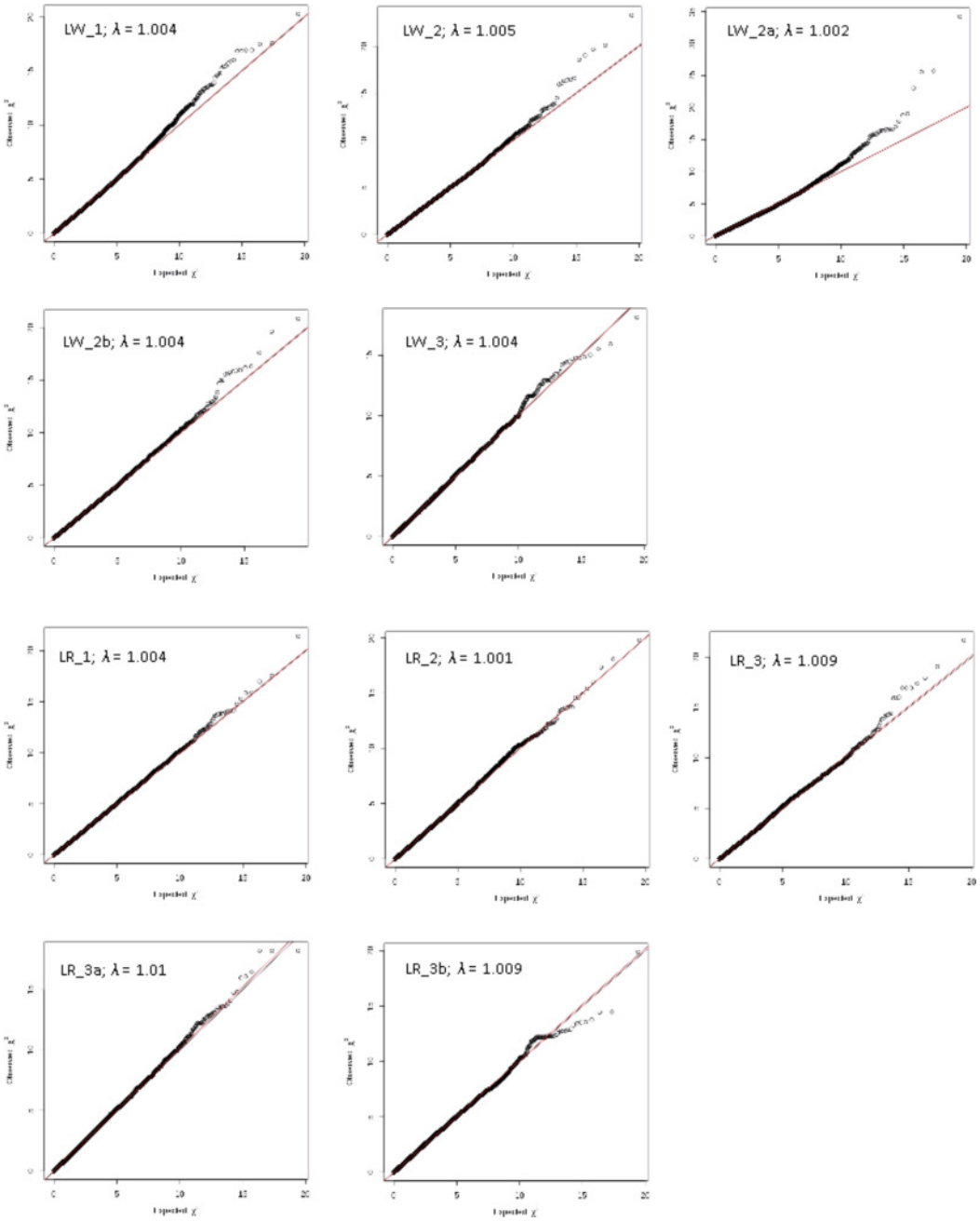

Figure 22: Q-Q plots of all association studies for all breed clusters

3 CHAPTER 3: GENOME-WIDE ASSOCIATION STUDY IN LARGE WHITE AND LANDRACE POPULATIONS REVEALING PLEIOTROPIC GENOMIC REGIONS FOR REPRODUCTION AND PRODUCTION TRAITS

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3.1 Abstract

Production traits like average daily gain (ADG), lean meat percentage (LMP) or backfat (BF) and number of piglets born alive per litter (NBA) are economically essential traits for pig meat production. In recent years, selection programmes of most pig breeding companies were focused on improving these trait complexes using efficient breeding tools like BLUP and genomic selection. In order to achieve sufficient genetic progress and to avoid undesirable genetic side effects, knowledge about the biological function and genetic relationships between all target traits should be improved. Against this background, the objective of our study was to clarify the genetic background of NBA, ADG, LMP and BF and to identify possible pleiotropic effects. Genome-wide association studies (GWAS) were performed using 3,496 Large White and Landrace pigs from herdbook and commercial breeding companies in Germany (2), Austria (1) and Switzerland (1). Illumina PorcineSNP60 BeadChip was used for animal genotyping. In a first step, data sets of each breeding organization were analysed separately with singe-trait analyses. Secondly, data of breeding organizations were combined and analysed. Because of population stratifications within and between breeds, clusters were formed using the genetic distances between the populations. In the third step, principal component analyses (PCA) were used which resulted in a number of principal components (PCs) reflecting phenotypic variance and covariance of all traits to test for pleiotropic effects. These PCs were used as phenotype in a univariate GWAS to verify the biological and physiological relationship between reproduction and production traits. In total, 71 chromosome-wide and four genome-wide significant markers affecting analysed traits were found in both breeds. Only one significant chromosome area for both breeds was detected on SSC12 affecting NBA and ADG. Four SNPs were found in more than one cluster. On SSC8 pleiotropic effect was detected for LPM and BF. Moreover, pleiotropic effects were found when significant SNPs were compared to previousl QTL detection by other studies.

Keywords: Pig; reproduction; production; Pleiotropy; GWAS

3.2 Introduction

In the last decades, strong selection pressure was mainly on reproduction and production traits in pig breeding. In comparison to reproduction traits, genetic improvements of production performance can be achieved faster because of high heritability of these traits. With respect to balanced breeding goals, the relationships between litter size and production traits are of particular interest. Comparing the results of several studies (Crump et al., 1997; Hermesch et al., 2000b; Imboonta et al., 2007; Merks and Molendijk, 1995; Noguera et al., 2002a; Rydhmer et al., 1992; Short et al., 1994; Tholen et al., 1996), the relationships between number of piglets born alive (NBA) and average daily gain (ADG) as well as NBA and carcass composition traits like lean content (LMP) or backfat (BF) were estimated in a wide range with partly conflicting signs (-0.42 to 0.23). In contrast to these heterogeneous results, the estimated genetic relationships between birth weight of the piglets (BW) are mostly favorable relative to production traits (Berard et al., 2008; Gondret et al., 2006; Gondret et al., 2005; Nissen et al., 2004; Poore and Fowden, 2004; Quiniou et al., 2002; Rehfeldt and Kuhn, 2006; Rehfeldt et al., 2008; Smith et al., 2007; Wolter et al., 2002), and consistently unfavorable regarding NBA (Beaulieu et al., 2010; Kerr and Cameron, 1995; Quiniou et al., 2002; Roehe, 1999b; Smit et al., 2013). From this follows, that there is at least a high risk of conflicting relationship between NBA and ADG. Furthermore this hypothesis is supported from a physiological point of view. Increased leanness in sows induces decreased reproduction performance due to deterioration in mobilizing body´s lipid resources (Johansson and Kennedy, 1983b; Kersey De Niese et al., 1983). Low birth weight induced by increased litter sizes is associated with lower postnatal growth rates and decreased piglet survival (Herpin et al., 2002; Milligan et al., 2002; Quiniou et al., 2002). These light weight piglets showed higher fat content and lower lean accretion in carcass compared to heavier littermates (Bee, 2004; Gondret et al., 2006; Kuhn et al., 2002; Powell and Aberle, 1980; Rehfeldt and Kuhn, 2006).

In general, information about possible pleiotropic effects for reproduction and production traits is limited. For genes like retinol binding protein 4 (*RBP4*) and insulin growth factor 2 (*IGF2*) influence on reproduction as well as on production traits has been described (Cheng et al., 2013; Munoz et al., 2010; Stinckens et al., 2009; Wang et al., 2006a). But more detailed information is necessary for an optimal combination of production and reproduction traits in selection programmes in order to avoid indirect negative effects.

In this study, genotype and phenotype information of 3,496 Large White (LW) and Landrace (LR) animals from four different European herdbook and breeding organizations were used to perform Genome-Wide Association Studies (GWAS) to identify possible pleiotropic effects between NBA and production traits. Therefore, single trait GWAS within and across breeding populations owned by several organizations were performed. Furthermore, multivariate analyses using principal component reflecting variance covariance structure for all analyzed traits, were performed to confirm overlapping effects from single trait analysis and find possible new pleiotropic effects. This method was recommended to use for multitrait detection of pleiotropic effects (Weller et al., 1996) which increased the power of QTL detection and accuracy (Gilbert and Le Roy, 2003).

3.3 Material and Methods

3.3.1 Animals and phenotype data

3,496 LW and LR pigs from commercial breeding companies and herdbook organizations in Germany (2), Austria (1) and Switzerland (1) were included in the study. Data set consisted of 2,202 (boars: 1,272, sows: 930) LW and 1,294 (boars: 806, sows: 488) LR animals born between 2002 and 2011.

Phenotypes used for GWAS were estimated breeding values (EBVs) for number of piglets born alive per litter (NBA), lean meat percentage (LMP), backfat (BF) and average daily gain during test period (ADG). Breeding values for BF was not available from every breeding organization (Table 10). Moreover, phenotyping for these traits, especially for LMP, varied between breeding organizations. This trait was recorded with AutoFOM (Org_1), FOM (Org_2), calculated with the “bonner” formula (Org_3) or from valuable parts from half carcasses (Org_4). EBVs were routinely estimated by the breeding organizations using a standard animal model including organisation specific fixed effects.

3.3.2 Genotype data and SNP quality control

The Illumina PorcineSNP60 Bead Chip (Ramos et al., 2009) was used to genotype tissue samples of the pigs in the laboratory Life & Brain GmbH, Bonn.

Following quality standards were used in quality check:

a) Minor allele frequency (MAF) < 0.01 %,

b) Call rate < 95 % and

c) Strong deviation from the Hardy-Weinberg-Equilibrium ($p < 10^{-3}$)

d) SNPs on sex chromosomes.

SNPs which did not pass the quality check were excluded from further analysis.

Quality control was performed as implemented in the GenABEL package (Aulchenko et al., 2007b) within defined population clusters and breeding organization cluster.

3.3.3 Population structure

Genetic distances between populations were visualized using multidimensional scaling (MDS) (Figure 18-Figure 20). These plots were used to define within and across population cluster. In a first step, each population of an organization was analysed separately. Because of genetic distances, the LR population of breeding organisation 2 was further divided into two sub-populations. These sub-populations were probably the result of migration of breeding animals in the recent past.

In a next step, within the LW and LR populations, five across organisation clusters were defined, containing animals from two to five breeding companies.

3.3.4 Genome-Wide Association Study

Using the data of the within and across organisation cluster, several Genome-Wide Association Studies (GWAS) for the estimated breeding values NBA, LMP, BF and ADG as dependent variables were realized. In addition, the information content of all breeding values was condensed into principal components (PCs). The corresponding multivariate PC procedure is an unsupervised method which condenses the EBVs into a set of representative, uncorrelated PCs by means of their variance covariance structure. Only PCs with loadings larger than |0.2| were considered for further analysis and interpretation. The relevance of each

EBV within a PC was quantified by their corresponding loading. The absolute values of these loadings were used to label the PC roughly according to their biological composition.

In order to control existing population stratification, a combined approach using the "Genome-wide Rapid Analysis using Mixed Models and Regression" (GRAMMAR) (Amin et al., 2007) combined with EIGENSTRAT (Price et al., 2006) was used for GWAS as implemented in the R-Package GenABEL (Amin et al., 2007; Aulchenko et al., 2007a; Aulchenko et al., 2007b).

In a first step, each phenotype (breeding value) was corrected for the fixed effect "breeding organization" and a polygenetic effect, separately. The random additive polygenic $(a_i \sim N(0, G \times \sigma^2_a))$ effect estimates the contribution from the polygene (breeding value) with G as the genomic kinship matrix and the additive genetic variance σ^2_a.

The genomic kinship (G_{ij}) was estimated by applying the method suggested by Astle & Balding (2009):

$$G_{ij} = \frac{1}{L}\sum_{l=1}^{L}\frac{(g_{l,i} - p_l)(g_{l,j} - p_l)}{p_l(1-p_l)} \qquad (1)$$

with L as the number of SNP, p_l as the allelic frequency at l-th locus (major allele) and $g_{l,j}$ / $g_{l,i}$ as the genotype of j-th / i-th individual at the l-th locus, coded as 0, 1/2 and 1, corresponding to the rare homozygous, heterozygous, and common homozygous genotype.

Ignoring the covariance between animals from one family can lead to a high number of false-positive SNPs. The residuals computed with GRAMMAR are corrected for polygenic relationships between the animals and can be used as a new phenotype in association analyses (Amin et al., 2007; Aulchenko et al., 2007a).

In a second step, these familial correlation-free residuals were included in a simple linear regression as new phenotype for association test. The phenotype for GWAS consisted of the pre-corrected EBVs from first step.

In order to verify remaining population stratification, the inflation factor λ, which depends on the squared original test statistic of the *i-th* SNP (T_i^2) was calculated as

$$\lambda = \frac{Median\,(T_i^2)}{0.4549}.$$

Aulchenko et al. (2007b) and Price et al. (2010) showed that an inflation factor λ in the range of 1.0 to 1.05 is an indicator of a sufficiently corrected population stratification which can be analysed with an acceptable risk of false-positive results. Preliminary results of our analysis showed that λ deviates considerably from this optimum. This implies that serious population stratifications still exist.

To correct for this existing population stratification PCs estimated from the genomic kinship were included in the model for association test as covariables (Aulchenko et al., 2007b; Price et al., 2006). With this method phenotype and genotype were adjusted for population stratification. The function "egscore" as implemented in the R-package GenABEL (Aulchenko et al., 2007b) was used to estimate PC and for the performance of association analyses. The number of necessary PC depends on the power to correct for cluster-specific population stratification and is listed in SI 23 and 24. The final number of used PC were chosen so that the inflation factor λ (Devlin and Roeder, 1999) was closest to 1. The quantile-quantile (Q-Q) plots illustrate the inflation factor and the observed (calculated) versus the expected (optimum, $\lambda = 1$) p-value for each SNP displayed by two regression lines. When population stratification was corrected sufficiently, no differences between the two lines should be visible.

The p-values of the SNP significance test were corrected using Bonferroni-adjustment to reduce the risk of false-positive associations. A threshold of 5 % was chosen for genome-wide and chromosome-wide significance.

Variance of the pre-corrected EBVs (σ^2_{y*}) explained by each SNP was calculated approximately using following formula:

$$r^2 = \frac{\chi^2_{1df}}{N - 2 + \chi^2_{1df}} \qquad (3)$$

with χ^2_{1df} as the test statistic for each SNP resulted from association test and N as the number of animals. This formula resulted from the transformation of a student's t-distribution into a z-distribution (Stuart and Ord, 2009). In our analysis, r^2 cannot be interpreted as the proportion of explained phenotypic variance of the traits – as is usually the case – because pre-corrected EBVs were analyzed instead of phenotypes.

For annotation of associated SNPs, Pig Sscrofa 10.2 (International Swine Genome Sequencing Consortium) (Archibald et al., 2010) was used. Ensembl BioMart (Flicek et al.,

2014; Kinsella et al., 2011) was used for the search of biologically relevant genes within a 2 Mb window around the significant region.

3.3.5 Analysis of pleiotropy

In order to detect possible pleiotropic effects between NBA and production traits PCA were performed. This is a commonly used method to reduce the dimensionality of data sets to a lower number of uncorrelated PCs. As proposed by Weller et al. (1996), this condensation step was performed from the phenotypic covariance matrix which was considered as the residual covariance matrix of the underlying data set. The transformation of *n* traits led to *p* phenotypically independent variables conducted from the components of the eigenvectors of the phenotypic covariance matrix. Each eigenvalue represents the part of phenotypic variability explained by the associated principal component variable (Gilbert and Le Roy, 2003).

Beside the condensation of independent variables, the PCA method was recommended to use for multitrait detection of pleiotropic effects. Weller et al. (1996) showed that the use of PCs increased the power of QTL detection and the accuracy (Gilbert and Le Roy, 2003). Because of this capability, PCs were used as dependent variables in our GWAS. Moreover, the composition of each PC was described by the loadings of the underlying EBVs, which are functions of the corresponding eigenvalues.

3.4 Results

3.4.1 Population structure

Genetic distances were visualized using MDS plots (Figure 23 - Figure 25) with each color representing animals of one specific breeding organization. Because of the large genetic distance between LW and LR population, these breeds were regarded as genetically disconnected. In addition, distinct genetic dissimilarities can be observed regarding the subpopulations of different breeding organizations. According to the overlapping parts in the MDS plots, three and two across organization clusters were defined in the LW and LR

population, respectively. In summary 10 intra- and five across- breed × organization clusters were defined.

3.4.2 Quality control

SNP quality control was performed within each cluster using the SNP information of the autosomes. Within breeding organizations only a few (five) animals fall below the threshold of at least 95% of valid SNP information (call rate animal). Because of the low number of animals (53) GWAS in cluster Org1_LR was not performed. However, these pigs were integrated in corresponding across organization cluster.

Minimum call rate of SNP marker was set to 95 %. The quantity of remaining genetic markers within cluster ranged from 37,616 to 45,300 in the LW and from 38,232 to 45,900 in the LR clusters (SI 32, SI 33 and SI 34).

3.4.3 Genome-Wide Association analyses

Possible population stratification has to be taken into account to ensure the power and accuracy of GWAS (Aulchenko et al., 2007b; Bouaziz et al., 2011; Pausch et al., 2012). Therefore, a varying number of PCs, depending on analyzed clusters and traits, were included in the statistical model of the association tests as covariates to avoid negative effects of population stratification. In this case, PC condensed the genetic relationships between the animals and corrected the phenotype as well as the genotype for existing population stratification.

The number of PC used in the GWAS of defined clusters ranged from 16 (Org3_LW) to 132 (Org1_LW) within the breeding organizations (SI 32) and from 55 (LW_2a) to 338 (LW_1) across the breeding organizations (SI 33). Number of PC were chosen so that the genomic inflation factor λ was close to one in all analyses, so that possibly existing populations stratifications did not adversely affect the validity of corresponding GWAS analysis.

Cluster specific Q-Q plots (Figure 26, SI 10-SI 31) illustrate the expected test statistics (independent variable) on observed test statistic (dependent variable) as regression lines. The slopes of these lines are in accordance to the calculated cluster specific inflation factor. Furthermore, Manhattan plots illustrate the p-value of the SNP association test for the target

trait according to the genomic positions. For instance, Manhattan plot for NBA from Org1_LW is given in Figure 26. All other Manhattan and resulting Q-Q plots are given as Supplementary Information (SI 10-SI 31).

Single Trait GWAS

For the breeds LW and LR, 28 (three) and 51 (one) chromosome-wide (genome-wide) significant SNPs were found for all traits when analysing all clusters, respectively. Regarding the two breeds similar numbers of significant SNPs for all traits were found (Table 11). If data sets from different breeding organisations were combined, relative to the within breed ×organisation analysis a lower number of SNPs were detected. Regarding the increased number of observation of the across breed × organisation analysis this result was unexpected. However, all genome-wide significant SNPs for BF (three) were found in across organisation cluster.

Comparing the results of both breeds or the results of different breed × organisation clusters, only four overlapping significant SNPs for one trait were found. Moreover, on SSC12, 16 and 18 SNPs for two different traits were detected within the same region in both breeds. All SNPs for NBA, ADG, LMP and BF found in univariate analysis are illustrated in Figure 27 and Table 13-Table 16. Ordered by trait, more details of all significant SNPs are given in the next sections.

NBA

In the LW breed, seven and three chromosome-wide significant SNPs were detected for NBA when analysing within or across organisation data sets. These QTLs are located on SSC5, 9, 10, 12, and 17 (Table 13). An interesting region was found for Org3_LW on SSC10 where 3 SNPs were clustered within a 2 Mb window. In the analysis of the combined LW data sets none of the tree detected SNPs (SSC5, 12 and 17) explained more than 4 % of σ^2_{y*} and none of the intra organization QTLs was confirmed.

In the LR population nine SNPs on five different chromosomes (SSC2, 5, 7, 16 and 18) were found only in the analysis of the intra-organization data sets. On SSC2 in Org2a_LR a SNP cluster consisting of three SNPs was found, which explained 9.4 % of σ^2_{y*}. The significant SNP on SSC16 in cluster Org2_LR was confirmed in its sub-cluster Org2b_LR (Table 13).

ADG

Within the LW × organisation cluster, significant SNPs for ADG were only found in cluster Org3_LW. In this cluster four chromosome-wide significant associated SNPs were identified on SSC7, 12 and two closely linked marker on SSC8 (Table 14). Four SNPs for ADG were found in the analysis of the combined cluster LW_1 (SSC3), LW_2 (SSC10, 16) and LW_2a (SSC13). However, these SNPs did not explain more than 2.9 % of σ^2_{y*}.

Within the LR breed, one genome-wide significant marker was detected for ADG in cluster Org3_LR located on SSC1 at 172.7 Mb and explaining 5.5 % of σ^2_{y*} (Table 14). Worth mentioning are two regions on SSC12 and SSC8. SSC12 (39.9 Mb) harbours 3 SNPs of Org2_LR and its sub cluster Org2b_LR within a 3 Mb window. Extending this region to 10 Mb, a significant QTL was also found within the LW breed. On SSC8 (Org3_LW), a linked QTL pair was found, which explained more than 10% of σ^2_{y*}.

Carcass traits LMP and BF

Within the LW breed and using the data from one breeding organization, four and one significant SNPs for LMP and BF found on chromosomes SSC2, 8 and 18 (Table 15 and Table 16). A region on SSC2 was found in Org1_LW which contained two significant SNPs. This region was confirmed by the results of the across organization analysis for LW_2.

When analyzing the combined cluster for LMP and BF, three and 12 significant SNPs were found (Table 15 and Table 16). Of particular importance were two genome-wide significant SNPs found in LW_2, which were associated with BF. These QTLs are detected on SSC5 in a closely linked chromosome position (68.3 Mb) and explain a relative small proportion of σ^2_{y*} (1.9-2.2 %). In a 2Mb window at the distal end of SSC8 three closely linked SNPs were identified for BF using the within (Org2_LW) or across organisation LW cluster (LW_1, LW_2a). The proportion of σ^2_{y*} ranged between 3.6–3.9 %. At the proximal end of SSC9 a pair of linked SNP was found for BF in LW_2a, which was responsible for 3.6-3.7 % of σ^2_{y*}.

Three SNPs which were detected in breed × organisation specific clusters were confirmed in combined LW clusters. As it has been mentioned above, one marker located on SSC2 was significant associated with LMP in LW_2 and Org1_LW and markers on SSC8 were associated with BF in Org2_LW, LW_1 and LW_2a. Furthermore, the SNP DIAS0002693 on SSC18 was detected for LMP in LW_2 and its connected sub cluster LW_2a.

Within the LR breed and using the within or across organization data sets, for BF in each case seven and for LMP 11 and one SNPs were identified on 11 different chromosomes. Almost all intra-organisation SNPs were positioned in none overlapping regions. The only exceptions were two regions on SSC13 and SSC16, which harbours four or two closely linked SNPs for BF (Org2b_LR) or LMP (Org2_LR). Regarding the across organisation cluster analysis, one genome-wide significant marker for BF was located on SSC2 at position 149.1 Mb, which was detected in data set LR_1. All other SNPs were on different chromosomes and explained between 1.7-4.4 % of σ^2_{y*}.

Because BF and LMP are both indicators of carcass composition, similar SNPs could be expected. However, only one marker on SSC8 was associated with LMP and BF in cluster Org2a_LR.

Multivariate GWAS

It has been shown in the previous section that there are almost no overlapping genomic regions with a joint impact on different traits. This could be the result of true missing underlying pleiotropic effects and/or insufficient statistical power of the analysis. Multivariate GWAS approaches might help to increase the statistical power. In this context a PC analysis was used to rearrange the information content of all available EBVs into a set of independent PCs, which are linear functions of all traits. PC analyses were only performed within breed × organization clusters and resulting PCs were used for GWAS. Some breeding organizations did not provide EBVs for BF. Therefore, the maximum number of calculated PCs ranged between three and four. A PC analysis using clustered data of more than one breeding organization was not performed, because definition of LMP was different and EBVs for BF was missing in some breeding organizations. Statistics of significant SNPs in LW and LR are given in Table 17-Table 18 and illustrated in Figure 28.

As can be seen in Table 17 and Table 18, 39.0-52.5 % of the EBV variance (σ^2_{PC*}) can be explained by the first PC in all data sets. The contribution of the second and third PC is of lower importance but explains normally 20 % of σ^2_{PC*}. Because of the low proportion of explained variance (below 10 % of σ^2_{PC*}) PC4 was excluded from further analysis. An overview about data sets used for multivariate analyses are given in SI 34.

The general composition of a PC can be characterized by the loadings of the underlying EBVs. The values and signs of these loading are heterogeneous (Table 17 and Table 18).

Estimated canonical correlations between PC and traits showed the same tendency and signs as loadings (SI 35 and SI 36).

In order to give some rough biological idea of the PC composition, the components were classified according to the loadings of the underlying EBVs. As a general rule, EBVs which had loadings above the heuristic absolute threshold of |0.2| and the sign of the remaining loadings were used for classification. All PCs, which were significantly influenced by SNPs were classified by their loadings into the following PC-qualifier groups.

- LMP/BF-
- NBA/ADG, NBA/ADG-
- NBA/LMP, NBA/LMP-
- (ADG/LMP), ADG/LMP-
- NBA/ADG/LMP, NBA/ADG/LMP-, NBA/ADG-/LMP, (NBA/ADG-/LMP-)

A minus (-) indicator means, that the affected traits had a different sign than the other traits. The loadings of LMP and BF had always the expected signs, with the exception of the group LMP/BF-, these PC members were combined into a single PC-qualifier subgroup (LMP). Because none of the first three PCs could be assigned into the PC-qualifier groups ADG/LMP and NBA/ADG-/LMP-, these groups were dropped from the following figures and tables.

Large White

In total 23 significant SNPs were detected within LW breed when GWAS was performed using PC as phenotype (Figure 28, Table 17). Only four of these QTL were also found in the univariate analyses using a single EBV.

Within Org1_LW, five significant SNPs were found for PC3 which is mainly influenced by NBA and ADG with opposite signs (PC_qualifier NBA/ADG-). One of these SNPs on SSC9 reached a genome-wide significant level and explained 3.1 % of σ^2_{PC*}.

Eight and three cluster specific SNPs were detected for the PC-qualifier groups NBA/ADG/LMP- or NBA/ADG-/LMP. For these antagonistic acting PCs interesting regions were located on SSC8 (NBA/ADG/LMP-) and SSC4. In addition, a SNP of type NBA/ADG- or NBA/LMP on SSC7 explained more than 10 % of σ^2_{PC*}.

SNPs for PCs, which comprised EBVs with non-conflicting signs (NBA/ADG, ADG/LMP), were found in the organizations Org2_LW and Org3_LW. An interesting SNP pair for these

PCs was found on SSC2, whereas on SSC17 a single SNP was identified which was close to genome-wide significance level and explained a considerable amount of σ^2_{PC*} (11.3%).

Landrace

In total, 67 chromosome-wide and 8 genome-wide significant SNPs were detected when analyzing LR breed with PC as phenotypes (Figure 28, Table 18). Eight of these SNPs were also detected in univariate analyses. Regarding the PC-qualifier groups, most (51) QTLs were found for PCs, which comprised EBVs with conflicting signs of the loadings.

For Org4_LR the PC-qualifier group NBA/ADG-/LMP was most important. For this PC-qualifier type, 15 chromosome-wide and six genome-wide QTLs were found on 10 different chromosomes. Beside the six genome-wide QTLs, regions on SSC1 and 11 are worth mentioning because they harbor three neighboring QTLs in a 2 Mb region, respectively.

