

The use of genetic markers to improve sow productive life and genetic abnormalities

by

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A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Genetics

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2008

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ABSTRACT

The driving force behind using molecular genetics in livestock selection programs is to improve the profitability of not only the genetic supplier but also their customers using the product. Traditional quantitative genetics have greatly helped the geneticists improve traits with higher heritabilities, but there is still room for advancements, especially in traits that are measured late in life, measured in only one sex, and traits with lower heritabilities. Two such traits that could benefit from the identification and use of molecular markers are genetic defects and sow reproductive life, or the length of time that a sow remains productive in the breeding herd. Both sow reproductive life and genetic defects are often overlooked by producers. The studies presented in this dissertation identify current culling reasons of commercial sows, the identification of genetic markers in growth pathways, genetic markers and their associations with sow productive life, and the use of genetic markers to try to isolate the specific genetic defect causing extra digits on pigs.

Analysis of removal reasons for commercial sows revealed that the culling reasons for today's modern sow are very similar to those existing before the massive rearrangement of the swine industry into the highly vertically integrated industry it is today. The primary culling reasons for sows in early parities were for reproductive and locomotive failure, while the main reported reason that sows in more advanced parities were culled was simply because of old age, even though most of these sows were still producing at very acceptable levels. The genetic mapping paper identified single nucleotide polymorphisms (SNPs) in six candidate genes for growth/longevity,

including *insulin-like growth factor binding protein 1 (IGFBP1)*, *insulin-like growth factor binding protein 2 (IGFBP2)*, *insulin-like growth factor II receptor (IGF2R)*, *beta-2 adrenergic receptor (ADRB2)*, *carnitine O-palmitoyltransferase I (CPT1A)*, and *organic cation/carnitine transporter 2 (Solute carrier family 22 member 5; SLC22A5)* and their location in the pig genome. These genetic markers were then incorporated with additional markers in research pertaining to genetic markers from growth related pathways and their association with sow productive life. Though no marker suggested that it was causative as none caused an amino acid change, the genetic markers for *C-C chemokine receptor 7 (CCR7)* and *CPT1A* showed the clearest and most consistent associations, regardless of analyses attempted, with sow productive life. Additionally, *CPT1A* and *IGFBP1* both were significantly associated with reproductive performance traits such as the total number of pigs born or the number of pigs born alive. The use of molecular genetics to identify the causative mutation pertaining to pigs having extra digits was not as successful. This project was hampered by the inability to obtain offspring from an affected individual as well as incomplete sequence of the swine genome. The results presented herein will enhance producers' abilities to increase the financial well being of their operations with reduced financial losses due to genetic defects and poor sow productive life.

CHAPTER 1. GENERAL INTRODUCTION

INTRODUCTION

The overall objective of any business is to continually be profitable regardless of the input and output costs. To accomplish this goal, businesses must: 1) be elastic to the increasing input costs, 2) increase the price of goods sold as needed, 3) increase output quantity, or 4) maximize efficiency. Swine businesses are no different than any other business entities in these regards except, like most other agriculture industries, they generally buy based on retail prices and sell on wholesale prices. Therefore, with minimal opportunities to control the price of the products they buy or the price of the market hogs they sell, swine producers must focus on either increasing output or maximizing efficiency within the system. A large increase in pigs/sow/year and the number of market hogs sold per farm were seen in the 1990s when the consolidation of the swine industry occurred. The pork industry was transformed from having many small family farms into today's industry, where most of the market hogs are produced by a limited number of swine companies. In attempts to maximize efficiency of the entire system, several of today's companies, such as Smithfield or Tyson Foods, are vertically integrated and therefore maintain ownership of the pigs from the breeding farms to the processed pork products they produce and sell to customers. This illustrates the so called conception to consumption theory. However, even within large vertically integrated systems, there are still many ways to improve efficiencies that have gone largely overlooked until recently. Two such ways are to improve the productive life of the sows that produce

the market hogs and another is to minimize the number of terminal animals born with genetic defects that either never produce a viable fetus, die at an early age after birth, or never reach acceptable market weight. These losses are a cost burden on the pork production system.

Traditional quantitative trait improvements in livestock populations are based on the measurement of traits and the prediction and use of estimated breeding values. Traditional quantitative genetics has produced significant improvements in growth, increased loin eye area, and decreased backfat in market swine (National Swine Registry, 2007a) and has also increased the number of pigs born alive in maternal lines (National Swine Registry, 2007b). The accuracy and resulting responses to selection from using estimated breeding values are based on the amount of genetic contribution of the trait of interest and the amount of quality information gathered from ancestors, progeny, full-siblings, or half-siblings. The genetic gains have been the greatest for traits with the highest heritabilities with diminishing returns on traits with larger proportions of the trait being controlled by environmental factors. Therefore, there is a need to increase the accuracy of phenotypic traits with lower heritabilities and this is a role that can and is being filled by the use of molecular genetics.

The use of restriction fragment length polymorphisms in genetic maps (Lander and Botstein, 1989) greatly enhanced researchers' abilities to identifying loci that control traits of economic importance. This became the driving force behind a new research area for the identification and mapping of "loci underlying a quantitative character" (Lynch and Walsh, 1998) known as quantitative trait loci

(QTL). A number of resource populations have been utilized for genome scans to identify QTL in pigs for traits such as growth and carcass traits (Andersson et al., 1994; Rohrer and Keele, 1998; Malek et al., 2001; Milan et al., 2002) and reproduction traits (Rohrer et al., 1999; Cassady et al., 2001). However, there has been limited use of resource populations to conduct genome scans that included genetic defects (Genini et al., 2004) and sow productive life, largely due to the enormous resources needed to obtain enough informative phenotypes. Another popular approach used today to identifying the genetic mutations responsible for the observed changes in phenotypes is the candidate gene approach (Rothschild et al., 1996). Candidate genes traditionally fall within one or more of the three primary categories. The three categories include: genes identified by mutational analysis in a closely related species, genes identified because of their known physiological function, and genes with biological functions of importance that fall within a particular region of interest on a chromosome (Rothschild and Soller, 1997). The first type of candidate gene is called a mutational candidate due to its selection being based on mutational analysis, such as a knock out study in mice, which resulted in an altered phenotype which could suggest a physiological role in a trait of interest. The second type of candidate gene is known as a biological candidate due to its selection being based on an understanding of the gene's biological function, generally acquired from other species whose genome sequence and comprehension is further advanced, such as the human, mouse, dog, and chicken (Venter et al., 2001; Waterston et al., 2002; Kirkness et al., 2003; International Chicken Genome Sequencing Consortium, 2004). The final type of candidate gene is known as a positional candidate gene

because its selection was based upon a previously isolated QTL region. Most often a candidate gene will be chosen that can be included into at least two of these categories, such as a gene that is chosen based on its location within a QTL region and its physiological role. The candidate gene approach typically uses single nucleotide polymorphisms, known as SNPs, as genetic markers in the identification of the causative mutation or QTN, as described by Mackay (Mackay, 2001).

