USING BEHAVIORAL AND GENOMIC TOOLS TO IDENTIFY PIGS SUITED FOR GROUP LIVING

By

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PUBLIC ABSTRACT

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In an effort to improve the welfare of pigs raised for meat, many producers are reducing the amount of individual housing practices for their animals. Pigs are social animals, and in the wild females would live within small family groups. However, it is challenging to replicate these social circumstances on modern farms, and aggression amongst individuals is common when new pigs are introduced. Breeding for pigs that are better adapted for living in groups is an additional way that farmers can improve the quality of life of their animals. Previous research suggests that there is the potential to breed for less aggressive pigs. This study aimed to better understand how much aggression is influenced by genetic versus environmental factors. This study also followed pigs as they aged to see if they were consistent in how they responded to exposure to new individuals. In addition, genetic relationships were estimated to determine if aggression and body composition traits were controlled by the same genetic elements. Results from this study indicate that aggression is controlled by genetics to an extent that selective breeding may significantly reduce fighting. We found a region on chromosome 11 responsible for a significant portion of variation in aggressiveness. As pigs aged, they appeared to be relatively consistent in their aggressiveness suggesting that selection can be made on traits at a young age and still have an impact on traits present later in life. Finally, it did not appear that aggression and the growth traits we examined were controlled by the same genes, meaning it may be possible to breed for reduced aggression without inadvertently harming valuable growth traits.

ABSTRACT

USING BEHAVIORAL AND GENOMIC TOOLS TO IDENTIFY PIGS SUITED FOR GROUP LIVING

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Increasing numbers of pigs are being housed in groups as producers transition away from use of gestation stalls. Remixing of pigs is a common management technique that can cause aggression between pigs as social groups are disrupted. This aggression is often intense and can lead to stress and injury, both of which are concerns for animal welfare and production efficiency. To reduce the amount and severity of aggression, changes to the environment as well as the genetics of the pig need to occur. This study used behavioral and genotypic tools to better understand the underlying genetic component to aggression in order to identify individuals best suited for group living environments. Previous research validated the use of skin lesions as an indicator trait of aggression. We estimated genetic parameters of skin lesions immediately following remixing with unfamiliar individuals, as well as 3 weeks following remixing, at 3 age groups in which mixing in a commercial setting may occur (at weaning, at move into grow-finish pens, or at sexual maturity). We estimated lesion score heritabilities at multiple ages and examined how these differed across multiple regions of the body. Genetic and phenotypic correlations between the various body regions within and across different ages were computed to compare underlying relationships. Genetic correlations between lesions and growth traits were also obtained. This study found moderate heritability estimates for lesion scores, suggesting that there is a significant portion of lesion score variation that can be attributed to genetic components. Genetic and phenotypic correlations were highest between periods closest together in time, and between body regions next to one another. Additionally, there were no undesirable

genetic correlations between growth traits and lesions suggesting that skin lesions can be genetically selected upon to reduce aggression without having adverse effects on growth rates or body composition traits. An additional step in furthering our understanding of the genetic mechanisms of aggression is identifying regions of the genome that are associated with aggressive traits. This study performed genome-wide association analyses on skin lesions and identified regions on chromosome 11, which were associated with accumulation of anterior and central skin lesions immediately following mixing into grow-finish pens. Furthermore, this study examined video observations to determine total time pigs were engaged in delivering and receiving damaging and non-damaging aggression. Heritabilities of many agonistic behaviors were estimated, and values were small to moderate and overall of lower magnitude than previously estimated for skin lesions. Genetic and phenotypic correlations between groups of behaviors and skin lesions were obtained at both 24 hours post-mixing and 3 weeks post-mixing. At mixing, reciprocal aggressive interactions were genetically and phenotypically positively correlated with lesions to the anterior and central regions of the body. Delivery of one-sided aggression was also positively correlated with anterior lesions. Genetic and phenotypic correlations 3 weeks post-mixing were difficult to interpret, potentially due to small population size and low numbers of lesions observed at this time period. In conclusion, this study provides further information about the underlying genetic components of aggression. This knowledge will help guide genetic selection to reduce levels of aggression by helping determine the most optimal traits to select for the greatest potential impact on genetic change while reducing any potentially negatively associated correlated traits.

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PREFACE

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KEY TO ABBREVIATIONS

°C = Degrees Celsius

AT = One-sided attack

B = Isolated bite

BLUP = Best linear unbiased prediction

B-mode = Brightness mode

BW = Body weight

CT = Connecticut

d = Day

FDR = False discovery rate

G = Genomic relationship matrix

GBLUP = Genomic best linear unbiased prediction

GWA = Genome-wide association

h = hour

h^2 = Heritability

HD = High density

HK = Head knock

Inc = Incorporated

IP = Inverse parallel pressing

kg = Kilogram

m = Meter

m = Number of loci

m^2 = Square meters

MI = Michigan

mL = Milliliter

n = Number of individuals

NE = Nebraska

NRC = National Research Council

PIC = Pig Improvement Company

PP = Parallel pressing

QTLs = Quantitative trait loci

RDF = Rest during fight

RF = Reciprocal fight

r_g = Genetic correlation

SD = Standard deviation

SNPs = Single nucleotide polymorphisms

SRUC = Scotland's Rural College

US = United States

USDA = United States Department of Agriculture

WF = Withdrawal from fight

wk = week

Z = Standardized marker matrix

CHAPTER 1: GENERAL INTRODUCTION

INTRODUCTION

Transition to group housing

The United States' swine industry is currently undergoing a significant shift in production practices as it transitions from individual to group housing of gestating sows. Multiple states, including Michigan, are currently in the midst of a multiple year phase out period, which will allow producers to make the necessary adaptations before the new requirements must be met. Societal pressures, both from legislative action and consumer purchasing preferences for more ethically raised meat products, have been driving forces behind this change (Tonsor et al., 2009). Recently, numerous prominent retailers and food service chains that purchase or distribute pork are requiring their suppliers to transition to group housing, and multiple states have passed legislation requiring increased space or social housing of their sows.

Group housing is not a new concept for producers, as pigs at other life stages are routinely group housed. Typically, pigs in modern production systems tend to be grouped based on functional characteristics (e.g., age, weight, stage of pregnancy, etc.) rather than kinship. Further, pigs are sometimes moved between groups over time, and a social order must be established in each new group. In these situations, aggressive flare-ups are common and often cause injuries and stress. It has been reported that immediately after mixing, pigs undergo a greater incidence of fighting resulting in wounds, scratches, and lameness (Estienne, 2006; Jansen, 2007). This culminates in group-housed pigs being at greater risk for lameness (Jensen, 2010) and ultimately culling (Engblom et al., 2007; Stein et al. 1990), thus, reducing the health and welfare of group-housed pigs as well as farm profitability. Additionally, multiple studies have indicated concern over sow production measures such as farrowing weight and litter birth

weight in group managed sows (Backus and Vermeer, 1997; Den Hartog et al., 1993; Peltoniemi et al., 1999).

These housing practices contrast greatly with how pigs in natural environments live. Pigs in the wild or feral state are extensively social animals and inhabit relatively large home ranges. Bands of a few closely related females and their offspring tend to live together, and aggression is infrequent (Wood-Gush, 1989). When aggression does arise, it is typically limited to mating periods or disputes over food sources or preferred lying places. To avoid aggression, pigs develop a social order and maintain a certain distance between each other, as well as use non-physical threats or submissive behaviors to maintain dominance hierarchies and social relationships (Wood-Gush, 1989).

Unfortunately, this transition to group-housing is not only a matter of changing physical housing systems by replacing individual sow stalls with group pens. Genetic selection decisions in the swine industry over the past several decades have further exacerbated this issue by focusing on economically important traits such as growth rate and meat quality on an individual animal basis. However, there is growing evidence that sows selected purely on this basis do not function or perform well in a group-housing environment (Rodenburg & Turner, 2012).

Gestating sows make up roughly 10 percent of the total US pig population and have not historically been group housed. The effects of the housing change may have a profound impact on the success of the pig industry. Specific worries related to gestating sows represent a substantial investment in both time and finances and have thus sparked the need for additional research on this topic.

Aggression following regrouping

In natural environments, groups of female pigs will form stable, linear hierarchies (Mauget, 1981). Typically, the more mature and heavier pigs occupy the highest places in the hierarchy, and aggression is rare as these groups remain consistent and there is plenty of space for avoidance of conflict. However, in commercial production pigs are grouped based upon similar weight making hierarchy formation difficult without large amounts of aggressive interactions. Meese and Ewbank (1973) found that the most intense aggression ceases within the first 24 hours and the hierarchy rank could be observed by 48 hours post mixing. Many techniques have been implemented to try to minimize aggression within these first 24 hours but with limited success. Some techniques such as restricting space (Hvozdik et al., 2002; Turner et al., 2000; Weng et al., 1998; Wiegand et al., 1994), mixing late in the day (Barnett et al., 1994; 1996), or administering chemicals to disrupt olfactory recognition (Friend et al., 1983) reduce aggression in the short term, but may actually increase levels of aggression in the long term due to poor initial hierarchy formation. Some additional techniques such as improving pen design (Edwards et al., 1993; McGlone & Curtis, 1985; Olesen et al., 1996; Weigand et al., 1994), having a boar present when mixing gilts (Docking et al., 2001; Grandin & Bruning, 1992), and allowing pre-exposure to increase familiarity before mixing (Pluske & Williams, 1996; Van Putten & Bure, 1997; Weary et al., 1999) do work to reduce aggression levels overall. However, these techniques however often require investment of additional labor and time, or costly pen reconfigurations, reducing the feasibility of implementation on large scale commercial facilities (Spoolder et al., 2009; Vermeer et al., 2001). An alternative method to reduce aggression that may be used in conjunction with improved pen design and management practices, or on its own,

is utilizing genomic information to select for individuals that are better suited for group housed environments.

Advancements in genetic selection

Animal evaluation and selection for breeding have traditionally relied on the collection of observable phenotypic and available pedigree information. An early method of artificial selection, the selection index theory, helped lay the foundation for modern day selection methods (Rutten et al., 2002). This method allowed for the simultaneous selection on multiple traits, weighted by importance, and lead to the development of our more advanced prediction models. Modern day prediction of breeding values, either through marker assisted selection or genomic selection, incorporate genomic information to make more accurate predictions. Advancements in genotyping technology have made obtaining genomic information on large populations with a relatively high density of makers, a reality.

Marker assisted selection works by allowing breeders to identify quantitative trait loci (QTLs) or regions of the genome that account for a significant level of phenotypic variation, and to use their genotypes at those markers to inform selection decisions. This has worked well in selection for traits with a small number of QTLs with large effect (e.g. markers in MC1R and KIT genes for coat color and stress related markers in RYR1 gene). However, success has been limited for traits in which the variance is accounted for by many QTL of small effect such as in growth or behavioral phenotypes. For example, when a population is screened for markers explaining a substantial portion of the genetic variance, the selected marker may only account for 1-2% of the total variance (Hayes, 2010). In order to account for this missing variance, an alternative method, genomic selection, has been developed which takes all markers in a training population into account simultaneously without any pre-screening for their significance

(Meuwissen et al. 2001). Genomic selection is more advantageous than marker assisted selection when selecting for complex traits due to its utilization of all markers that have a potential effect, including those that would not meet the significance threshold in a screening for marker assisted selection. This helps ensure unbiased marker-effect estimates and capture of small effects (Jia & Jannink, 2012). Using a reference population one can predict the breeding value (sum of all marker effects) of un-phenotyped animals. One caveat to genomic selection is that a relatively large number of animals in the reference population is necessary for accurate predictions; however, large-scale affordable genotyping is making this a feasible option for many breeders.