In sub cluster Org2b_LR, interesting genome regions on SSC3, 9 and 10 were found for PCs, which were mainly influenced by NBA and ADG. For group NBA/ADG, three and 12 linked SNPs were located on SSC12 and SSC3, whereas in the opposite group NBA/ADG- a cluster of nine QTLs was identified on SSC10.

PC3 in Org3_LR and Org4_LR reflects an antagonistic relationship between NBA and LMP. Interesting regions for the PC-qualifier groups NBA/LMP- (Org4_LR) and NBA/ADG/LMP- (Org4_LR) were identified on SSC10 (two SNPs) and SSC17 (one genome-wide significant) SNP) and SSC18 (three SNPs).

3.5 Discussion

3.5.1 Differences in phenotyping and EBV procedure

In this study, EBVs which were estimated routinely by the breeding organizations were used as phenotypes. Although more or less standardized methods in phenotyping and estimating breeding values were used, some differences between breeding organizations complicate the interpretation of the results.

Depending on the breeding company, ascertaining of traits was different. Some breeding organization counted NBA directly after birth, others within one or two days. Consequently, EBVs for NBA from different breeding organizations are more or less influenced by genes

which might have an impact on postnatal piglet survival. In particularly the interpretation of SNPs for LMP is problematic because marked differences in phenotyping existed. In some organizations LMP was estimated by carcass grading systems like AutoFOM and FOM. In other breeding companies LMP was calculated via regression formulae by means of linear carcass measurements ("Bonner" formula) or carcass cut weights ("Forchheimer" formula). EBVs for BF were only estimated routinely by two breeding organizations. The underlying BF phenotype was measured either directly at the surface of the splitted carcass or via ultrasound-scan measurements from living breeding animals.

Besides phenotyping, differences in the EBV estimation procedure might have an impact on the GWAS results. All breeding companies have used a standard animal model with relevant fixed effects and have treated NBA as an uncorrelated trait. However, breeding values for production traits were estimated using a multivariate model. This was performed within each breeding organization under the assumption of different heritabilities and correlations between the production traits. Moreover accuracies of the EBV might be different not only because of different genetic parameters, but also because of different testing schemes. Some breeding companies used results of station tested slaughter sibs or progenies of the selection candidates. In other organizations, traits like ADG and BF were directly recorded on living selection candidates. These records were augmented by carcass information from culled relatives with inferior performance in ADG and BF. The culling process results into a skewed distribution of ADG and BF.

Because of these considerable deviations in phenotyping and in the EBV estimation procedure, EBVs of different breeding organization cannot be seen as completely identical traits/EBVs although they were equally labeled. Hence, differences in SNP detection might not be only the result of differences in the genetic architecture of the population but also of the result of deviating phenotypes and applied EBV procedures. The high number of significant SNPs found for LMP might result due to different phenotyping and stand for individual traits. Moreover, some significant SNPs which resulted from GWAS with combined data sets of different breeding organizations should be interpreted with caution.

3.5.2 Biological relevance of significant markers

Because of the high number of detected association in this study, we will not discuss the biological relevance of all significant SNPs in detail, but focus on genome-wide significant markers, SNPs and chromosome regions with significant effects across breeds or populations/clusters, and SNPs with potential pleiotropic effects. Comparison of present results with previously detected QTLs were made using the pigQTL database (Hu et al., 2013).

Biological relevance of genome-wide significant markers

In total, 13 genome-wide significant SNPs were detected in all analyses.

One genome-wide significant SNP (ALGA0016635) identified in the present study was detected on SSC2 at position 149.1 Mb for BF in cluster LR_1. At this chromosome position a significant QTL for BF at mid-back was detected by Guo et al. (2008) in a Meishan × Large White population. This SNP is located within protocadherin beta 15 (*PCDHB15*), whose function is not clarified yet. Moreover, steroid receptor RNA activator (*SRA*) was mapped on SSC2 within the 2 Mb window around the detected SNP. It was shown with *SRA*-/- mice that *SRA* plays an important role in regulating adipose tissue mass and function in mice (Liu et al., 2014). *SRA*-/- mice showed lower fat mass and increased lean content in comparison to other *SRA* genotypes.

Also for BF, two genome-wide significant SNPs were found in LW_1. These two markers were located on SSC5 at 68.3 Mb. Several other previously reported QTLs or associations for BF have been mapped within this SNP region. Harmegnies et al. (2006) found a suggestive QTL for BF between 6th and 7th rib at this position. One additionally suggestive QTL for average BF thickness measured with ultrasound was found at this position on SSC5 (de Koning et al., 2001). This genome-wide significant SNP was located within the confidence interval of the previously reported QTL for BF thickness between 3rd and 4th rib in a Iberian × Landrace experimental cross by Fernandez et al. (2012). Furthermore, several associations for average BF thickness and BF at last rib were detected on SSC5 (Fan et al., 2011; Li et al., 2011; Onteru et al., 2013). Close to the SNP region the gene phospholipase A2, group 6 (*PLA2G6*) was mapped. This is a potential important gene, which is discussed to be involved in fat metabolism in mice (Cheon et al., 2012; Ueno et al., 2011).

Biological relevance of significant markers across breed

Comparing results of GWAS across both breeds, three chromosome regions were identified with significant effects on at least two different traits.

Possible pleiotropic SNPs found in both breeds were found on SSC12 between 38.6-39.9 Mb. This region harbours significant SNPs associated with NBA in Org4_LW and ADG in Org2_LR. SSC12 is an important chromosome for the reproduction trait NBA because of the located candidate genes like N-acetyltransferase 9 (*NAT9*), growth factor receptor-bound protein 2 (*GRB2*) and solute carrier family 9, subfamily A, member 3 regulator 1 (*SLC9A3R1*). The significant associated SNP was not located within any of these candidate genes. However, previously reported QTLs for number of stillborn piglets were located within this chromosome region (Onteru et al., 2012). Additionally, one QTL affecting ADG was detected by de Koning et al. (2001) within this region and unc-45 homolog B (*UNC45B*, also known as *CMYA4*) was mapped at position 41.5 Mb. This gene is a potential candidate gene for BF (Xu et al., 2008). Noteworthy, SNPs from two distinguishing breeding organizations and both breeds were detected as significant at this chromosome position for two different traits. It can be concluded, that this chromosome region influence growth traits as well as litter size in two different breeding organizations. This assumption was supported by the results of the multivariate GWAS. Two markers which were significant associated with ADG in Org2_LR were also found when performing PC analyses. With this method, PC2 was significant associated with high loadings for NBA and ADG (-0.71 and -0.69, respectively).

Another significant across breed chromosomal region with pleiotropic effects was detected on SSC16 at 70.5-71.2 Mb. In this region one SNP for ADG in LW_2 and two SNPs associated with LMP in Org3_LR were found. These three SNPs were located within previously detected associations and QTLs for ADG (Edwards et al., 2008; Fontanesi et al., 2014; Ruckert and Bennewitz, 2010) and LMP (Wimmers et al., 2006). These results indicate that this chromosomal region may affect both breeds and traits which can be interpreted as pleiotropic effects of this chromosome segment on ADG and LMP.

Moreover, on SSC18 one SNP affecting NBA was detected at 59.6 Mb in Org4_LR. At position 60.5 Mb one significant SNP for LMP was detected in cluster Org2_LW. This might be an indicator for pleiotropic effects of this chromosome region. However, there are only markers within this area. Further investigations should be performed to clarify the biological relevance of SSC18 on NBA and LMP.

Except these three chromosome regions on SSC12, 16 and 18, no overlapping areas for the traits across the breeds were found, which is also illustrated in Figure 27.

Biological relevance of significant markers across populations within breed

As shown in Figure 23, within the LW and LR breed distinct genetic clusters can be observed. This was also detected in a previously performed study (Bergfelder-Drüing et al., 2015). These differences might be the result of different breeding goals and breeding activities, so that the identification of identical QTL is not self-evidently. However, cluster LW_2 and its sub-cluster LW_2a as well as the Org2_LR and its sub-clusters Org2a_LR and Org2b_LR partly contain the same animals, so that overlapping SNPs for these clusters can be expected. Overall, six identical significant SNPs were detected within different clusters of the same breed (Figure 27).

On SSC2 the SNP ASGA0084451 at 33.6 Mb was associated with LMP in the overlapping clusters LW_2 and Org1_LW. This marker was located within previous reported QTL for LMP (Nezer et al., 1999; Tortereau et al., 2010), BF (Ai et al., 2012; de Koning et al., 2001; de Koning et al., 2000; Lee et al., 2003; Liu et al., 2008a; Ruckert et al., 2012; Thomsen et al., 2004; Tortereau et al., 2010) and ADG (Lee et al., 2003; Ruckert and Bennewitz, 2010). Moreover, at 32.8 Mb follicle stimulating hormone beta (*FSHB*) was mapped in swine. An influence of *FSHB* on litter size has been reported by several authors (Wang et al., 2006b; Zhao et al., 1998). This chromosome region has an influence on all analyzed production traits as well as on litter size.

On SSC8, one chromosome-wide significant QTL for BF was found in clusters LW_1 and its independent sub-clusters LW_2a and Org2_LW (ASGA0040385). However, the biological relevance of this chromosomal region is unknown up to now.

In cluster Org2_LR and its sub-cluster Org2b_LR one chromosome-wide significant SNP (ASGA0072103) on SSC16 was associated with NBA. In cluster Org2a_LR, which is also part of cluster Org2_LR, ASGA0072103 was excluded from the analysis due to a low MAF.

The SNP DIAS0002692 on SSC18 at 34.4 Mb was detected for LMP in cluster LW_2 and its sub-cluster LW_2a. The gene protein phosphatase 1, regulatory (inhibitor) subunit 3A (*PP1R3A*) was mapped at position 34.4 Mb in swine. A disruption of *PP1R3A* results increased weight gain and obesity in mice (Delibegovic et al., 2003). However, this effect is not yet known in swine.

In summary, it can be concluded that most of the detected SNPs were breed and population specific. SNPs which were detected in more than one cluster were always found in a direct sub-cluster. The only exception is SNP ASGA0040385 on SSC8 which was detected in cluster LW_2a and in Org2_LW consisting of animals of only one breeding organization. This organization is not part of the cluster LW_2a which consisted of three breeding organizations.

Biological relevance of significant markers within populations and breed

Beside across breed and cluster, a large number of QTLs were found only in a specific population and its corresponding sub-cluster. QTLs detected within important candidate regions found in swine will be discussed in the following section.

One genome-wide significant marker was found on SSC1 at 172.7 Mb when analysing ADG in Org3_LR. This SNP was located within a QTL and associations for ADG were reported by Onteru et al. (2013) who used 1,400 pigs from the divergently selected ISU-RFI lines. Moreover, at position 178.5 Mb the gene melanocortin 4 receptor (*MC4R*) was mapped. This gene is a potential candidate gene for growth and muscularity in pigs (Stinckens et al., 2009).

On SSC4 the gene amylo-alpha-1 (*AGL*) was mapped at position 129.7 Mb. Han et al. (Han et al., 2010) found higher growth rates for two *AGL* genotypes in a LR x Jeju black pig F_2 population. Next to this gene two markers were significant associated with ADG in Org4_LR (131.4 Mb).

Additionally, on the p-arm of SSC2, SNPs associated with LMP and BF were found in our analyses. At this position insulin-like growth factor 2 (*IGF2*) was mapped. *IGF2* is a known candidate gene for BF and LMP (Stinckens et al., 2009; Van den Maagdenberg et al., 2008; Vykoukalova et al., 2006).

3.5.3 Significant SNPs with potential pleiotropic effects, detected in univariate analysis

The influence of one gene on more than one trait is called pleiotropy (Bolormaa et al., 2014; David et al., 2013; Solovieff et al., 2013). Solovieff et al. (2013) distinguished between biological (one gene with a direct biological influence on at least two traits), mediated (one phenotype is causally related to another phenotype) and spurious pleiotropy (a genetic variant was falsely identified to be associated with more than one phenotype).

According to this definition, in the present study only one mediated pleiotropy marker (ASGA0092531) was found on SSC8, which was associated with BF and LMP in cluster Org3_LR. Moreover, this marker was also found in Org3_LR with multivariate approach for PC1 (PC_ADG/LMP/BF-). LMP was partly estimated by different BF measurement, so that LMP is causally related to BF which has been discussed as mediated pleiotropy.

Regarding biological pleiotropy, only rough indicators were found in our analysis by comparing overlapping genome regions.

On SSC12, one chromosome region (38.6-39.9 Mb) showed significant influence on NBA as well as on ADG. It can be concluded, that this region had pleiotropic effects on the reproduction trait NBA and on the production trait ADG in both breeds. Additionally, at 42.7-43.9 two markers were associated with ADG and LMP in two organization specific LR cluster (Org2b_LR and Org4_LR) indicating potential biological pleiotropic effects of this region.

On SSC16 at 70.5-71.2 Mb one SNP for ADG in LW_2 and two SNPs associated with LMP in Org3_LR were found indicating pleiotropic effects of this region.

In the following section, results of the present study are compared with results found in literature in order to identify potential pleiotropic SNPs across different studies. In total, eight SNPs were found to be associated with one of the analysed traits in the present study and with additional traits analysed by other authors.

The gene *IGF2* is a known candidate gene for BF and LMP (Stinckens et al., 2009; Van den Maagdenberg et al., 2008; Vykoukalova et al., 2006) as well as for litter size (Munoz et al., 2010) indicating possible pleiotropic effects of *IGF2*. We detected two SNPs affecting LMP in clusters Org4_LR and LR_1 located within the potential *IGF2* region (p-arm of SSC2). One of the SNPs (ASGA0084451) was also associated with LMP in cluster LW_2 and Org1_LW.

One SNP (ALGA0016635) on SSC2, which was genome-wide significant associated with BF in cluster LR_1 in the present study was also found to be associated with adrenal weight in German Landrace pigs as revealed in a previous GWA study performed by Murani et al. (2012). These results are indicating pleiotropic effects across breeds of this marker for BF as well as for adrenal weight in pigs.

On SSC5, three markers, which were found to be associated with reproduction or production traits, were detected in previous GWA studies. The SNP M1GA0007072 associated with

NBA in LW_2 was also associated with ADG in Italian LW pigs found by Fontanesi et al. (2014). The genetic distances between the used LW populations can still be large, but the probability that this marker has an impact on both traits is present. For both genome-wide significant SNPs (MARC0036560 and ALGA0032500) on SSC5 detected in LW_1 for BF, pleiotropic effects were found. The marker MARC0036560 was found to be associated with overall leg action in commercial female pigs analyzed by Fan et al. (2011). Moreover, both SNPs were found to have a significant effect on front leg pastern reported by Rothschild (2010) who used 820 commercial females, genotyped with Illumina porcine 60K BeadChip. Both, leg soundness as well as BF are key components for pork profitability. The association of the two SNPs with BF, as well as with leg soundness, illustrates the importance of the consideration of pleiotropic effects in commercial breeding programs.

On SSC7, pleiotropic effects were found for marker CASI0006750. This SNP was chromosome-wide significant for NBA in Org2_LR in the present study and also significant for vertebral number in Chinese and Western pigs in a previously study (Fan et al., 2013).

In the present study on SSC12 the SNP ALGA0119023 was chromosome-wide significant for BF in Org2a_LR. This marker also found to be associated with intramuscular fat content and marbling in a three-generation resource population of LW boars and Minzhu sows (Luo et al., 2012). This can be interpreted as possible pleiotropic effect between BF, intramuscular fat content and marbling.

On SSC18, one SNP associated with LMP in Org1_LW was found at 56.4 Mb. Within the 2 Mb window insulin-like growth factor binding protein 3 (*IGFBP3*) was mapped. It is known that this gene influences meat quality and carcass traits (Wang et al., 2009). Moreover, it has been shown that *IGFBP3* has an effect on follicular development in swine (Ongeri et al., 2004). This underlines that genes exist with pleiotropic effects on more than one trait.

3.5.4 Significant SNPs with potential pleiotropic effects, detected in multivariate analysis

In comparison to a single trait analysis, a multivariate GWAS increases the QTL detection power and the precision in mapping pleiotropic QTL (Bolormaa et al., 2014; Jiang and Zeng, 1995; Knott and Haley, 2000; Sorensen et al., 2003; Xu et al., 2009). This holds particularly in the situation when investigating highly correlated traits or heritability of one of the traits

affected by the QTL is low (Sorensen et al., 2003). However, the interpretation of results is difficult when analyzed traits are correlated, which might lead to correlated sampling errors (Bolormaa et al., 2010). In order to overcome this problem, the principal component method could be beneficial, which was described as a more powerful approach in comparison to a single trait analysis (Gilbert and Le Roy, 2007; Klei et al., 2008). Applying this approach, traits of interest are condensed to a number of linear combinations of uncorrelated PC (Bolormaa et al., 2010), which reflect the (co)variance matrix of the underlying traits.

Pleiotropy is responsible for genetic and phenotypic correlations that can be detected between complex traits where a locus influences different traits (Cheverud, 2001). In this study LMP and BF were jointly analyzed. According to autocorrelations between these traits, pleiotropic effects can be expected.

It was suggested by several authors to analyze just the first PC because this explained the majority of variation (Liu et al., 1996; Mahler et al., 2002). Olsen et al. (1999) demonstrated that the second and following PCs can identify the highest phenotypic proportion explained by a genetic marker. This is an agreement with a recent study published by Aschard et al. (2014). They concluded that the second and following PCs which normally explain a small amount of the phenotypic variance might harbor a substantial part of the total genetic variation. These PCs seemed to be very powerful when QTL effects are opposite to positively correlated traits. In this study, the first three PCs were investigated and analyzed to enable the identification of substantial part of the total genetic association.

In our analysis we have found 98 significant SNPs for PC components, which were highly influenced by different traits. Of particular importance are significant genomic regions of PCs containing traits with relevant controversial loading signs. Such PCs were found in several organizational clusters for the trait combinations NBA and LMP or NBA and ADG indicated by a minus in the corresponding PC-qualifier (NBA/LMP-; NBA/ADG-) (Table 17-Table 18, Figure 28).

The association study of data set Org3_LR for PC3 (NBA/LMP-) resulted in three chromosome-wide significant SNPs and one additional genome-wide significant marker located on SSC17 at 47.2 Mb. Similar results were also found in literature, where QTLs for NBA (Schneider et al., 2012b) and BF (Fontanesi et al., 2012b) were identified within this region. These findings demonstrate the importance of antagonistic pleiotropic relationships between reproduction and production.

On SSC10 (38.0 to 39.3 Mb) nine linked SNPs were significantly associated with PC3 in Org2b_LR. Indicated by the corresponding loadings, PC3 was most important for NBA and ADG with opposite signs (NBA/ADG-). Within this chromosome area, several QTLs affecting reproduction like number of corpora luteum, NBA and number of mummified piglets have been detected (Onteru et al., 2012; Rohrer et al., 1999; Schneider et al., 2012b). Additionally, QTLs for ADG have been found in this region (Knott et al., 1998; Liu et al., 2007). The porcine aquaporin 7 (*AQP7*) (SSC10, 37.3-37.4 Mb) is involved in adipose tissue enlargement and glucose homeostasis (Hibuse et al., 2005). *AQP7* knockout mice showed higher growth rates because of reduced energy expenditure and accumulation of fat (Hibuse et al., 2005). Moreover, it has been shown that *AQP7* plays a role during post-implantation due to increased expression after embryo attachment (Peng et al., 2011; Richard et al., 2003). Altogether, this chromosome region has pleiotropic effects on both traits. However, indicated by opposite signs of loadings (PC_NBA/ADG-) might lead to antagonistic breeding success when selection is based on this chromosome region.

On SSC3 (131.19 to 132.89 Mb), 12 neighbored SNPs were significant associated with PC2 in Org2b_LR. This PC is dominated by NBA and ADG (NBA/ADG). Lipin-1 (*LPIN1*) was mapped on SSC3 (133.6 Mb) which is involved in adipose tissue mass and energy metabolism in mice. Variations in *LPIN1* levels induced extreme forms of obesity due to higher weight gain and increased body weight as well as significant higher body fat mass (Phan and Reue, 2005). Furthermore, within this chromosome region, QTL for number of stillborn piglets (Onteru et al., 2012) and association for ADG on test (Li et al., 2011) have been found. The influence of *LPIN1* on weight gain (Phan and Reue, 2005), previously reported QTL/association for ADG and number of stillborn piglets and the high loadings of ADG and NBA indicated possible pleiotropic effects.

One (SSC9), six (SSC1, 2, 4 and14) and two (SSC15) genome-wide significant markers were identified for PCs of various organizations, which were assigned to the PC-qualifier groups NBA/ADG-, NBA/ADG/LMP- and NBA/LMP-. As has been shown by other authors (e.g. Duthie et al., 2008; Lee et al., 2003; Sanchez et al., 2006), these QTLs were located within regions, which had a partly known impact on ADG or body weight, but not on fertility traits. The antagonistic pleiotropic relationships illustrated that a further investigation of these chromosome regions might be helpful for a successful improvement of both trait complexes.

3.6 Conclusion

In our data sets a distinct genetic stratification between different pig breeds and pig sub-populations was detected. This result could be expected, because several commercial pig populations from competing pig breeding organisations with different breeding goals were analysed. In order to avoid inflation of false positive SNPs, the statistical analyses were performed within breeds and various, more or less overlapping sub-clusters. In addition, the statistical models contained correction factors, which account for existing population stratification. The resulting λ- values were close to an optimum value of 1.0 and indicated that the applied techniques sufficiently corrected the existing population stratification.

Detected SNPs were population specific and only confirmed in analysis of sub-clusters. In comparison to the sub-cluster analysis, the analysis of across breeding organization cluster yielded to a lower amount of significant SNPs. From this follows, that combining data sets was not beneficial. This might be explained by the higher amount of PCs which were needed to adjust for population stratification sufficiently and eliminated a substantial proportion of genetic variance. However, most genome-wide significant SNPs for carcass composition traits were found in across organization data sets, so that depending on the trait a combination of data might be useful.

Pleiotropic effects are particularly important to understand the genetic background of all traits included in the breeding objective and to avoid negative side effect in correlated traits.

Comparing the results of univariate GWAS, pleiotropic effects for only one single SNP associated with BF and LMP were found. The picture was slightly different, when the results of our studies were compared with genomic regions described in literature. A few overlapping genomic regions on SSC 12, 16 and 18 were significantly associated with different production traits in pigs of both breeds within the present study.

In general, it remains questionable if the statistical power of our univariate analyses to detect pleiotropic effects was sufficient. In this regard, a multivariate approach based on PC technique could be beneficial. In our study a high amount of SNPs were found for PC which reflect the variance covariance of the EBVs as dependent variables. In many PCs, the underlying EBVs were weighted with opposite signs. For these PCs a considerable number of significant SNPs were found, emphasizing the theoretical potential of PCA for detecting pleiotropic effects.

In order to achieve the targets of a well-balanced breeding objective with fitness, reproduction and production traits, more attention should be given to these potential pleiotropic regions. This holds in particular when applying efficient selection tools like genomic selection.

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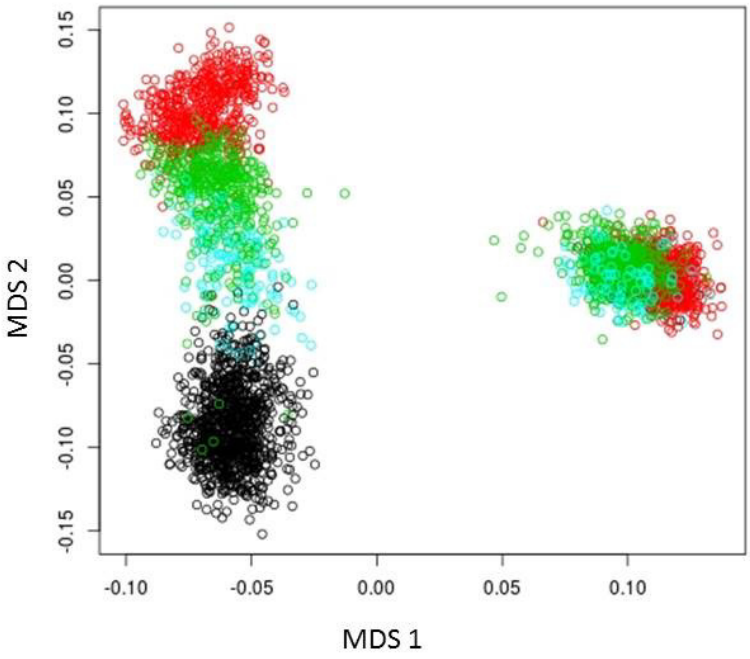

Figure 23: MDS Plot of Landrace (left) and Large White (right) populations of four European breeding companies

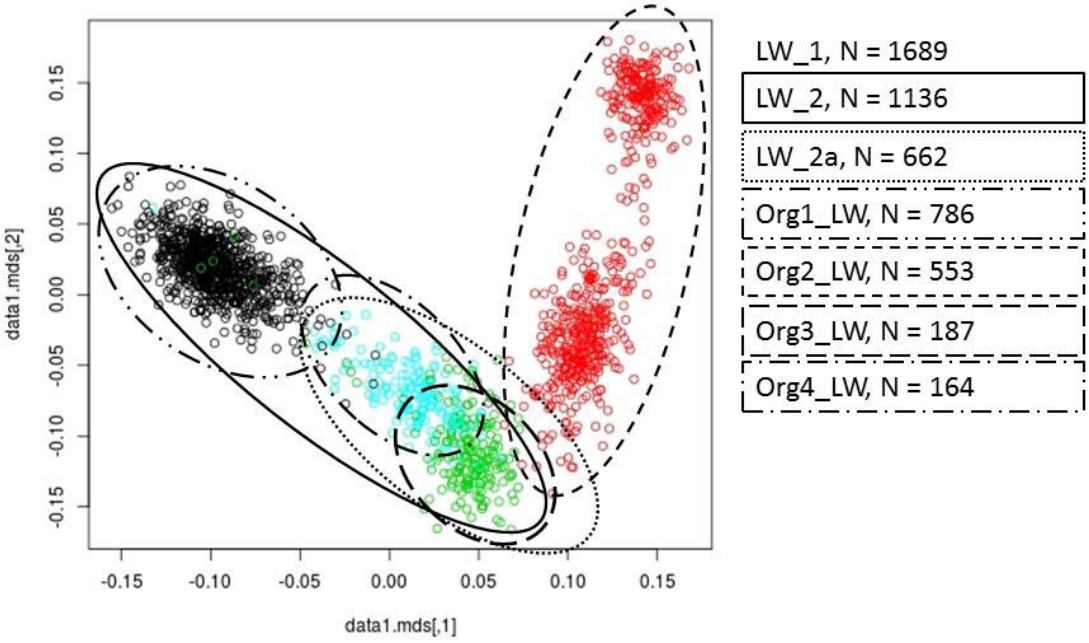

Figure 24: MDS plot of Large White population, each colour represents one breeding company, circles indicate different clusters

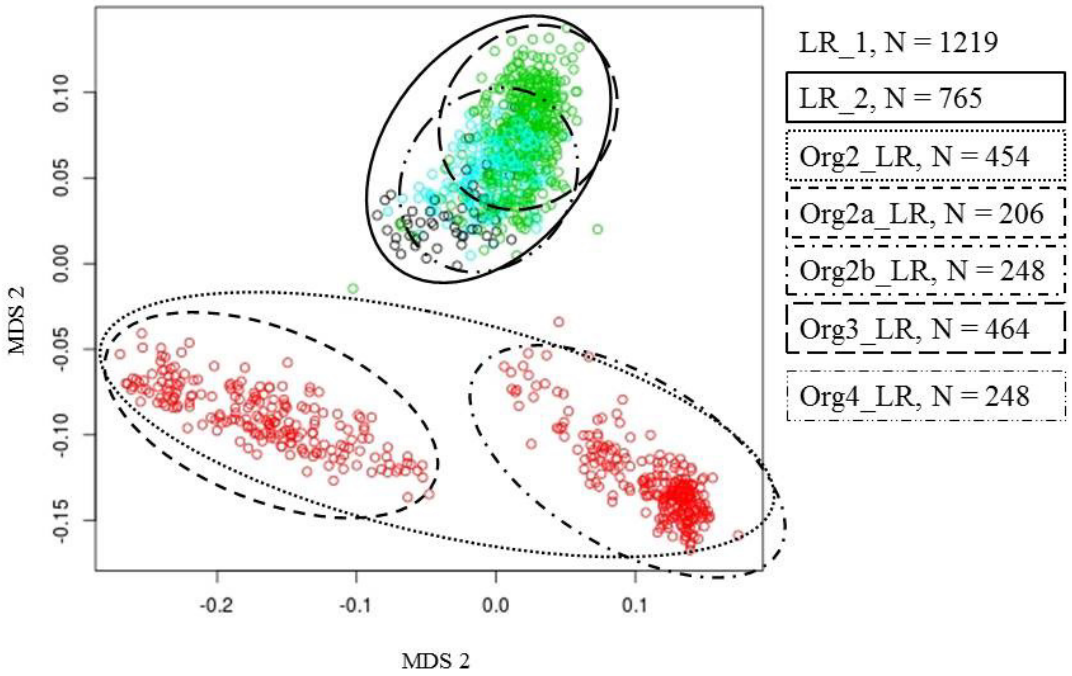

Figure 25: MDS Plot of Landrace population, each colour represents one breeding company, circles indicate different clusters