The desired outcome of the identified SNPs from the candidate gene studies is to identify a marker associated with a phenotype of interest that can be incorporated into selection schemes using marker-assisted selection (MAS). The greatest advantages, in terms of improved accuracies and response to selection, of using MAS over traditional quantitative genetics are for traits with relatively lower heritabilities, those measured late in life, those measured in only one sex, and those measured post harvest (Meuwisen and Goddard, 1996). The incorporation of individual animal's marker genotypes into selection schemes can be accomplished by use of a selection index (Lande and Thompson, 1990) or by the use of best linear unbiased prediction (Fernando and Grossman, 1989). The effectiveness of using a marker for MAS depends on the linkage of the marker with the true cause of the phenotypic trait of interest. Markers that are in population wide equilibrium are the least effective markers to use as their association must be tested within each family (Dekkers and Hospital, 2002). Markers that are in population wide linkage disequilibrium with the QTL or those that identify the functional mutation are the most sought after markers for use in MAS. Markers that identify the functional

mutation can be used without concern of linkage phase differences between different populations affecting the outcome of the desired selection index.

The productive life of sows is influenced by many different factors beyond genetic control, such as the health of the sow herd, the flooring used, the amount of feed allotted to each sow, fertility related traits, reproductive output, and the most varied component being that of management. Because of the intricacies of this trait, such as low heritability, sex limited expression, and measured late in life, this trait is a prime example of a trait that could benefit from the incorporation of genetic markers into MAS. While the productive life of sows is undoubtedly quantitative in nature, the time and resources required for a proper genome scan on this trait make it unfeasible. Therefore the candidate gene approach is the desired method to identify genetic markers that explain a proportion of the phenotypic variance of the trait.

One of the major benchmarks used to identify the productiveness and subsequent profitability of swine operations is the number of pigs born per sow per year. Increasing the number of pigs born per sow per year is a fairly straight forward way to decrease the fixed costs associated with a swine operation. However, more importantly than simply the number of pigs born alive, the pigs must be born healthy and grow at the same rate as their contemporaries. If the pigs are not healthy, require additional assistance by the farmer, or additional time to reach market weight, additional expenses are accrued and often go unnoticed by the producer until it is really too late to mitigate the effects. A detriment to pigs either not being born alive or not reaching market weight at the same time as their contemporaries is

the occurrence of genetic defects. Embryonic lethal genetic defects are unknowingly selected against in selection indexes by selection for increased number of pigs born alive. However, most other genetic defects are eliminated from the breeding farms by simply removing families where these genetic defects arise. While this approach helps control the number of animals in the population with the undesired phenotype, this approach is not a foolproof or effective way to eliminate all carriers in this population and it also eliminates many breeding animals that are superior individuals and actually don't carry the undesirable allele. Therefore, identifying genetic markers for these genetic defects would greatly enhance producers' abilities eliminate the undesired phenotype. Genome scans within families with genetic defects combined with candidate gene analysis can be incorporated together to identify genetic markers that are associated with the undesired traits and thus allow producers to manage them.

The work presented here identifies genetic markers identified via the candidate gene approach for sow productive life and candidate genes combined with a genome scan for polydactyly in swine. The present research focused on the use of genetic markers to enhance sow productive life is the first attempt known to the authors. The ultimate goal of the research summarized herein is for the genetic markers to be incorporated into selection programs to improve sow productive life, while also laying the groundwork for the elimination of pigs that are polydactyl.

RESEARCH OBJECTIVES

Due to the ever increasing size of the human population and the important role that swine fill in providing a quality protein source to much of the world, keeping swine production operations sustainable is extremely important. The objective of Chapter 2 is to provide an overview of advances in the swine genome and their impact on the swine industry. The goal of Chapter 3 is to isolate genetic markers in candidate genes involved in growth and their subsequent location in the swine genome. The intention of Chapters 4 and 6 are to provide insights on the first known associations of genetic markers with sow productive life. The aim of Chapter 5 is to provide an understanding of why today's modern commercial sows are leaving the breeding herd and to identify them so that producers can focus their attention on sow retention. The purpose of Chapter 7 is to show the inheritance of a polydactyl phenotype in pigs. Although, these projects vary in overt themes, they all pertain to the overall goal of identifying and utilizing genetic markers to maximize the efficiency of swine operations.

THESIS ORGANIZATION

The remainder of this chapter provides the literature review that coincides with the research conducted herein. The subsequent elements of this thesis are organized into individual papers that have either been accepted for publication or are in preparation for publication. Chapter 2 is a manuscript that was published in *Genome Dynamics* 2:86-96. This work titled "Cracking the genomic piggy bank:

identifying the secrets of the pig genome,” was written by Benny Mote under the direction of Max Rothschild and is a review of the current state of the swine genome. The manuscript comprising Chapter 3, “SNP detection and linkage mapping of pig genes involved in growth,” was published in *Animal Genetics* 37:295-296. This research and manuscript was conducted and written by Benny Mote under the supervision of Max Rothschild. . The intention of Chapter 4, entitled “The holy grail for pigs: candidate genes affecting sow productive life” is to provide insight on the first known associations of genetic markers with sow productive life. The research was conducted by Benny Mote under the supervision of Max Rothschild. Timo Serenius and Ken Stalder contributed suggestions on analysis. The manuscript comprising Chapter 5 “Current commercial sows: reproduction culling and mortality,” has been submitted for publication in the *Journal of Animal Science*. The research was conducted by Benny Mote under the supervision of Max Rothschild. John Mabry assisted with data extraction from PigChamp™ files and contributed suggestions on traits to analyze. Ken Stalder contributed to many components of this research from data collection, data analysis, and manuscript preparation. The research presented in Chapter 6, titled “Identification of genetic markers for productive life in commercial sows,” is in preparation for submission to the *Journal of Animal Science*. This research was conducted by Benny Mote under the direction of Max Rothschild. Ken Koehler provided valuable insights on survival analysis. John Mabry assisted with data extraction from PigChamp™ files and contributed suggestions on traits to analyze. Once again, Ken Stalder was instrumental in several aspects of this manuscript from data collection to final analysis and

submission. The manuscript titled “Polydactyl inheritance in the pig,” is in preparation for submission to Journal of Heredity. This research was conducted by Benny Mote and supervised by Max Rothschild. Additional assistance was provided by Liviu Totir and Rohan Fernando regarding the implementation and use of the Elston-Stewart algorithm. Chapter 8 summarizes the conclusions and resulting implications that were drawn from the projects described in chapters 2 through 7.

LITERATURE REVIEW

Sow longevity

Sow longevity is a complex trait and has multiple definitions depending on who is asked. Sow longevity can be considered either the number of days that a sow remains in the breeding herd or the number of litters that a sow produces. Both the number of days and number of litters that a sow has can easily be measured, but they don’t take into account the number of non-productive days that a sow has. It is uneconomical to simply keep a sow so that she reaches some predetermined parity or age. A sow must produce an acceptable number of offspring with a minimal number of non-productive days. Though the true life span of pigs is probably closer to 20 years (Classic Encyclopedia, 2008), the average number of parities that today’s commercial female produces is approximately 3.4 parities (PigCHAMP, 2007). Therefore, the average sow is only a little older than two years of age on average when she is removed from the breeding farm. While it is unrealistic to expect the productive life of sows to be near their true life span, there is still

considerable room for improvement in sow productive life (SPL) and hence improved efficiency and profitability.