Aggression is a complex trait and likely controlled by many SNPs – each contributing only a small portion of the total variance. Genomic selection provides an opportunity to understand the genetic basis of aggression due to its ability to simultaneously estimate many marker effects. Previous studies examining aggression have relied solely on pedigree information. Pedigrees however can only estimate the amount of shared genetic material between individuals. Our research aims to improve breeding value estimates for aggression with the inclusion of genetic marker data, allowing us to obtain the actualized genomic relationships between individuals in a population.

Genetics of behavior

The majority of genetic research on pigs has been historically directed towards understanding economically important production traits, such as growth rates and carcass quality. Pressure to alter housing systems in pigs has increased the importance of studying alternative traits, such as behavior, that have the potential to significantly impact productivity in group-housed systems. The estimation of genetic parameters has recently been conducted on a wide range of pig behavioral traits ranging from aggression to maternal care. Many of these

studies have shown that seemingly complex behavioral traits do have a significant level of heritability. For example, damaging aggressive behavior in pigs is associated with several moderately heritable traits, such as skin lesions (h^2=ranging from 0.20 to 0.26) as demonstrated by Turner et al. (2009) and chronic tail biting (h^2=0.27) (Breuer et al., 2005). In studies estimating genetic parameters of feeding behaviors, Labroue et al. (1997) found daily feed intake to be highly heritable (h^2=0.42). Von Felde et al. (1996) examined an array of feeding related behaviors and found that feeding rate, feed intake per visit, number of visits, time per visit, and time per day in the feeder showed high heritabilities of 0.44, 0.51, 0.43, 0.42, and 0.43 respectively. An additional study using pigs as a model to study obesity traits in humans identified a potential candidate gene, MC4R, associated with feed intake traits (Kim et al., 2000). Løvendahl et al. (2005) also estimated genetic parameters of aggressive interactions and found moderate heritabilities related to performing aggressive behaviors (h^2=0.17 and h^2=0.24) with weaker heritability estimates for receiving aggression (h^2=0.06 and h^2=0.04). While these heritabilities were lower than heritabilities of key traits described in previous studies, they still show potential for genetic selection. These past studies indicate that complex behavioral traits possess heritability estimates within similar ranges to production traits in which successful improvement through genetic selection has occurred. This is promising for the incorporation of aggressive traits into genetic selection schemes to improve welfare and production in group-housed pigs.

GWA background

Advancements in genotyping technologies have allowed researchers to explore for associations of quantitative trait loci (QTL) with behavioral traits through genome-wide association analyses (GWA). Genome-wide association studies scan the genome, or a region of

the genome, and identify markers which contribute to a significant portion of the phenotypic trait variance. There are several cases of successful genome-wide association analyses and marker assisted selection implementation in livestock, including swine. Studies have ranged from analysis of meat quality traits to identifying SNPs that play a role in disease susceptibility. For instance, Luo et al (2012b) were able to identify 45 SNP significantly associated with several meat quality traits. Another GWA study aimed at identifying SNP associated with reproductive traits such as farrowing characteristics and found 124 significant QTL (Schneider, 2012). Do et al. (2013) found 16 moderate SNPs for feeding behavior. Houston et al. (2005) found a QTL that affected daily feed intake on chromosome 2. Zhang et al. (2009) identified a total of 8 QTL with significant associations to feed consumption on 5 total chromosomes. Désautés et al. (2002) found significant gene effects relating to stress response on chromosomes 1 and 17. Yet another study has utilized GWA to aid in the discovery of QTL related to disease and immune function and identified 62 genome-wide significant SNPs (Luo, 2012a). In order for genomic selection and genome-wide association to be implemented in pig breeding programs, high density SNP genotypes are required to identify markers within linkage disequilibrium with traits of interest. By utilizing Illumina's 70,000 SNP chip for swine, a sufficiently high density of SNPs can be obtained to successfully perform genome wide association studies and genomic selection (Ramos, 2009). These studies examining the genetic components of behavior have shown promise for future research in this field. In addition, the successful implementation of genome-wide association scans for behavioral traits show opportunities for candidate gene identification in future research, leading to much broader understanding of the underlying genetic control of aggression in pigs.

Lesion scores and aggression

Colleagues from Scotland's Rural College (SRUC) were the first to quantify the extent of individual variability and short-term repeatability of aggressive behavior in pigs (Erhard, 1997). Since that time, they have gone on to find significant heritabilities for a number of aggressive behavioral traits and developed a way to score the skin lesions resulting from fighting that can predict what forms of behaviors are occurring (Turner et al., 2006; 2008; 2009; 2010). For example, duration of time spent in reciprocal fighting and delivering non-reciprocated aggression within 24 hours post-mixing have heritabilities of 0.43 and 0.35 respectively, which are a similar magnitude to heritabilities reported for growth traits. Locations of skin lesions have also been shown to correlate with the type of aggressive interaction that occurred. Lesions on the anterior region of the body were highly associated with reciprocal fighting and the receipt of non-reciprocal aggression ($r_g = 0.67 \pm 0.04$ and $r_g = 0.70 \pm 0.11$, respectively). Lesions located on the center and rear were highly associated with the receipt of non-reciprocal aggression ($r_g = 0.80 \pm 0.05$, 0.79 ± 0.05) (Turner, 2008). Total accumulation of skin lesions within 24 hours post mixing has a heritability of 0.22 (Turner, 2010). Although this heritability is lower than those for duration of fighting, it is still greater than many traits commonly under selection. The heritability of skin lesions and their high associations with agonistic behaviors make them an ideal candidate to be used as a proxy trait for aggression.

CHAPTER OVERVIEW

The goal of Chapter 2 was to further validate lesion score heritabilities in grow-finish aged pigs and to obtain estimates on 2 additional age groups, newly weaned pigs and mature gilts. These heritability estimates were obtained using a genomic relationship matrix 24 hours post-mixing and 3 weeks post-mixing. We also wanted to gain a better understanding about how lesions on different regions of the body were related, and to assess if pigs are consistent in their response to mixing as they age. To meet these objectives, phenotypic and genetic correlations were calculated between lesions on regions on the body, and between ages of pigs. Finally, to ensure skin lesions were not detrimentally associated with production traits we obtained genetic correlations between lesions and traits of weight gain, backfat thickness, and loin muscle area.

The overall goal of Chapter 3 was to identify regions of the genome associated with skin lesion traits. We performed genome-wide association analyses for skin lesions on three body regions across 3 ages of pigs.

Chapter 4 aimed to assess the relationship between skin lesions and durations of observed agonistic behavior. Heritability estimates were first obtained for the behavioral traits. Secondly, genetic and phenotypic correlations were calculated between skin lesions and durations of observed behaviors 24 hours post-mixing and 3 weeks post-mixing. Also, to examine the relatedness of different types of aggression, correlations of marginal residuals were reported.

OBJECTIVES

Specifically, the objectives of this dissertation were:

1. Estimate heritabilities of skin lesions across three regions of the body and across 3 ages of pigs. Obtain phenotypic and genetic correlations of skin lesions between body regions and

between age groups. Obtain genetic correlations between skin lesions and production traits of: growth rate, backfat thickness, and loin muscle area.

2. Perform genome-wide association analyses on skin lesion traits at mixing.

3. Estimate heritabilities of agonistic behaviors observed 24 hours post-mixing and 3 weeks post-mixing using data obtained from video observations of grow-finish pigs. Obtain phenotypic and genetic correlations between skin lesions and duration engaged in agonistic behaviors. Calculate correlations of marginal residuals between specific types of agonistic interactions.

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CHAPTER 2: ESTIMATION OF GENETIC PARAMETERS FOR LESION SCORES AND GROWTH TRAITS IN GROUP-HOUSED PIGS

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ABSTRACT

Pigs housed in groups are remixed with unfamiliar individuals, which can trigger aggressive interactions, potentially compromising animal welfare. Skin lesions are a reliable indicator trait of aggression and are moderately heritable, suggesting that aggression may be reduced through selection. This study estimated genetic parameters of skin lesions of pigs at multiple life stages, explored genetic correlations of skin lesions between age groups and body location, and studied the relationship between skin lesions and production traits of commercial importance. A population of 1,079 Yorkshire pigs was strategically remixed into new groups of familiar and unfamiliar animals at 3 life stages (weaning, grow–finish, and mature gilts). Skin lesions (fresh, bright red cuts) were counted immediately prior to mixing and 24 h and 3 wk after mixing across 3 body regions: anterior, central, and caudal. Weights were recorded prior to each mixing event. Prior to slaughter, backfat thickness and loin muscle area were determined using ultrasound. Univariate analyses were performed to obtain heritability estimates of lesion scores. Bivariate analyses were performed with response variables being skin lesions, weight gain per life stage, backfat thickness, or loin muscle area, depending on the relationship of interest, to obtain correlations. Lesion score heritabilities ranged from 0.10 to 0.40 and were significant ($P < 0.05$). Heritability was highest for lesions on the anterior region of the body for 24 h and 3 wk after mixing. Lesions to the central and caudal areas showed the highest genetic correlation at each stage of production, whereas those to the anterior and caudal regions had the lowest correlation. The highest genetic correlation was found between the mature gilt and grow–finish stages, whereas the weaning and mature gilt stages had the lowest correlations. Genetic

correlations between lesions and production traits were not significantly different from 0 for weight gain and backfat thickness, but loin muscle area was negatively correlated with lesions ($P = 1.17 \times 10^{-4}$, $P = 2.30 \times 10^{-5}$, and $P = 6.08 \times 10^{-4}$ for anterior, central, and caudal lesions, respectively). These results are promising for the industry because they suggest that pigs selected for reduced lesions will show increased loin muscle area without negative effects on growth. Alternatively, selection for these production traits would not increase lesions.

INTRODUCTION

Increasing numbers of pigs are being housed in groups, particularly due to shifts in legislative requirements and consumer concerns about pig welfare. Although providing greater freedom of movement, commercial group-housing environments regularly result in competition for feed access and social groups may be disrupted as part of routine management in an environment where escape from conflict is difficult. Aggression at regrouping is typical and often intense, at least until social rank is established (Spoolder et al., 2000; Turner et al., 2009). A pig may be mixed with unfamiliar conspecifics multiple times throughout its life including at weaning as a piglet, transition to grow–finish pens, after selection, during gestation, and again following each farrowing, subjecting them to potentially numerous stressful aggressive encounters. Natural individual variability in response to regrouping exists among pigs, and when less aggressive pigs are housed together, less fighting is observed (Erhard et al., 1997). Therefore, if aggression is heritable, the selective breeding of less aggressive pigs could reduce the severity of this challenge. Multiple studies have estimated heritabilities of aggressive behaviors and found heritabilities of magnitudes similar to those of traits commonly incorporated into commercial breeding programs (Løvendahl et al., 2005; Turner et al., 2008, 2009; D'Eath et

al., 2009). Skin lesion scores at the grow–finish stage have been shown to be highly associated with aggressive interactions and possess moderate heritabilities (Turner et al., 2008). Although the total lesion count is usually not associated specifically with either receipt or delivery of aggression, several previous papers (Turner et al., 2006a, 2008, 2009) have shown that accumulation of lesions in the rear part of the body are phenotypically and genetically correlated to receiving aggression, whereas high lesion counts in the anterior part of the body are positively correlated with reciprocal fighting and delivering aggression in nonreciprocal interactions. Therefore, lesion scores in specific areas of the body can provide information about both quantity and type of aggression interaction, not simply whether a pig has received aggression.

This study estimated genetic parameters of skin lesion scores in different parts of the body as a validated proxy of aggression received or delivered (depending on body location) across multiple life phases of pigs and explored genetic correlations between skin lesions across multiple body locations within and across age groups. In addition, phenotypic and genetic correlations between lesion scores and production traits are reported.

MATERIALS AND METHODS

All animal protocols were approved by the Institutional Animal Care and Use Committee (Animal Use Form number 01/14-003-00).