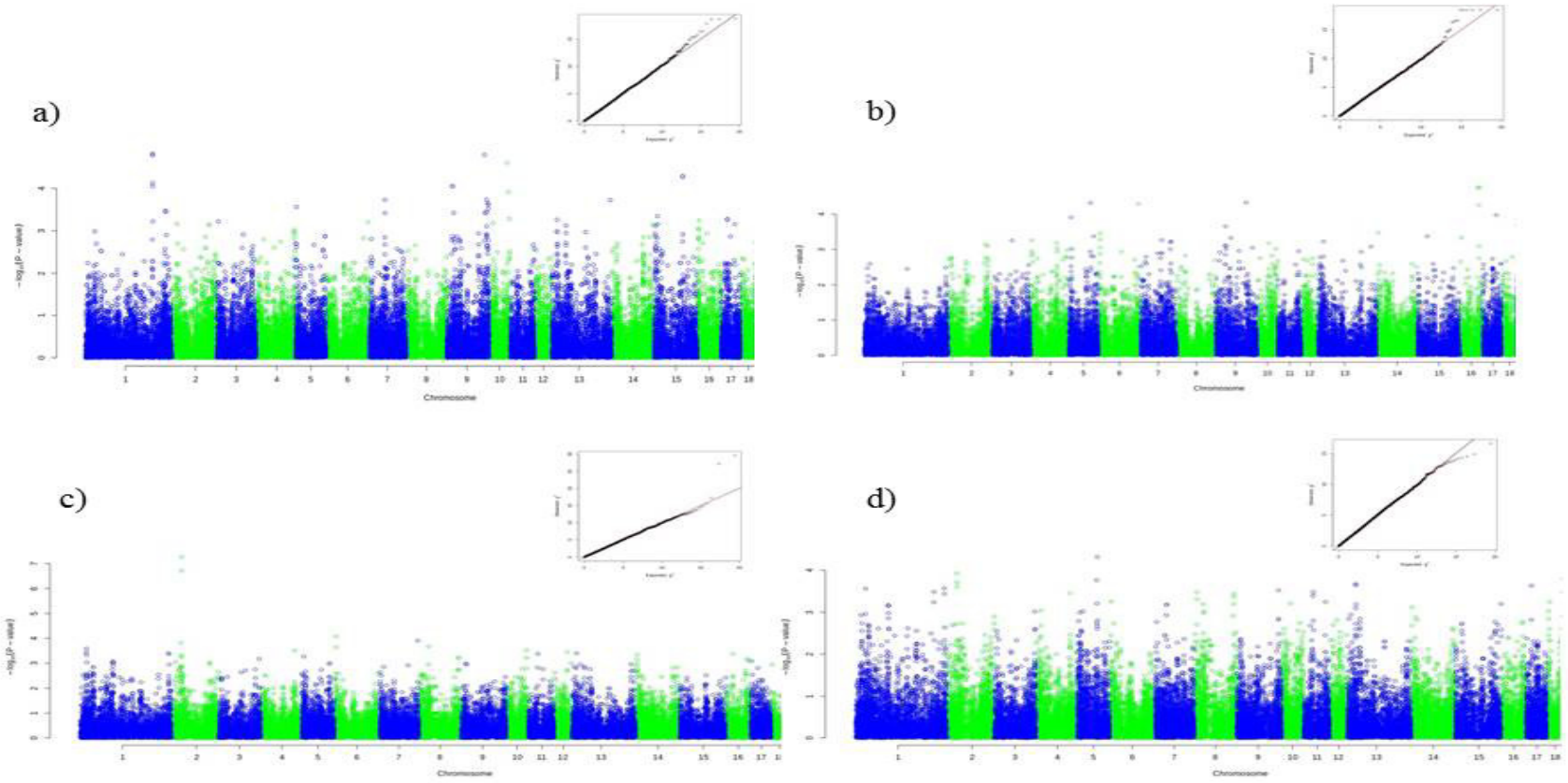

Figure 26: Manhattan and resulting Q-Q plot of Genome-Wide Association Study for NBA in Org1_LW

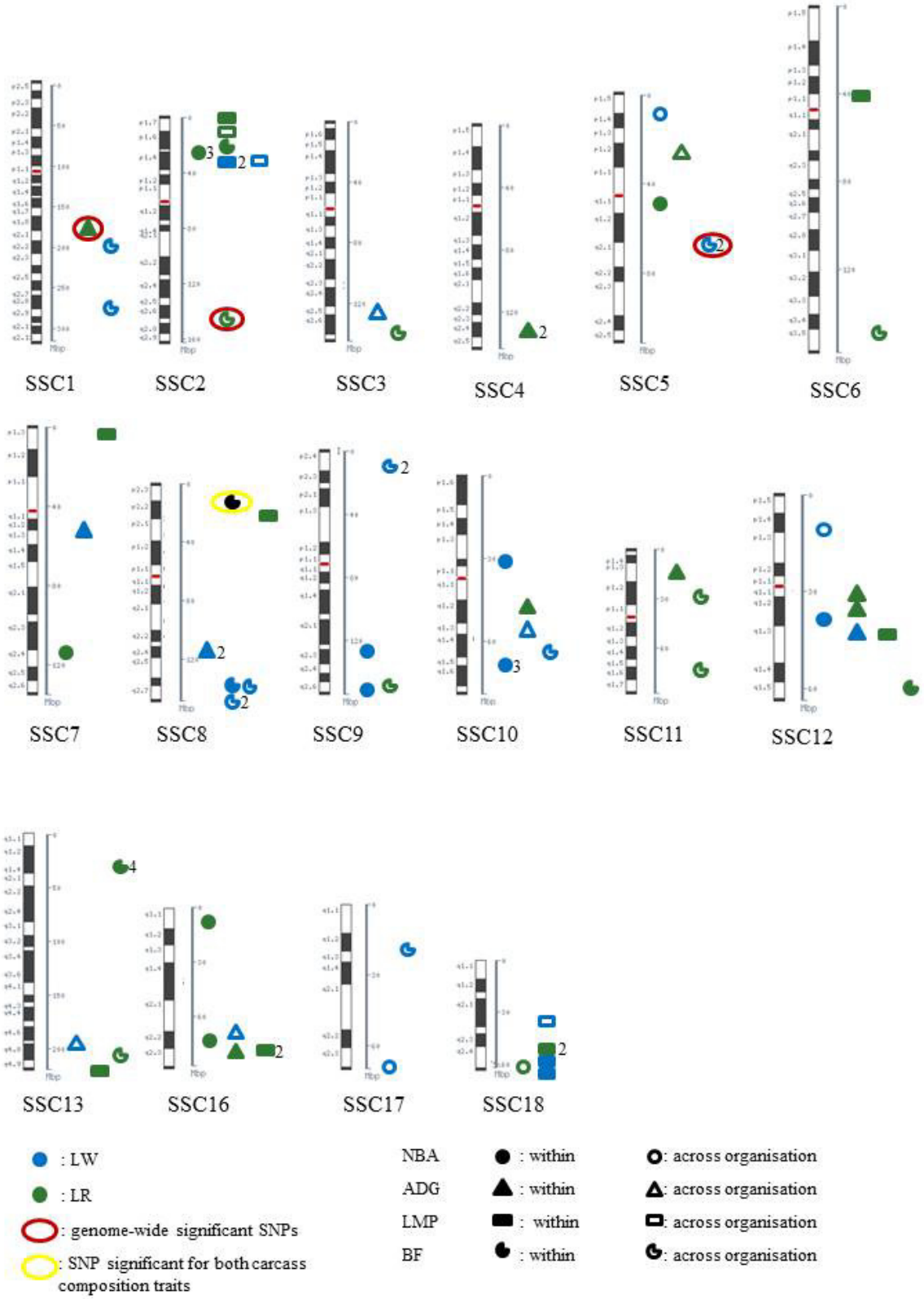

Figure 27: Detected SNPs for all traits within and across organizations and breeds in univariate analyses

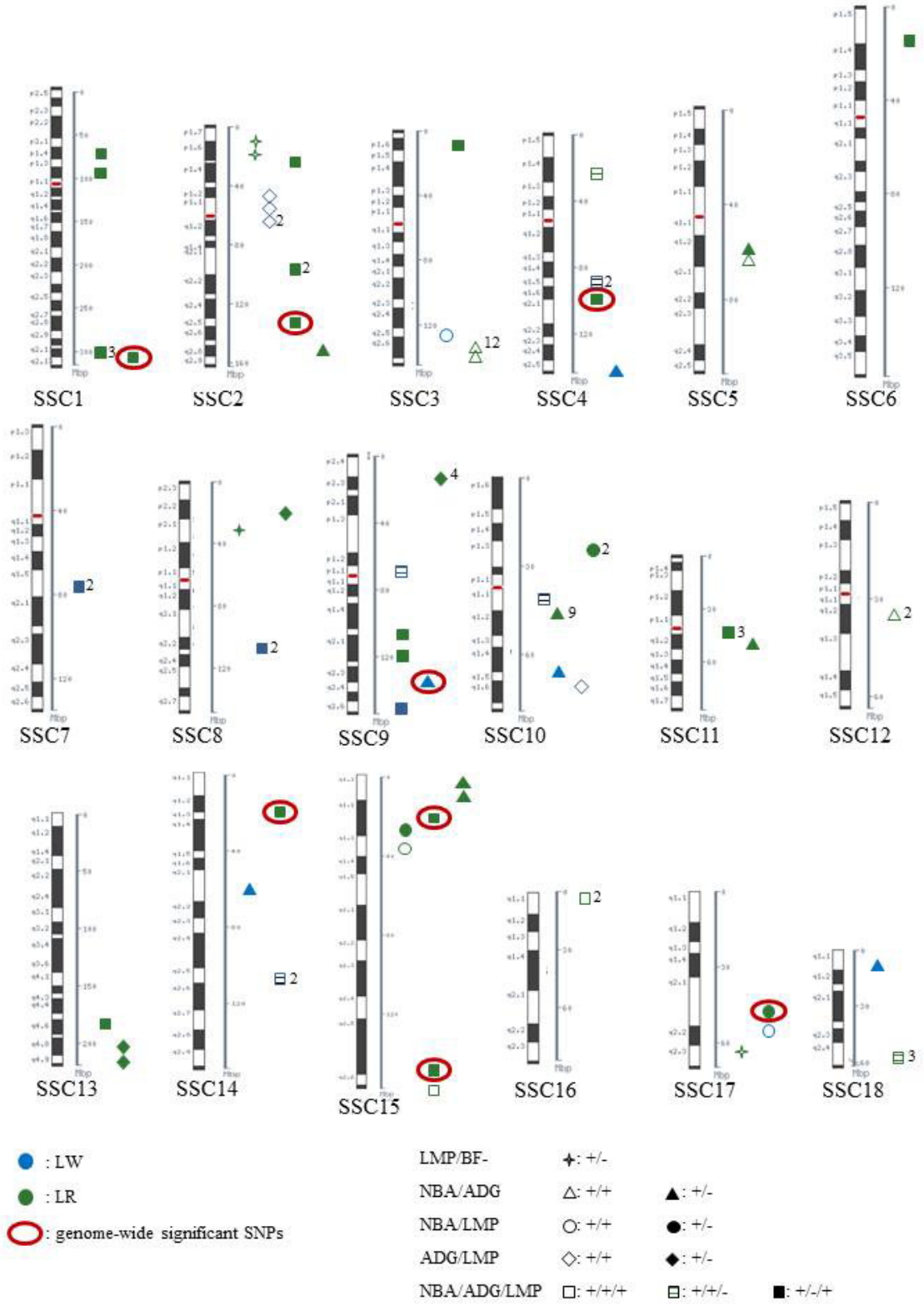

Figure 28: Detected SNPs for all traits within organizations and breeds in multivariate analyses

Table 10: Number of available estimated breeding values for each trait from breeding organization

Trait / Organisation	**Org_1**	**Org_2**		**Org_3**		**Org_4**	
	LW	**LW**	**LR**	**LW**	**LR**	**LW**	**LR**
NBA/ADG/LMP	786	553	454	187	464	164	248
BF	786	553	454	-	-	-	-

NBA = number of piglets born alive; ADG = average daily gain; LMP = lean meat percentage; BF = backfat

Table 11: Number of chromosome-wide and genome-wide SNPs found within and across breed × organisation data set depending on trait and breed

Cluster		**Breed × organisation**		
		Within	**Across**	**Σ**
Trait	Breed			
NBA	LW	7[a)]	3[a)]	10[a)]
	LR	9[a)]	0	9[a)]
ADG	LW	4[a)]	4[a)]	8[a)]
	LR	8[a)]+1[b)]	1[a)]	9[a)]+1[b)]
LMP	LW	4[a)]	3[a)]	7[a)]
	LR	11[a)]	1	12[a)]
BF	LW	1[a)]	10[a)]+2[b)]	11[a)]+2[b)]
	LR	7[a)]	6[a)]+1[b)]	13[a)]+1[b)]
Σ		51[a)]+1[b)]	28[a)]+3[b)]	89[a)]+4[b)]

a) Chromosome-wide significance, b) genome-wide significance

Table 12: Number of identified chromosome- and genome-wide significant QTLs in multivariate analysis

		Cluster				
		LW				
PC-qualifier	**Signs**	**Org1_LW**	**Org2_LW**	**Org3_LW**	**Org4_LW**	**Σ**
LMP/ BF						**0**
NBA/ ADG	**+/+**					**0**
	+/-	$4+1_{(3)}$ [a)]				**4+1**
NBA/ LMP	**+/+**		$1_{(2)}$	$1_{(2)}$		**2**
	+/-					**0**
ADG/ LMP	**++**		$5_{(3)}$			**5**
	+/-					**0**
NBA/ ADG/ LMP	**+/+/+/**					**0**
	+/+/-		$3_{(1)}$	$4_{(1)}$	$1_{(1)}$	**8**
	+/-/+			$3_{(1)}$		**3**
Σ		4+1	9	8	1	**22+1**

continued

Table 12 continued: Number of identified chromosome- and genome-wide significant QTLs in multivariate analysis

		Cluster					
		LR					
PC-qualifier	**Signs**	**Org2_LR**	**Org2a_LR**	**Org2b_LR**	**Org3_LR**	**Org4_LR**	**Σ**
LMP/ BF		$4_{(1)}$					**4**
NBA/	**+/+**	$2_{(2)}$	$1_{(2)}$	$13_{(2)}$			**16**
ADG	**+/-**	$3_{(3)}$		$11_{(3)}$			**14**
NBA/	**+/+**					$1_{(2)}$	**1**
LMP	**+/-**				$3+1^{b)}{}_{(3)}$		**3+1**
ADG/	**++**						**0**
LMP	**+/-**		$1_{(1)}$	$6_{(1)}$			**7**
NBA/	**+/+/+/**				$3_{(1)}$		**3**
ADG/	**+/+/-**					$4_{(3)}$	**4**
LMP	**+/-/+**					$15+7_{(1)}$	**15+7**
Σ		9	2	30	6+1	23+4	**70+5**

a) Subscript: Identifier of nominal PC; b) Number of chromosome- + genome-wide significant (p_{gen} <0.05) QTLs

Table 13: Statistic of significant SNPs for NBA (univariate analyses)

SNP	SSC	Pos (bp)	N[A]	MAF[B]	Var[C]	p-value[D]	Cluster
H3GA0006388	2	27612628	206	0.36	9.6	0.03	
ASGA0095823		27622712	206	0.36	9.6	0.03	Org2a_LR
H3GA0052945		27622773	206	0.36	9.6	0.03	
M1GA0007072	5	844337	1136	0.15	1.6	0.04	LW_2
DRGA0005424		5121445	464	0.02	3.8	0.04	Org3_LR
CASI0006750	7	115511369	454	0.04	4.1	0.04	Org2_LR
ALGA0105307[1]	9	136882252	786	0.09	2.5	0.02	Org1_LW
ASGA0101949		149272587	187	0.03	11.9	0.007	Org3_LW
ASGA0047248	10	31482003	164	0.24	11.9	0.01	Org4_LW
H3GA0030744		70551436	187	0.25	11.3	0.007	
MARC0089035		70581666	187	0.25	11.3	0.007	Org3_LW
ASGA0106280		70642357	187	0.24	10.1	0.02	
DRGA0011611	12	12115648	662	0.40	3.1	0.008	LW_2a
CASI0006976		38628506	164	0.08	10.4	0.04	Org4_LW
ASGA0072103	16	6470509	454	0.01	4.9	0.003	Org2_LR
			248	0.01	8.6	0.009	Org2b_LR
ALGA0091714		77421054	464	0.02	4.6	0.007	Org3_LR
ALGA0106137	17	68990915	662	0.02	2.7	0.03	LW_2a
INRA0056206	18	59600842	247	0.46	7.5	0.02	Org4_LR

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] σ^2_{y*} = Variance of the pre-corrected EBVs (Var, %), [D] nominal p-value and corresponding significant thresholds: **genome-wide significant ($p_{gem} < 0.05$)

Table 14: Statistic of significant SNPs for ADG (univariate analyses)

SNP	SSC	Pos (bp)	N[A]	MAF[B]	Var[C]	p-value[D]	Cluster
ALGA0006523	1	172748312	463	0.01	5.8	0.001**	Org3_LR
ASGA0083506	3	125625241	1687	0.13	1.1	0.04	LW_1
ALGA0028834	4	131377049	248	0.05	7.7	0.04	Org4_LR
ALGA0028847		131562487	248	0.05	8.6	0.01	
MARC0093090	5	27814032	764	0.27	2.5	0.02	LR_2
ASGA0033595	7	52462626	185	0.01	10.2	0.04	Org3_LW
ALGA0049088	8	114685369	185	0.01	10.4	0.03	Org3_LW
ASGA0039589		116982940	185	0.01	10.4	0.03	
MARC0018208	10	49911155	248	0.47	9.3	0.003	Org4_LR
MARC0074336		55089008	1134	0.10	1.6	0.03	LW_2
DIAS0002204	11	52440612	454	0.08	3.9	0.03	Org2_LR
ALGA0066285	12	39878968	454	0.08	4.5	0.008	Org2_LR
M1GA0027257		39929071	454	0.05	4.5	0.008	
ASGA0083415		42766036	248	0.88	7.3	0.03	Org2b_LR
CASI0009186		49529323	185	0.01	9.7	0.03	Org3_LW
DRGA0017470	13	199196126	660	0.22	2.9	0.03	LW_2a
MARC0019021	16	70553486	1134	0.01	1.6	0.03	LW_2
H3GA0047153		78001888	248	0.84	8.4	0.008	Org2b_LR

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] $\sigma^2_{y^*}$ = Variance of the pre-corrected EBVs (Var, %), [D] nominal p-value and corresponding significant thresholds: **genome-wide significant ($p_{gem} < 0.05$)

Table 15: Statistic of significant SNPs for BF (univariate analyses)

SNP	SSC	Pos (bp)	N[A]	MAF[B]	Var[C]	p-value[D]	Cluster
ALGA0007361	1	210049844	1339	0.63	1.4	0.04	LW_1
H3GA0004300		283667732	507	0.40	4.4	0.01	LW_2a
ASGA0008883	2	9260718	454	0.41	4.4	0.03	Org2_LR
ALGA0016635		149106718	507	0.12	4.7	0.003**	LR_1
ASGA0102422	3	138489340	504	0.15	3.9	0.02	LR_1
MARC0036560	5	68326348	1136	0.42	2.2	<0.001**	LW_1
ALGA0032500		68352730	1136	0.23	1.9	<0.001**	
ASGA0030100	6	147923367	507	0.39	3.7	0.03	LR_1
ASGA0092531	8	24808952	206	0.23	9.1	0.03	Org2a_LR
ASGA0040385		144325319	553	0.51	3.4	0.04	Org2_LW
			1339	0.55	1.7	0.003	LW_1
			507	0.52	3.8	0.02	LW_2a
ALGA0122246		145633710	507	0.47	3.9	0.02	LW_2a
ALGA0109684		145660794	507	0.47	3.9	0.02	
H3GA0026296	9	9019463	507	0.43	3.6	0.04	LW_2a
ASGA0041339		9503803	507	0.44	3.7	0.03	
H3GA0028483		144214338	507	0.11	4.3	0.001	LR_1
ASGA0048429	10	61174479	786	0.36	2.4	0.02	LW_2
ASGA0050525	11	28330740	507	0.57	3.9	0.01	LR_1
ASGA0083653		74666729	507	0.47	4.4	0.003	
ALGA0119023	12	59454215	206	0.29	8.9	0.02	Org2a_LR
ALGA0112365	13	26282228	248	0.28	8.3	0.02	Org2b_LR
ALGA0120574		26310854	248	0.28	8.3	0.02	
DIAS0004377		26392676	248	0.28	8.3	0.02	
ALGA0068910		26426245	248	0.28	8.3	0.02	

continued

Table 15 continued: Statistic of significant SNPs for BF (univariate analyses)

SNP	**SSC**	**Pos (bp)**	**N[A]**	**MAF[B]**	**Var[C]**	**p-value[D]**	**Cluster**
ASGA0059911		208161183	507	0.49	4.6	0.004	LR_1
H3GA0048041	17	21777697	507	0.26	3.5	0.03	LW_2a

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] σ^2_{y*} = Variance of the pre-corrected EBVs (Var, %), [D] nominal p-value and corresponding significant thresholds: **genome-wide significant ($p_{gem} < 0.05$),

Table 16: Statistic of significant SNPs for LMP (univariate analyses)

SNP	SSC	Pos (bp)	N[A]	MAF[B]	Var[C]	p-value[D]	Cluster
ALGA0110785	2	251447	248	0.19	8.0	0.02	Org4_LR
DIAS0001270		1881100	1218	0.56	1.7	0.02	LR_1
ASGA0084451		33623071	1134	0.05	8.0	0.04	LW_2
			786	0.05	5.1	0.01	Org1_LW
H3GA0006478		33812534	785	0.05	2.9	0.006	Org1_LW
ALGA0035288	6	42414764	199	0.15	9.4	0.04	Org2_LR
ASGA0030419	7	1127100	206	0.20	8.9	0.04	Org2a_LR
ASGA0092531	8	24808952	206	0.23	8.8	0.04	Org2a_LR
MARC0063481		29095960	454	0.21	3.9	0.03	Org2_LR
MARC0087562	12	43983692	248	0.15	6.9	0.04	Org4_LR
ALGA0073949	13	212744285	248	0.07	8.0	0.01	Org2b_LR
ALGA0091322	16	71090979	454	0.09	3.9	0.03	Org2_LR
ALGA0091330		71187160	454	0.06	4.2	0.01	
DIAS0002692	18	34468417	1134	0.02	1.5	0.04	LW_2
			660	0.02	2.5	0.04	LW_2a
H3GA0051028		51699670	463	0.38	3.7	0.04	Org3_LR
H3GA0051040		51774186	463	0.51	4.0	0.02	
ASGA0080347		56474257	786	0.57	2.2	0.04	Org1_LW
M1GA0023446		60537055	553	0.71	3.1	0.04	Org2_LW

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] $\sigma^2_{y^*}$ = Variance of the pre-corrected EBVs (Var, %), [D] nominal p-value and corresponding significant thresholds: **genome-wide significant ($p_{gem} < 0.05$)

Table 17: Statistic of significant SNPs in LW (multivariate analyses)

SNP	SSC	Pos_bp	N[A]	MAF[B]	Var[C]	p_value[D]	Cluster	PC
NBA/ADG-								
DRGA0005371	4	137303297	786	0.28	2.4	0.04	Org1_LW	PC3
ALGA0105307[1]	9	136882252		0.1	3.1	0.002**		
M1GA0025846	10	65385183		0.14	2.3	0.02		
ASGA0060745	14	6086000		0.37	2.4	0.04		
ALGA0107449	18	12234417		0.37	2.1	0.04		
NBA/LMP								
MARC0114647	3	127967288	549	0.19	3.4	0.03	Org2_LW	PC2
ALGA0095385	17	51911178	185	0.28	11.3	0.007	Org3_LW	PC3
ADG/LMP								
MARC0053324	2	489542	553	0.09	3.4	0.04	Org2_LW	PC3
M1GA0024950	2	519058		0.08	3.7	0.01		
MARC0066239	2	609757		0.08	3.7	0.01		
ALGA0104042	2	609952		0.08	3.7	0.01		
ALGA0059909	10	69946023		0.42	3.4	0.02		
NBA/ADG-/LMP								
ALGA0049088[1]	8	114685369	185	0.01	10.3	0.03	Org3_LW	PC2
ASGA0039589[1]	8	116982940		0.01	10.3	0.03		
ASGA0101949[1]	9	149272587		0.03	10.3	0.03		
NBA/ADG/LMP-								
ASGA0045843	10	4273933	553	0.17	3.4	0.02	Org2_LW	PC1
DIAS0002359	14	10090367		0.15	3.5	0.03		
DIAS0003616	14	10090367		0.15	3.5	0.03		
H3GA0013165	4	85206499	185	0.03	11.8	0.01	Org3_LW	
H3GA0013168	4	85227529		0.03	11.8	0.01		

continued

Table 17 continued: Statistic of significant SNPs in LW (multivariate analyses)

SNP	SSC	Pos_bp	N[A]	MAF[B]	Var[C]	p_value[D]	Cluster	PC
ASGA0034482	7	79384961		0.02	11.4	0.01	Org3_LW	PC1
ALGA0042588	7	79525239		0.02	11.4	0.01		
ASGA0045543	9	73102952	164	0.05	11.4	0.04	Org4_LW	

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] $\sigma^2_{PC}{}^*$ = Proportion of total variance explained by each PC (Var, %), [D] nominal p-value and corresponding significant thresholds: **genome-wide significant ($p_{gem} < 0.05$), [1] = SNP was also significant in univariate analyses

Table 18: Statistic of significant SNPs in LR (multivariate analyses)

SNP	SSC	Pos_bp	N[A]	MAF[B]	Var[C]	p_value[D]	Cluster	PC
LMP/BF-								
DIAS0003578	2	8352062	454	0.39	4.2	0.03	Org2_LR	PC1
ASGA0008883[1]	2	9260718		0.41	4.6	0.01		
MARC0063481[1]	8	29095960		0.02	4.4	0.01		
MARC0014510	17	63277542		0.03	4	0.02		
NBA/ADG								
ALGA0066285[1]	12	39878968	454	0.05	3.9	0.03	Org2_LR	PC2
M1GA0027257[1]	12	39929071		0.05	3.9	0.03		
ALGA0032465	5	67994769	206	0.55	9.6	0.01	Org2a_LR	PC3
MARC0027808	3	131191678	248	0.01	8	0.02	Org2b_LR	PC2
MARC0070460	3	131302875		0.01	8	0.02		
MARC0016367	3	131339947		0.01	8	0.02		
MARC0033632	3	131859366		0.01	8	0.02		
MARC0048071	3	131906132		0.01	8	0.02		
ALGA0021483	3	132222276		0.01	8	0.02		
ALGA0021485	3	132242749		0.01	8	0.02		
ASGA0016597	3	132275049		0.01	8	0.02		
INRA0011766	3	132351391		0.01	8	0.02		
ALGA0021498	3	132557454		0.01	8	0.02		
MARC0027455	3	132885104		0.01	8	0.02		
ALGA0021539	3	132895691		0.01	8	0.02		
MARC0024866	3	138933442		0.01	8	0.02		
NBA/ADG-								
ALGA0032106	5	62251465	454	0.12	4	0.03	Org2_LR	PC3
ASGA0100021	11	52875626		0.53	3.8	0.04		
ALGA0083564	15	7775107		0.04	4.1	0.03		

continued

Table 18 continued: Statistic of significant SNPs in LR (multivariate analyses)