Current analysis of the commercial sow herd shows that 42% of the females that enter the farm wean 30 or fewer pigs before they are culled and 94% are culled before they wean 57 pigs (Anil and Deen, 2007). PigCHAMP™ records (PigCHAMP, 2007) for 2005 reveal that culling rates averaged 51%, with the poorest 10% of farms averaging 64%. Additionally, these records indicate sow mortality rates approached 9% and the worst 10% of farms having mortality rates over 13%. These high replacement rates can cause a downward spiral in herd performance in systems with undersized multiplication efforts, since a heavy demand for replacement gilts may result in sub-standard gilts entering the breeding herd. An often overlooked component of having these high replacement rates is that there is a larger than ideal proportion in the herd of parity one females. Parity one females' offspring are typically slower growing and endure more health related problems when compared to offspring from older sows due to the older sows acquired immunity (Moore, 2001).

The early removal of a sow or premature death have both economic and welfare ramifications for the commercial swine industry. The length of a sow's productive life on a farm is one of the most important components contributing to the economic success of swine production. The growing percentage of sows being culled for involuntary reasons, such as locomotion problems, reproductive failure, or death, causes many females to be culled before they reach their most productive parities, parities 3 through 5 (Koketsu et al., 1999). Culling and mortality rates on

this scale cause many breeding females to be removed from the breeding herd before they have produced their third parity, an age when most females recover their investment costs (Stalder et al., 2000; Stalder et al., 2003). It has been demonstrated that swine operations with lower replacement rates are usually more profitable than those with high replacement rates (Faust et al., 1992; Faust et al., 1993; Rodriguez-Zas et al., 2003). Using standard net present value calculations for a farrow to finish operation, such as a purchase price of \$200 per gilt, an average number born alive/litter of 10.2, 8.5 pigs sold per litter, and an average price of 44 \$/CWT for market hogs, an increase in net present value of \$77.38 per sow could be realized if an operation could increase litters per sow from three to four (Stalder *et al.*, 2000). Using the same purchase price and number born alive/litter as the farrow to finish operations along with an average price per head of \$28 for segregated early weaned (SEW) pigs, and marketing 9 pigs per litter, the net present value per sow would increase by \$45.59 if a sow would have four parities instead of three for a farrow to wean operation (Stalder *et al.*, 2003). Therefore, an increase of one tenth (i.e. 0.1 more litters) in average parity farrowed per sow would raise the profit in the U.S. alone by approximately \$15,000,000 per year.

Early studies on sow longevity were only conducted up to either sow parity three (Rozeboom et al., 1996) or four (Moeller et al., 2004). These studies provided some understanding as to why sows leave the herd in early parities, but did not provide insight into reasons why some sows can thrive well beyond four parities. However, more recent analyses have extended the focus to include later parities as well. Tarres et al. (2006) examined exterior traits and their effects on sow longevity

in purebred Landrace sows. Additional work has recently been released on the length of productive life in Swedish crossbred sows (Engblom et al., 2007). This work used survival analysis to determine the hazard for removal for various removal reasons such as reproductive disorders, lameness, and mortality.

The reasons that sows leave the breeding farms have remained relatively constant over time. The two most prominent reasons causing sows to be removed from the breeding herd are reproductive deficiencies (D'Allaire et al., 1987; Friendship et al., 1986; Stone, 1981; Lucia et al., 2000) and locomotive problems (Anil et al., 2005; Kirk et al., 2005; Stone, 1981; Taranti and Morrison, 2006). Both of these reasons appear to affect a higher percentage of sows in early parities, while culling for old age is a primary culling reason for removal of sows that have had more than the average number of litters (D'Allaire et al., 1987; Lucia et al., 2000). Heart failures and torsion of abdominal organs have been the predominant reasons for the death of sows (Chagnon et al., 1991; D'Allaire et al., 1991; Kirk et al., 2005). There is also a substantial number of gilts that entered the breeding herd but are culled before having an attempted mating because of lameness and reproductive problems (Stone, 1981) and thus never enter in many record keeping systems. An often unnoticed benefit of sows that reach advanced parities is that they not only produce more pigs over their lifetime simply because they produce more litters, but they also have more pigs born per litter (Lucia et al., 2000).

Sow productive life is a complicated trait to analyze since it is a combination of several different traits such as age at puberty, reproduction, structure, and management decisions. Reproductive traits are low to moderately heritable (Roehe

and Kennedy, 1995; Holl and Robison, 2003) and have low repeatability across parities, although some managers still cull sows for poor reproductive performance based on a single record.

It has been shown that gilts bred at the optimal time point of 221-240 days of age typically are more productive in regards to both number of parities and size of litters when compared to gilts that don't reach puberty in a timely manner (Babot et al., 2003; Engblom, et al., 2007). In addition to reaching puberty at an early age, getting a gilt to farrow her first litter (Goodwin, 2002) and have a short wean to first service interval following her first litter (Tantasuparuk et al., 2001) have rather a large impact on longevity and lifetime pig production. Additionally, there is a large human error in the culling process itself, as the culling reason listed for many sows that are culled can be inaccurate such as when a female is culled for being a non-breeder when she was in fact bred (Knauer et al., 2007). All of this results in lowering the heritability of SPL (Serenius and Stalder, 2004) making it hard but not impossible to achieve genetic progress using traditional quantitative genetic approaches. Therefore, any information that molecular markers could add to selection decisions for SPL would greatly enhance the financial well being of swine operations.

Genome scans for longevity

Genome scans have been conducted to discover QTL affecting lifespan in *Drosophila* (Mackay, 2002; Geiger-Thornsberry and Mackay, 2004). Results from *Drosophila* studies also have unveiled genotype by environment and sex by environment interactions for QTL affecting lifespan (Vieira et al., 2000).

Caenorhabditis elegans (*C. elegans*) has also proved useful in QTL research for longevity with recombinant-inbred lines (Ayyadevara et al., 2001) and mutagenesis work (Kagan et al., 1997) advancing the knowledge of lifespan in this species. Additional species have also been the subject of QTL studies for longevity as well. These include studies for human longevity (Puca et al., 2001), human long healthy life (Reed et al., 2004), mouse longevity (Huang, et al., 2006), as well as studies for direct herd life for number of days in the herd (Kuhn et al., 2003) and indirect measures of longevity such as the number of lactations (Buitenhuis et al., 2007) in dairy cattle. The studies in dairy cattle are probably more related to sow productive life since they are both measures of how long the animals remain at productive levels in dairy operations.