Data set

The experimental population was housed at the Michigan State University Swine Teaching and Research Center, East Lansing, MI. This study followed 1,093 purebred Yorkshire pigs (548 gilts and 545 barrows) from weaning to market across 8 replicates (120 pigs in replicate 1, 133 pigs in replicate 6, and 140 pigs in replicates 2, 3, 4, 5, 7, and 8). A commercial

diet meeting or exceeding the NRC (2012) nutritional requirements for each phase of development was consumed by pigs ad libitum and delivered through self-feeders with no more than 4 pigs per feeder space within the nursery and no more than 10 pigs per space in the grow–finish/mature gilt pens. Water was provided by a single nipple drinker in each pen (nipple with cup in grow–finish pens and gilt pens). Nursery rooms were initially temperature controlled to 26.6°C and gradually decreased to 18.3°C by the end of the weaning phase. Pens in the nursery were 1.78 by 1.18 m (0.21 m2/pig) with fiberglass-gated pens and rounded metal flooring. Grow–finish/mature gilt pens were 4.83 by 2.44 m (0.79 to 0.98 m2/pig) with painted metal sides and slatted concrete floors. Ventilation to all rooms was provided with a negative pressure system with variable speed fans. An average of 8 h of light per day using fluorescent lighting was provided in both housing types. Pigs received iron and ceftiofur crystalline-free acid (Excede; Pfizer Animal Health, New York, NY) injections and were ear notched for identification within 48 h of birth. Boars were surgically castrated between 7 to10 d of age using a scalpel. Cross-fostering between litters was not performed, contrary to standard industry practice.

Regrouping

Pigs were strategically regrouped at 3 different stages: weaning (mean of 21.5 ± 2.3 d of age), grow–finish (mean of 67.1 ± 3.0 d of age), and mature gilt (approximately 100 kg and mean of 147.9 ± 6.3 d of age). Similar proportions of familiar vs. unfamiliar pen mates were maintained across pens, and BW variation was minimized within pen. At weaning, pigs were moved from conventional farrowing pens into single-sex groups of 10 composed of pairs and triplets of littermates. Grow–finish groups consisted of 13 to 15 pigs in 3 to 5 different nursery pens (groups of 3 to 5 familiar pigs from the nursery). Familiarity at this stage was defined as

sharing a previous pen with no regard to whether they were littermates. Gilts were regrouped a final time into pens at approximately 100 kg to emulate breeding replacement groups. Gilts were moved into pens previously occupied by barrows in the same room in groups of 12 to 15. Each gilt pen contained 2 to 6 grow–finish pen mates from a total of 3 to 5 different grow–finish pens. In all cases, pens alternated by sex to limit interactions of pigs with potential future pen mates by contact through the pen walls.

Lesion scoring

The total number of skin lesions was counted immediately prior to regrouping, 24 h following regrouping, and 3 wk following regrouping. Lesion scoring was performed by 3 trained observers and consisted of counting the total number of lesions. A lesion was counted when it was a single and continuous scratch, regardless of severity, and was fresh (<24 h old). Fresh lesions were judged on the basis of redness and development of scabbing. Lesions were recorded by location on the body (anterior, central, and caudal), because as previously discussed, accumulation of skin lesions in the anterior part of the body has been genetically and phenotypically correlated with the delivery of 1-sided aggression and with a pig's involvement in reciprocal fighting, whereas the accumulation of skin lesions in the posterior part of the body is more correlated with receiving aggression (Turner et al., 2006a, 2008, 2009).

Production traits

Weights were collected prior to regrouping to aid in allocation to new pens and again at time to market. Backfat thickness and loin muscle area were estimated using noninvasive B-mode ultrasound (Aloka SSD-500V; Hitachi Aloka Medical America, Inc., Wallingford, CT) by a single, trained individual.

Genotyping and data editing

Whole blood samples were obtained at 7 wk of age for DNA isolation. Two 10-mL vacutainers (heparin coated, 158 United States Pharmacopeia units) of whole blood were collected per individual using a jugular venipuncture. At this time, pigs were given an additional, larger ear tag for visual identification. A total of 1,082 individuals were genotyped using the GeneSeek Genomic Profiler for Porcine HD version 1 commercial BeadChip (Neogen Corporation – GeneSeek Operations, Lincoln, NE). Initial genotyping returned 68,516 markers. To control for sources of error, SNP with greater than 10% missing data were removed from the analysis (n= 5,390). Similarly, 2 animals that had >10% missing SNP were removed. The X chromosome was also excluded (n = 3,305) as well as markers with a minor allele frequency of <5% (n = 7,426), leaving a total of 52,925 SNP from 1,079 animals for analysis. The total percentage of missing genotypes was minimal (0.003%), and therefore, a naïve imputation tactic was performed by replacing missing genotypes with the expected allelic dosage of the SNP.

Estimation of genomic relationship matrix

The genomic relationship matrix, **G**, was computed following procedures set forth by VanRaden (2008). Genotypes were expressed as allelic dosage and stored in marker matrix **M**, with dimensions n (number of individuals) by m (number of loci). The marker matrix **M** was standardized by first subtracting the expected allelic dosages from each SNP and dividing by the square root of a common expected variance of allelic dosage, setting the mean values of allele effects to 0 and its variance to 1. From here, **G** was computed by multiplying **Z** (the standardized marker matrix) by its transpose, producing an element matrix containing estimates of the realized genomic relationship between any 2 animals.

Prediction model

For estimation of variance components, genomic best linear unbiased prediction models (genomic BLUP; Mrode, 2014) were fitted using the gwaR package in R (https://github.com/steibelj/gwaR; accessed 1 May, 2017) as follows:

$$\boldsymbol{Y = Xb + u + Z_p p + e,}$$

in which **y** is the vector of log-transformed lesion scores with incidence matrix **X**; **b** is the vector of fixed effects of sex (gilt or barrow), replicate (8 levels), observer (9 levels following mixing, 3 levels for established groups), and weight as a covariate; **u** is a random vector of genetic additive effects and $\mathbf{u} \sim N(\mathbf{0}, \mathbf{G}\sigma u^2)$, in which **G** is the genomic relationship matrix and σu^2 is the additive genetic variance; **p** is the vector of random contemporary group effects and $\mathbf{p} \sim N(\mathbf{0}, \mathbf{I}\sigma p^2)$, in which **I** is the identity matrix and σp^2 is the pen to pen variance; **Zp** is the incidence matrix relating **y** to **p**; and **e** is a vector of residual effects and $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma e^2)$, in which **I** is the identity matrix and σe^2 is the residual variance.

The response variables were post-mix lesion scores (with pre-mix lesions as an additional covariate included in the model) or total number of skin lesions present 3 wk after mixing (in an "established" group) broken down by location on the body. To better approximate a normal distribution, lesions were transformed ($y = \log_e (1 + \text{lesion count})$).

To examine covariance and correlations of lesion scores between different body locations, bivariate genomic BLUP models were used. Models were jointly fit to anterior and central, central and caudal, and anterior and caudal lesions within each of the 3 stages. Models included effects similar to those of the univariate analysis, expanded to include all interactions of fixed effect with trait, and a bivariate Gaussian distribution was assumed for all random effects.

To examine covariance and correlations of lesion scores between stages, similar bivariate genomic BLUP models were fit to lesions at the same body location between each of the 3 stages (i.e., weaning anterior lesions with grow–finish anterior lesions or weaning caudal lesions with mature gilt caudal lesions, etc.).

Additional bivariate models were jointly fit to lesion scores and each of the production traits. For growth, we used weight gain within stage (weaning and grow–finish) by subtracting their entry weight from their exit weight. Backfat thickness and loin muscle area were also analyzed with grow–finish stage lesions. Weight gain and loin muscle area traits were scaled prior to analyses to avoid inconsistent estimations due to drastic differences in scale between the production traits and the transformed lesion scores. Rescaling was performed by dividing each of the variables by their SD. After fitting these models, estimates of variance components, $\hat{\sigma}_u^2$, $\hat{\sigma}_e^2$, and heritabilities as well as the covariance and phenotypic, genotypic, and residual correlations were obtained.

RESULTS AND DISCUSSION

Heritability

Skin lesion scores have been shown to be associated with aggressive interactions, and location of lesions on the body has been associated with engaging in delivery of aggression and reciprocal fights (primarily anterior lesions) or receiving aggression (primarily caudal lesions; Turner et al., 2008, 2009). Heritabilities (h^2) of lesion counts at mixing (lesions accumulated within 24 h of remixing) and stable time points (lesions present 3 wk after mixing) are reported in Table 2.1. All estimates of h^2 of lesions at each stage and body location were deemed significant ($P < 0.05$) through likelihood-ratio testing. Apart from lesions on the central

region of the body at the weaning age group, heritability at mixing was higher than at the corresponding stable time point. This contrasts with previous studies that have reported larger heritability estimates in established groups than immediately after mixing in grow–finish aged pigs (Turner et al., 2009; Desire et al., 2015a, 2016). An explanation for this difference could be due to the relatively low count and large SD of lesions present at the stable stage (Table 2.8). Although the relative magnitude of the heritability estimates of lesion scores for post-mixing and stable stage values differed from those previously published, the overall magnitudes of our estimates were similar to those from the previous studies. Our heritability values estimated for gilts and barrows were also similar in magnitude to skin lesion heritabilities estimated in mature boars (h^2 = 0.31) at time of slaughter (Parois et al., 2015). These parameter estimates suggest potential for selective breeding, because a significant proportion of the variation for lesion traits can be attributed to additive genetic effects. Anterior lesions had the largest heritabilities for each age group at mixing and in the established setting, suggesting the greatest potential for genetic change through targeted selection of lesions to this body region.

Correlation of lesion scores

Lesion scores at different body locations have been linked to distinct types of aggressive interactions. For instance, lesions on the anterior portion of the body are strongly associated with participation in reciprocal fights, whereas lesions on the caudal portion of the body are associated with retreat from an agonistic interaction (Turner et al., 2006a). Genetic and phenotypic correlations were obtained among lesion counts for the 3 body locations within each production stage (Table 2.2). Genetic correlations were larger than their corresponding phenotypic correlations. Correlations between central and caudal lesions scores were of the highest magnitude, whereas correlations between anterior and caudal lesions were lowest for

each of the 3 stages. The high correlations between central and caudal lesions suggest that selection for either trait may have an associated effect on the other. Turner et al. (2009) found similar results, with estimates of genetic correlations between central and caudal lesions close to 1. Genetic correlations among lesion scores at different body locations measured 3 wk after mixing were also positive and followed a pattern similar to those estimated for mix lesions, with correlations between central and caudal lesions being strongest (Table 2.3).

Most research examining genetic correlations of lesions has focused on the grow–finish age group. However, Stukenborg et al. (2012) examined Pearson correlations of aggressive behaviors between different age groups and found nonsignificant correlations between weaned and growing pigs and intermediate correlations between growers and gilts. Our research included pigs in the nursery and mature gilts to investigate whether pigs were consistent in their response to remixing as they age (results shown in Table 2.4) using skin lesions as a proxy of aggression. Again, we found genetic correlations were stronger than the corresponding phenotypic correlations. The highest genetic correlation was found between the mature gilt and grow–finish stages, whereas the lowest correlations were between weaning and mature gilt stages. These results followed our expectation that age groups closest together in time would have the highest correlations. Scheffler et al. (2016) similarly found that phenotypic correlations decreased as the difference in age increased and found the lowest correlations between weaning and mature gilt stages when estimating genetic and phenotypic correlations of aggressive behaviors. In the present study, the correlation between weaning and mature gilts was positive and moderate for anterior lesions, suggesting that lesions at a young age could be predictive of lesions obtained later in life. However, the correlations among caudal lesions at these stages were very low, and lesions, in general, at the weaning stage may result from high amounts of play fighting, so

repeated scoring throughout life may be necessary. Similar trends were also found 3 wk after mixing and can be found in Table 2.5.