SNP	SSC	Pos_bp	N[A]	MAF[B]	Var[C]	p_value[D]	Cluster	PC
ALGA0117251	2	155274718	248	0.63	8	0.02	Org2b_LR	PC3
ASGA0047572	10	38009455		0.04	7.4	0.03		
ALGA0102748	10	38055678		0.04	7.4	0.03		
MARC0003765	10	38220149		0.04	7.4	0.03		
ALGA0058399	10	38616182		0.04	7.4	0.03		
ALGA0058396	10	38655801		0.04	7.4	0.03		
ALGA0058412	10	38986300		0.04	7.4	0.03		
ASGA0047602	10	39029551		0.04	7.4	0.03		
MARC0027577	10	39164815		0.05	7.8	0.01		
CASI0005931	10	39345211		0.05	7.8	0.01		
SIRI0001081	15	955995		0.04	8	0.01		
NBA/LMP								
ASGA0102757	15	35152470	248	0.05	8.3	0.01	Org4_LR	PC2
NBA/LMP-								
H3GA0029603	10	26483406	463	0.08	3.9	0.03	Org3_LR	PC3
ALGA0057837	10	26717528		0.17	3.8	0.04		
ASGA0105547	15	24791984		0.04	4.9	0.004		
ALGA0095171	17	47215770		0.01	5.3	0.001**		
ADG/LMP-								
ASGA0092531	8	24808952	206	0.09	8.9	0.04	Org2a_LR	PC1
ALGA0119624	9	16771021	248	0.11	7.6	0.04	Org2b_LR	PC1
ASGA0083435	9	16798794		0.11	7.6	0.04		
MARC0046852	9	16840372		0.11	7.6	0.04		
ASGA0099434	9	16865632		0.89	7.6	0.04		
ALGA0073634	13	205238035		0.11	8	0.03		
ALGA0073949[1]	13	212744285		0.06	8.2	0.02		

continued

Table 18 continued: Statistic of significant SNPs in LR (multivariate analyses)

SNP	SSC	Pos_bp	N[A]	MAF[B]	Var[C]	p_value[D]	Cluster	PC
NBA/ADG/LMP								
DRGA0015653	15	150422151	463	0.06	5.1	0.003	Org3_LR	PC1
ALGA0124436	16	5537597		0.16	4.3	0.01		
MARC0079512	16	5540458		0.16	4.3	0.01		
NBA/ADG-/LMP								
ALGA0003988	1	74309279	248	0.02	8	0.04	Org4_LR	PC1
INRA0003242	1	99869938		0.02	9.5	0.006		
M1GA0001790	1	303984121		0.02	8.1	0.04		
ALGA0010917	1	305823994		0.01	9.5	0.006		
MARC0102908	1	305915437		0.01	9.5	0.006		
ASGA0098979	1	308111095		0.03	12.5	0.001**		
MARC0030386	2	9260718		0.03	7.7	0.03		
ALGA0106685	2	93152911		0.03	7.7	0.03		
ALGA0014427	2	93753764		0.03	7.7	0.03		
INRA0009745	2	133098875		0.01	11	0.001		
MARC0002694	3	92786470		0.02	8.5	0.01		
ALGA0026453	4	94746928		0.01	9.8	0.002		
MARC0059485	6	18457265		0.01	7.8	0.03		
DIAS0002592	9	113613936		0.01	9.9	0.001		
ASGA0044308	9	121797557		0.01	8.1	0.02		
ALGA0062149	11	49293536		0.01	9.5	0.002		
MARC0103945	11	49340727		0.01	9.5	0.002		
ALGA0062164	11	49959584		0.01	9.5	0.002		
MARC0037806	13	186878981		0.01	8.6	0.01		
MARC0110512	14	28732033		0.02	10.3	0.001**		
ALGA0102740	15	15275590		0.02	12.6	0.001**		

continued

Table 18 continued: Statistic of significant SNPs in LR (multivariate analyses)

SNP	SSC	Pos_bp	N[A]	MAF[B]	Var[C]	p_value[D]	Cluster	PC
ASGA0084747	15	146590572		0.01	10.1	0.001**		
NBA/ADG/LMP-								
H3GA0012414	4	30430591	247	0.36	7.8	0.03	Org4_LR	PC3
ASGA0080432	18	59520434		0.61	9.2	0.002		
INRA0056206[1]	18	59600842		0.48	9.1	0.002		
ASGA0080436	18	59739721		0.62	8.2	0.008		

[A] Number of analysed animals, [B] minor allele frequency (MAF), [C] $\sigma^2_{PC}{}^*$ = Proportion of total variance explained by each PC (Var, %), [D] nominal p-value and corresponding significant thresholds: **genome-wide significant ($p_{gem} < 0.05$), [1] = SNP was also significant in univariate analyses

Table 19: Loadings and proportion of total variance explained by PCs in LW

Cluster	PC	PC-qualifier	NBA	ADG	LMP	BF	Var (%)
Org1_LW	**PC1**	LMP/BF-	0,17	0,07	-0,69*	0,69*	46,6
	PC2	NBA/ADG	-0,66*	-0,73*	-0,09	0,15	27,9
	PC3	NBA/ADG-	-0,73*	0,68*	-0,06	0,05	21,4
Org2_LW	**PC1**	NBA/ADG/LMP-	0,26*	0,49*	-0,56*	0,62*	52,5
	PC2	NBA/LMP	-0,96*	0,09	-0,24*	0,12	23,1
	PC3	ADG/LMP	0,07	-0,83*	-0,53*	0,14	17,3
Org3_LW	**PC1**	NBA/ADG/LMP-	0,64*	0,42*		-0,64*	41,4
	PC2	NBA/ADG-/LMP	-0,27*	0,91*		0,32*	31,6
	PC3	NBA/LMP	0,71*	-0,03		0,70*	27,0
Org4_LW	**PC1**	NBA/ADG/LMP-	-0,66*	-0,64*		0,38*	44,3
	PC2	NBA/ADG/LMP	0,22*	0,31*		0,92*	31,5
	PC3	NBA/ADG-	0,71*	-0,69*		0,06	24,2

* illustrate the different biological background and the resulting PC-qualifier

Table 20: Loadings and proportion of total variance explained by PCs in LR

Cluster	PC	PC-qualifier	NBA	ADG	LMP	BF	Var (%)
Org2_LR	**PC1**	LMP/BF-	-0,06	0,14	-0,69*	0,70*	44,2
	PC2	NBA/ADG	-0,71*	-0,69*	-0,12	-0,04	35,9
	PC3	NBA/ADG-	-0,69*	0,70*	0,11	-0,09	13,8
Org2a_LR	**PC1**	ADG/LMP-	0,06	0,22*	-0,68*	0,69*	47,1
	PC2	NBA/ADG-	-0,84*	0,54*	0,09	0,003	25,8
	PC3	NBA/ADG	-0,54*	-0,81*	-0,16	0,16	23,1
Org2b_LR	**PC1**	ADG/LMP-	-0,06	0,24*	-0,68*	0,69*	43,8
	PC2	NBA/ADG	0,73*	0,66*	0,15	-0,02	31,4
	PC3	NBA/ADG-	-0,68*	0,70*	0,18	-0,13	17,4
Org3_LR	**PC1**	NBA/ADG/LMP	0,59*	0,54*		0,59*	50,5
	PC2	NBA/ADG-/LMP	-0,43*	0,84*		-0,34*	26,1
	PC3	NBA/LMP-	-0,68*	-0,06		0,73*	23,4
Org3_LR	**PC1**	NBA/ADG-/LMP	-0,23*	0,74*		0,63*	39,0
	PC2	NBA/LMP	0,85*	-0,16		0,50*	35,3
	PC3	NBA/ADG/LMP-	0,47*	0,64*		-0,59*	25,6

* illustrate the different biological background and the resulting PC-qualifier

4 CHAPTER 4: GENERAL DISCUSSION

In modern breeding programs one of the latest promising tools to achieve defined breeding goals is the application of genomic selection (GS). GS is a selection method that based on SNP information and was introduced by Meuwissen et al. (2001). From the perspective of competing animal breeding organization, accuracy of GS to predict gEBVs of young, untested selection candidates is most important. In addition, it is useful to understand the genetic background of important genes in order to avoid unexpected antagonistic side effects due to pleiotropic effects on important trait complexes. In order to gain such information, GWAS are beneficial tools.

The success of GS and GWAS depends mainly on the number of animals with accurate EBVs and genomic data (Goddard and Hayes, 2009; Pausch, 2013; VanRaden et al., 2011). However, the number of available pigs in a single breeding organization is limited. In order to increase the number of individuals, different breeding organizations located in Germany, Austria and Switzerland selected boars and sows of Large White and Landrace breeds for a combined analysis within the pigGS-project ("pigs – Neue Beiträge zur Optimierung der Schweinefleischproduktion"). Generally, a larger number of animals increase theoretically the power/accuracy of statistical analysis like GWAS or GS (Goddard and Hayes, 2009; Pausch, 2013; Spencer et al., 2009).

The aim of the recent work was to apply GWAS across the whole data set and within populations of particular breeding organizations, in order to proof if a combined analysis is useful and to identify biologically relevant chromosome regions affecting NBA (see chapter 2). In a second approach, beside NBA, the production traits ADG, LMP and BF were investigated in order to identify pleiotropic regions (see chapter 3).

4.1 Analysis of combined populations

Combining phenotypic and genetic data from different breeding organization with different genetic background leads to statistical problems in the detection of QTLs. The arising problems and the applied statistical tools described in chapter 2 and 3 are discussed in the next two sections.

4.1.1 Controlling population stratification using statistical methods

In order to avoid negative consequences of population stratification, in both GWAS analysis, a combined approach using GRAMMAR and EIGENSTRAT was used.

The GRAMMAR method was applied to control population stratification mainly caused by familiar structure (Amin et al., 2007). To avoid an increase of false-positive association due to accumulation of pedigree information, methods used in this thesis (GRAMMAR approach) considered the genomic "true" relationship between animals by calculating environmental residuals free from familial correlations. These produced familial correlation-free residuals were used as new phenotypes. The model for association test was further extended by PC as fixed covariables estimated by the genomic kinship matrix reflecting the axes of genetic variation (method EIGENSTRAT) (Aulchenko et al., 2007b; Price et al., 2006). Theses PCs were used to adjust the phenotype and the genotype for population stratification.

The GWA studies performed only with GRAMMAR approach in Chapter 2 and 3 did not result in a sufficient correction for existing strong population stratification (data not shown). This was indicated by extreme high λ values. After performing the adjustment of phenotype and genotype for population stratification with differing number of PCs, λ -values were in an adequate range.

During the EIGENSTRAT step in some clusters a noticeable high number of PCs was needed in order to remove population stratification sufficiently. However, in such a situation there is a high risk to eliminate substantial proportion of genetic variation which might be important for QTL detection. Against this background it can be doubted, that λ should be the only useful criteria to identify the number of PCs, which were integrated into the statistical model. Using statistical methods and bioinformatic tools which are not that rigorous to remove a large amount of genetic variation, might identify additionally SNPs of biologically relevance. On the other hand, when population stratification is not taken into consideration, higher number of false-positive associations might be detected.

4.1.2 EBVs as phenotypes in association studies

Accurate recorded phenotypes are most important prerequisite for successfully association studies. However, the recording of reproduction traits was difficult because many environmental effects mask the genotype. Phenotype recording of traits like ovulation rate or

prenatal survival raises challenges for breeding companies. As a consequence, Barendse et al. (2011) emphasized that precisely defined phenotypes were indispensable for the replication or association signals in validation populations. This might be a reason why QTLs with a high fraction of trait variation can remain undetected in a validation data set without well-defined phenotypes.

In the present studies GWAS were performed using EBVs for NBA and production traits as phenotype. Using EBVs for GWAS is advantageous, because many phenotypes cannot be measured in the selection candidate itself. In addition, EBVs are corrected for systematic environmental effects. Furthermore, when EBVs are based on a large number of progeny records, they lead to a high estimation accuracy and are therefore highly heritable (Becker et al., 2013). From this followed that using EBVs considerably increased the power to detect QTL (Goddard and Hayes, 2009) and allowed the detection of QTLs even for traits with low heritability in small populations (Pausch et al., 2011).

These advantages were of high importance in the present studies, because heritability of NBA is rather low. EBVs for NBA used in the analysis based on at least 20 phenotyped progenies, so that it can be concluded that the power of QTL detection is sufficiently high.

EBVs accumulate family information, which supposed to cause an inflation of false-positive associations in association studies when EBVs are used as phenotypes in GWAS (Ekine et al., 2010). To avoid this, EBVs can be de-regressed which means that the contribution of relatives is removed from the EBV (Garrick et al., 2009). However, daughter yield deviations (DYD) are proposed to be the best phenotype for QTL analyses in pedigreed populations (Hoeschele and Van Raden, 1993; Thomsen et al., 2001). These DYDs reflect the real average of the progeny and are thus not distorted by information of ancestors (VanRaden and Wiggans, 1991). These observations were confirmed by Pausch et al. (2012) who first performed GWAS with DYDs for udder clearness and then repeated the GWAS using EBVs. They found no significant differences between the two approaches. One reason might be the high heritability of the EBVs for udder clearness.

Within analyses performed in chapter 2 and 3, it was not possible to use DYD as phenotypes because EBVs of every relative was not available for each genotyped animal.

4.2 The usefulness of a analysis across all breeding organizations

In the present data sets a distinct genetic stratification between different pig breeds and pig sub-populations was observed. This result could be expected, because several commercial pig

populations from competing pig breeding organizations with different breeding goals were analyzed. In order to avoid inflation of false positive QTLs, the statistical analyses were performed within breeds and various, more or less overlapping sub-clusters. In addition, the statistical models contain correction factors, which account for existing population stratification. The resulting λ-values were close to an optimum value of 1.0 and indicated, that the applied techniques sufficiently corrected the existing population stratification.

Detected SNPs were population specific and only confirmed in analysis of independent sub-clusters. In comparison to the sub cluster analysis, the analysis of across breeding organization cluster yield to a lower amount of significant QTLs. From this follows, that combining genetic heterogeneous data sets, which is typical for different pig breeding organizations, was not beneficial. This might be explained by the higher amount of PCs which were needed to adjust for population stratification sufficiently and eliminates a substantial proportion of genetic variance. However, most genome-wide significant SNPs for carcass composition traits were found in across organization data sets, so that depending on trait a combination of data might be useful.

4.3 Comparison of the results from different QTL analyses

During the last years, a large number of QTLs and identified candidate genes influencing reproductive traits have been identified. Thereby, complementary approaches were used to identify genetic markers. Physiological candidate genes comprised genes with known impact on the trait of interest. They were scanned for polymorphism and further tested for association with variation in the trait (Fernandez-Rodriguez et al., 2011; Jiang et al., 2001; Rothschild et al., 1996; Rothschild et al., 2000; Short et al., 1997; Vallet et al., 2005). The second approach used unbiased genome scans with anonymous markers like microsatellites or SNPs to identify QTLs affecting the trait of interest (Bidanel et al., 2008; Cassady et al., 2001; de Koning et al., 2001; Ding et al., 2009; Holl et al., 2004; King et al., 2003; Onteru et al., 2012; Onteru et al., 2011; Rathje et al., 1997; Rodriguez et al., 2005; Rohrer et al., 1999; Tribout et al., 2008; Wilkie et al., 1999).

The evaluation of all studies from literature showed some overlapping results. However, remarkable differences in QTL location, estimated effect size and magnitude of the QTL or candidate gene have been identified. The observed differences can be traced back to the experimental design and categorized into five aspects: Genetic diversities in populations,

breeds and crosses, recording of phenotypes, number of analysed animals, number and kind of markers, quality control criteria, and applied statistical model and method and significance thresholds.

Diversities in populations, breeds and crosses can lead to considerably differences in GWAS results. Genes or significant SNPs which were present in one population or breed might not be present or significant in another population or breed. Genetic variation and differences of environmental influences may enhance or inhibit gene effects (Buske et al., 2006a). Such diversities within breeds have been detected in the present study. As it has been already discussed, the transferability of a QTL from one population to another one is by no means guaranteed. This could be explained by differences in linkage phases between investigated markers and the trait of interest or the marker is not segregating in one population. It is known, that the genetic effects might differ if they are estimated in purebred or crossbred populations (Spotter et al., 2009).

The identification of a QTL associated with the trait of interest in independent populations is absolutely essential to validate this QTL (Liu et al., 2008b). Moreover, the identification of one QTL in two different populations suggests that this locus influence the trait owning a relevant biological genetic variation slightly than to confusing effects such as artefacts of population stratification (Becker et al., 2013; Buske et al., 2006a). QTLs which have not been confirmed in another population may be produced by population specific rare allelic variants or due to multiple testing just by chance (Buske et al., 2006a).

Differences in recording of phenotypes like applied measurement techniques or time points of recording led to varying phenotypes, which might be influenced by different genes. As an example of our study, BF and LMP are both indicators of carcass composition, but LMP comprises different traits like carcass cut weights or muscle depths. Another example is the time point of NBA recording. NBA results, recorded immediately after birth are less influenced by genes which have an impact on piglet survival compared to NBA recorded one or two days after birth. Results of GWAS with those different phenotypes can hardly be compared.

Another influencing factor is the number of individuals used in a GWAS. In general, a large number of animals with available genotypes, as well as phenotypes, was required to obtain accurate marker effects (Hickey et al., 2013). In the present studies within the breeds LW 2,272 to 507 across and 786 to 164 within breeding organizations and LR 1,598 to 53 across and 454 to 206 animals within breeding organizations were included into analyses. Relative to

other GWAS study this amount of animal is relative high. However, because of population stratification a combined analysis of the whole data sets is only partially possible as it has been shown in chapter 2. Beside the size of the investigated population, the number of markers and the source of markers are important for the outcome of a GWAS. In the situation of SNP genotyping using chip or array technologies the power of QTL detection markedly depends on an acceptable LD between SNP and QTL (Goddard and Hayes, 2009). When the distance between SNPs is too far, markers might not be in LD with the QTL that remains unobserved.

In chapter 2 and 3 all pigs were genotyped with the porcine Illumina 60k Chip (Ramos et al., 2009). The remaining number of SNPs depended on the quality criteria which were applied on the genotypic data. Although standard parameters were used, the number of SNPs varied between subcluster and breeds. Excluding SNPs and animals in the quality check were mainly population specific. Population specific rare variants might be removed from data sets when the chosen MAF threshold is too stringent. One Marker, which was highly significant associated with analysed trait in one population, might be excluded from data set due to MAF in another population.

In order to increase the resolution of SNP markers next generation sequencing technologies shouled be applied. This would allow to identify directly the presumable functional mutation.

The choice of a particular statistical GWAS model depends on the investigated population, the source and number of genetic marker, the recorded phenotype and the marker effects that should be estimated within the study. In section 4.2.1 the consequences of unconsidered population stratification has been described. When existing population stratification was not taken into account the number of false-positive increased.

In order to correct for multiple testing different statistical parameters like Bonferroni correction, q-value or false-discovery rate are estimated. It has been discussed that the Bonferroni-correction is too stringent (Han et al., 2009) especially when polygenic traits are analyzed that are influenced by numerous of small marker effects (Andersson and Georges, 2004).

4.4 Detection of pleiotropic effects using univatiate and multivariate genome-wide association analysis

In general, in the situation of pleiotropy, selection for one trait might lead to unfavourable negative side effects on another trait. From a statistical point of view, using multivariate approaches in comparison to an univariate analysis would increase the QTL detection power and the precision in mapping QTLs (Bolormaa et al., 2014; Jiang and Zeng, 1995; Knott and Haley, 2000; Sorensen et al., 2003; Xu et al., 2009). In our analysis we applied a PC analysis in order to condense the information content of different EBV into 3 uncorrelated PCs. The composition of these PCs can be characterised by the values and signs of the corresponding PC member variables (EBVs). As described in Chapter 3, many QTLs were found in the GWAS analysis for these PCs, which were not detected in the univariate GWAS. This result could be explained by the increased power oft the multivariate approach. Moreover, most PCs are dominated by more than one EBV. Because of the controversial loading signs of these EBVs, it can be speculated that many hidden antagonistic effects between analysed reproduction and production traits exist. These effects might be very important for balanced breeding programmes which try to improve negatively correlated trait complexes. As a heuristic approach, in GS, SNPs in pleiotropic regions can be weighted (upvalue or devalue) depending on their impact on the selection trait. This strategy could lead to an improved selection success and could avoid possible negative side effects on other traits.

In general, multivariate analyses to detect pleiotropic effects are more consistent with biological basis in comparison to cross-trait comparison of single trait GWAS. Additionally, multivariate methods where associations of several traits are verified with one single test reduce the number of performed association tests and therefore reduce the problem of multiple testing (Chavali et al., 2010; Klei et al., 2008{Zhu, 2009 #1815).

Up to the present, different methods for multivariate GWAS have been proposed which were derived from univariate methods in some cases. Galesloost et al. (2014) distinguished between direct, indirect and univariate-based methods. Direct methods model the genetic variant directly on the trait of interest without alteration of the general nature and format of the trait and are implemented into several programs like SNPTEST (Marchini et al., 2007), BIMBAM (Guan and Stephens, 2008; Stephens, 2013) and the R package MultiPhen (O'Reilly et al., 2012). In indirect methods the dimension of the trait is reduced. Therefore, linear combination of traits which maximize the covariance between the genetic variant and all traits are extracted or PC are used (Galesloot et al., 2014). Univariate-based methods use

for example meta-analysis where correlation structure between analyzed traits is taken into consideration. Another approach is the comparison of results of univariate GWAS. Here the problem of multiple testing is a disadvantage.

No one of these methods can be titled as the golden standard which works for every scenario (Galesloot et al., 2014). Choice of method and corresponding power of the analyses depends on the data set, aim of the study, existing stratification and the correlation between the traits.

4.5 Further steps

The enlargement of the data set can be a further step. Therefore, homogenous generations should be chosen to reduce population stratification. Moreover, it might be more promising to genotype sows instead of boars because sows are closer to the actual generation and the trait is part of the female reproduction complex. Genes which are actually present in the breeding population would be capture by genotyping animals from the present population. On the other side, due to higher number of progenies, boars have EBVs with higher accuracy. On the other hand, the trait of interest is measured on sows.

The exchange of breeding stock of cooperating breeding organizations in order to increase the number of animals simultaneously to decrease the effect of population stratifications will help to increase the data set and the power of GWAS.

Furthermore, genotyping of animals with HD chips or the use of next generation sequencing can be a further step. An enlargement of the SNP panel might be more useful to detect possible QTLs more accurate. These steps, the enlargement of the data set and SNP panel might lead to detection of further locis with small effects on NBA.

GWAS with other multivariate approaches for comparison should be investigated.

In the future, well and exact defined phentotypes across breeding organizations should be implemented into routine phenotyping (“Phenotype is King”). Better comparability can be achieved of the results of QTL analyses within different breeding organizations of those standardized and well defined phenotypes.

5 CHAPTER 5: CONCLUSION

Distinct genetic stratifications between LW and LR breeds and corresponding sub-populations were detected within this thesis. Populations of competing breeding organizations with different goals were combined so that these stratifications could be expected. The statistical analyses were performed within breeds and various more or less overlapping sub-clusters to avoid inflation of false positive SNPs. Moreover, corrections factors accounting for population stratification were included in statistical model. These methods corrected the existing population stratification sufficiently indicated by optimal λ- values of 1.0.

In this thesis, enlargement of data sets did not consequently led to more identified associations between SNPs and analyzed traits. Higher amounts of correction for population stratification were needed within those analyses. This might explain the lower number of significant SNPs because a substantial proportion of genetic variation is eliminated with this correction. Most of genome-wide significant SNPs were found in across organization data sets which do not support the hypothesis above. It seems that the benefit of combining data sets depends on the analyzed trait.

Applying a GWAS with animals genotyped with Illumina 60k Chip resulted in the investigation of genetic background of NBA and the production traits. These detected SNPs were population specific and only confirmed in analysis of direct sub-clusters. In Chapter 3, a few overlapping genomic regions on SSC 12, 16 and 18 were significant associated with different production traits in pigs of both breeds. Moreover, associated SNPs detetcted in this chapter 3 were detected within previously reported regions influencing NBA. Additionally, novel associations were identified for all traits.

Pleiotropic effects are particular important to understand the genetic background of all traits included into the breeding objective and to avoid negative side effect in correlated traits. Within this thesis possible pleiotropic effects for LMP and BF were detected for a single marker located on SSC8 within the study. Moreover, furher pleiotropic effects have been found when the results of our studies were compared with genomic regions described in previously articles.

In general, it remains questionable if the statistical power of our univariate analyses to detect pleiotropic effects was sufficient. In our study a high amount of SNPs were found with multivariate PCs approaches. Considerable numbers of significant SNPs for PC with opposite loadings of EBVs were detected. These results underline the theoretical potential of PCA for detecting pleiotropic effects and the requirement of consideration of those antagonistic relationships in order to achieve the targets of a well-balanced breeding objective with fitness,

reproduction and production traits particular in the situation when applying efficient selection tools like genomic selection.

6 CHAPTER 6: SUMMARY

The number of piglets born alive (NBA) per litter is one of the most important reproduction traits due to its influence on production efficiency pig breeding. The main reason for removing sows from herd is poor performance in NBA (Hoge and Bates, 2011). In the recent past, pig breeding organizations have been focused on the breeding of sows with high number of NBA in order to generate higher profits in piglet production (de Koning et al., 2001; Geisert and Schmitt, 2002; Hanenberg et al., 2001; Lewis et al., 2005). Despite low heritability (h^2) and the complex genetic basis, considerable genetic progress has been achieved for NBA in many breeding organisations. However, antagonistic relationships within reproduction trait complexes (piglet survival, IBW) and between reproduction and later growth performance and carcass traits were reported (Brien, 1986; Haley et al., 1988; Roehe and Kalm, 2000). These antagonistic relationships must be clarified in detail because reproduction and production trait complexes are responsible for the economic profit in swine production (Rothschild et al., 1996).

In order to improve the biological and genetic knowledge of NBA and to identify possible pleiotropic effects between NBA and production traits, Genome-Wide Association Studies (GWAS) were performed. In total, 4,012 Large White (LW) and Landrace (LR) pigs from herdbook and commercial breeding companies in Germany, Austria and Switzerland were genotyped with the Illumina PorcineSNP60 BeadChip. EBVs of NBA and the three production traits average daily gain (ADG), lean meat percentage (LMP) and backfat (BF) were used as phenotypes. These EBVs were routinely estimated by the breeding organizations.

Two different GWAS studies were conducted. The aims of the first study were a) to reveal genetic similarities and differences between LW and LR populations of competing breeding organisations, b) to identify significant associated SNPs for NBA, and c) to clarify the biological relevance of these significant markers. Considerable differences between LW and LR populations of the competing breeding organizations were found, so that all GWAS were performed within each breed. In addition, because of population stratification within the two breeds, five further sub-clusters were formed within each breed, which were analysed separately. These sub-clusters comprised genetic similar pigs from one to three breeding organisation. In total, GWAS of all clusters resulted in 17 significant markers affecting NBA. Most of these markers were found within regions with already known influence on female reproduction or previously reported QTLs detected for litter traits. However, only a few (four) of these QTLs were found in more than one sub cluster. From this can be concluded that in

each sub-population litter size is influenced by different alleles and it is questionable if the combination of genetically divergent sub-populations is a useful strategy for detecting relevant QTL or improving the accuracy of genomic selection.

The second GWA study is focused on the detection of possible pleiotropic effects for NBA and production traits (LMP, ADG, BF) using the data sets described above. To identify possible pleiotropic effects, two different approaches were performed. First, univariate GWAS were performed using breeding organization overlapping clusters and within the specific LW and LR population of each organization. Results of these analyses were compared. Moreover, multivariate PCA were used for the detection of pleiotropic effects to increase the power of SNP detection. With this approach, EBVs were condensate to PCs reflecting all traits which were then used as new phenotype for GWAS. Because of differences in the estimation of breeding values and the number of available EBVs within each breeding organization, multivariate GWAS was performed within each breeding organization. In total, with univariate approach 79 significant QTLs were identified which were positioned on all chromosomes of the pig genome. However only one significant SNP located on chromosome 8 was found, which were identical for LMP and BF. Furthermore, regarding a two Mb window, only three overlapping regions on SSC 12, 16 and 18 for two traits and across the breeds were identified. Based on the multivariate GWAS 98 significant markers for uncorrelated PCs were identified. Ten of these markers were already detected with univariate GWAS. The PCs were dominated by two or three different EBVs, which showed partly opposite signs of the corresponding loadings indicating an antagonistic relationship. These findings demonstrate that a considerable number of genomic regions might have an (antagonistic) pleiotropic effect on production and reproduction traits. This is a valuable information to achieve the defined objectives of a balanced breeding objective in pig breeding programs. Moreover, with multivariate approach a higher number of significant markers were detected. This underlines the higher power of QTL detection with pleiotropic effects using multivariate approach.