In model organisms such as *C. elegans*, *Drosophila*, and mouse, QTL studies for longevity are much more realistic to conduct in terms of time and economics. Researchers have many more tools available to help them identify QTL regions. The uses of balancer chromosomes (Geiger-Thornsberry and Mackay, 2004), deficiency mapping (Pasyukova et al., 2000), and mutagenesis screens (Kagan et al., 1997) are tools that are simply not available to longevity analyses in livestock species. Additionally, researchers working with model organisms have the advantages of being able to conduct rapid selection experiments to increase longevity (Valenzuela et al., 2004), accomplish large scale microarray analyses (Lai et al., 2007), and have large numbers of animals (n=51,778) (Nuzhdin et al., 2005) for a fraction of the costs and time that would be required for livestock species. Further advantages for researchers with model organisms include that their results

are less skewed by the uncontrolled environmental factors such as housing, health, and simple management factors (such as culling decisions) involved in livestock longevity. One final major difference between researchers working with model organism verses those working with livestock is the trait that is being analyzed itself. In model organisms, the trait that is being analyzed is life span where in livestock the trait is productive life. Therefore, in livestock the goal isn't simply to have animals live for a long time, but is actually to have the animals remain productive longer. To date, there are no direct QTL studies in swine for sow productive life, although there have been QTL studies for reproductive traits (Cassady et al., 2001; Rohrer et al., 1999; Wilkie et al., 1999) that are components of sow productive life. Without QTL experiments for sow productive life, there are no positional candidate genes. Therefore, all candidate genes must be based on the biological roles of genes found in other species.

Candidate gene approach

The use of the candidate gene approach has proven effective in identifying markers associated with phenotypic traits of interest. It has been used to find the causative mutation creating the stress related syndrome in the pig (Fujii et al, 1991) by cross referencing the information gathered from humans over to a similar phenotype found in pigs. The Estrogen Receptor (ESR) gene has been shown to be associated with increased litter size (Rothschild et al., 1996). Kim et al. (2000) used the research regarding the melanocortin 4 receptor (*MC4R*) gene's effects on appetite in humans and mice to identify a mutation in the porcine *MC4R* that changed growth and backfat phenotypes in pigs (Kim et al., 2000). Additional research in pigs have

found functional mutations in calpastatin (Ciobanu et al., 2004), insulin-like growth factor 2 (Jungerius et al., 2004), and thyroxin-binding protein (Nonneman et al., 2005) for meat quality, fatness, and testis weight, respectively. The candidate gene approach is used extensively in human disease studies, such as in the identification of *TFII-I* as a candidate gene for Williams syndrome (Danoff et al., 2004), human leukocyte antigen-G as a candidate gene for asthma (Nicolae et al., 2005), choline acetyltransferase as a candidate for Alzheimers disease (Cook et al., 2005), and isolation of the zinc finger protein as a candidate gene for ischemic heart disease (Stene et al., 2006). Thus it can be observed that using the candidate gene approach has helped identify genes involved in many phenotypes from multiple species.

Longevity lessons learned from model organisms

Results of genome scans and candidate gene studies in model organisms have primarily suggested a limited number of pathways that influence life span or longevity. These pathways include those that serve to mimic caloric restriction or retard growth, that serve to reduce stress and oxidative damage, and those that are involved in immune response. The “longevity pathway” that attracts the most attention from researchers studying longevity is the insulin/IGF-1 pathway (Richardson et al., 2004; Warner, 2005; Wolkow et al., 2002). *C. elegans* was the first organism to offer insight into the insulin/IGF-1 pathway when a recessive mutation was identified that resulted in a 40% increase in mean lifespan (Friedman and Johnson, 1988). These general results were later replicated in both *Drosophila* (Tatar et al., 2001) and mice (Bluher et al., 2003).

This increased longevity isn't always without consequences. Reproduction in some species appears to be negatively correlated with longevity due to competition for the same resources (Partridge et al., 2005) and this is expressed by the resulting delayed or reduced fecundity seen in long lived *C. elegans* with mutations in the insulin/IGF-like signaling pathways (Freidman and Johnson, 1988). Mutations that serve to mimic caloric restriction are thought to retard aging (Barger et al., 2003) though caloric restriction doesn't always correspond to increased longevity (Mockett et al., 2006) and therefore several propose that caloric restriction doesn't universally increase lifespan (Le Bourg and Rattan, 2006; Shanley and Kirkwood, 2006).

The insulin/IGF-like pathways are thought to have an additional benefit to aging, which is their role in resistance to oxidative stress (Brown-Borg, 2003). Additional research has shown that the Snell dwarf mouse responded differently to oxidative stressors than their wild type littermates (Madsen et al., 2004). Iuchi et al. (2007) showed that mice with a deficiency of SOD1, which is required to suppress oxidative stress, led to increased erythrocyte vulnerability and triggered an immune response. Genes involved in immune response are thought to have beneficial effects early in life but detrimental effects later in life (DeVeale et al., 2004). Collectively, there are a limited number of genetic pathways that have been suggested to play a role in longevity in multiple organisms. In model organisms, the alleles associated with leaner phenotypes or associated with reduced caloric intake are often the preferred allele for longevity (Tatar et al., 2003). However, it has been shown that gilts that are leaner have a tendency to be removed from the herd sooner (Stalder et al., 2005). It is highly probable that these same genes that are important

for longevity in model organisms could still prove beneficial to SPL, though the so called longevity allele in model organisms could be the detrimental allele for SPL, but must be accessed on a case by case basis. An example of this is where the beneficial allele in model organisms is associated with reduced caloric intake, but lactating sows must consume enough feed to maintain enough body condition to breed back after weaning a litter.

Genetic abnormalities

Genetic defects are not the first thing that comes to mind when livestock producers look to increase profit margins and efficiency of the operation. In species such as cattle with a limited number of offspring per pregnancy, genetic defects that result in loss of pregnancy or result in unviable progeny have a greater impact on producers' financial well being and are thus realized and acted on sooner than species that have litters of offspring. Chondrodysplasia, often mislabeled as dwarfism, has shown a large impact in Australian Dexter cattle (Harper et al., 1998). In Angus cattle in the United States, an recent increase of dwarfism that was originally seen in the 1940s and 1950s, severely impacted the producers who were known or thought to have this disease in their herd as they were blacklisted by fellow breeders. This relatively recent occurrence prompted researchers to identify the causative mutation behind dwarfism so that Angus breeders could use a genetic marker to eliminate the mutation from their breeding herd if they chose to do so (Koltes, 2007). Dwarfism related abnormalities are not only found in cattle. They have also been identified in the Texel sheep breed in New Zealand (Thompson et al., 2005). An embryonic mortality disease in cattle was found to be related to the

deficiency of uridine monophosphate synthase and was labeled DUMPS (Shanks and Robinson, 1989). Holstein cattle have also been found to have a disorder labeled as complex vertebral malformation (Thomsen et al., 2006). In addition to these monogenic genetic defects, polygenic genetic defects have also been identified that affect livestock species such as entropion, epidermolysis bullosa, and muscular dystrophy (Basrur and Yadav, 1990).