Correlation of lesion scoring traits with production traits

When selecting for reduced aggression, it is critical to avoid inadvertent selection for reduced growth that may occur due to antagonistic correlations between aggressiveness and productivity. There is concern that less aggressive pigs may not compete as effectively for access to feed, thus leading to lower growth rates and less optimal carcass composition traits. We calculated weight gain in the weaning and grow–finish pens and assessed backfat and loin muscle area at exit from the study to examine genetic correlations between these growth traits and lesion scores (Table 2.6). (Estimating heritability of production traits was not a goal of this paper, but these values are included in Table 2.7 as a point of interest.) Genetic correlations between weight gain and lesion scores were close to 0 or slightly negative, as in the case of weight gain and weaning caudal or grow–finish anterior lesions. However, no correlations between weight gain and lesion scores were statistically significant. Backfat and lesions were slightly positively, but not significantly, correlated. Turner et al. (2006b) similarly found nonsignificant genetic correlations between lesions and weight gain (daily) and between lesions and backfat depth. Desire et al. (2015b) also reported low, nonsignificant genetic correlations between lesions and backfat at the grow–finish stage. Moderate significant negative correlations (and therefore favorable) were found between loin muscle area and lesions in our study (Table 2.6), although previously, Desire et al. (2015b) reported no significant correlations between these traits for grow–finish pigs. Desire et al. (2015b) also examined genetic correlations between lesions and lifetime daily gain and found low, nonsignificant correlations with anterior lesions but moderate, significant correlations with central and caudal lesions, which indicated that pigs

that received the most lesions in these areas were genetically predisposed to grow at the fastest rate. Although this is contradictory to our results, it is important to note that lesions to the anterior of the body have the highest associations with initiating aggressive interactions, and individuals with high lesion scores for central and caudal lesions may be victims of bullying behavior and not necessarily engaging in aggression by choice. Furthermore, Desire et al. (2015b) found near-0 phenotypic correlations between lesions and growth, indicating a detachment between these 2 traits. Our results, therefore, continue to support the idea that selection for fewer lesions can lead to lower rates of aggression without negatively affecting growth. The nonsignificant, low genetic correlations between growth rate and lesions and between backfat and lesions indicate that these traits are not genetically correlated and therefore can be independently selected for. The negative moderate correlations between loin muscle area and lesions suggest that selection for reduced lesions could lead to a correlated desirable increase in loin muscle area, and vice versa.

In conclusion, our estimates of genetic parameters for lesion scores were similar to those presented in previous studies. These findings validate the genetic selection potential of lesions in grow–finish pigs and provide evidence that lesions are similarly heritable in differing age groups, similar to previously published studies. Using lesion scores as a proxy for aggression as done in our study has practical potential, because scoring can be quickly performed without extensive training or equipment, although an automated scoring method would be ideal for large-scale breeding programs. The study demonstrates the early lifetime benefits of selecting on lesions, particularly with regard to those received to the anterior portion of the body, where positive genetic correlations were estimated across a period of 126 d in rapidly developing animals. We obtained genotypic and phenotypic correlations across body regions of grow–finish pigs

comparable to those presented in previous research. Our study also further explored the relationship between lesion scores and production traits, obtaining further evidence of the potential to reduce aggression by selecting on lesions at any point in growth without compromising commercial production traits. In addition, we found new evidence supporting strong genotypic and phenotypic correlations for lesion scores across different stages of pigs, suggesting that lesions obtained at a young age could be predictive of lesions obtained later in the growth phase. Despite environmental effects across months of time as pigs grow and lack of detail captured with lesions, lesion scoring can be a simple and repeatable measure breeders can use as a selection tool to reduce aggression in group housed pigs.

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APPENDIX

Table 2.1. Heritability estimates for skin lesions accumulated at mixing or present three wk post-mixing in a stable group along with their standard errors (in parentheses) and P-values.

		At Mixing		3 Wk Post-Mixing	
Stage	Location	Heritability	*P*-Value	Heritability	*P*-Value
Weaning	Anterior	0.26 (0.05)	1.04E-14	0.25 (0.05)	2.40E-20
	Central	0.21 (0.05)	8.65E-11	0.25 (0.05)	1.38E-16
	Caudal	0.22 (0.05)	1.05E-11	0.18 (0.05)	1.63E-10
Grow-finish	Anterior	0.32 (0.06)	5.56E-19	0.18 (0.05)	8.01E-10
	Central	0.15 (0.04)	4.57E-08	0.14 (0.04)	1.31E-07
	Caudal	0.16 (0.04)	9.14E-08	0.10 (0.04)	8.07E-05
Mature gilt	Anterior	0.40 (0.09)	1.61E-10	0.18 (0.07)	1.21E-04
	Central	0.28 (0.09)	8.35E-07	0.13 (0.07)	6.48E-03
	Caudal	0.26 (0.08)	4.74E-09	0.10 (0.07)	1.55E-02

Table 2.2. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) of lesion scores scored at mixing among locations on the body within each production stage. Standard errors are reported in parentheses.

Mix		Weaning			Grow-finish			Mature gilt	
Trait	Anterior	Central	Caudal	Anterior	Central	Caudal	Anterior	Central	Caudal
Anterior		0.91 (0.04)	0.83 (0.07)		0.84 (0.06)	0.81 (0.08)		0.89 (0.06)	0.72 (0.12)
Central	0.70 (0.02)		0.97 (0.04)	0.70 (0.02)		0.95 (0.05)	0.72 (0.03)		0.95 (0.07)
Caudal	0.51 (0.03)	0.67 (0.02)		0.52 (0.03)	0.69 (0.02)		0.51 (0.04)	0.67 (0.03)	

Table 2.3. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) of lesion scores accumulated three wk post-mixing among locations on the body within each production stage. Standard errors are reported in parentheses.

Stable				Weaning			Grow-finish			Mature gilt	
Trait	Anterior	Central	Caudal	Anterior	Central	Caudal	Anterior	Central	Caudal		
Anterior		0.89 (0.06)	0.83 (0.09)		0.79 (0.11)	0.84 (0.13)		0.85 (0.20)	0.29 (0.32)		
Central	0.60 (0.02)		0.89 (0.06)	0.52 (0.02)		0.86 (0.15)	0.44 (0.04)		0.93 (0.23)		
Caudal	0.49 (0.03)	0.63 (0.02)		0.39 (0.03)	0.42 (0.03)		0.34 (0.04)	0.47 (0.04)			

Table 2.4. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) of lesion scores accumulated at mixing among the production stages within each location on body. Standard errors are reported in parentheses.

Mix	Anterior			Central			Caudal		
Trait	Weaning	Grow-finish	Mature gilt	Weaning	Grow-finish	Mature gilt	Weaning	Grow-finish	Mature gilt
Weaning		0.76 (0.10)	0.63 (0.13)		0.64 (0.14)	0.43 (0.17)		0.15 (0.18)	-0.08 (0.19)
Grow-finish	0.26 (0.03)		0.75 (0.10)	0.19 (0.03)		0.60 (0.18)	0.10 (0.03)		0.58 (0.17)
Mature gilt	0.21 (0.04)	0.37 (0.04)		0.13 (0.04)	0.22 (0.04)		0.07 (0.04)	0.19 (0.04)	

Table 2.5. Genetic correlations (above diagonal) and phenotypic correlations (below diagonal) of lesion scores accumulated three wk post-mixing among production stages within each location on body. Standard errors are reported in parentheses.

Stable	Anterior			Central			Caudal		
Trait	Weaning	Grow-finish	Mature gilt	Weaning	Grow-finish	Mature gilt	Weaning	Grow-finish	Mature gilt
Weaning		0.62 (0.14)	0.25 (0.21)		0.52 (0.16)	0.57 (0.22)		0.71 (0.20)	0.84 (0.21)
Grow-finish	0.13 (0.03)		0.66 (0.19)	0.16 (0.03)		1.00 (0.19)	0.06 (0.03)		0.90 (0.33)
Mature gilt	0.09 (0.04)	0.15 (0.04)		0.17 (0.04)	0.14 (0.04)		0.06 (0.04)	0.06 (0.04)	

Table 2.6. Genetic correlations among lesion scores accumulated at mixing in the weaning and grow-finish stage (by location of lesions on the body) and the production traits of weight gain, backfat, and loin muscle area. Standard errors are reported in parentheses.

Trait	Weight gain	P-value	Backfat	P-value	Loin muscle area	P-value
Weaning anterior	2.00E-03 (0.14)	5.00E-01				
Weaning central	-0.05 (0.15)	3.76E-01				
Weaning caudal	-0.20 (0.15)	9.72E-02				
Grow-finish anterior	-0.16 (0.14)	5.00E-01	0.13 (0.11)	1.25E-01	-0.48 (0.12)	1.17E-04
Grow-finish central	-0.08 (0.18)	5.00E-01	0.23 (0.14)	5.71E-02	-0.65 (0.14)	2.30E-05
Grow-finish caudal	-0.06 (0.17)	5.00E-01	0.05 (0.14)	5.00E-01	-0.51 (0.15)	6.08E-04

Table 2.7. Heritability estimates for production traits along with their standard errors (in parentheses) and P-values.

Trait	**Heritability**	***P*-Value**
Weight gain during weaning stage	0.32 (0.05)	8.10E-24
Weight gain during grow-finish stage	0.18 (0.04)	1.57E-16
Backfat	0.58 (0.06)	3.06E-55
Loin muscle area	0.34 (0.06)	4.57E-22

Table 2.8. Means and standard deviations of lesion counts are presented for anterior, central, caudal, and total body lesions.

	Anterior		Central		Caudal		Total	
Trait	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Weaning pre-mix lesions	5.01	4.83	3.12	3.51	2.33	2.82	10.46	9.49
Weaning post-mix lesions	30.30	25.87	18.32	18.91	13.67	13.25	62.28	53.56
Weaning 3 wk post-mix lesions	9.27	15.42	6.97	10.80	4.43	7.12	20.67	31.23
Grow-finish pre-mix lesions	11.72	7.91	16.21	10.98	9.32	6.45	37.25	22.69
Grow-finish post-mix lesions	54.55	33.75	48.03	33.55	24.02	17.82	126.60	76.01
Grow-finish 3 wk post-mix lesions	8.20	7.73	6.20	6.18	3.30	3.16	17.70	14.53
Mature gilt pre-mix lesions	7.50	5.78	5.09	4.64	4.27	3.55	16.86	11.37
Mature gilt post-mix lesions	66.92	1.22	41.47	29.82	29.12	20.39	137.51	78.51
Mature gilt 3 wk post-mix lesions	16.07	27.46	8.81	15.18	7.58	11.38	32.45	50.76

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CHAPTER 3: GENOME-WIDE ASSOCIATION ANALYSIS OF LESION SCORES IN GROUP-HOUSED PIGS

Wurtz K.E., Siegford J.M., Ernst C.W., Raney N.E., Bates R.O. & Steibel J.P.

ABSTRACT

Aggression in group-housed pigs is a welfare concern, which can negatively affect production. Skin lesions are reliable indicators of aggression, and are moderately heritable, suggesting that selective breeding may reduce aggression. To further understand the genetic control of behavioral traits, such as the aggressive response to regrouping, associated single nucleotide polymorphisms (SNP) can be identified within the genome, and the region in which these SNP are located can be related to known genes. To investigate SNP associated with aggression, 1,093 purebred Yorkshire pigs were strategically remixed into new groups of familiar and unfamiliar animals at 3 life stages and lesion scores were recorded. Genomic best linear unbiased prediction (GBLUP) models were fitted for each trait. The genetic additive effect was obtained from a genetic relationship matrix constructed from the 52,925 SNP. SNP effects and their variances were estimated from the GBLUP objects. SNP that were associated with a significant portion of the trait variance were identified for lesions to the anterior and central (3 SNP, FDR<5%) portions of the body in grow-finish pigs. These SNP were located on chromosome 11, suggesting chromosome 11 contains a region explaining variation in lesion scores that should be further explored to identify genes underlying biological control of aggression.