7 CHAPTER 7: REFERENCES

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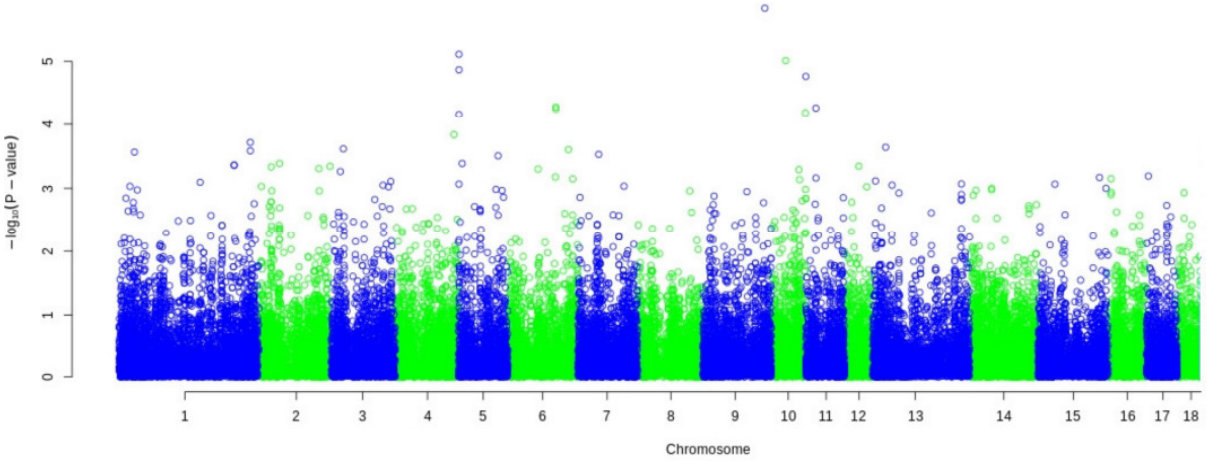
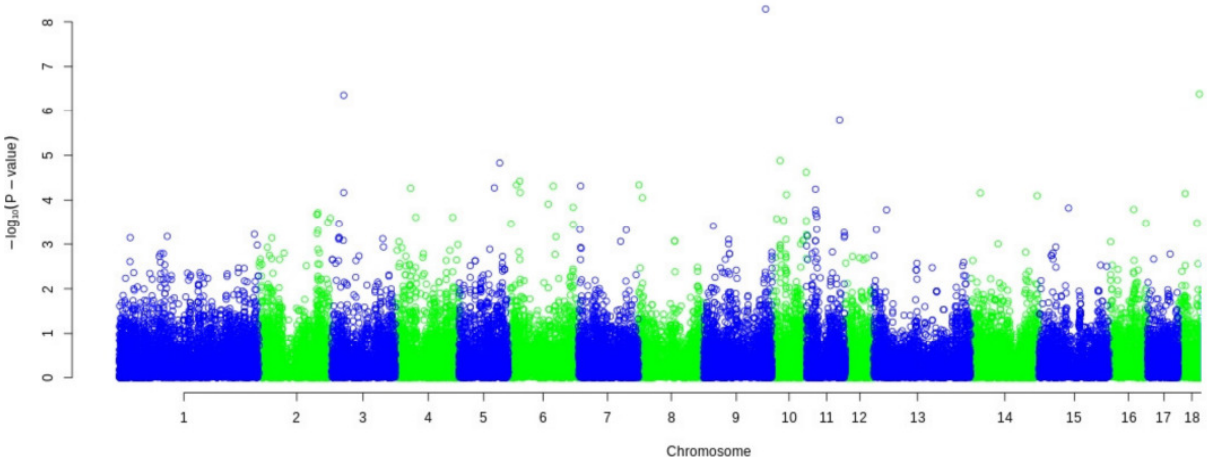
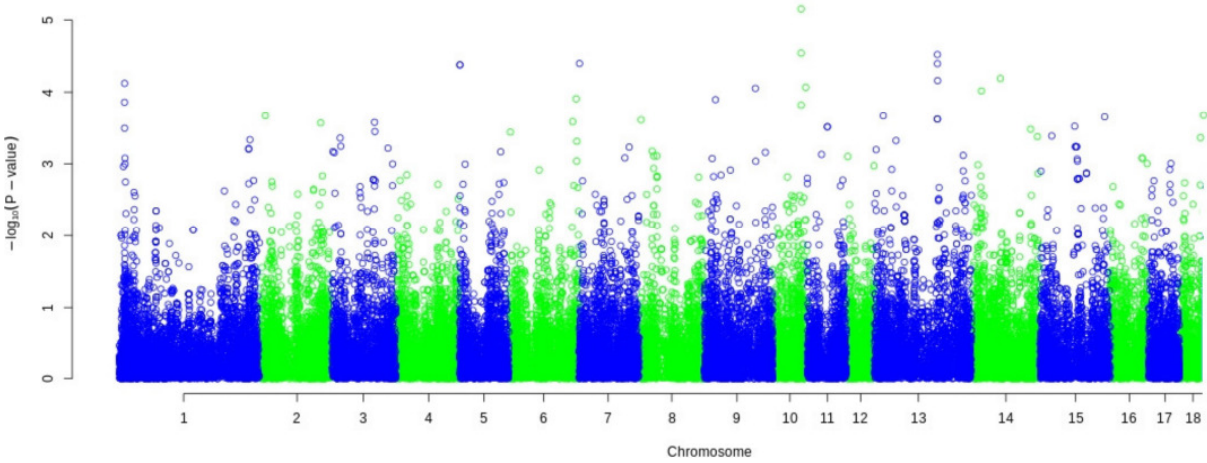
8 CHAPTER 8: APPENDIX



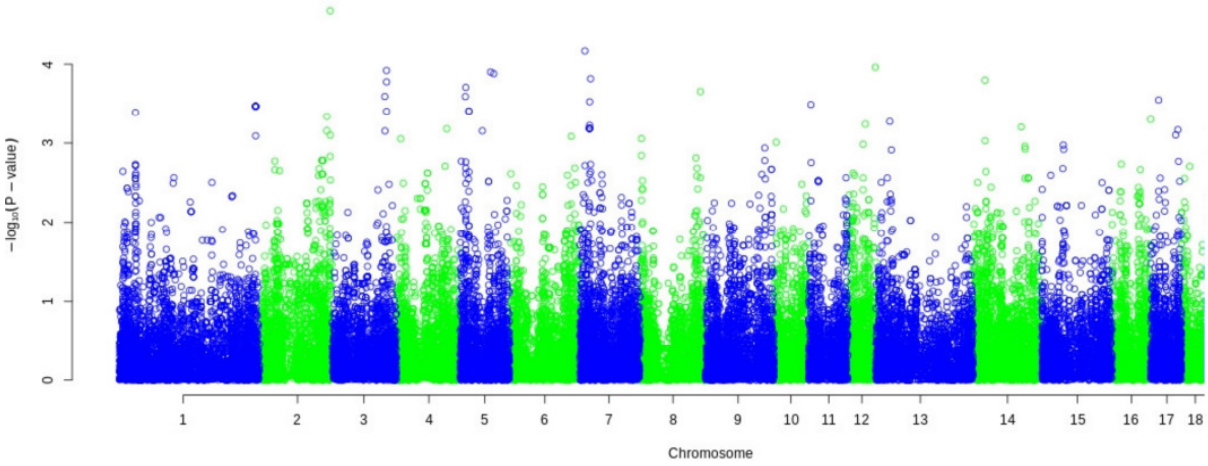
SI 1: Manhattan plot of Genome-Wide Association Study for NBA in LW_2



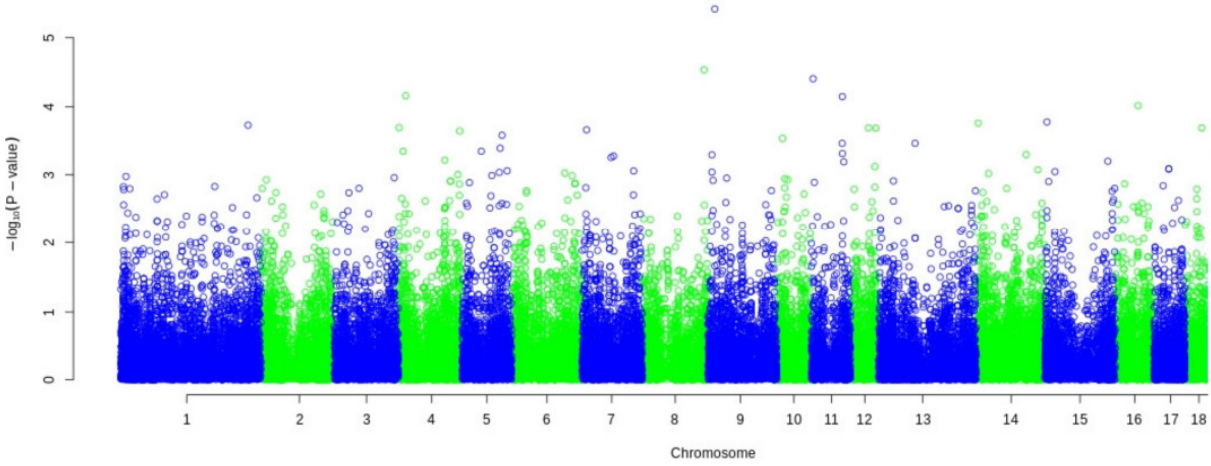
SI 2: Manhattan plot of Genome-Wide Association Study for NBA in LW_2a



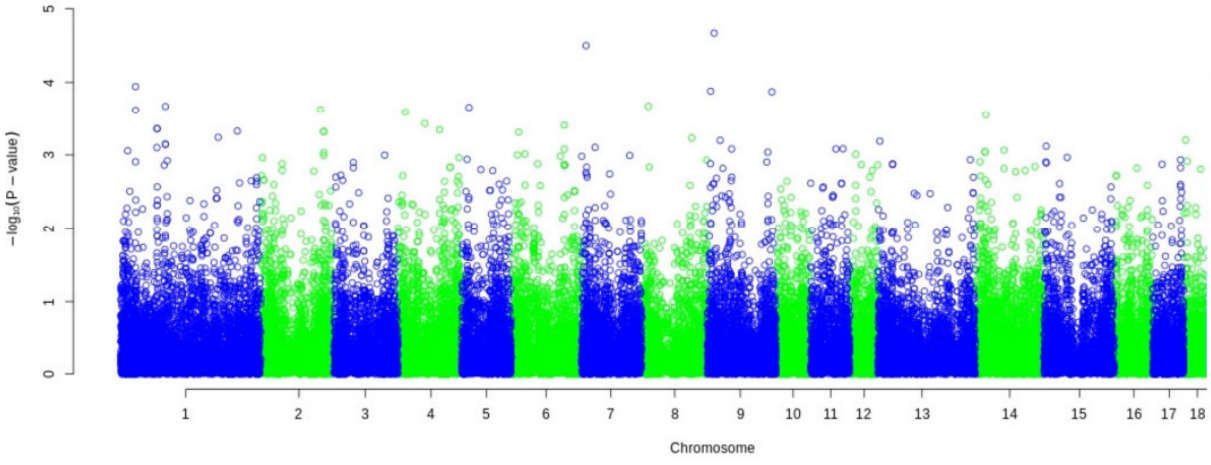
SI 3: Manhattan plot of Genome-Wide Association Study for NBA in LW_2b



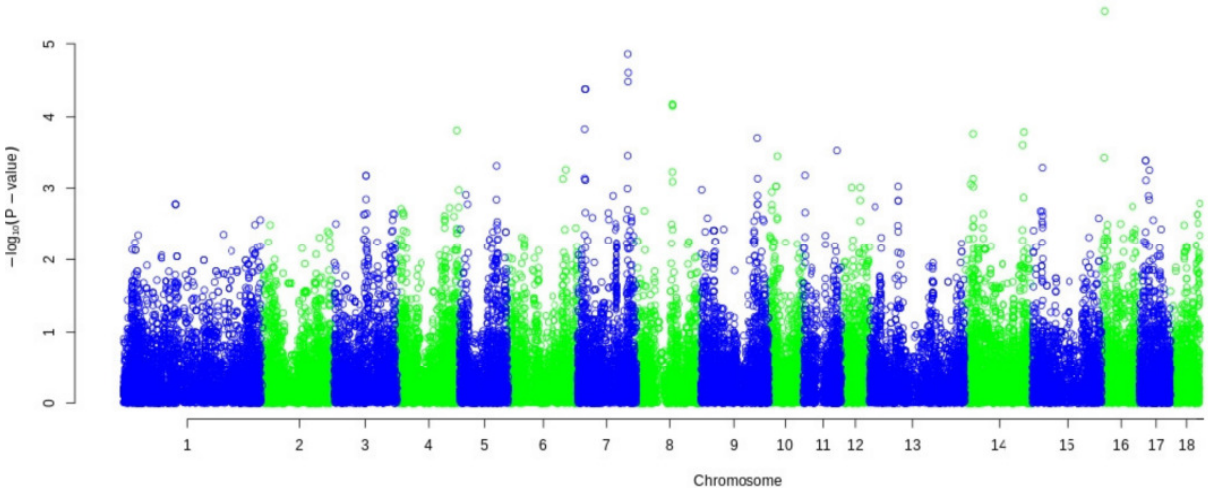
SI 4: Manhattan plot of Genome-Wide Association Study for NBA in LW_3



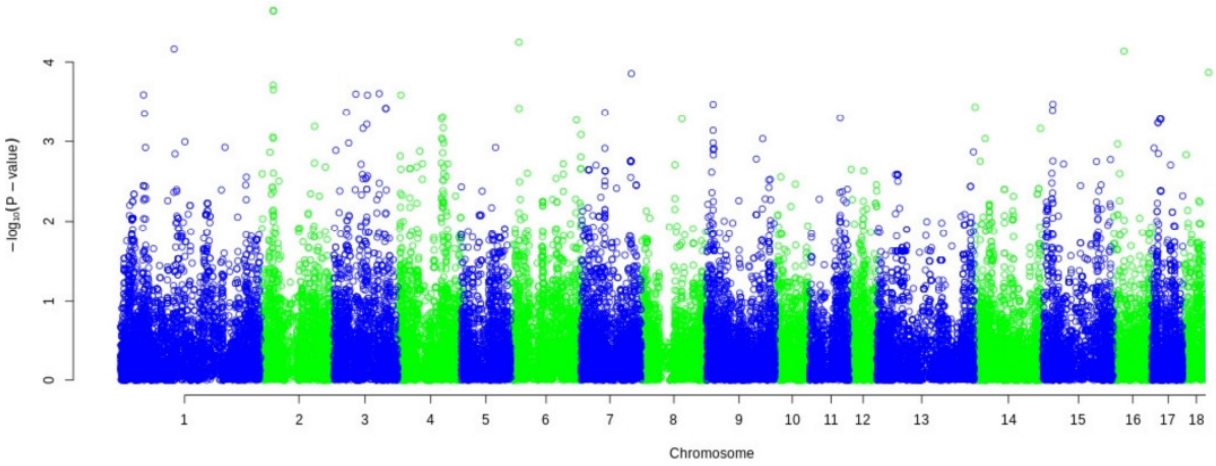
SI 5: Manhattan plot of Genome-Wide Association Study for NBA in LR_1



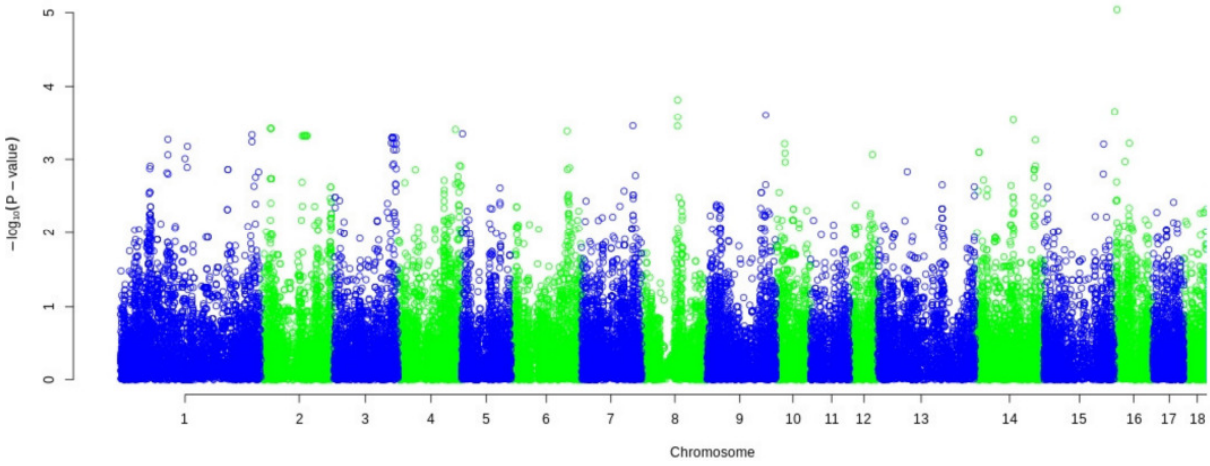
SI 6: Manhattan plot of Genome-Wide Association Study for NBA in LR_2



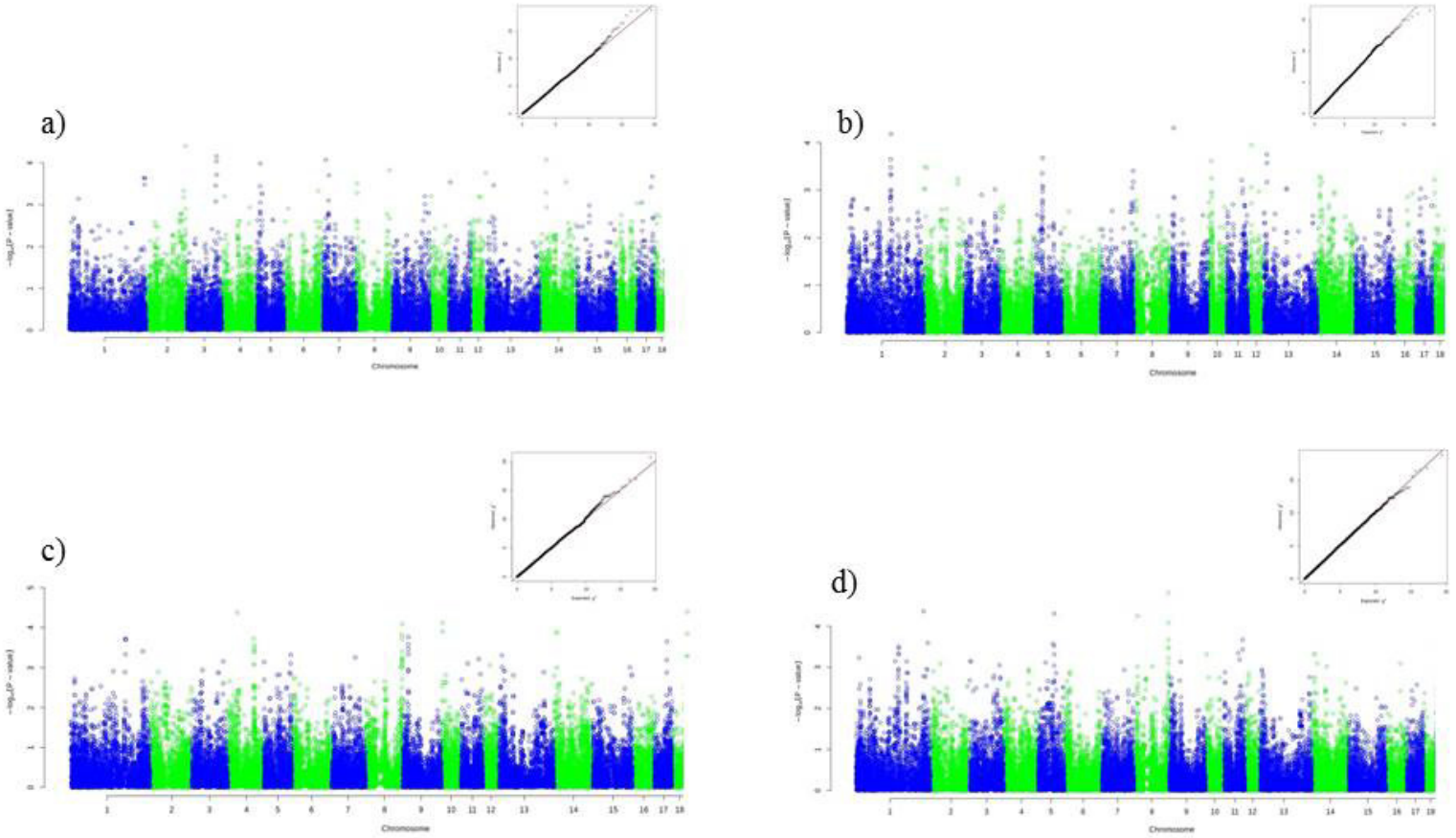
SI 7: Manhattan plot of Genome-Wide Association Study for NBA in LR_3



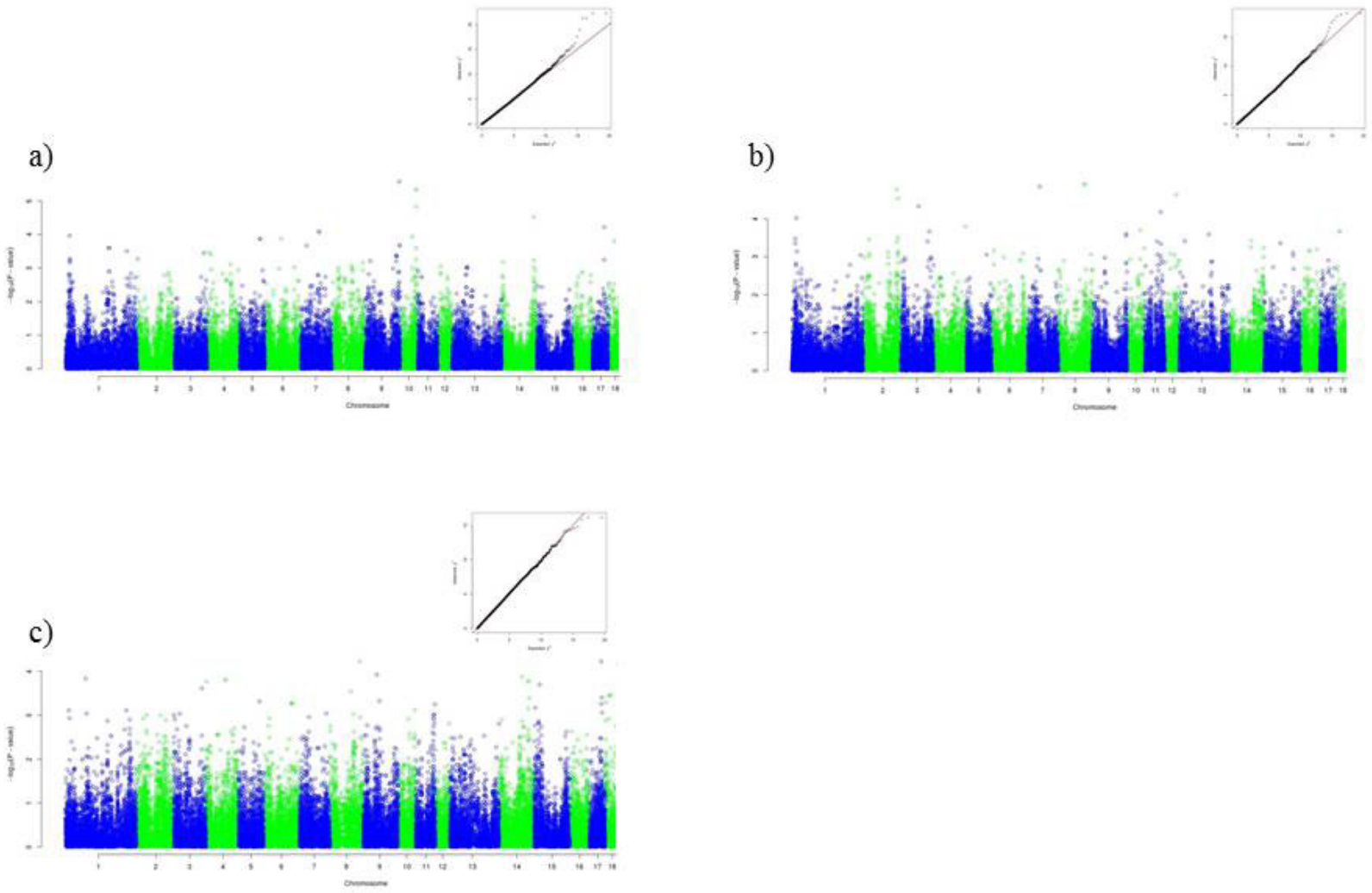
SI 8: Manhattan plot of Genome-Wide Association Study for NBA in LR_3a



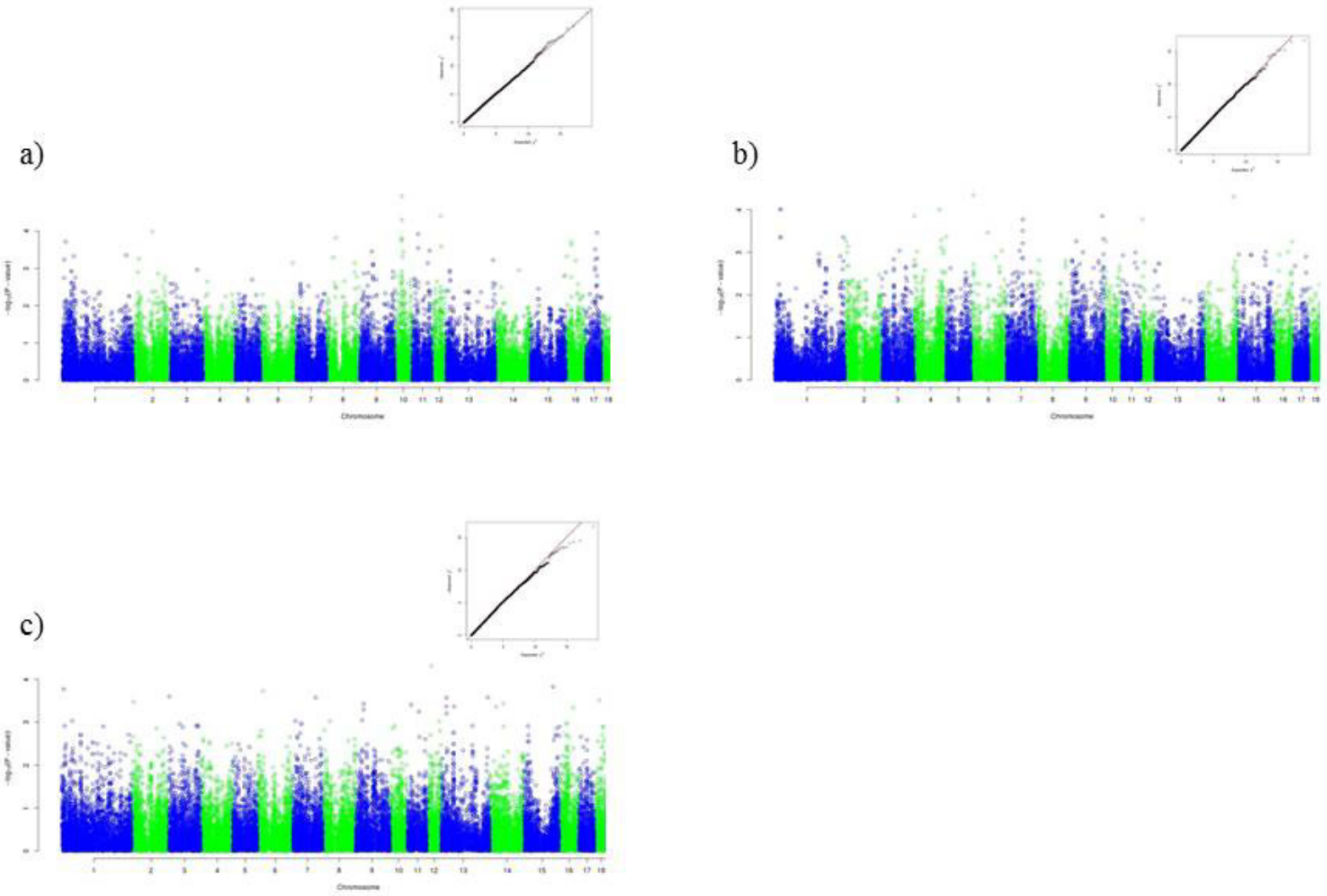
SI 9: Manhattan plot of Genome-Wide Association Study for NBA in LR_3b