Although the high reproduction rates of swine can cause a recessive embryonic lethality to go unnoticed, a recessive lethal gene linked to the SLA has been identified in pigs (Renard and Vaiman, 1989). Fertility problems caused by a genetic defect have also arisen in a line of Finnish Yorkshires (Sukura et al., 2005). This abnormality results in severe tail malformation of individual sperm, where the tail is two-thirds shorter than that of normal spermatozoa. Scrotal hernias (Grindflek et al., 2006) and cryptorchidism (Amann and Veeramachaneni, 2006; Rothschild et al., 1988) in pigs have also been identified as genetic in nature. Since they are both thought to be under multigenic control, they are not easily removed from breeding populations. Both scrotal hernias and cryptorchidism are thought to be the result of the incorrect closure of the inguinal ring and have shown to have a slight genetic correlation with each other (Mikami and Fredeen, 1979). These two genetic abnormalities are a severe inconvenience in the swine industry, prompting the added cost and labor of a minor surgery to repair as well as the increased risk of death following surgery. A structural defect that has been shown to be genetic in nature is arthrogryposis multiplex congenita (Genini et al., 2004). This defect is presumed to be autosomal recessive and causes permanent joint contractures at

birth in pigs. A less severe form of a structural defect seen in pigs is one that causes pigs to be born with spraddle or splay legs (Holl and Johnson, 2005). This is one of the most common congenital defects observed in pigs (Partlow et al., 1993). These pigs have impaired mobility early in life, hence decreasing their ability to suckle and receive colostrum. This condition also results in an increased incidence of piglet crushing by the sow due to their inability to move out of her way when she lies down.

Another defect known to affect pigs is preaxial polydactyly and has been reportedly observed since at least 1938 (Hughes, 1938) in purebred pigs. This defect causes the creation of extra digits on the inside of the foot. Although this defect doesn't initially cause mobility impairment, when the extra digit grows to the point where it reaches the ground, pressure is placed abnormally on the limb and can lead to lameness. This genetic abnormality has been characterized in other species as well such as chicken (Huang et al., 2006) and mouse (Lettice et al., 2002), and has been identified in conjunction with recessive congenital anomalies in humans such as Bardet-Biedl syndrome (Davis et al., 2007), Ellis van Creveld syndrome (Chakraborty et al., 2007), Meckel syndrome (Baala et al., 2007), Pallister-Hall syndrome (Kang et al., 1997), and Joubert syndrome (Chance et al., 1999).

Any genetic abnormality that causes compromised immune response, increases farm labor requirements, surgeries, decreased mobility, decreased growth, or death has an economic impact on the financial well being of livestock operations. A vast majority of these defects do not have a specific quantified

economic value associated with them. Undoubtedly, any genetic test that results in their elimination from the breeding population would be welcomed by livestock producers.

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CHAPTER 2. CRACKING THE GENOMIC PIGGY BANK: IDENTIFYING SECRETS OF THE PIG GENOME

A paper published by Genome Dynamics 2:86-96.

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Abstract

Though researchers are uncovering valuable information about the pig genome at unprecedented speed, the porcine genome community is barely scratching the surface as to understanding interactions of the biological code. The pig genetic linkage map has nearly 5,000 loci comprised of genes, microsatellites, and amplified fragment length polymorphisms markers. Likewise, the physical map is becoming denser with nearly 6,000 markers. The long awaited sequencing efforts are providing multidimensional benefits with sequence available for comparative genomics and identifying single nucleotide polymorphisms for use in linkage and trait association studies. Scientists are using exotic and commercial breeds for quantitative trait loci scans. Additionally, candidate gene studies continue to identify chromosomal regions or genes associated with economically important traits such as growth rate, leanness, feed intake, meat quality, litter size, and disease resistance. The commercial pig industry is actively incorporating these markers in marker-assisted selection along with traditional performance information to improve said traits. Researchers are utilizing novel tools including pig microarrays along with advanced bioinformatics to identify new candidate genes, understand gene function,

and piece together gene networks involved in important biological processes.

Advances in pig genomics and implications to the pork industry as well as human health are reviewed.

1. Introduction

The pig was most likely one of the first animals to be domesticated over 7,000 years ago with pork now representing forty-three percent of red meat consumed in the world [1]. There are 330 'non-extinct' breeds of swine [2] worldwide with pictures and/or information listed for 72 breeds of swine at

<http://www.ansi.okstate.edu/breeds/swine/> [3]. Currently, the commercial pork industry primarily only uses six of these breeds for pork production as either purebreds or in synthetic crosses. The pig has more recently expanded its role to humankind beyond that of just a protein source and is proving to be a very useful biological model to study many human diseases and conditions with great potential to serve as a source for organ transplants to humans as evidenced by the present use of pig heart valves for transplants.

Coordinated efforts to better understand the pig genome were initiated in the early 1990s with the development of the international PiGMap gene mapping project [4] as well as projects by the USDA and US agricultural universities [5, 6]. These projects were structured in such a way that they included cooperation and collaborations by many different institutes. In the United States, the position of a Pig Genome Coordinator was created to facilitate collaborative efforts between scientists from both state and private universities as well as with that from federal labs that operate cooperatively in a Swine Genome Technical Committee, which has been

meeting yearly since 1994. The Committee works to increase collaborative efforts, share information and advice the Coordinator. Most recently, an international pig genome sequencing committee was formed to lead efforts to find funding and to initiate the sequencing of the pig genome. Formation of these consortiums has allowed the status of the pig genome along with the development in functional genomics to advance rather quickly over the last decade.

2. Mapping Efforts

There were two significant linkage maps published by the mid 1990s [4,5]. The initial published linkage map contained about 1200 markers [5]. Since that time, progress in growth of the linkage map has slowed though new gene markers such as microsatellites, amplified fragment length polymorphism (AFLPs), and single nucleotide polymorphisms (SNPs) have been continuously identified and mapped with limited integration into linkage maps taking place. Recently a large number of additional markers have been added to the on line version of the map (<http://www.genome.iastate.edu/maps/marcmmap.html>). There now totals nearly 1,600 genes and 3,300 markers in the public database (www.thearkdb.org/browser?species=pig) developed by the Roslin Institute. An AFLP map in progress with 2300 AFLPs is likely to be added to the PiGMap linkage map sometime in the near future. Combining all of these markers from the above mentioned maps and databases, now totaling over 7,000, allow composite maps to be roughly sketched together.

Due to the development of techniques and resources such as chromosome painting [7], a pig somatic cell hybrid panel [8], and a 7,000 rad radiation hybrid (RH)

panel (ImpRH) [9, 10], integration of the linkage, physical, and cytogenetic maps has made great progress [11]. The density of the RH map is growing very rapidly, now nearly 6,000 markers comprised of microsatellites and over 2,000 expressed sequenced tags (ESTs). Many of the ESTs are orthologs of human genes and therefore can be used in comparative mapping efforts. While these resources are still being employed, there was development and now use of an even more powerful RH map with the 12,000 rad RH map [12] that allows for even greater precision for mapping within and across species. Using the ImpRH panel as a template, a new comparative map has been constructed that far exceeds anything to date with an average spacing between comparative anchor loci at 1.15 Mb based on the human genome sequence [13]. Thus, the incorporation of these valuable tools continue the rapid development of an extensive comparative map which has made it possible for accelerated identification of genes controlling variation in traits of interest that have been identified by quantitative trait loci (QTL) studies or candidate gene association tests.