MATERIALS AND METHODS

It is not uncommon for group-housed pigs to be regrouped with unfamiliar individuals at multiple points throughout their lives. This disruption results in aggressive interactions until a social hierarchy is established within the new group. This aggression is often stressful and

intense, and can potentially lead to illness or injury. Aggressiveness in pigs varies greatly between individuals and is a heritable trait (D'Eath 2009; Desire et al. 2015, 2016; Turner et al. 2006b, 2008, 2009; Wurtz et al. 2017). Skin lesions are a reliable predictor of levels of aggression, and have been shown to be moderately heritable as well (Desire et al. 2015, 2016; Turner et al. 2006b, 2008, 2009; Wurtz et al. 2017). The location of the lesions on the body is associated with different types of aggressive encounters, with lesions on the anterior of the body associated with pigs engaging in reciprocal aggression, and lesions on the caudal region associated with retreat from bullying or one-sided attacks (Turner et al. 2006a). This is important to note as it means that the number of lesions an individual pig has not only reflect aggression it received from other pigs but also how much aggression that individual delivered to other pigs. This study aimed to identify genetic regions associated with lesion scores in pigs through genome-wide association analyses (GWA). Identifying significant genetic regions linked to lesion scores is an early step in identifying causal genes, furthering our understanding of the genetic control of aggression, so that steps may be made to reduce the negative welfare and production losses associated with regrouping pigs.

Our study followed 1,093 purebred Yorkshire pigs housed at the Michigan State University Swine Teaching and Research Center, East Lansing, MI, from weaning to market across eight contemporary groups or replicates (Rep 1: n = 120, Rep 6: n = 133, Reps 2, 3, 4, 5, 7, 8: n = 140). Pigs were fed a commercial diet meeting or exceeding the National Research Council (NRC 2012) nutritional requirements for each stage of development. Feed was delivered using self-feeders with no more than four pigs/space within the nursery and no more than 10 pigs/space in the grow-finish/mature gilt pens. Water was provided by a single nipple drinker in each pen (nipple with cup in grow-finish/mature gilt pens). Nursery pens housed 10 pigs and

were 1.78 m x 1.18 m with fiberglass-gated sides and rounded metal slat flooring. Grow-finish/mature gilts pens housed 13-15 pigs and were 4.83 m x 2.44 m with metal-gated sides and fully slatted concrete floors. All pens consisted of single-sex groups (gilts or barrows).

Pigs were strategically regrouped into pens with a mix of familiar and unfamiliar conspecifics at three ages: weaning (mean: 21.5 ± 2.3 d of age), grow-finish (mean: 67.1 ± 3.0 d of age), and mature gilt (~100 kg, mean: 147.9 ± 6.3 d of age). Groups were formed to keep similar proportions of familiar/unfamiliar pen mates across pens and to minimize body weight variation within pens. Weaned pigs were housed with pairs or triplets of littermates. Grow-finish pens consisted of groups of 3-5 familiar (shared a previous pen) pigs. The mature gilt pens consisted of groups of 2-6 familiar (grow-finish pen mates) pigs.

As a proxy to the quantification of the amount of aggression occurring within the first 24 h following regrouping, when aggression is oftentimes the most intense, skin lesion counts by body region were collected immediately prior to regrouping, and 24 h after regrouping following the recommendations of Turner et al (2006a).

Whole blood samples were collected at seven wk from each pig for DNA isolation. Individuals were genotyped using the GeneSeek Genomic Profiler for Porcine HD ver 1 commercial BeadChip (Neogen Corporation – GeneSeek Operations, Lincoln, NE). To control for sources of error, markers or animals that had greater than 10% missing data were removed from the analysis. In addition, markers with a minor allele frequency of < 5% were removed. Finally, the X chromosome was excluded, leaving a dataset of 52,925 single nucleotide polymorphisms (SNP) for 1,079 animals for analyses. Naïve imputation was utilized to account for missing genotypes (0.003%) by replacement with the expected allelic dosage of the SNP.

Genotypes were expressed as allelic dosage for each locus for each individual and stored in marker matrix ($\boldsymbol{M}$). $\boldsymbol{M}$ was standardized (to matrix $\boldsymbol{Z}$) by first subtracting the expected allelic dosages from each SNP and dividing by the square root of a common expected variance of allelic dosage, setting the mean values of allele effects to zero and its variance to one. The genomic relationship matrix ($\boldsymbol{G}$) was computed following procedures set forth by VanRaden (2008) by multiplying $\boldsymbol{Z}$ by its transpose, producing an element matrix containing estimates of the realized genomic relationship between each animal in the population.

To estimate variance components, genomic best linear unbiased prediction models (Genomic BLUP) (Mrode 2014) were fit using the gwaR package in R (https://github.com/steibelj/gwaR). The response variables were log-transformed (Y = $\log_e$ (1 + lesion score)) post-mix lesion scores at each body location. Fixed effects included sex (gilt or barrow), replicate (eight levels), lesion scorer (nine levels), and body weight and pre-mix lesions as covariates. Pen was included as a random effect, as well as a random vector of genetic additive effects obtained from the genomic relationship matrix.

Genome-wide association analyses were performed following Gualdron Duarte et al. (2014) for each trait. The model fit was:

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{a} + \boldsymbol{e},$$

Where $\boldsymbol{y}$ is the vector of log transformed post-mix lesion scores, $\boldsymbol{\beta}$ are fixed effects of sex, replicate, lesion scorer, and covariates of body weight and pre-mix lesions, $\boldsymbol{a} \sim \boldsymbol{N}(\boldsymbol{O}, \boldsymbol{G}\sigma_{\boldsymbol{a}}^{\boldsymbol{2}})$ are the random genetic additive effects, and $\boldsymbol{e} \sim \boldsymbol{N}(\boldsymbol{O}, \boldsymbol{I}\sigma_{\boldsymbol{e}}^{\boldsymbol{2}})$ is a vector of random error terms.

The vector of SNP effects, $\boldsymbol{g}$, was obtained by linearly transforming the breeding values, $\boldsymbol{a}$, obtained as follows:

$$\boldsymbol{g} = \boldsymbol{Z}'\boldsymbol{G}^{-\boldsymbol{1}}\boldsymbol{a}$$

Association testing was performed following Gualdron Duarte et al. (2014) to derive appropriate standard errors and to obtain frequentist p-values for the association of each SNP with the phenotype of interest.

RESULTS AND DISCUSSION

Three SNP (FDR < 5%) associated with phenotypic records were identified for lesions to the anterior and central portions of the body in grow-finish pigs. These SNPs were located on chromosome 11 (ALA0061562, ALGA0061574, and MIGA0026237). Manhattan plots were created by plotting the logarithmically transformed p-values versus the corresponding SNP genomic position (Figure 3.1). There were no significant SNPs associated with the remainder of traits tested (Figure 3.2). For all significant association peaks, a confidence interval for their position was estimated following Casiró et al. (2017). The peak marker associated with anterior lesions explained 6.43% of the phenotypic variance, while the peak marker associated with central lesions explained 5.97%. For both markers, the B alleles were associated with lower lesion scores. These association peaks are presented in Table 3.1 along with their significance and confidence intervals, as well as the annotated porcine genes (Sus scrofa 10.2 assembly from Ensembl (Yates et al. 2016)) included within the confidence intervals. Two genes are located within the 95% confidence interval (CI) for anterior lesions for grow-finish pigs, A-kinase anchoring protein 11 (*AKAP11*), and diacylglycerol kinase eta (*DGKH*). *DGKH* is also contained within the 95% CI for central lesions for grow-finish pigs, in addition to a novel protein coding gene (*ENSSSCG00000029837*). The *DGKH* gene has been identified as a risk haplotype for bipolar disorder in humans (Weißflog 2016). Bipolar disorder patients show increased levels of aggression (Ballester 2012, Perroud 2011), making this association particularly interesting given

our focus on understanding social aggression in pigs. Together, these results suggest that chromosome 11 contains a region explaining variation in lesion scores, and further exploration should be conducted to determine if genes in this region are involved in the underlying biological control of aggression. While this study could benefit from a larger sample size to increase the power to detect significance, it is promising that significant markers are being identified for the complex trait of aggression, and for the potential of selective breeding or for identifying alternative therapies to decrease aggression within group-housed pigs.

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APPENDIX

Table 3.1. Peak position markers, their location, SNP, q-value, effect on phenotype, lower and upper boundary of the 95% confidence interval, and symbols of annotated genes in the region.

Trait	Peak Marker	SSC	Pos	q-value	Effect of B allele[1]	% var[2]	95% CI lower limit	95% CI upper limit	Genes contained in region
Anterior grow-finish lesions	ALGA0061562	11	25593848	3.87E-07	-	6.43	25432289	25755407	*AKAP11*[a], *DGKH*[b]
Central grow-finish lesions	ALGA0061574	11	25705345	2.77E-07	-	5.97	25471772	25938918	*DGKH*[b], *ENSSSCG00000029837*[c]

[a] A-kinase anchoring protein 11, [b] Diacylglycerol kinase eta, [c] Novel gene
[1] Whether the B allele of peak SNP increases or decreases phenotype, [2] % of phenotypic variance explained by peak marker genotype

Figure 3.1. Manhattan plots for anterior and central lesions at mix for grow-finish pigs. The x-axis corresponds to chromosomal position, the y-axis represents the -$\log_{10}(P)$ value, and the green line indicates the genome-wide significance threshold (FDR<0.05).

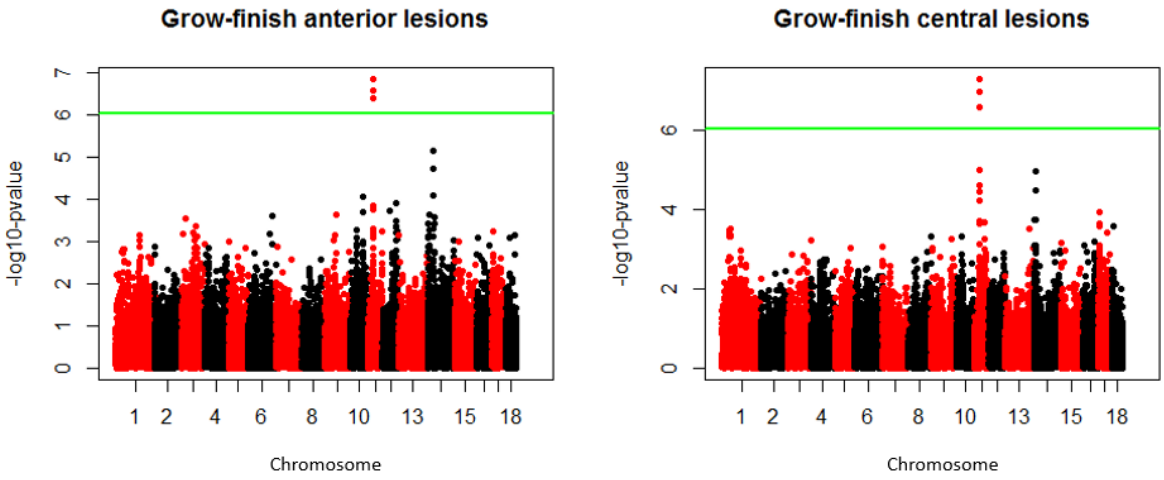


Figure 3.2. Manhattan plots for the remainder of traits tested where markers did not reach the genome-wide significance threshold (FDR<0.05).