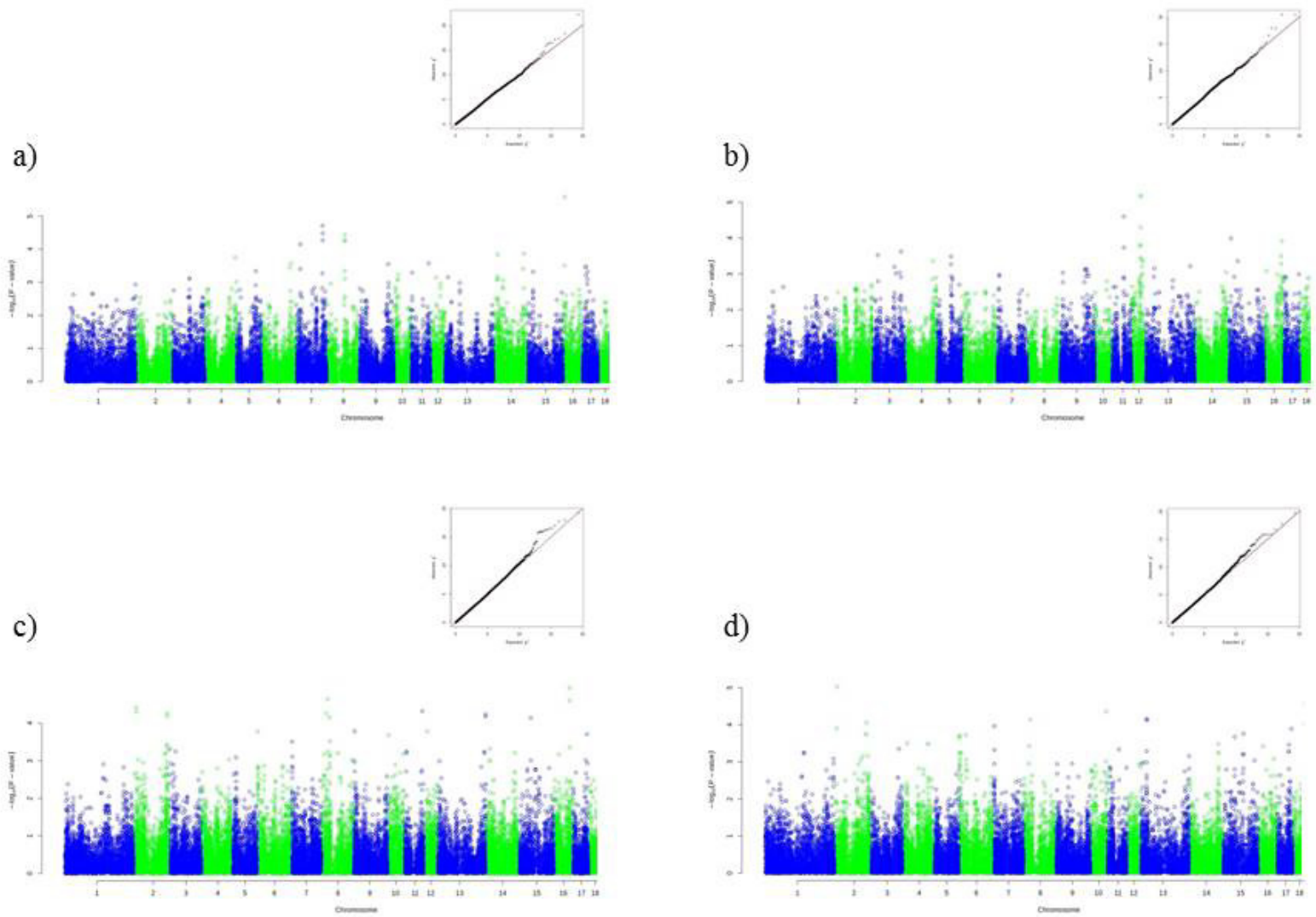
SI 10: Manhattan Plots and corresponding Q-Q plots of Org2_LW for a) NBA, b) ADG, c) LMP and d) BF



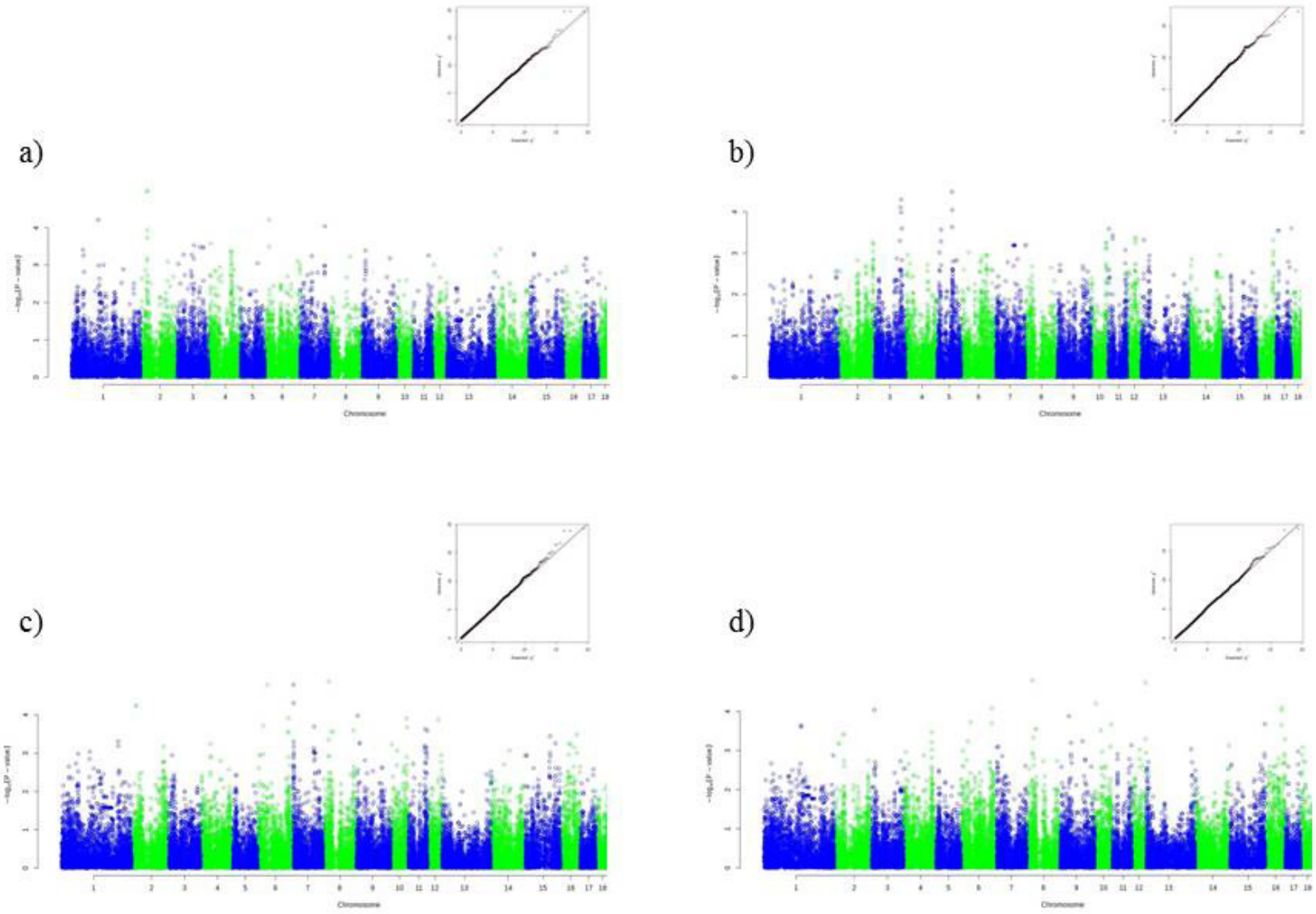
SI 11: Manhattan Plots and corresponding Q-Q plots of Org3_LW for a) NBA, b) ADG and c) LMP



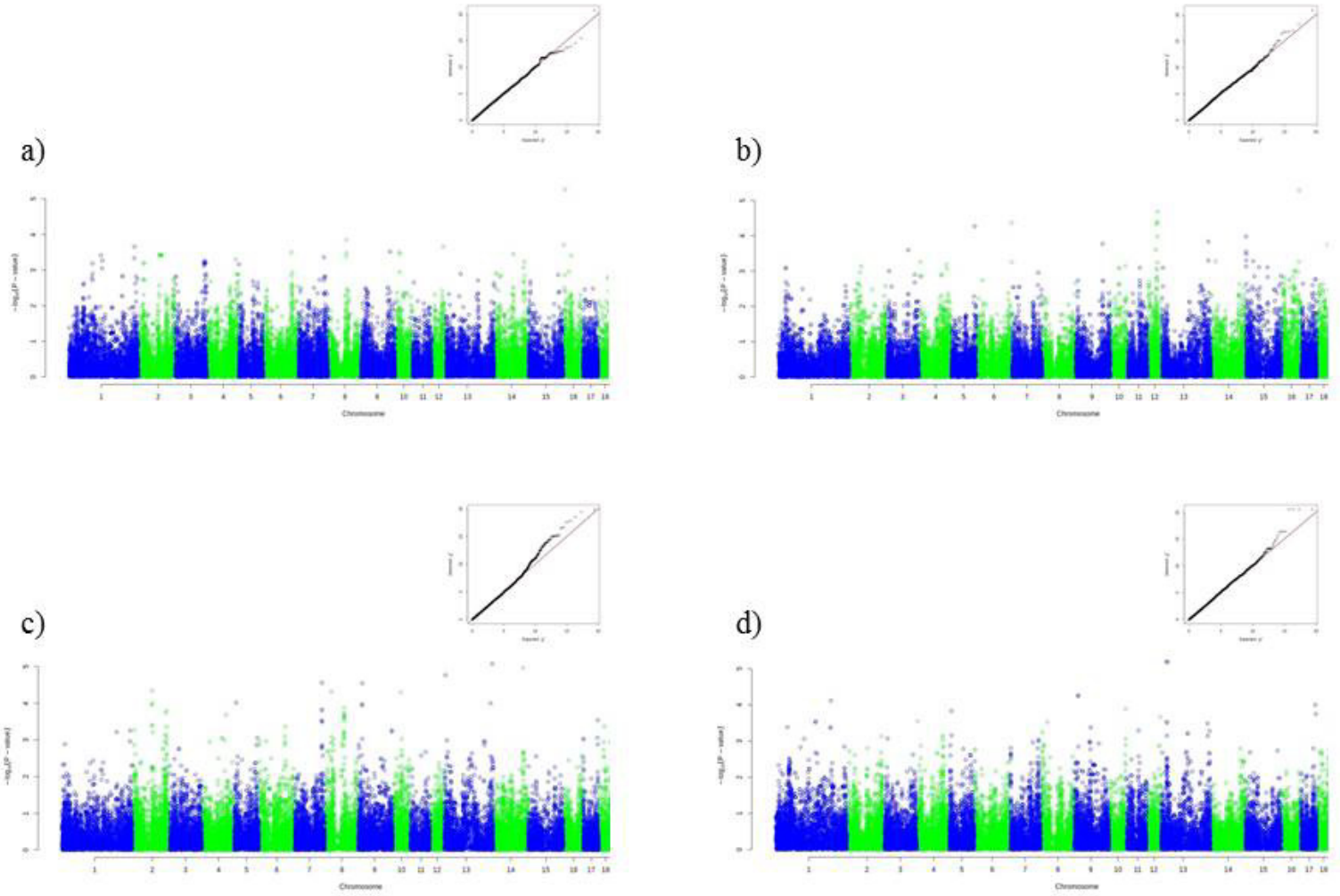
SI 12: Manhattan Plots and corresponding Q-Q plots of Org4_LW for a) NBA, b) ADG and c) LMP



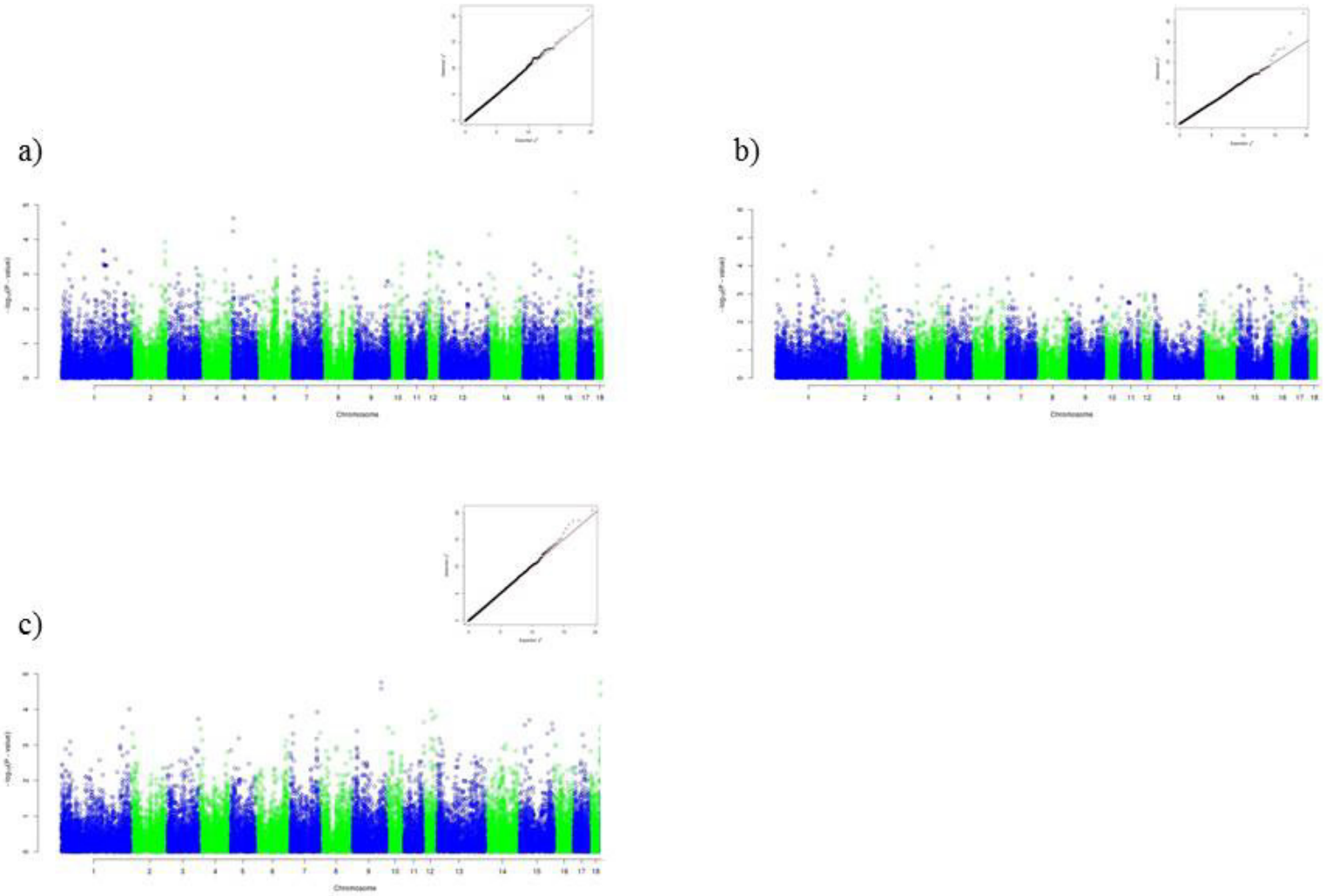
SI 13: Manhattan Plots and corresponding Q-Q plots of Org2_LR for a) NBA, b) ADG, c) LMP and d) BF



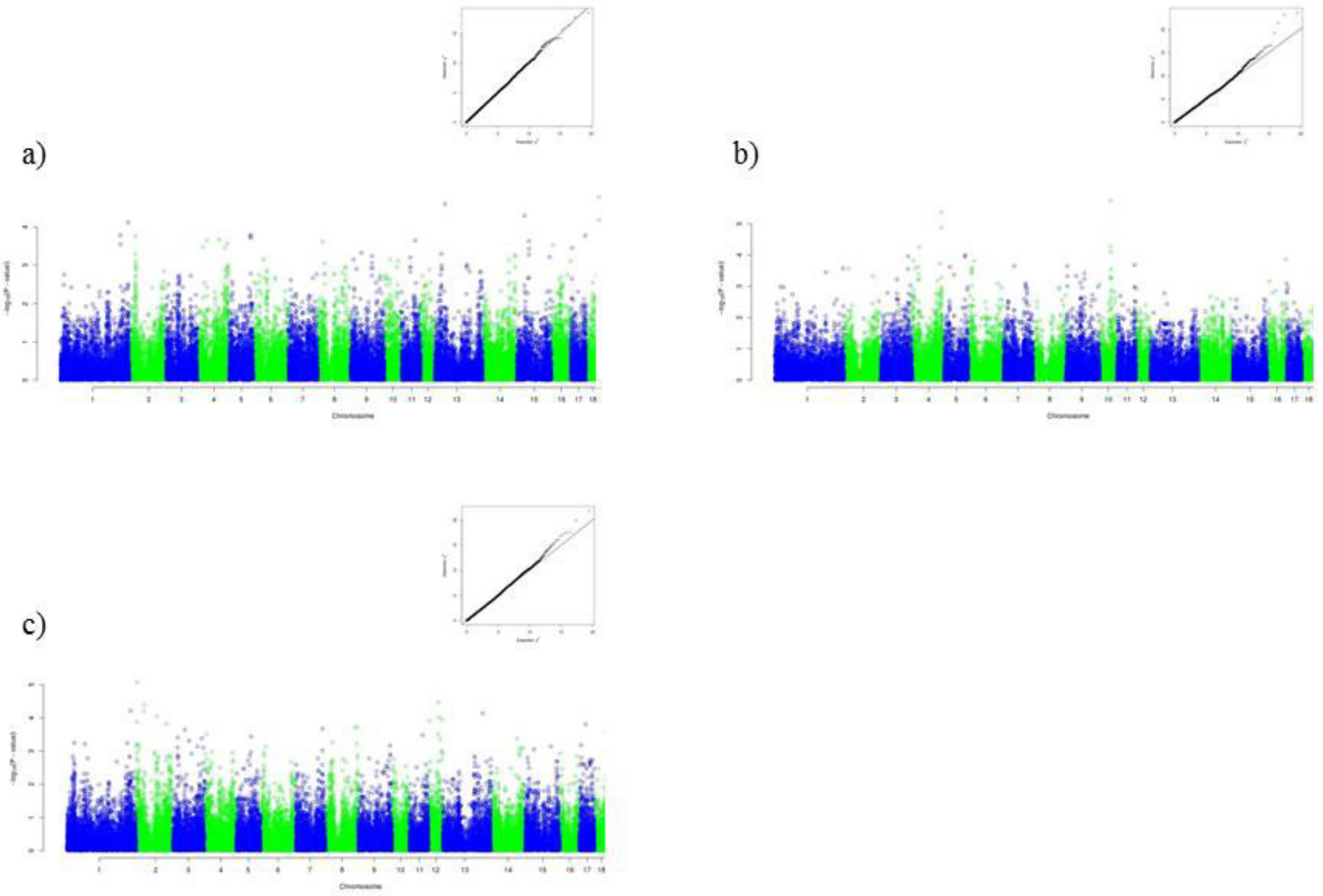
SI 14: Manhattan Plots and corresponding Q-Q plots of Org2a_LR for a) NBA, b) ADG, c) LMP and d) BF



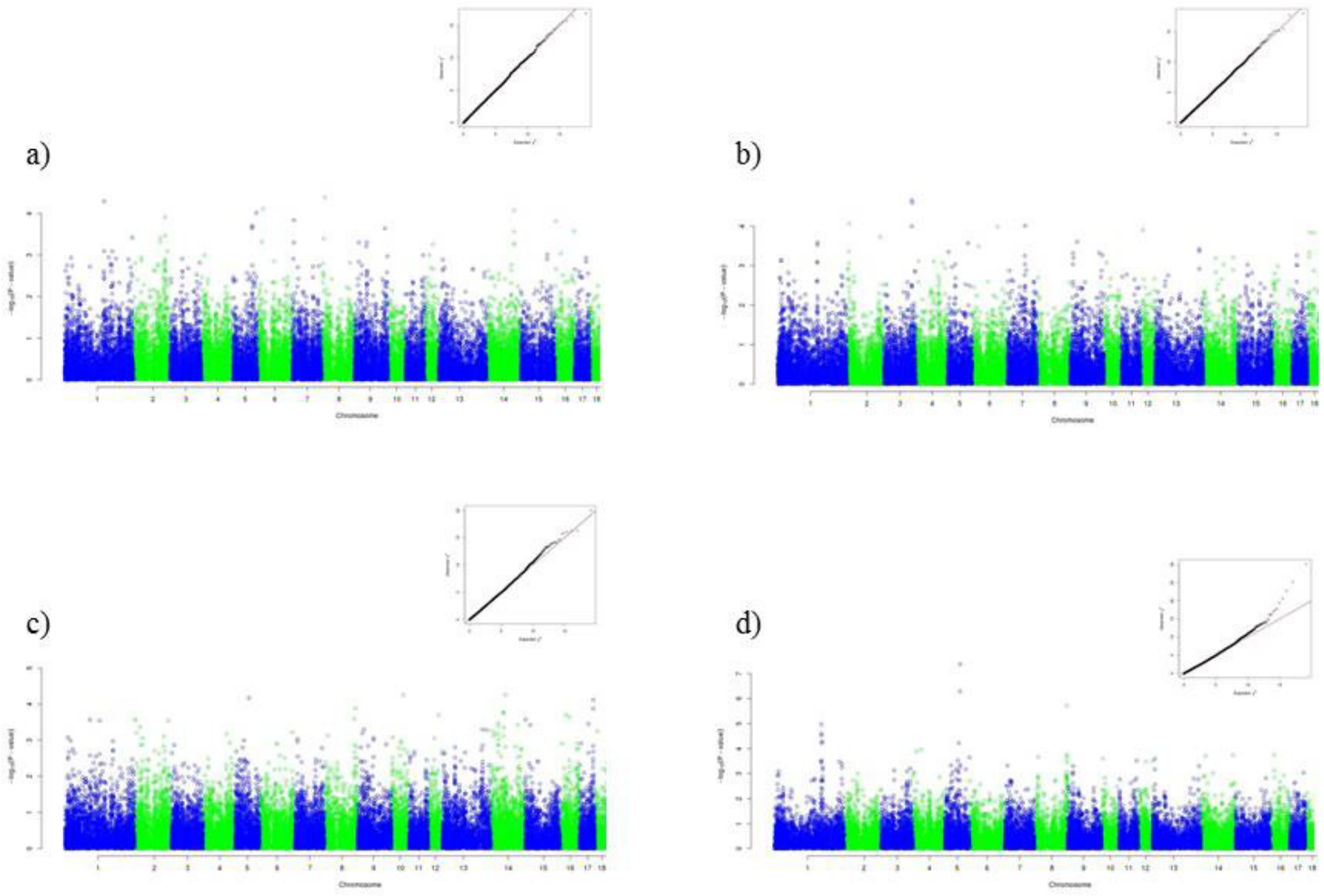
SI 15: Manhattan Plots and corresponding Q-Q plots of Org2b_LR for a) NBA, b) ADG, c) LMP and d) BF



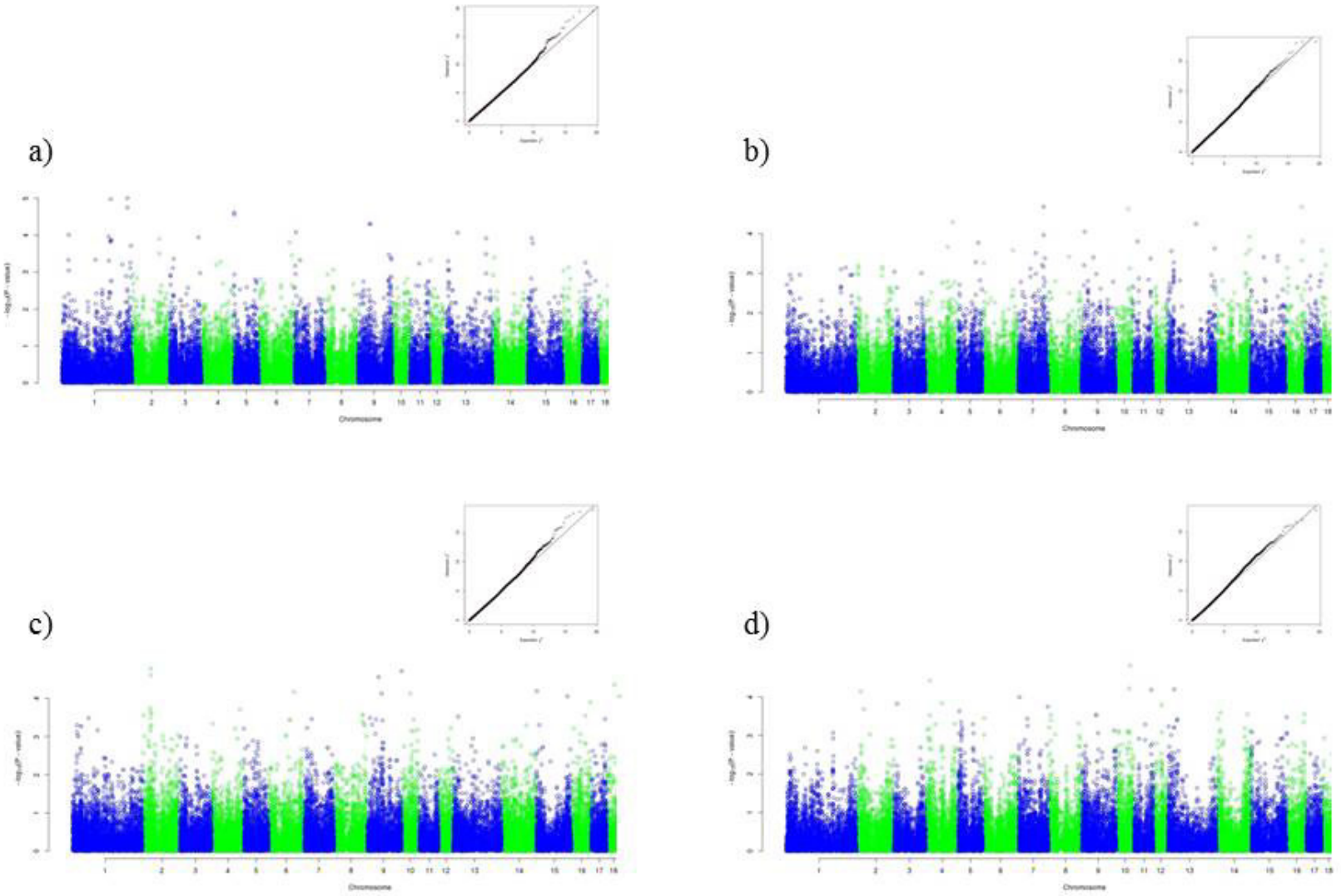
SI 16: Manhattan Plots and corresponding Q-Q plots of Org3_LR for a) NBA, b) ADG and c) LMP