3. Databases

Databases play a vital role providing the tools needed for future genomic discoveries. Substantial pig bioinformatics efforts have been undertaken by the Roslin Institute, Scotland (www.thearkdb.org) and to a lesser extent in the US (www.genome.iastate.edu, <http://www.animalgenome.org/>) supporting pig genome efforts as well as displaying the gene maps [14]. PiGBASE, obtainable through the aforementioned sites, has several useful tools including references for pig gene mapping with over 1,250 citations in the database along with gene maps comprised

of nearly 5,000 loci as of October 2005. Additionally, the cytogenetic map of the pig, available at <http://www.toulouse.inra.fr/lgc/pig/cyto/cyto.htm>, as well as the RH panel map at <http://www.toulouse.inra.fr/lgc/pig/RH/Menuchr.htm> are valuable tools for the pig genome community. A human-pig comparative map can be obtained on the web at <http://www.toulouse.inra.fr/lgc/pig/compare/compare.htm>. To streamline the efforts of many researchers interested in trait discovery, a new database called PigQTLdb (<http://www.animalgenome.org/QTLdb/>) has been constructed that combines all the published QTL information into one searchable database and allows the user to search by either chromosome, trait, or key words from the publications [15]. To date, there are 1,239 QTL representing 235 different traits from 93 publications available on PigQTLdb. An additional database has recently come online at http://www.ncbi.nlm.nih.gov/SNP/snp_batchSearch.cgi?org=9823&type=SNP called the PigSNP database that contains 6,441 SNPs (October, 2005) identified through various methods. This database not only provides information about the SNP, but also contains valuable flanking sequence that allows users to design SNP tests for multiple genotyping platforms.

4. QTL and Candidate Genes

Factors affecting pork's efficient production are vitally important as are traits that affect consumer preferences and pork consumption. The most important traits for pork production in the finishing phase are lean growth, feed intake, and pig survival. Arguably, the two most economically important traits to the financial bottom line of pork production are reproductive traits and disease resistance. There are

several reproductive traits of interest to the pig industry with the two most important being the number of pigs weaned per sow per year and the other more overlooked trait being the reproductive life of the sow herself. Several research groups have conducted research that has clearly shown genetic variation for these traits.

Though consumers are most concerned about the degree of fatness or carcass merit as well as pork quality, pork producers must also pay attention to the ever-growing demand by consumers that the pigs be grown without the use of antibiotics as growth promoters and in facilities that are more animal friendly. Additionally, pork producers must do all of this while becoming more environmentally friendly by having pigs reduce feed wastage, improve feed efficiency, and produce waste that contains less phosphorous.

Many QTL experiments were undertaken by using linkage maps to help determine regions underlying traits of importance to the pig industry. Researchers have identified over 1,200 QTL affecting most traits by using both commercial and exotic pig breeds with various population structures. Due to limitations regarding experimental design and classification of phenotypes, QTL associated with immune response traits and disease resistance has been sparse. Such phenotypes may find gene expression approaches to be more beneficial to unearthing the genes likely to be associated with disease resistance.

Researchers in the swine genomics community, having learned from their counterparts in other species regarding imprinted genes, have expanded their projects to also target imprinted and parent of origin effects. One such region lying on chromosome 2 has been intensively investigated [16] with *IGF2* being implicated

in causing a major effect on muscle mass. Georges and colleagues employed a cleverly designed haplotype sharing strategy analysis combined with marker assisted segregation analysis to position the QTL within a 500kb region. After investigating 180 SNPs residing in the 500kb region, the quantitative trait nucleotide (QTN) was identified. This work shows the need for carefully calculated analysis of entire gene regions and individual genes with the appropriate animals and phenotypic information. Now that such methodology has been developed, further analysis in other chromosomal locations will likely identify more imprinted regions.

An alternative approach to QTL scans is candidate gene analyses. They have been employed using biological or mutational candidate genes from other species to investigate a variety of traits. A substantial number of candidate genes have shown significant associations with many traits important to swine production. Four genes (*ESR*, *PRLR*, *RBP4*, *FSHB*) identified to date have shown significant association for litter size with effects ranging from 0.25 to over 1 pig per allele per gene copy with variations depending on breed background. Over 20 genes have been examined in multiple laboratories for growth and backfat traits with *MC4R* showing the most promise. A *MC4R* mutation has shown significant association with a reduction in feed intake with less backfat or faster growth depending on which allele is inherited. Extensively reviewed meat quality genes (*HAL*, *RN*) have been reported and genetic markers identified within these genes allow for genetic testing therefore allowing producers to remove the alleles deleterious to meat quality. Additional genes including *PRKAG3* and *CAST* have been shown to be associated with changes in pH and tenderness. Several candidate genes or gene regions (*K88*,

FUT1, *SLA*, *NRAMP*) have been identified to be associated with differences in immune response or disease resistance with *FUT1* being currently used to reduce post weaning diarrhea in commercial pork production. Recently, a polymorphism has been identified as showing an association with resistance to K88 *E. coli* [17]. Additional genes such as *KIT* and *MC1R* have been used by breeding companies to produce pigs that are white in color, a phenotype that is preferred by commercial meat packing companies. Commercial pig breeding companies are combining these genetic markers with traditional performance information in marker-assisted selection programs to identify and select individuals that have the most genetic potential. Though marker-assisted selection programs are in their infancy, the effectiveness of this approach has shown varied but promising results such as the pork industry's ability to greatly reduce the number of pigs exhibiting porcine stress syndrome often associated with a mutation in the Ryanodine Receptor which can be selected against by using the HAL 1843TM genetic marker. Other markers in use include *ESR*, *MC4R*, *PRKAG3* for example. Breeding companies are refining their strategies to allow early implementation of newly discovered genetic markers.

Two computer programs have been created to help researchers more precisely identify QTL. The first program, QTL Express [18], is a user-friendly web-based interface that first identifies Identity-By-Descent (IBD) probabilities for all chromosomal locations from multiple marker data and then fits a linear regression model to the phenotypes. Populations that are suitable for QTL Express are either a halfsib outbred population or a F_2 population derived from a cross between either inbred or distinct outbred lines. Users can choose between a one QTL model or a

two QTL model for the genetic component while also allowing for fixed effects, covariates, and known QTL can be fitted through the use of cofactors. Qxpak [19] is a methodology and software package used to locate QTL that was recently released in 2004. Qxpak uses the framework of mixed model statistics to provide a more flexible platform than previously used programs. It allows for multiple trait and multiple QTL analyses from experiments involving various populations, be it a cross between inbred lines, a within population study, or a mixture of populations. Qxpak can also be used to help determine causative SNPs by large association studies between SNPs and the traits of interest.

As the QTL regions become more clearly identified, positional candidate gene analyses are being employed to elucidate the causative mutation. An example of such is the identification of QTN in *PRAKAG3* that shows association with pH, drip loss and meat color. Another example is the QTN in *CAST* that shows association with tenderness scores as measured by trained consumer panels that have tested the meat after cooking. Through the continued use of programs such as QTL Express and Qxpak, efforts to exploit QTL maps from various crossing experiments combined with a more accurate comparative map will allow for additional positional candidate genes to be targeted leading to the discovery of the QTN effecting various traits.