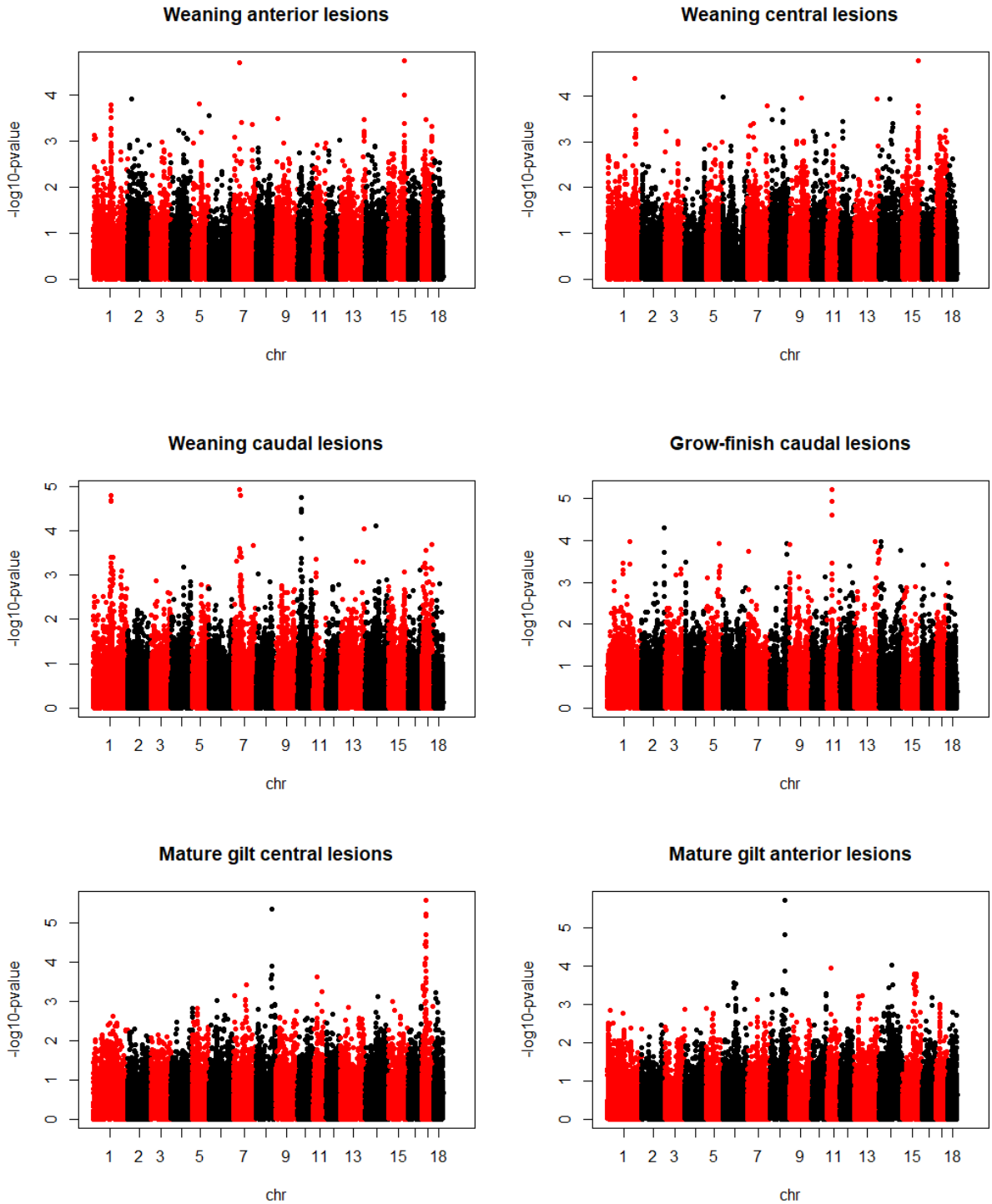
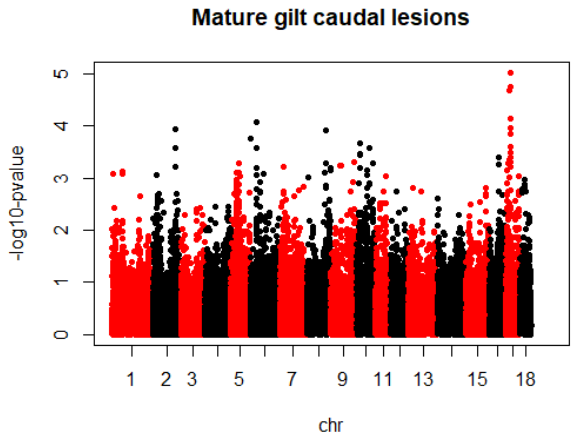


Figure 3.2 (cont'd)



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CHAPTER 4: ESTIMATION OF GENETIC PARAMETERS OF AGONISTIC BEHAVIORS AND THEIR RELATION TO SKIN LESIONS IN GROUP-HOUSED PIGS

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ABSTRACT

Aggression in group-housed pigs following mixing with unfamiliar animals is a pressing welfare and production issue in the swine industry. Genetic selection for pigs better suited for group living has been proposed, however a better understanding of the underlying genetic components of aggression and their relationship to easily measured phenotypic traits is necessary before widespread adoption into breeding programs. Our study used lesion scores as a readily measured proxy for assessing level and type of aggression. We performed genetic and phenotypic correlations of these lesion scores with aggressive behavior durations obtained through video observation. Observations were conducted on 229 purebred Yorkshire pigs immediately following mixing into new pens with unfamiliar animals and on 389 individuals 3 weeks following remixing to allow for stable observations after the establishment of group hierarchies. Correlations of marginal residuals were generated to observe relationships among different behaviors. To examine phenotypic and genetic correlations between behaviors and lesions, bivariate analyses were performed using a genomic best linear unbiased prediction model. Fixed effects were composed of sex and replicate, covariates were composed of weight and pre-mixing lesions, and pen and genetic additive effect were evaluated as random effects. The response variables were log-transformed post-mixing lesions and log-transformed duration of time spent engaging in specific aggressive behaviors. A genetic relationship matrix was constructed using genotypes from 52,925 SNP. Similar univariate models were fitted for each behavior to estimate heritabilities. Behaviors of both inverse and parallel pressing, were highly

correlated with reciprocal fights, and thus grouped for further analyses. Pigs that avoided aggression though the use of subtler non-damaging behaviors, such as head knocks, showed more involvement in damaging aggression later on in established groups. Heritability estimates of behaviors were generally low and not significant at mixing, while moderate heritabilities were estimated at the 3 week post-mixing period. At mixing, reciprocal aggressive interactions were genetically and phenotypically positively correlated with lesions to the anterior and central regions of the body. Delivery of one-sided aggression was also positively correlated with anterior lesions. Genetic and phenotypic correlations 3 week post-mixing were difficult to interpret, potentially due to small population size and low numbers of lesions observed at this time period. This knowledge will help guide genetic selection to reduce levels of aggression by helping determine the most optimal traits to select for the greatest potential impact on genetic change while reducing any potentially negatively associated correlated traits.

INTRODUCTION

Aggression in group housed pigs is a topic of great interest from both production and welfare perspectives. Researchers are working to better understand the genetic basis of aggression and its relation to economically important traits (D'Eath et al., 2009; Desire et al., 2015a; Turner et. al., 2006a; Wurtz et al., 2017). However, a barrier to studying aggression effectively is the large of time and labor required to decode large amounts of video data. To circumvent this barrier, counts of skin lesions have been proposed as a proxy for aggression. This study examined the phenotypic and genetic correlations between skin lesions and duration of aggressive behaviors to further validate this substitution. The phenotypic and genetic correlations were also estimated among three classes of aggressive interactions; reciprocal fights,

delivering one-sided aggression, and receiving attacks with skin lesions. In addition, the heritabilities of these behavioral interactions were estimated. The correlations between skin lesions and behavioral observations were estimated for two observational periods: immediately following regrouping and three weeks post grouping in a more established social setting. By better understanding the underlying genetic components to aggressive behaviors and their relationship to lesions in different social structures, better decisions regarding selection of pigs for breeding can be made. Improved selection should also help reduce the amount of acute aggression observed after mixing and chronic social stress that may persist in unstable groups over time.

MATERIALS AND METHODS

Population

All animal protocols were approved by the Institutional Animal Care and Use Committee (AUF# 01/14-003-00).

An experimental population housed at the Michigan State University Swine Teaching and Research Center, East Lansing, MI was utilized for this study. The population of 1,093 purebred Yorkshire pigs was followed from weaning to market and consisted of eight replicates. Additional information on housing and management practices may be found in Wurtz et al. 2017.

Regrouping

Pigs were strategically placed into new social groups when moved from nursery pens into grow-finish pens at an average of 67.1 (± 3.0 d) days of age. Similar proportions of familiar vs unfamiliar pen mates were maintained across pens and body weight variation was minimized within pen. Pens consisted of multiple groups of 3 to 5 previously familiar pigs (i.e. those from

the same nursery pen) totaling 13-15 pigs per grow-finish pen. Familiarity was defined based on nursery housing regardless of whether pigs were littermates. All groups were single sex, and location of pens in rooms alternated by sex.

Lesion scores

Lesion scores were obtained from the entire population of pigs immediately prior to mixing, 24 h following mixing, and three weeks following mixing. Lesion scoring was performed by three trained observers and consisted of counting the total number of lesions. A lesion was counted when it was a single, fresh, continuous scratch (< 24 h old). Fresh lesions were judged on the basis of redness and development of scabbing. Lesions were recorded by location on the body (anterior, central, and caudal).

Genotypic data

A total of 1,082 individuals were genotyped using the GeneSeek Genomic Profiler for Porcine HD ver 1 commercial BeadChip (Neogen Corporation – GeneSeek Operations, Lincoln, NE). Data cleaning procedures and the steps taken to obtain the genomic relationship matrix for further analyses are outlined in detail in Wurtz et al. 2017.

Behavioral data

Video cameras (Clinton Electronics VF540) connected to a multichannel digital video recorder were mounted above each pen. Pigs were marked on their backs with a non-toxic marker for individual identification. Continuous video decoding took place for five hours immediately following regrouping and for four hours the next morning. At three weeks post regrouping pigs were recorded for 4 hours in the afternoon (13:00 – 17:00). Data collected immediately after mixing was used to demonstrate the pigs' response to social change, while

data collected 3 weeks later provided information on the pigs' social behavior in an established group. A subset of the total population was fully observed at the time of this study; this subset consisted of 299 individuals 24 hours post-mixing, from 22 total pens, and 389 individuals 3 weeks post-mixing, from 29 total pens.

A team of undergraduate students identified pre-determined aggressive social interactions on recorded video. A set of reference video clips were used to train and evaluate the accuracy and reliability of each student. Students were required to obtain at least an 85% match to the reference video key before they were allowed to begin decoding project video. Every student's reliability was re-checked every 4 months by having students re-decode a segment of their already completed work to ensure consistency (intra-observer reliability) and an additional clip to compare with the rest of the viewers in the lab to ensure uniform decoding (inter-observer reliability). In addition, random segments of completed video data were checked by a different viewer to ensure at least 85% coincidence in decoded data among viewers. If the data from a student failed to meet the 85% accuracy criteria, the entire video segment was re-decoded.

Recorded behaviors were grouped into two classes: damaging aggression and non-damaging aggression. Damaging aggression included behaviors such as one-sided attacks (AT) and reciprocal fights (RF). Non-damaging aggression included head knocks (HK), isolated bites (B), inverse parallel pressing (IP), parallel pressing (PP), rest periods during a reciprocal fight (RDF), and withdrawing at the end of a reciprocal fight (WF). A detailed ethogram of these behaviors can be found in Table 4.1. Durations of behaviors in seconds and the pigs involved in the interactions were recorded. For rapid, instantaneous behaviors, such as bites or head knocks, the duration recorded was one second per instance.