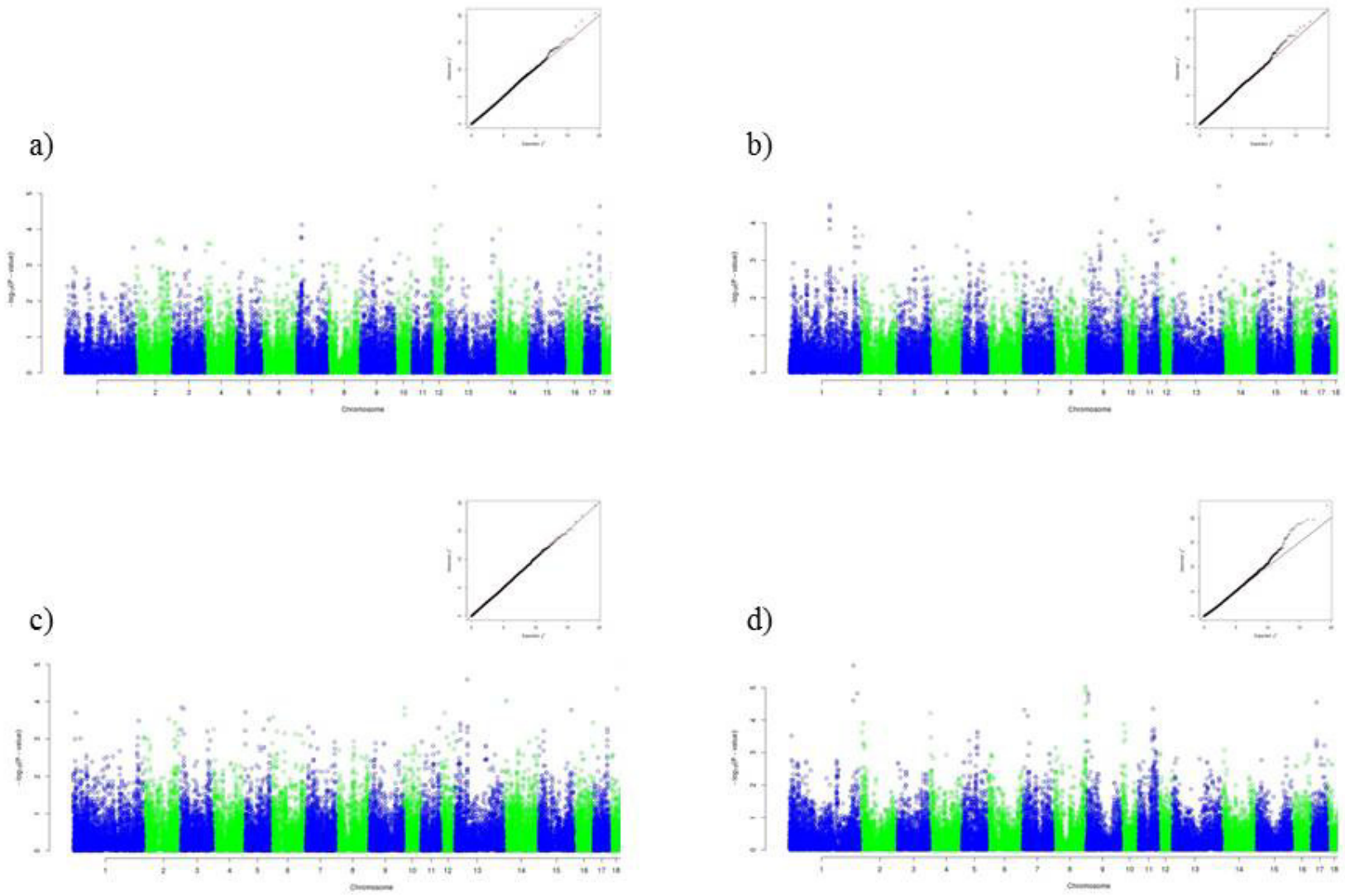
SI 17: Manhattan Plots and corresponding Q-Q plots of Org4_LR for a) NBA, b) ADG and c) LMP



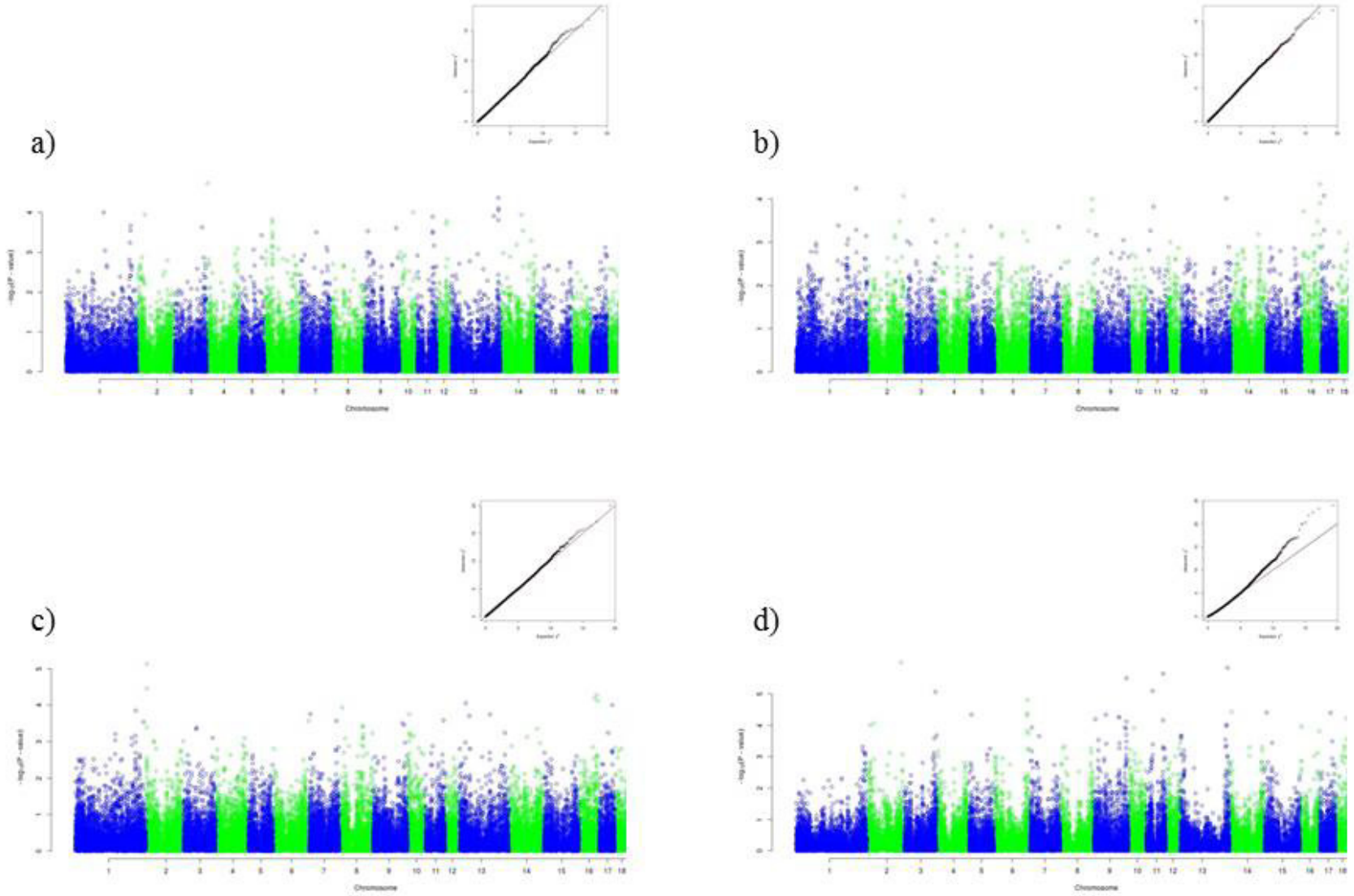
SI 18: Manhattan Plots and corresponding Q-Q plots of LW_1 for a) NBA, b) ADG, c) LMP and d) BF



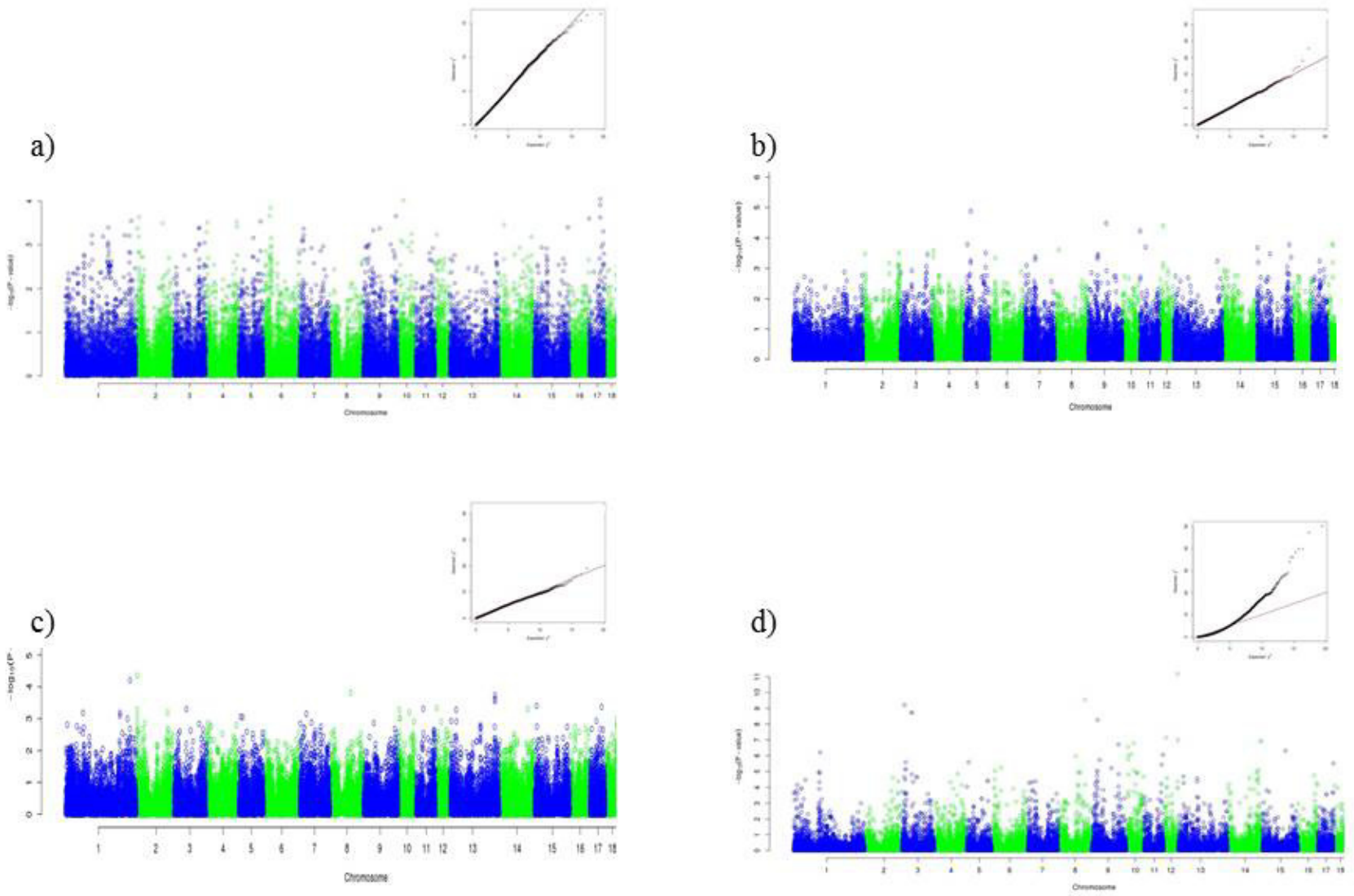
SI 19: Manhattan Plots and corresponding Q-Q plots of LW_2 for a) NBA, b) ADG, c) LMP and d) BF



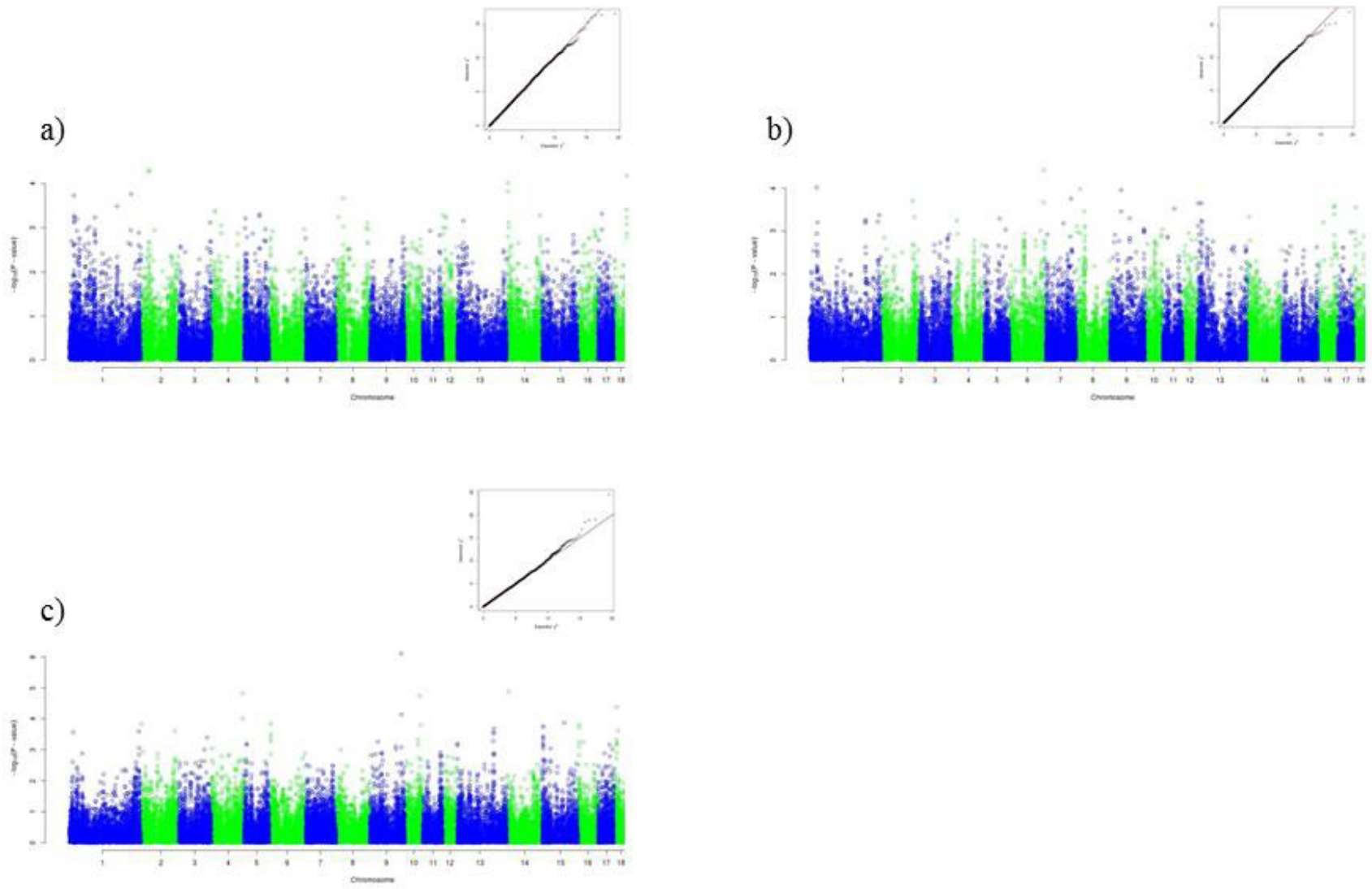
SI 20: Manhattan Plots and corresponding Q-Q plots of LW_2a for a) NBA, b) ADG, c) LMP and d) BF



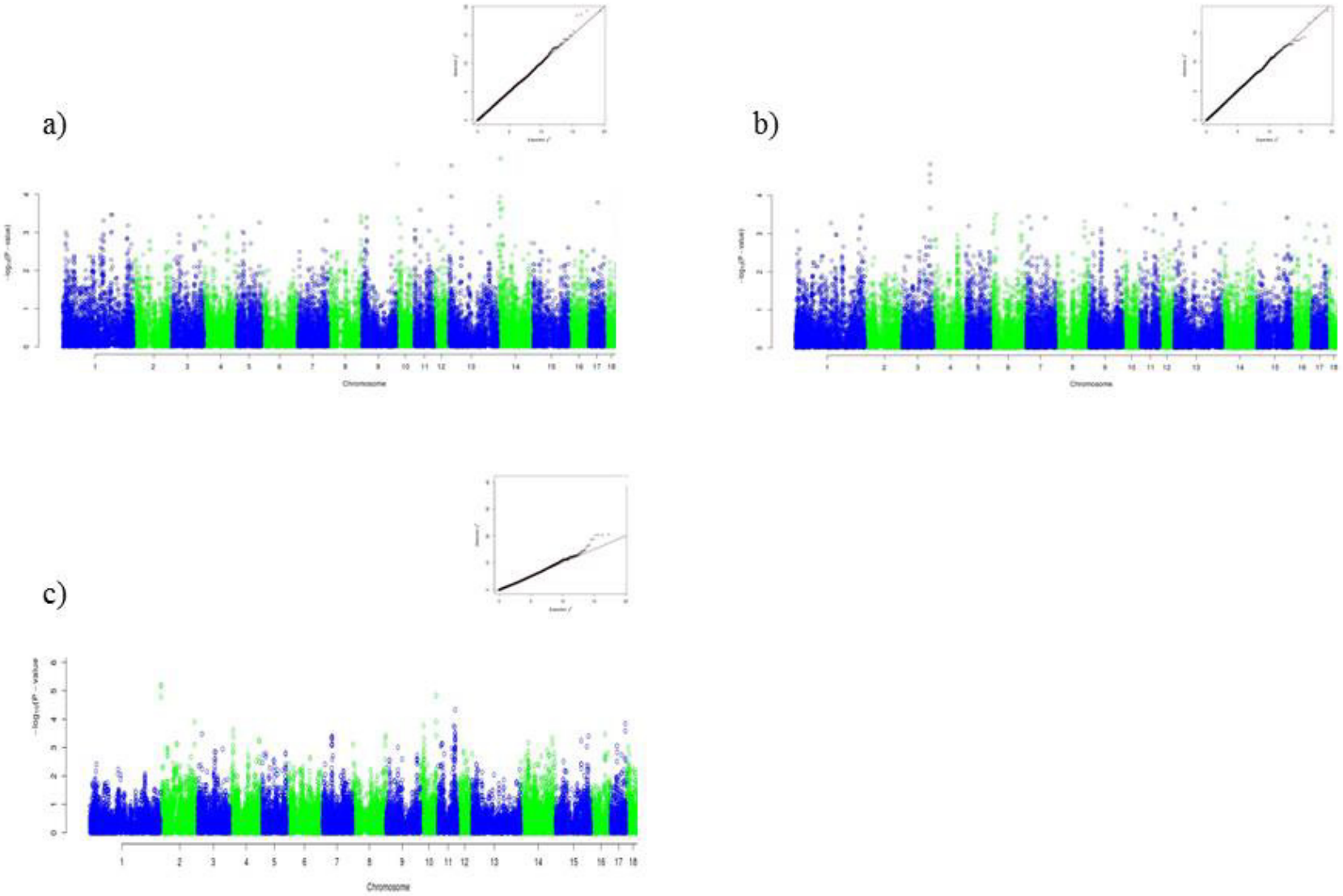
SI 21: Manhattan Plots and corresponding Q-Q plots of LR_1 for a) NBA, b) ADG c), LMP and d) BF



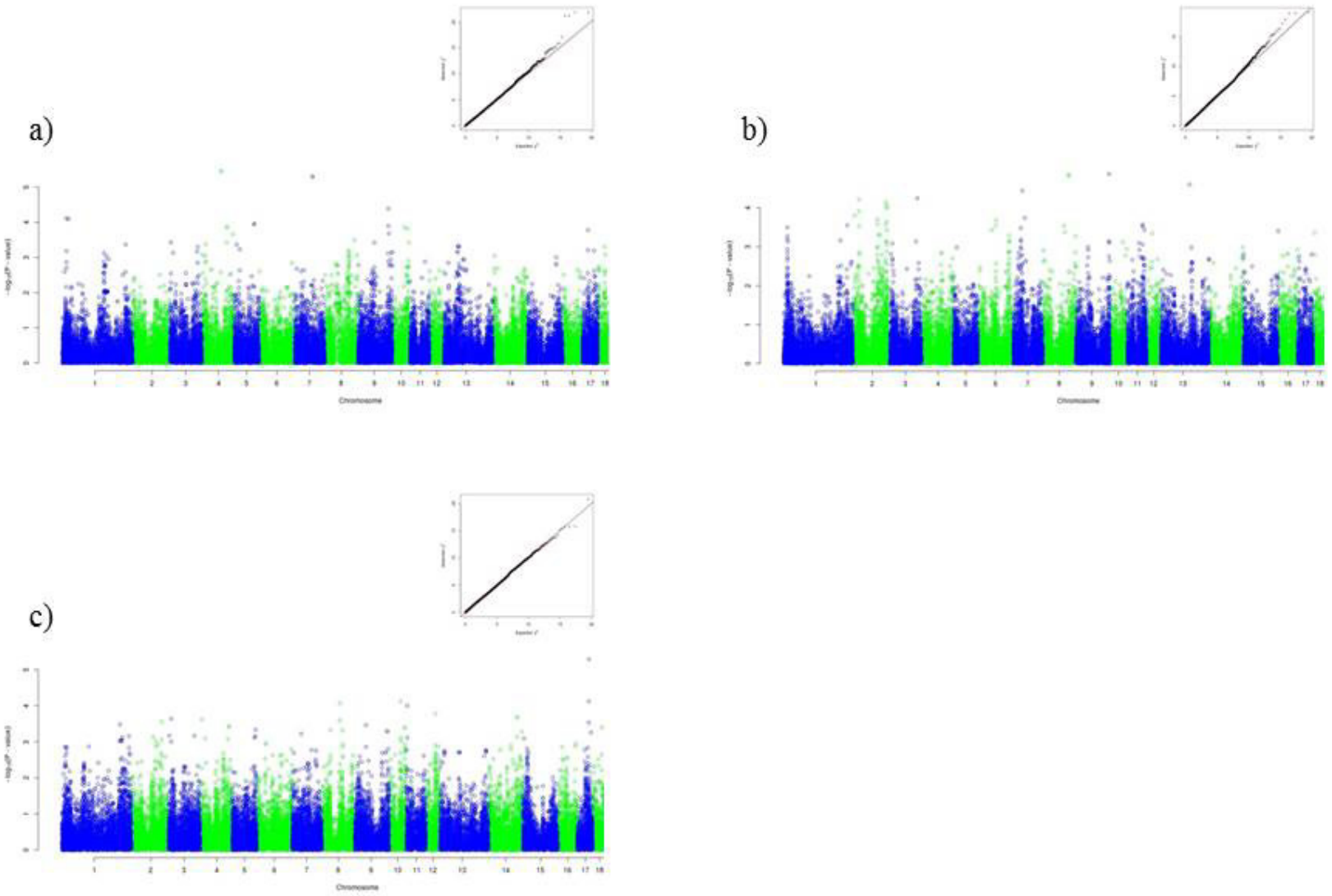
SI 22: Manhattan Plots and corresponding Q-Q plots of LR_2 for a) NBA, b) ADG, c) LMP and d) BF



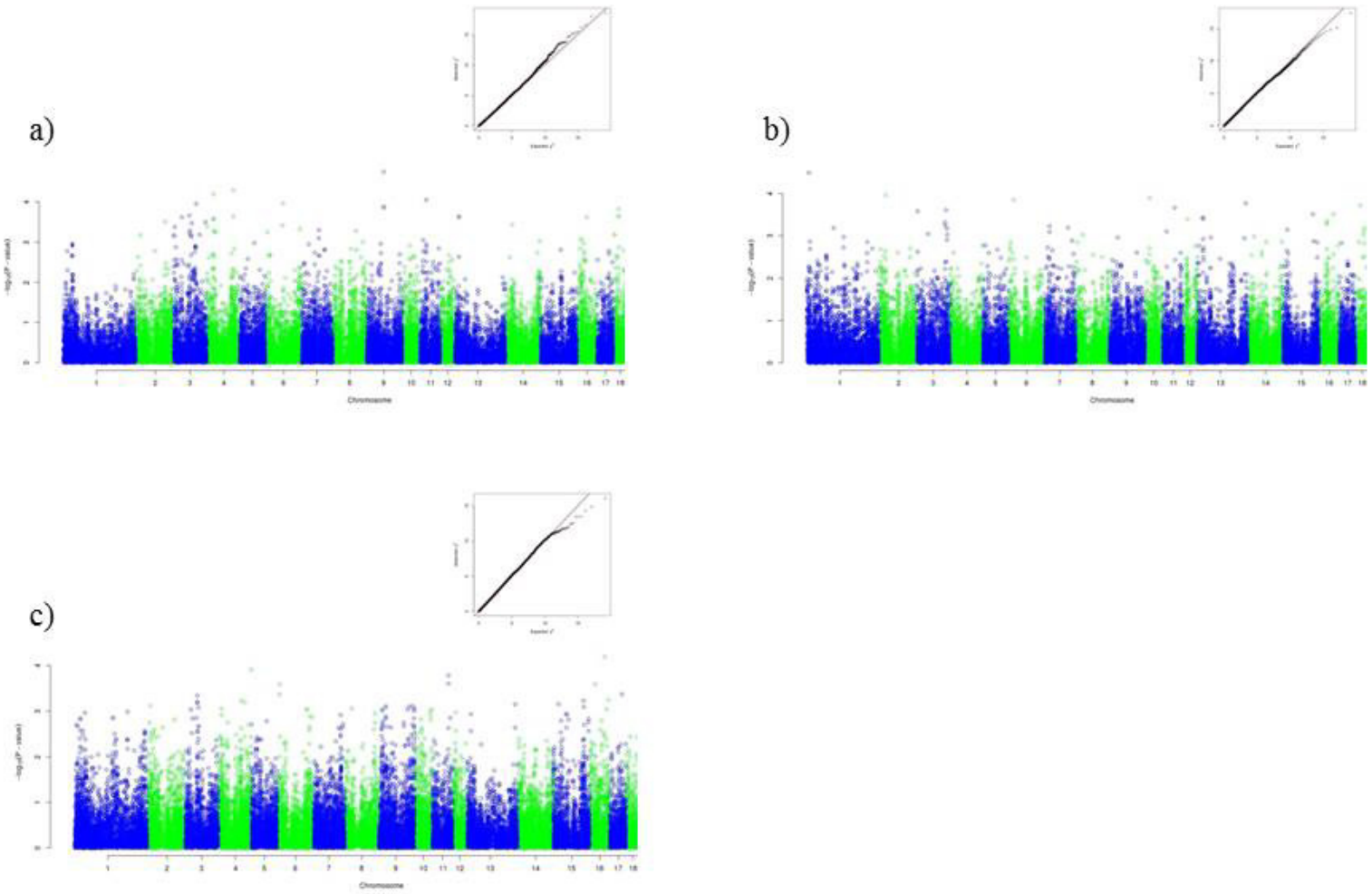
SI 23: Manhattan Plots and corresponding Q-Q plots of Org1_LW for a) PC1, b) PC2 and c) PC3



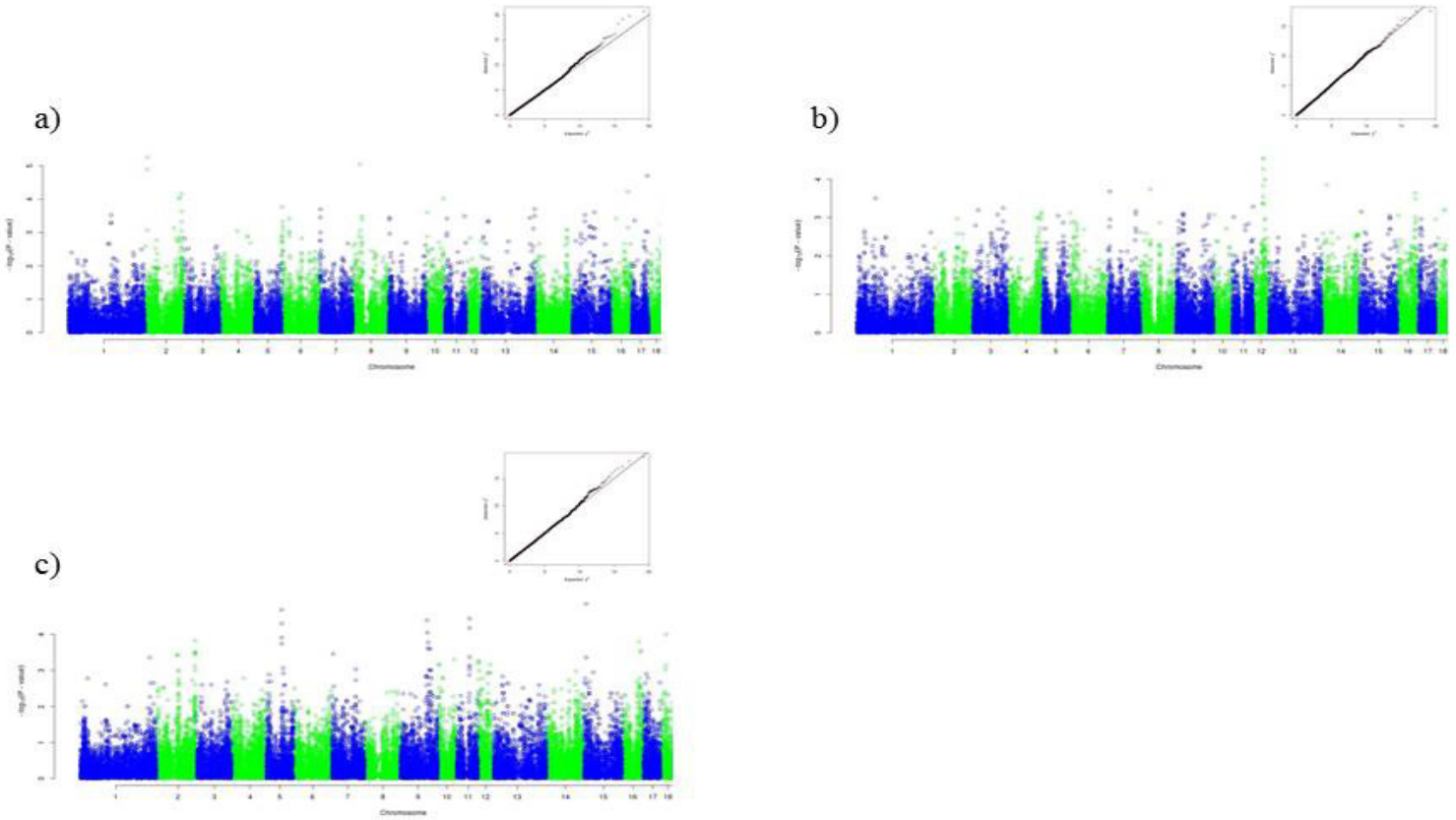
SI 24: Manhattan Plots and corresponding Q-Q plots of Org2_LW for a) PC1, b) PC2 and c) PC3