5. Sequencing

After the completion of the human genome sequence, the intent of the NIH was to sequence other species, especially those organisms that can be a useful model for human conditions. Ongoing analysis of the porcine genome has provided

strong evidence of the high similarity to that of the human genome such as in chromosomal organization with $2n=38$ including both meta- and acrocentric chromosomes, size of the genome being ~3 billion base pairs, and complexity. A swine genome community effort produced a 'White Paper' [20] for consideration by NHGRI that outline the role pigs play in agriculture and as biological models for humans. This White Paper received solid backing from all sectors of the swine community and served to give the pig genome initially a 'high priority status' for sequencing from NHGRI.

Efforts to sequence the pig genome have come from many fronts using multiple approaches. Sequences for the pig genome have been generated from ESTs of cDNA clones from varying tissues, the sequencing of candidate genes, and more recently large scale genomic sequencing efforts from the swine genome community. The largest of the EST projects published to date are the 66,245 ESTs produced by Fahrenkrug and colleagues [21] and the 21,499 sequences produced from reproductive tissues by a consortium of research groups [22]. Genbank now contains 671,752 pig sequences and the January 2005 release of TIGR combined them to contain 38,781 clusters with 65,000 singletons. Most recently, the efforts of the Sino-Danish generated ~3.84 million shotgun sequences of the pig genome resulting in a 0.66X coverage of the porcine genome translating to 48% of the pig genome being sequenced by this project [23]. In addition to simply providing sequence information, the Sino-Danish sequencing effort continued to prove that the pig coevolved more closely with humans than mice and should therefore serve as a more applicable biological model to humans.

For the past 3 years, collaboration has taken the form of an “International Swine Genome Sequencing” committee, which is active in pushing the pig genome sequencing agenda. Sequencing efforts are taking place at the Sanger Institute through this international collaboration composed of many different laboratories. The sequencing approach is to utilize a minimal tiling path (MTP) of bacterial artificial chromosomes (BACs) constructed from previous landmarks, BAC end sequences, and restriction fingerprinting of over 300,000 BACs from 4 BAC libraries consisting of all 18 autosomes, and the X chromosome [24]. It is also planned that gene rich regions of the Y chromosome will be sequenced. A hybrid sequencing approach is being used to generate a draft 6X coverage of the pig genome from 3X coverage of BACs comprising the MTP and a 3X coverage of the whole genome shotgun sequencing produced from various labs worldwide. The use of a MTP will greatly enhance the sequencing efforts thus reducing the time needed to produce the complete genome compared to that of the human and mouse. Initial estimates place a draft sequence of the pig genome to be completed by 2007. Funding will be provided in part by the USDA, which has committed \$10 million to this project, and contributions from the Sanger Institute and other labs. The sequencing of the porcine genome will enhance pig researchers ability to amplify their desired targets within chromosomal regions without having to design primers based off of the sequences from other organisms. Additionally, the USDA recently issued an RFA for functional genomics that is only available to scientists that are working with species whose genome has been sequenced at 5X coverage, which further

emphasizes the importance of sequencing the pig genome for researchers focusing on swine.

6. Functional Genomic Analysis

Researchers are still a long way from fully understanding the physiological complexity of the pig transcriptome. Researchers are utilizing expression studies to more completely understand certain genes and gene pathways that control traits of economical importance. Early expression studies in pigs used techniques such as Northern analysis and differential display RT-PCR while more recent projects have incorporated quantitative real time PCR to determine mRNA levels for genes effecting traits of interest. Though extremely useful, these techniques are limited by the number of genes that can be examined at a time. Other approaches have included the use of limited numbers of trait specific cDNA on macroarrays [25]. Initially human arrays were used for expression studies since there was no large scale pig cDNA array. These experiments proved to be a valuable resource as reproducibility was high between humans and pigs, thus providing more evidence as to the relatedness of the two species. The large numbers of pig ESTs generated has lead to large scale expression analysis using materials derived solely from the pig. Pomp and colleagues [26,27] used cDNAs derived from ovary and follicular RNA from animals of either a line of pigs selected for high litter size (the Nebraska index line) or their control line and co-hybridized them with 4,600 follicle derived probes to study gene expression patterns related to reproductive efficiency. Other projects, such as two large scale efforts in Europe, are also ongoing. The first European Community supported project is called PathoCHIP

(<http://www.pathochipproject.com>) and uses cDNA spotted arrays for disease organism and immune response genes while the second project called QualityPorkGENES (www.qualityporkgenes.com) looks at co-expressed genes related to meat quality traits. Additional cooperative efforts lead by the US Pig Genome Coordinator and a committee of interested scientists has generated a first stage cDNA or oligo spotted array. The array is a 13,000 element oligo array produced with the QIAGEN Array-Ready Oligo Set for the Pig Genome (version 1.0) and the Pig Genome Oligo Extension Set (version 1.0). The array contains a total of 10,665 spotted 70-mer probes representing 10,665 *Sus Scrofa* gene sequences with a hit to human, mouse, or pig gene transcript. This microarray has been validated and is currently being employed by multiple research groups to help determine gene(s) and gene pathways involved in many traits [28]. A second such array is in development with 20,000 probes and in addition Affymetrix has a pig chip with 23,935 probe sets. These tools will greatly enhance the swine community's understanding the porcine's genome full complexity.

7. Pigs as Biological Models

There has been continued interest in the pig as a biological model for human biology and a recent CRISP search (October 2005) showed that over 400 active grants using pigs as models have received funding from NIH. The use of pigs in these grants covers many research areas such as the use of pigs as models for more effective vaccines and immune therapy for Hepatitis C virus, models for chronic wound repair often seen in humans with Type 2 diabetes, models for cystic fibrosis, and also in whole pig heart xenotransplantation with chimeric donor pigs.

Research using pigs as animal models of human conditions has covered a vast array of disciplines such as nutrition, digestive physiology, kidney function, heart function, diabetes, obesity, and skin formation and healing. With the growing evidence of the close relatedness of the pig to that of the human, evidenced by the sequence analysis of the Sino-Danish project [23], the extent of biomedical projects using the pig could be expected to grow in the future.

Shortages of human tissues and organs available for transplantation have created interest in xenotransplantation and the pig is the preferred donor due to its size and comparative physiology. Recent concerns about retroviruses and difficulties producing transgenic pigs meeting the standards required for safe transplantation have slowed the progress in the use of the pig for xenotransplantation and caused some companies to scale back the active research in this area.

8. Conclusions

With the ongoing sequencing and array efforts, the pig genomic community does not need to rely solely on the developments from other organisms such as the human, mouse, and rat any longer. It is also a valid argument that efforts from the pig genome sequence and arrays could be used to identify elements in other organisms that cannot be isolated until comparative analysis takes place. The sequencing and expression analyses have offered new insights into the biological intricacy of the pig. The large-scale gene and trait identification/mapping show continued progress with the expectation that more gene tests will continually be offered to the pig industry. The discoveries in the labs have quickly found their way

to the farms as the commercial pig companies continue to employ these technological advances. Further discoveries and enhanced understanding of the complexity of the pig genome will simply boost the pig's role in providing a sustainable source of protein worldwide as well as its valuable role in biomedical research.