For estimation of genetic and phenotypic correlations, behaviors were grouped into three categories. This was done to help account for the rare occurrences of withdrawals from fights and rests during reciprocal fights observed 24 hours post-mixing and 3 weeks post-mixing, and rare occurrences of pressing behaviors observed 3 weeks post-mixing. The first group entitled "Reciprocal" consisted of reciprocal fights, inverse-parallel pressing, and parallel pressing, all of which involved 2 or more pigs actively engaged in delivering aggression. The second group entitled "Receive Attacks" consisted of the total duration that an individual was receiving one-sided attacks. Lastly, the third group, "Deliver One-sided" consisted of durations single pigs spent delivering one-sided attacks, delivering isolated bites, and delivering head knocks. To adjust for skewness present in the data (large numbers of observations of short duration), all behavioral duration data were log transformed ($\log_{10}$(1+observation)).

Statistical models

To obtain variance components for analyses genomic best linear unbiased prediction models (Genomic BLUP) (Mrode, 2014) were fitted using the gwaR package in R (https://github.com/steibelj/gwaR) as follows:

$$\boldsymbol{Y} = \boldsymbol{Xb} + \boldsymbol{u} + \boldsymbol{Z_p p} + \boldsymbol{e}$$

Where, $\boldsymbol{Y}$ = the vector of total duration of behavior in seconds (log-transformed); $\boldsymbol{b}$ = vector of fixed effects of sex (gilt or barrow), replicate (eight levels), and weight as a covariate; $\boldsymbol{X}$ was the incidence matrix relating observations to levels of fixed effects; $\boldsymbol{u}$ = random vector of genetic additive effects; $\boldsymbol{p}$ = vector of random pen effects; $\boldsymbol{Z_p}$ was the incidence matrix relating **Y** to $\boldsymbol{p}$;

and **e** was a vector of residual effects. The following distributions were assumed for random quantities in the model:

- $\boldsymbol{u} \sim N(\mathbf{0}, \boldsymbol{G}\sigma_u^2)$, where $\boldsymbol{G}$ = genomic relationship matrix
- $\boldsymbol{p} \sim N(\mathbf{0}, \boldsymbol{I}\sigma_p^2)$, where $\boldsymbol{I}$ = identity matrix
- $\boldsymbol{e} \sim N(\mathbf{0}, \boldsymbol{I}\sigma_e^2)$, where $\boldsymbol{I}$ = identity matrix

To estimate covariance and correlation of lesion scores at different body locations with observed behavioral durations, bivariate genomic BLUP models were used. Models were fit jointly to log-transformed lesions at each of the three body locations to log-transformed duration of engagement in reciprocal fights, delivering one-sided aggression, or receiving attacks both 24 hours post-mixing and 3 weeks post-mixing. Models included similar effects as the univariate analysis, expanded to include all interactions of fixed effect with trait and a bivariate Gaussian distribution was assumed for all random effects.

To examine covariance and correlations between durations of different classes of behaviors, similar bivariate genomic BLUP models were fit to each possible pair of behavioral durations (log-transformed). After fitting these models, estimates of variance components, $\hat{\sigma}_u^2$, $\hat{\sigma}_e^2$, and heritabilities were obtained, as well as the covariance and phenotypic, genotypic, and residual correlations.

Due to the small size of the dataset, which could limit the inferential power to estimate genetic and phenotypic correlations using bivariate GBLUP models, Pearson correlations of marginal residuals from the model were also estimated to examine the phenotypic relationships between observed aggressive behaviors, while accounting for fixed effects of sex, replicate, and weight.

RESULTS AND DISCUSSION

Heritabilities of behavioral traits

Heritabilities were generally low and not significant at 24 hours post-mixing (Table 4.2). Moderate heritabilities were estimated for receiving attacks, involvement in inverse pressing, and for the combined reciprocal interaction category (RF, IP, and PP), where inverse pressing and the combined reciprocal interactions were deemed significant ($P<0.05$). Larger behavioral heritabilities were estimated 3 weeks post-mixing with reciprocal fighting, general involvement in attacks, delivering and receiving attacks, bites, the combined reciprocal category, and the combined delivering aggression category all having moderate and significant heritabilities ($P<0.05$). Heritability estimates 24 hours post-mixing from this study were substantially lower than previously reported in Turner et al. (2008, 2009) for reciprocal aggressive interactions and the delivery of one-sided aggression. However, similar magnitudes of heritability were obtained for the receipt of one-sided aggression. This discrepancy may be due to Turner et al. (2008, 2009) only including damaging aggressive behavior, while this study grouped non-damaging aggressive behaviors such as pressing in the analyses. The present study is also reporting data from a much smaller population, especially at 24 hours post-mixing, which may hinder the ability to properly estimate genetic variances needed to obtain heritability values. An additional rational for smaller heritability estimates at 24 hours post-mixing than 3 weeks post-mixing is due to the overall pen-level amounts of aggression observed. At 24 hours post-mixing, aggression is generally frequent and intense, which often forces all pigs within the pen to become involved due to limited space as they are unable to avoid interaction. However, typically there are much lower instances of aggression observed 3 weeks-post-mixing (Jensen and Wood-Gush, 1984; McCort and Graves, 1982). The interactions that do occur at this time period may be

deliberate and targeted. Analyzing interactions at this point may allow us to better capture individual personality differences, therefore, increasing our success in identifying genetic variance associated with the three classes of aggression we identified (reciprocal, deliver one-sided, receive attack).

Bivariate analyses of behavioral and lesion traits

Genetic (Table 4.3) and phenotypic (Table 4.4) correlations were estimated between skin lesions and behavior durations at both 24 hours post-mixing and 3 weeks post-mixing time points. Previous studies have examined the correlations between 24 hours post-mixing and stable lesions to assess associations between short- and long-term aggressiveness (Desire 2015a; Wurtz 2017), and correlations between stable group and post-mixing lesions with aggressive behaviors (immediately post-mixing) have been observed (Desire 2015b). This study examined those relationships, but with the addition of behavioral observations collected 3 weeks post-mixing and correlations of these behaviors examined with both post-mixing and 3 weeks post-mixing skin lesions.

At 24 hours post-mixing, high positive genetic correlations were identified between duration of reciprocal interactions and number of anterior lesions. Delivering aggression more often was more highly genetically correlated with more anterior and middle lesions. Estimated genetic correlation between receiving aggression and lesions was of small magnitude and with proportionately large standard errors, suggesting non-significance. Phenotypic correlations of behaviors with lesions at 24 hours post-mixing were low with relatively large standard errors. Positive correlations were identified between reciprocal interactions and anterior lesions, as well between delivery of one-sided interaction with anterior lesions. This is in accordance with findings in other populations that found higher numbers of anterior lesions to be more associated

with engagement in reciprocal interactions or delivery of bullying (Turner et al., 2006b, Turner et al., 2008; Turner et al., 2009). However, our study did not replicate their findings of caudal lesions being more highly correlated with the receipt of aggression.

At 3 weeks post-mixing, we found reciprocal interactions to be negatively genetically correlated with front and rear lesions. A similar pattern was observed with receiving attacks. These correlations are difficult to explain, but may be an artifact of fewer lesions counted at the 3 weeks post-mixing period. Phenotypic correlations were close to zero and appeared to be non-significant based upon assessment of standard error values (proportionately large standard errors compared to estimated correlation values).

Genetic and phenotypic correlations were also calculated between lesions obtained at 24 hours post-mixing with behaviors observed 3 weeks post-mixing, and between behaviors observed 24 hours post-mixing and lesions present 3 weeks post-mixing to identify potential predictive traits to aid in selection decisions. If significant correlations are observed, selection decisions made early after mixing could impact levels of aggression present later in established groups. While Desire et al. (2015b) found negative phenotypic correlations with aggressive behaviors at 24 hours post-mixing and skin lesions 3 weeks later, our analyses did not return similar results. These non-significant correlations could be attributed to the relatively small study size, with insufficient power to identify any associations, if present. The results however, are included in Tables 4.2 and 4.3 for comparison.

The varying results between genetic and phenotypic correlations between our results raises an important point when implementing selection. Selection on observed phenotypes may not yield the most optimal results, as they may not follow the same proportions of associated genetic variation (Cheverud, 1988). Based upon heritability estimates obtained by this research,

it appears that selection upon number and location of lesions may have greater potential for reducing aggression than by selection on observed behavioral responses.

Correlations of marginal residuals

Correlations of marginal residuals at 24 hours post-mixing (Table 4.5) and at 3 weeks post-mixing (Table 4.6) are reported. At 24 hours post-mixing, involvement in attacks (AT) was highly correlated in general with involvement in aggressive interactions. Isolated bites (B) were negatively correlated with withdrawals from fights; potentially suggesting that those individuals delivering a lot of isolated bites were less likely to lose a fight and be forced to retreat. There were negative correlations between head knocks (HK) and pressing behaviors and head knocks (HK) and reciprocal fights (RF). This may be due to pigs successfully using non-damaging, subtle interactions to avoid engagement in damaging aggression. Inverse pressing (IP) and parallel pressing (PP) were both highly correlated with reciprocal fights (RF), which was expected as pressing typically did not occur outside of reciprocal fighting bouts. In the established, more stable groups (3 weeks post-mixing), similar patterns were observed with attacks (AT), pressing (IP and PP), and reciprocal fights (RF). We were not able to observe the relationship between B and WF in the stable groups as there were no instances of WF. There were also no observed instances of RDF during this period. An unexpected moderate positive correlation was obtained between head knocks (HK) and general involvement in attacks (AT). However, this is consistent with findings of Desire et al. (2015b) who showed pigs who avoid aggression at mixing show increased involvement in aggression later on due to failure of hierarchy formation.

These findings also support our decision to group certain behaviors. The high correlations between pressing and reciprocal fights reported here further validate the decision to group these behaviors into a general “reciprocal” interaction category.

Conclusions

This knowledge will help guide genetic selection to reduce levels of aggression by helping determine the most optimal traits to select for the greatest potential impact on genetic change while reducing any potentially negatively associated correlated traits. These preliminary results suggest that genetic variation in individuals’ response to mixing may be difficult to estimate 24 hours post-mixing when aggression is intense. It may be more efficient to select pigs based upon their interactions in a more established group setting, targeting individuals involved in chronic aggression. Anterior skin lesion’s high positive genetic correlation with engagement in reciprocal aggression and delivery of one-sided aggression make them a good candidate trait to target in selection schemes. Finally, selection decisions should not be made based upon phenotypic observations and correlations alone. Underlying genetic relationships and correlations may differ from their phenotypic counterparts, making them a key component in genetic selection decision making.

APPENDIX

Table 4.3. Genetic correlations between classes of aggressive behavior and skin lesions at both 24 hours post-mixing and 3 weeks post-mixing time points. Standard errors are reported in parentheses.

		Behavior 24 h post-mixing			Behavior 3 wk post-mixing		
		Reciprocal[1]	Deliver One-sided[2]	Receive AT	Reciprocal[1]	Deliver One-sided[2]	Receive AT
Lesions 24 h post-mixing	Anterior	0.84 (0.46)	0.69 (0.37)	-0.10 (0.30)	0.32 (0.22)	0.31 (0.19)	0.32 (0.27)
	Central	0.50 (0.42)	0.61 (0.66)	-0.07 (0.36)	0.40 (0.25)	0.52 (0.21)	0.64 (0.30)
	Rear	0.48 (0.37)	0.30 (0.59)	-0.04 (0.34)	0.30 (0.25)	0.35 (0.22)	0.23 (0.32)
Lesions 3 wk post-mixing	Anterior	0.05 (0.34)	-0.38 (0.22)	0.32 (0.34)	-0.60 (0.30)	-0.24 (0.22)	0.10 (0.31)
	Central	-0.28 (0.35)	-0.30 (0.24)	0.02 (0.36)	-0.07 (0.29)	0.26 (0.24)	0.03 (0.34)
	Rear	0.05 (0.39)	0.18 (0.25)	-0.13 (0.39)	-0.35 (0.31)	-0.30 (0.25)	-0.21 (0.37)

[1]RF + IP + PP, [2]Deliver AT + B + HK

Table 4.4. Phenotypic correlations between classes of aggressive behavior and skin lesions at both 24 hours post-mixing and 3 weeks post-mixing time points. Standard errors are reported in parentheses.