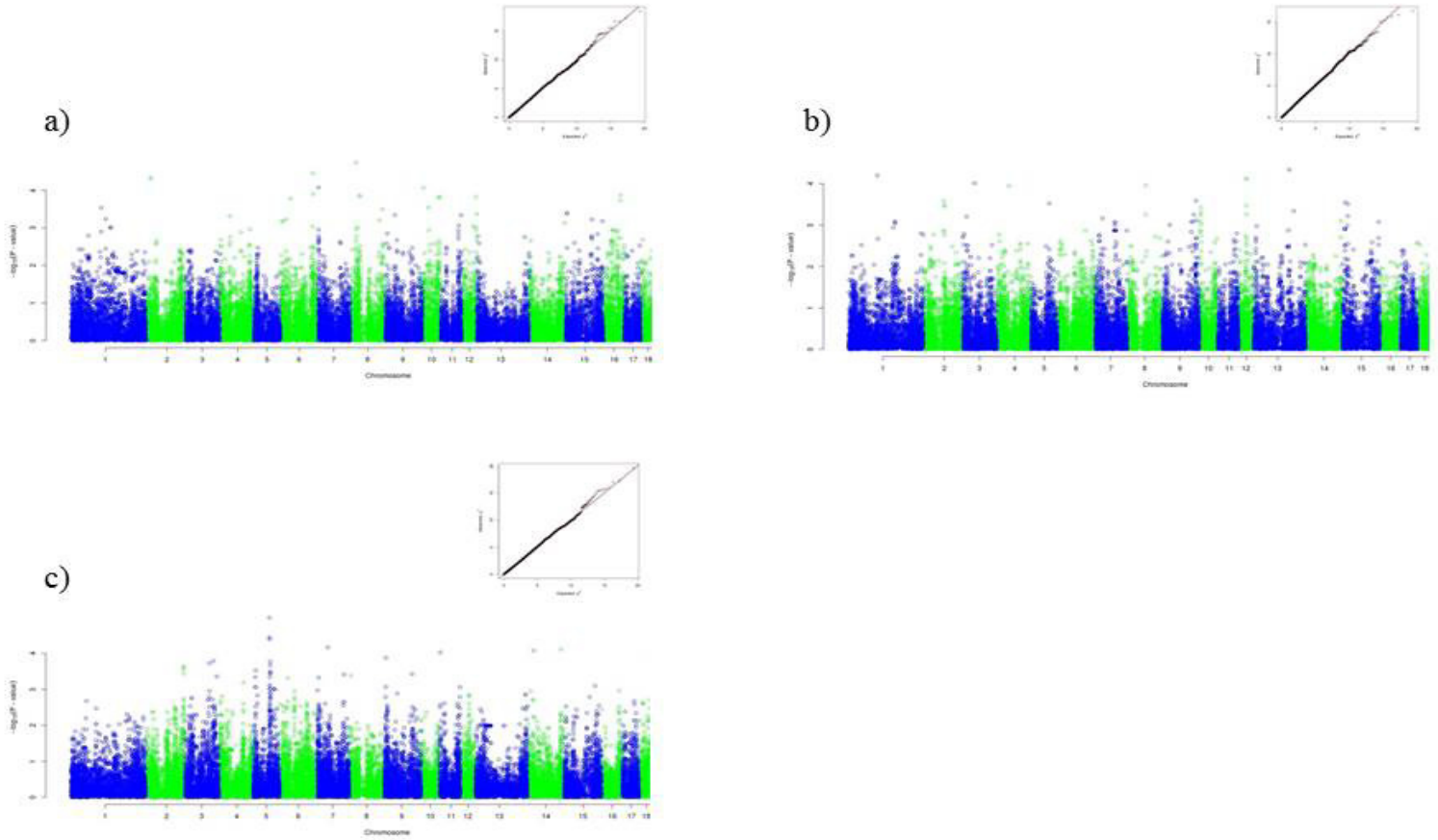
SI 25: Manhattan Plots and corresponding Q-Q plots of Org3_LW for a) PC1, b) PC2 and c) PC3



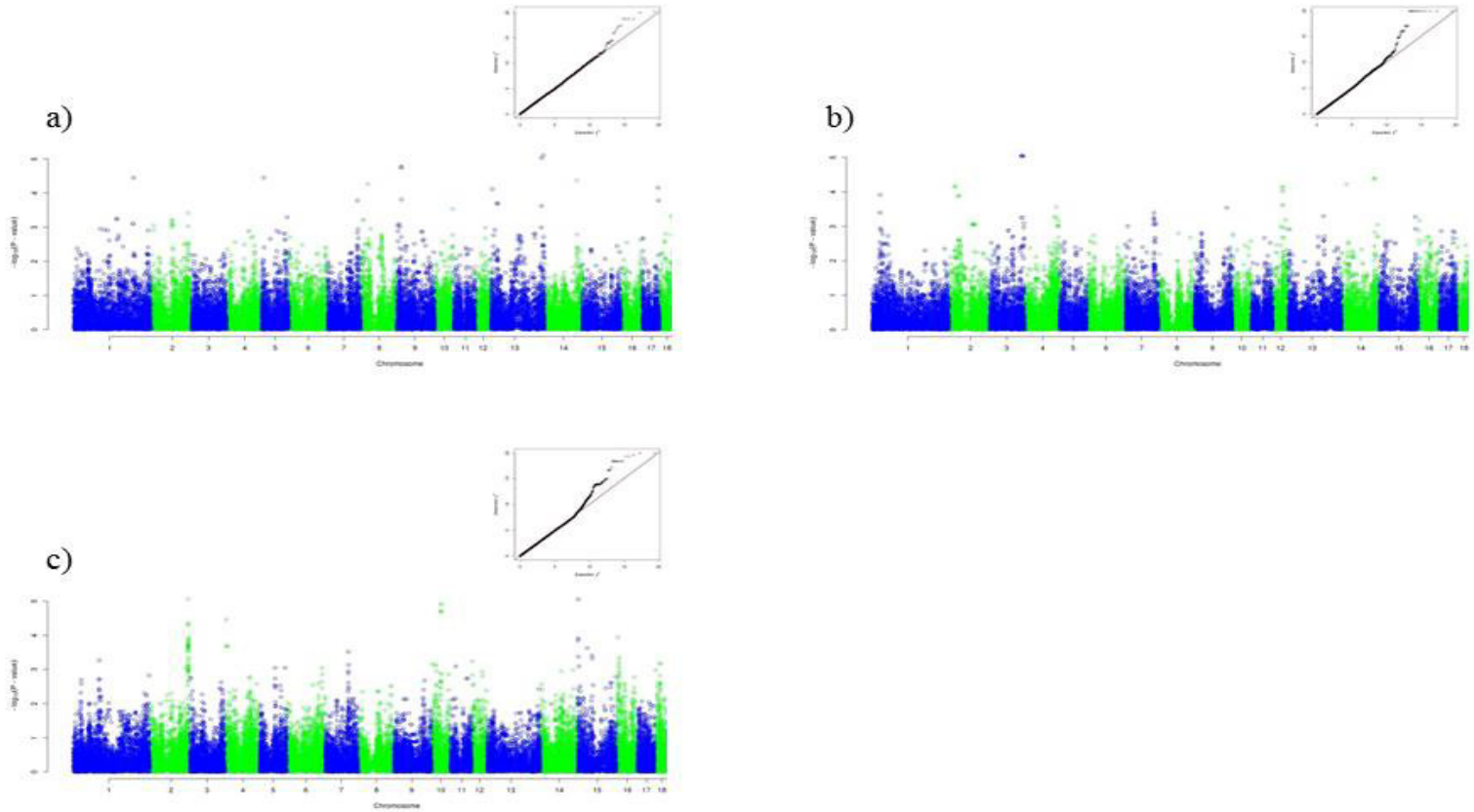
SI 26: Manhattan Plots and corresponding Q-Q plots of Org4_LW for a) PC1, b) PC2 and c) PC3



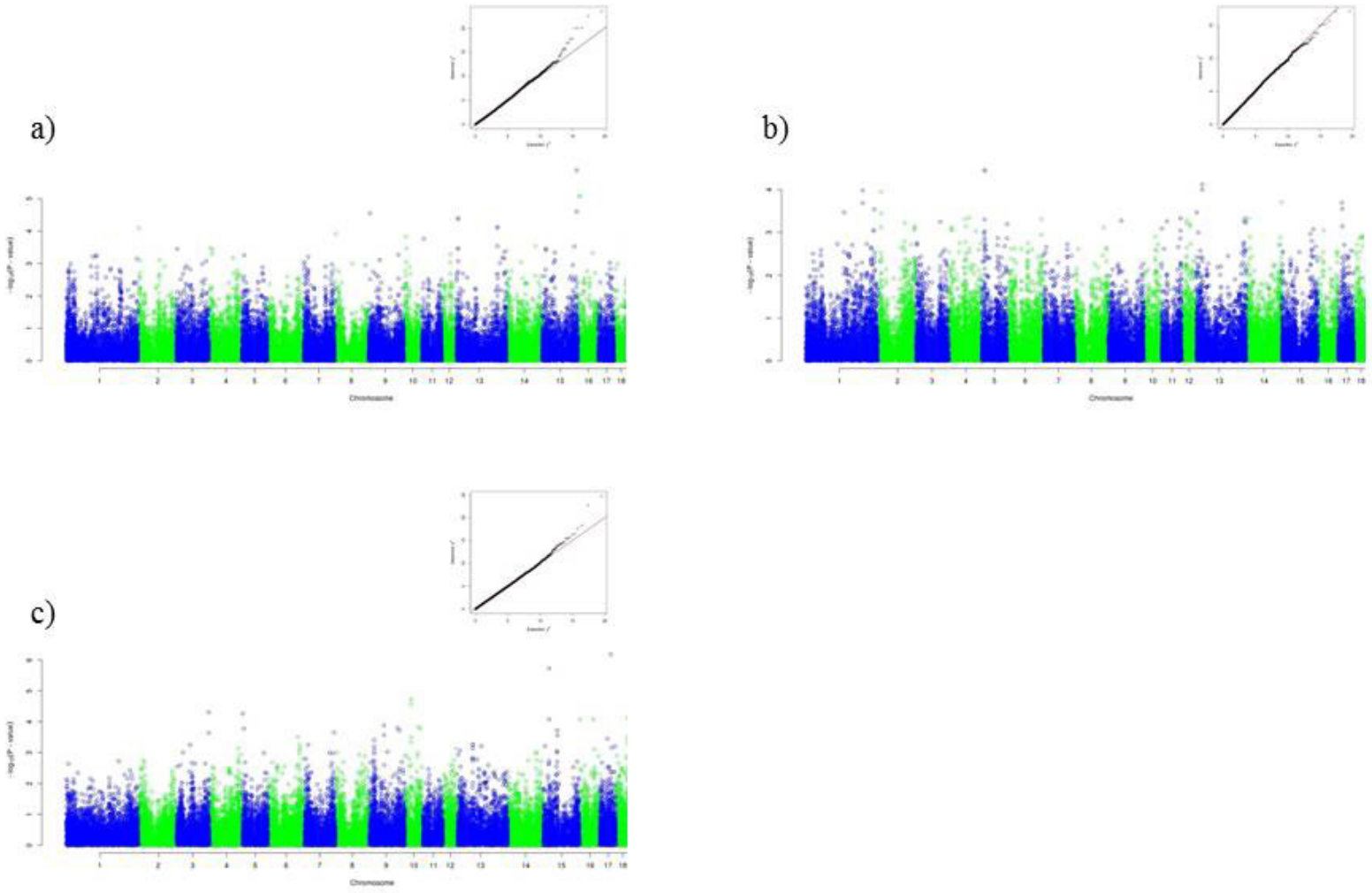
SI 27: Manhattan Plots and corresponding Q-Q plots of Org2_LR for a) PC1, b) PC2 and c) PC3



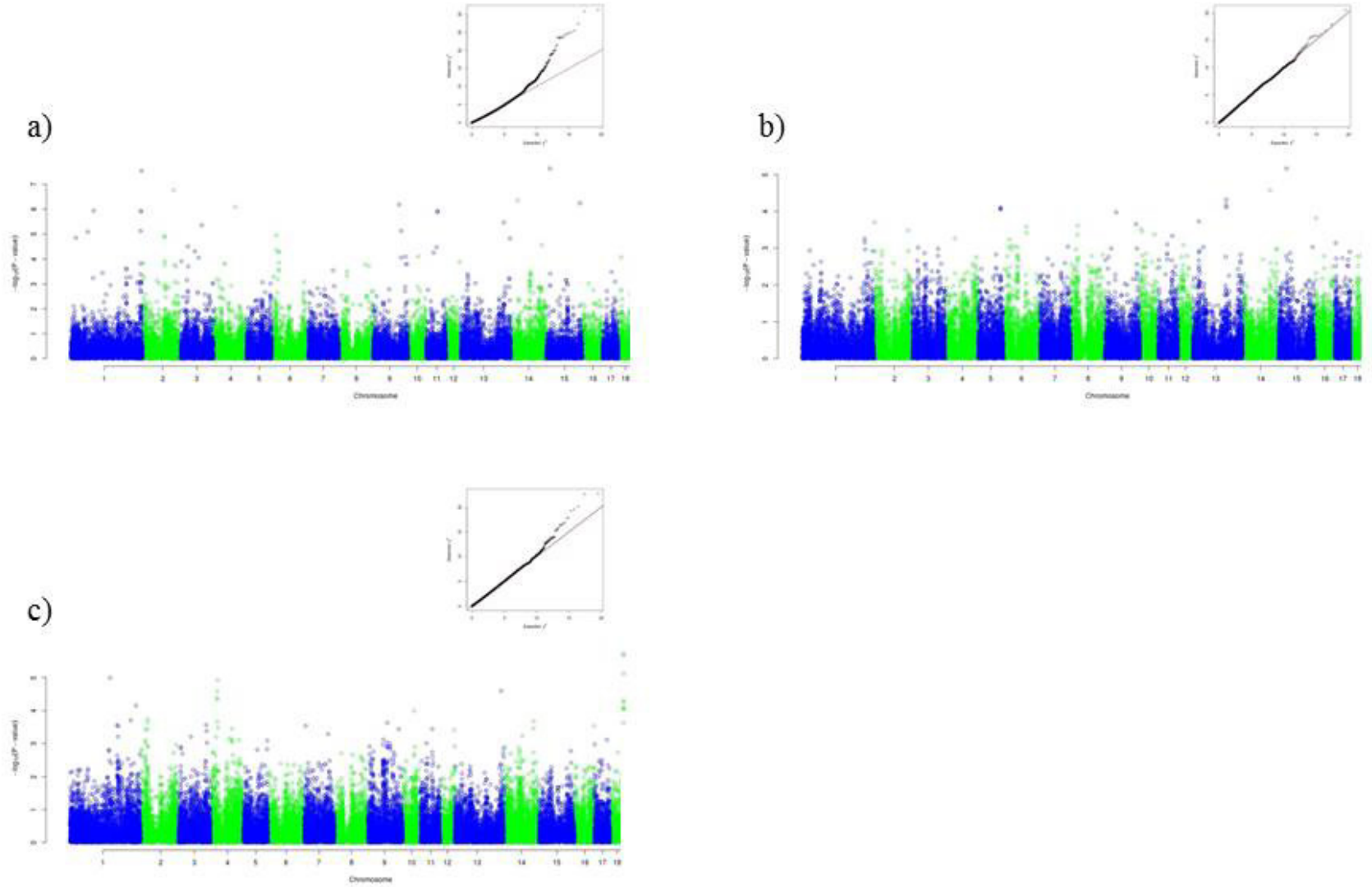
SI 28: Manhattan Plots and corresponding Q-Q plots of Org2a_LR for a) PC1, b) PC2 and c) PC3



SI 29: Manhattan Plots and corresponding Q-Q plots of Org2b_LR for a) PC1, b) PC2 and c) PC3



SI 30: Manhattan Plots and corresponding Q-Q plots of Org3_LR for a) PC1, b) PC2 and c) PC3



SI 31: Manhattan Plots and corresponding Q-Q plots of Org4_LR for a) PC1, b) PC2 and c) PC3

SI 32: Data sets for single-trait association analyses within breeding organizations

Data set	N animal	N-marker	PC	λ	Trait
Org1_LW	786	44.395	132	1.004	NBA
			101	1.004	ADG
			109	1.001	LMP
			108	1.004	BF
Org2_LW	553	42.429	113	1.004	NBA
			75	1.001	ADG
			74	1.009	LMP
			75	1.005	BF
Org3_LW	187	45.033	16	1.008	NBA
			19	1.003	ADG
			23	1.004	LMP
			-	-	BF
Org4_LW	164	45.300	20	1.01	NBA
			21	1.003	ADG
			17	1.01	LMP
			-	-	BF
Org2_LR	454	40.210	79	1.003	NBA
			61	1.003	ADG
			60	1.008	LMP
			66	1.009	BF
Org2a_LR	206	42.617	27	1.02	NBA
			21	1.003	ADG
			21	1.006	LMP
			30	1.009	BF
Org2b_LR	248	43.234	23	1.004	NBA
			26	1.005	ADG
			19	1.004	LMP
			20	1.01	BF

SI 32 continued: Data sets for single-trait association analyses within breeding organizations

Org3_LR	464	45.900	72	1.001	NBA
			91	1.008	ADG
			84	1.005	LMP
			-	-	BF
Org4_LR	248	38.232	38	1.003	NBA
			35	1.01	ADG
			38	1.002	LMP
			-	-	BF

*= Numbers of chromosome-wide and genome-wide significant associated SNPs with corresponding trait (p>0.05 %); N animal = number of animals used for the analysis; N marker = number of markers used for analysis after quality control and without SSCX and SSCY; PC = number of principal components; λ = inflation factor; MAF = minor allele frequency; $\sigma^2_{y^*}$ = Variance of the pre-corrected EBVs

SI 33: Data sets for single-trait association analyses in breeding organization overlapping clusters

Data set	N animal	N marker	PC	λ	Trait
LW_1	1689	37.616	338	1.004	NBA
	1687		306	1.001	ADG
	1687		250	1.002	LMP
	1339		275	1.001	BF
LW_2	1136	43.289	182	1.004	NBA
	1134		158	1.003	ADG
	1134		116	1.0004	LMP
	786		280	1.002	BF
LW_2a	662	42.457	100	1.001	NBA
	660		87	1.003	ADG
	660		55	1.001	LMP
	507		157	1.003	BF
LR_1	1219	40.184	248	1.003	NBA
	1218		264	1.003	ADG
	1219		177	1.001	LMP
	497		413	1.006	BF
LR_2	765	45.691	148	1.004	NBA
	764		127	1.005	ADG
	764		137	1.004	LMP
	53		-	-	BF

*= Numbers of chromosome-wide and genome-wide significant associated SNPs with corresponding trait (p>0.05 %); N animal = number of animals used for the analysis; N marker = number of markers used for analysis after quality control and without SSCX and SSCY; PC = number of principal components; λ = inflation factor; MAF = minor allele frequency; $\sigma^2_y{}^*$ = Variance of the pre-corrected EBVs

SI 34: Data sets for multivariate association analyses within breeding organizations

Data set	N animal	N marker	PC	λ	Trait
Org1_LW	786	44.395	109	1.003	PC1
			136	1.004	PC2
			93	1.004	PC3
			129	1.001	PC4
Org2_LW	553	42.429	67	1.003	PC1
			96	1.004	PC2
			87	1.002	PC3
			89	1.001	PC4
Org3_LW	187	45.033	18	1.02	PC1
			16	1.009	PC2
			16	1.001	PC3
Org4_LW	164	45.300	13	1.01	PC1
			17	1.01	PC2
			22	1.008	PC3
Org2_LR	454	40.210	61	1.005	PC1
			70	1.009	PC2
			68	1.004	PC3
			64	1.001	PC4
Org2a_LR	206	42.617	24	1.008	PC1
			28	1.01	PC2
			17	1.01	PC3
			32	1.009	PC4
Org2b_LR	248	43.234	20	1.02	PC1
			25	1.01	PC2
			19	1.009	PC3
			30	1.004	PC4

SI 34 continued: Data sets for multivariate association analyses within breeding organizations

Data set	N animal	N marker	PC	λ	Trait
Org3_LR	464	45.900	82	1.003	PC1
			86	1.002	PC2
			82	1.001	PC3
Org4_LR	248	38.232	8	1.001	PC1
			37	1.01	PC2
			38	1.006	PC3

*= Numbers of chromosome-wide and genome-wide significant associated SNPs with corresponding trait (p>0.05 %); N animal = number of animals used for the analysis; N marker = number of markers used for analysis after quality control and without SSCX and SSCY; PC = number of principal components; λ = inflation factor; MAF = minor allele frequency; $\sigma^2_y{}^*$ = Proportion of total variance explained by each PC

SI 35: Canonical correlation between PCs and traits in LW

Cluster	Trait	PC1	PC2	PC3
Org1_LW	**NBA**	0.24	-0.69	-0.68
	ADG	0.10	-0.77	0.63
	LMP	-0.95	-0.10	-0.06
	BF	0.94	0.16	0.05
Org2_LW	**NBA**	0.38	-0.92	0.06
	ADG	0.70	0.09	-0.69
	LMP	-0.81	-0.23	-0.44
	BF	0.89	0.11	0.12
Org3_LW	**NBA**	0.72	-0.27	0.64
	ADG	0.47	0.88	-0.02
	LMP	-0.71	0.31	0.63
Org4_LW	**NBA**	-0.76	0.22	0.61
	ADG	-0.75	0.31	-0.59
	LMP	0.44	0.89	0.05

SI 36: Canonical correlation between PCs and traits in LR

Cluster	Trait	PC1	PC2	PC3
	NBA	-0.08	-0.85	-0.52
	ADG	0.19	-0.83	0.52
	LMP	-0.92	-0.14	0.08
	BF	0.93	-0.05	-0.07
Org2a_LR	**NBA**	0.09	-0.85	-0.52
	ADG	0.31	0.55	-0.78
	LMP	-0.94	0.09	-0.15
	BF	0.95	0.003	0.15
Org2b_LR	**NBA**	-0.08	0.82	-0.56
	ADG	0.32	0.74	0.59
	LMP	-0.89	0.17	0.15
	BF	0.92	-0.03	-0.11
Org3_LR	**NBA**	0.73	-0.38	-0.57
	ADG	0.67	0.73	-0.05
	LMP	0.73	-0.29	0.61
Org4_LR	**NBA**	-0.25	0.88	0.42
	ADG	0.81	-0.17	0.57
	LMP	0.68	0.52	-0.52

9 CHAPTER 9: DANKSAGUNG

Mein herzlicher Dank gilt Prof. Dr. Karl Schellander für die Überlassung des Themas der vorliegenden Dissertation, sowie Prof. Dr. Christian Looft für die Übernahme des zweiten Gutachters.

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BERUFLICHE FORTBILDUNG

September 2013	Teilnahme und Vortrag bei der Vortragstagung der Deutschen Gesellschaft für Züchtungskunde e.V. und der Gesellschaft für Tierzuchtwissenschaften e.V, Göttingen, Deutschland
Juni / Juli 2013	Kurs: Genomic Selection in Livestock“, The Graduate School, Wageningen Institute of Animal Science, Wageningen, Niederlande
April 2013	Kurs: „Principles of Genomic Selection“, Leibniz Institute for farm animal biology, Dummerstorf, Deutschland
September 2012	Teilnahme am Genetisch-Statistischen-Ausschuss, Mariensee, Deutschland
	Teilnahme und Vortrag bei der Vortragstagung der Deutschen Gesellschaft für Züchtungskunde e.V. und der Gesellschaft für Tierzuchtwissenschaften e.V, Halle, Deutschland
August 2011	Teilnahme und Präsentation eines Poster bei European Federation of Animal Science (EAAP), Stavanger, Norwegen

10 CHAPTER 10: PUBLIKATIONEN, ANGEFERTIGTE PRÄSENTATIONEN UND VORTRÄGE

Bergfelder S, Grosse-Brinkhaus C, Looft C, Cinar MU, Jüngst H, Schellander K, Tholen E (2011): Genetic analysis of fertility and growth traits in a Duroc x Pietrain Ressource population. 62nd Annual Meeting of European Federation of Animal Science (EAAP), 29.8.-2.9.2011, Stavanger, Norway, Book of Abstracts, 17 (Abstr)

Bergfelder S, Grosse-Brinkhaus C, Looft C, Cinar MU, Jüngst H, Schellander K, Tholen E (2012): Quantitative und molekulargenetische Analyse von Geburtsgewicht und Aufzuchtsmerkmalen in einer Duroc x Pietrain Kreuzungspopulation. Vortragstagung der Deutschen Gesellschaft für Züchtungskunde e.V. und der Gesellschaft für Tierzuchtwissenschaften e.V., 12./13.9.2012, Halle, Deutschland. Tagungsband: B09 (Proc)

Grosse-Brinkhaus C, **Bergfelder S**, Tholen E (2012a): Genome wide association analysis of the QTLmas2012 data investigating pleiotropy. 16th QTL-Mas Workshop, 24./25.5.2012, Alghero (Sardinien), Italy. Proceedings (Abstr)

Bergfelder S, Große-Brinkhaus C, Looft C, Uddin M J, Schellander K, Tholen E (2013): Populationsübergreifende GWAS für ein Fruchtbarkeitsmerkmal in Mutterlinien beim Schwein. DGfZ/GfT-Gemeinschaftstagung, 4./5.9.2013, Göttingen, Deutschland, Tagungsband: B7 (Proc)

Ni G, Haberland A, **Bergfelder S**, Große-Brinkhaus C, Erbe M, Lind B, Tholen E, Simianer H (2013): Accuracy of genomic selection in a substructured population of Large White pigs. 64th Annual Meeting of the European Federation of Animal Science, 26.-30.8.2013, Nantes, France, Book of Abstracts: 236 (Abstr)

Simianer H, **Bergfelder S**, Haberland A, Große-Brinkhaus C, Guiyan N, Lind B, Tholen E (2013): Genomische Selektion bei Mutterlinien - das Projekt pigGS. 9. Schweine-Workshop, 19./20.02.2013, Uelzen, Deutschland, DGfZ-Schriftenreihe ; 62: 128-36

Große-Brinkhaus C, **Bergfelder S**, Tholen E (2014): Genome wide association analysis of the QTL MAS 2012 data investigating pleiotropy. BMC Proceedings 8, Suppl 5: S2

Bergfelder-Drüing S, Grosse-Brinkhaus C, Lind B, Erbe M, Schellander K, Simianer H and Tholen H (2015): A Genome-Wide Association Study in Large White and Landrace pig populations for number piglets born alive. PLoS ONE 10(3): e0117468. doi: 10.1371/journal.pone.0117468