		Behavior 24 h post-mixing			Behavior 3 wk post-mixing		
		Reciprocal[1]	Deliver One-sided[2]	Receive AT	Reciprocal[1]	Deliver One-sided[2]	Receive AT
Lesions 24 h post-mixing	Anterior	0.36 (0.06)	0.21 (0.06)	0.17 (0.06)	0.09 (0.06)	0.06 (0.06)	0.04 (0.06)
	Central	0.29 (0.06)	0.09 (0.06)	0.16 (0.07)	0.10 (0.07)	0.07 (0.07)	0.05 (0.07)
	Rear	0.24 (0.07)	0.09 (0.06)	0.11 (0.07)	0.11 (0.07)	0.08 (0.08)	0.08 (0.07)
Lesions 3 wk post-mixing	Anterior	-0.08 (0.07)	-0.05 (0.07)	-0.01 (0.07)	0.10 (0.07)	0.13 (0.07)	0.17 (0.06)
	Central	-0.04 (0.06)	-0.07 (0.06)	0.07 (0.06)	0.08 (0.06)	0.10 (0.06)	0.04 (0.06)
	Rear	-0.06 (0.07)	-0.05 (0.07)	-0.01 (0.07)	0.00 (0.07)	0.07 (0.07)	0.01 (0.07)

[1]RF + IP + PP, [2]Deliver AT + B + HK

Table 4.5. Pearson correlations of marginal residuals 24 hours post-mixing of log-transformed duration of behaviors.

Behavioral Trait	AT	B	HK	IP	PP	RF	WF	RDF	Deliver AT	Receive AT	Recip-rocal[1]	Deliver One-sided[2]
AT	1											
B	0.35	1										
HK	0.1[NS]	0.34	1									
IP	0.45	0.11[NS]	-0.18	1								
PP	0.5	0.2	-0.15	0.68	1							
RF	0.54	0.16	-0.14	0.84	0.71	1						
WF	0.12	-0.14	0.04[NS]	0.12	0.15	0.11[NS]	1					
RDF	0.13	0.08[NS]	0.09[NS]	0.13	0.13	0.11[NS]	0.02[NS]	1				
Deliver AT	0.73	0.29	-0.02[NS]	0.53	0.5	0.62	0.14	0.12	1			
Receive AT	0.68	0.17	0.06[NS]	0.15	0.21	0.21	0.11[NS]	0.06[NS]	0.14	1		
Reciprocal[1]	0.54	0.17	-0.13	0.94	0.76	0.93	0.12	0.13	0.59	0.21	1	
Deliver One-sided[2]	0.79	0.54	0.29	0.42	0.43	0.51	0.09[NS]	0.11	0.86	0.23	0.5	1

[1]RF + IP + PP, [2]Deliver AT + B + HK, [NS]Denotes non-significance with $P > 0.05$

Table 4.6. Pearson correlations of marginal residuals 3 weeks post-mixing of log-transformed duration of behaviors.

Behavioral Trait	AT	B	HK	IP	PP	RF	Deliver AT	Receive AT	Reciprocal[1]	Deliver One-sided[2]
AT	1									
B	0.37	1								
HK	0.4	0.04^{NS}	1							
IP	0.11	0.17	0.09^{NS}	1						
PP	0.11	0.12	0.11	0.2	1					
RF	0.44	0.3	0.26	0.31	0.12	1				
Deliver AT	0.8	0.37	0.32	0.08^{NS}	0.12	0.38	1			
Receive AT	0.79	0.24	0.34	0.14	0.10^{NS}	0.37	0.35	1		
Reciprocal[1]	0.43	0.34	0.28	0.59	0.33	0.89	0.36	0.36	1	
Deliver One-sided[2]	0.62	0.39	0.83	0.08^{NS}	0.11	0.36	0.63	0.38	0.37	1

[1]RF + IP + PP, [2]Deliver AT + B + HK, [NS]Denotes non-significance with $P > 0.05$

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CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

DISCUSSION

Current group-housing practices in the pig industry present some welfare and production concerns. By mixing unfamiliar pigs of similar weights into restricted space, outbursts of aggressive interactions occur. This aggression can be intense and lead to injury. If aggression persists it could lead to issues related to chronic stress. For the time being, the majority of group housed pigs are destined for market, so the amount of potential mixing is minimized. However, with legislation and market pressure to provide gestating sows with increased space and social opportunities, the number of potential mixings with unfamiliar pigs greatly increases. In addition, if gestating sows are exposed to chronic stress from continued disrupted social hierarchies, reproductive success could be hindered. These new challenges for industry are taxing, however, with additional research information can be obtained and disseminated to breeders and producers to reduce the negative impacts to animal welfare and production efficiency. Measuring aggression through video observations is costly, both in time and money, thus methods such as measuring skin lesion counts have been developed and implemented to obtain more rapid results. Lesion scoring is relatively quick to perform, and can give a quick snap shot view of levels of aggression both at a pen level, and on an individual basis. Furthermore, by examining the location of lesions on the body the type of interactions can be deduced.

Past research has estimated heritabilities of lesion scores using pedigree-based relationship matrices (Desire et al., 2015; 2016; Turner et al., 2006; 2008; 2009). Chapter 2 estimated lesion score heritabilities using genomic relationship matrices to further validate past results and to obtain improved accuracy of predictions through the use of actualized genetic

relationships amongst individuals. We estimated higher lesions score heritabilities at mixing than in established social groups, which contradicted what past studies have found; however, we concluded this was likely due to the generally low number of lesions present in the stable group. Overall, our heritability estimates were of similar magnitudes to those previously reported, provided further validation to the scientific literature of the genetic contribution underlying agonistic behaviors associated with receiving and delivering skin lesions. In addition, we obtained heritability estimates from two additional age groups of pigs (newly weaned pigs and mature gilts), in which estimates have not been reported previously in the literature. We found similar heritability estimates for skin lesions between these age groups, suggesting that the genetic component to skin lesions remains consistent regardless of age or production stage. To further investigate changes in aggressiveness as pigs age, phenotypic and genetic correlations were obtained, providing new evidence supporting strong correlations across different stages of pigs, suggesting that lesions obtained at weaning could be predictive of how pigs will behave later in life in a breeding population. Results from Chapter 2 provide evidence that, despite environmental effects across time as pigs age and inherent errors associated with measuring lesions, lesion scoring can be an efficient and repeatable phenotypic measure that can be incorporated into selection schemes to reduce the amount of aggression observed at mixing.

In addition, Chapter 2 focused on addressing the concern surrounding impacts on production efficiency by selecting for more docile pigs. We estimated genetic correlations between skin lesions and the traits of growth rate, back fat thickness, and loin muscle area. We did not find any significant correlations between skin lesions and growth rate and back fat thickness. However, we did identify a significant negative correlation with loin muscle area, suggesting that selection for reduced skin lesions may lead to increased loin muscle area.

Building upon the evidence supporting the underlying genetic control of aggression and its ability to be selected upon, we worked to identify which regions on the pig genome are responsible for the phenotypic variation present in our population. This is an early step in identifying causal genes responsible for increased aggressive responses, which will further our understanding of the biological mechanisms related to high levels of observed aggression. Chapter 3 discusses the genome-wide association studies that were performed for pigs at multiple life stages, for lesions located at three body regions. We identified significant peaks for grow-finish stage pigs for anterior and central skin lesions on chromosome 11. In both cases, peaks were located in similar regions of the chromosome. Investigation into annotated genes within these regions identified genes that warrant further study into their biological functioning and whether they could contribute to the expression of aggressive behaviors. While Chapter 3 will benefit from increased sample size to increase significance, it was promising to identify similar regions for multiple, complex traits.

Additional knowledge to help guide optimal genetic selection was obtained in Chapter 4. Larger heritability estimates for duration of aggression were obtained 3 weeks post-mixing, suggesting that selection on behaviors observed in stable groups may hold the greatest potential for genetic change though selection. This study also identified strong positive genetic and phenotypic correlations between anterior skin lesions with involvement and delivery of damaging aggression.

FUTURE DIRECTIONS

A useful follow up to Chapter 2 would be to examine genetic correlations between aggression and additional important production traits, such as feed efficiency. Practical

implications, i.e. lack of electronic feeders, did not make it possible for us to obtain individual consumption data. Growth rates within phase, backfat (lean growth), and loin muscle area (saleable yield) are important, however there may be producer interest in the genetic relationship between feed efficiency and aggression. I hypothesize that pigs that can better adapt to group living environments, by successfully forming social hierarchies and avoiding long-term chronic stress, would have lower residual feed intake levels, therefore making them more efficient producers. Residual feed intake would provide producers with a more rounded picture of how well their animals are thriving and growing. More scientific literature that shows the benefits of selection on reduced aggression for efficiency would further incentivize the industry to include aggressive behavioral measures in their selection schemes.

Our genome-wide association studies were only able to identify two areas of the genome associated with lesion scores. This was most likely due to a limited population size. Aggression is a complex trait influenced by many markers, each contributing to a small portion of the phenotypic trait variation. In the future, I would like to increase sample size to increase power to detect significant markers through analyses on combined populations in which similar traits have been measured. Population structures can be compared by assessing heritabilities, genetic variances, and residual variances. If there is no strong evidence suggesting heterogeneity in population structure, joint analysis can be performed. However, if there is significant heterogeneity in variance components between populations, meta-analyses may be performed. One sensible advantage to meta-analyses is that raw data will not need to be shared, which is a critical factor when collaborating with commercial industry.

Finally, decoding of video data was the biggest obstacle faced during this research. The amount of time invested into training student labor, as well as time and money spent to decode

recorded video is one of the greatest limitations to behavioral research and to incorporate behavioral phenotypes in breeding programs. The next logical step to advance behavior research and to incorporate behavioral data into precision livestock farming requires automated behavioral phenotyping. Technology exists to track pig activity levels within pens and to monitor their resource use; however, tracking individual animals within groups is challenging as it is difficult to identify features distinguishing between pigs. An additional challenge for automated technologies to replace manual decoding is the ability to determine the precise behavior that is occurring, for example detecting the difference between a series of head knocks (non-damaging one-sided interaction) versus a series of bites (damaging, one-sided or reciprocal interaction). However, if this technology can be successfully developed and validated, the amount of data used in behavioral research could increase exponentially. Instead of obtaining snap shots of behavior such as recording at two time points as we did during this research, we could track the pigs throughout the entire stabilizing period following mixing. This could lead to new insights into group stability and hierarchy formation in group-housed animals. Additionally, cameras could capture video around the clock on farms to provide producers with real time data about the levels of aggression occurring, and even information on the health status of individuals. With individual tracking, software could potentially identify when an individual deviates from its normal repertoire, alerting the farmer of potential welfare or production concerns.

The research outlined in this dissertation provides greater insight into the genetic component to aggression in group-housed pigs. Further evidence that aggressive behaviors, as well as skin lesions, are controlled significantly by the genetic make-up of the pig. This information is promising for the incorporation of aggressive behavioral traits into breeding programs. The strong correlations, both phenotypically and genetically between skin lesions and

aggressive behaviors suggest that selection on skin lesion scores would indeed lead to changes in levels of observed aggression. In addition, the concern about inadvertently harming growth and production traits was addressed and optimistic correlations between skin lesions and production traits were observed. These results provide promising information for the future of breeding for pigs more behaviorally suited for group-living environments.