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CHAPTER 3. SNP DETECTION AND LINKAGE MAPPING OF PIG GENES INVOLVED IN GROWTH

A paper published in Animal Genetics 37:295-296

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Source/description: With the high cost of both feed and many of today's modern pork facilities, profitability relies upon how quickly a group of pigs can reach final market weights. Therefore, genes involved in growth and growth pathways are of vital interest to the swine industry. Herein we describe the SNP detection and subsequent linkage mapping of six genes in growth pathways, including *insulin-like growth factor binding protein 1 (IGFBP1)*, *insulin-like growth factor binding protein 2 (IGFBP2)*, *insulin-like growth factor II receptor (IGF2R)*, *beta-2 adrenergic receptor (ADRB2)*, *carnitine O-palmitoyltransferase I (CPT1A)*, and *organic cation/carnitine transporter 2 (Solute carrier family 22 member 5; SLC22A5)*.

Primer sequences: Pig sequences for *ADRB2*, *CPT1A*, *IGFBP1*, *IGFBP2*, and *IGF2R* (GenBank accession nos. AF000134, AF288789, AB053605, AF120326, and AF339885 respectively) and human sequence for *SLC22A5* (GenBank accession no. BC012325) were queried from the NCBI nucleotide database (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide&itool=toolbar>) and used to design primers for each gene using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Primer sequences, gene location of primers,

amplified fragment sizes and SNP locations are given in Table 1. Standard ingredients and conditions for PCR reactions were used.

SNP detection and polymorphisms: Single nucleotide polymorphisms were detected using a pooled DNA sample from two Berkshire sires and three pooled samples from nine Yorkshire dams of the Iowa State University's Berkshire x Yorkshire resource population¹. The sequence data were analyzed using Sequencher software (Gene Codes Corporation, Ann Arbor, MI). PCR-RFLP tests were created for each gene and conducted by combining 3 μ L of PCR product and 3 units of gene specific enzyme (see Table 1), in 10 μ L volumes. The digested PCR products were separated on 3.5% agarose gels stained with ethidium bromide. Genotypes for each gene fragment were called based on the banding pattern listed in Table 1. Identified SNPs were deposited into dbSNP (49857704-49857709).

Mapping: The ISU Berkshire x Yorkshire resource population was genotyped using each PCR-RFLP test. Two point and multipoint linkage analyses of the genotype results were completed using CRI-MAP software². The most relevant LOD scores are shown for each gene in Table 2 along with the flanking gene order.

Comment: Gene locations for all markers mapped to the expected location and order based on the human/pig comparative map³. Further analysis of these candidate genes for growth is ongoing.

Acknowledgements: The authors thank Dusty Loy for laboratory assistance. Support was provided in part by a USDA National Needs Fellowship, Sygen

International, the Iowa Agriculture and Homes Economics Experiment Station, State of Iowa and Hatch funding.

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Gene	Primer sequences 5'-3' (Forward/Reverse)	Location of primers	Fragment size	Position and type of SNP	Restriction enzyme	Digestion temperature	Fragment sizes (bp) of alleles	dbSNP local identifier
<i>ADRB2</i>	GTCTTCCCTGAAGGCCTATG/ CTCCCCTGTGAATCAGTGCT	exon 1	195	25bp, C/T	<i>BsaXI</i>	37 °C	T: 195 C: 151, 30, 14	49857704
<i>CPT1A</i>	AGCTCTAGTTGGTTGTGGAATC/ CATACCTGGGCTTCTTCCTG	intron 11/ exon 12	350	87bp, C/T	<i>BstNI</i>	60 °C	T: 299, 51 C: 212, 87, 51	49857705
<i>IGFBP1</i>	CATCAAGAAGTGGAGGTGAGG/ TGTTTTCAAGTTCTACCCCTTGA	exon 2/ intron2	423	316bp, C/T	<i>BtsI</i>	37 °C	C: 423 T: 312, 111	49857706
<i>IGFBP2</i>	GGAACCTGCTCACCCCTTGTC/ CATACCTGGGCTTCTTCCTG	intron 2	361	135bp, A/T	<i>EaeI</i>	37 °C	A: 361 T: 232, 129	49857707
<i>IGF2R</i>	GTCCGGCCATTAGGAAGAAG/ TTCTTTCTCTTTCTCTGGGTGC	exon 17/ intron 17	491	359bp, C/T	<i>BsII</i>	55 °C	C: 341, 150 T: 215, 150, 126	49857708
<i>SLC22A5</i>	CCTGCCCTACATTCTCATGG/ CACTCTGGGGCTTTCTTCAC	exon 9/ exon 10	539	235bp, C/G	<i>HaeIII</i>	37 °C	C: 374, 165 G: 304, 165, 70	49857709

Table 1 Primers, primer locations, amplicon size, location of SNP, digestion requirements, and banding patterns for porcine SNPs.

Gene	Porcine Chromosome (SSC)	Marker	LOD Score	Distance From gene to marker (cM)	Map order with distances (cM)
<i>ADRB2</i>	2	S0404	63.63	5.7	S0404 - (1.5) - S0036 - (4.2) - <i>ADRB2</i>
		S0036	53.70	4.2	
<i>CPT1A</i>	2	SW2623	53.80	4.0	SW2623 - (4.0) - <i>CPT1A</i> - (25.6) - SW2445
		SWC9	28.90	16.4	
<i>IGFBP1</i>	18	<i>IGFBP3</i>	60.95	1.1	<i>IGFBP3</i> - (1.1) - <i>IGFBP1</i> - (7.7) - S0177
		S0177	39.26	7.7	
<i>IGFBP2</i>	15	<i>IGFBP5</i>	75.63	0.6	SW120 - (2.1) - <i>IGFBP2</i> - (0.6) - <i>IGFBP5</i>
		SW120	59.48	2.1	
<i>IGF2R</i>	1	SW1515	16.72	12.1	<i>IGF2R</i> - (12.1) - SW1515 - (17.4) - S0316
		S0316	3.72	29.5	
<i>SLC22A5</i>	2	SW1408	61.90	12.9	SW2157 - (9.7) - <i>SLC22A5</i> - (8.8) - S0565
		SW1844	55.81	15.3	

Table 2 Map assignments in the pig for six growth genes.

CHAPTER 4. THE HOLY GRAIL FOR PIGS: CANDIDATE GENES AFFECTING SOW PRODUCTIVE LIFE

A paper published in the 8th World Congress on Genetics Applied to Livestock Production. Belo Horizonte, Brazil. August 2006.

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INTRODUCTION

The length of a sow's productive life (SPL) on a farm is one of the most important components contributing to the economic success of swine production. The growing percentage of sows being culled for involuntary reasons such as locomotion, reproductive failure or death causes many females to be culled before they reach their most productive parities and before the investment costs of those females have been fully recovered. The early removal of sows or premature death increases sow replacement rates and has both economic and welfare ramifications for the commercial swine industry.

Using standard net present value calculations for a farrow to finish operation such as a purchase price of \$200 per gilt, an average number born alive/litter of 10.2, 8.5 pigs sold per litter, and an average price of 44 \$/CWT for market hogs, an increase in net present value of \$77.38 per sow could be realized if an operation could increase litters per sow from three to four (Stalder *et al.*, 2000). For a farrow to wean operation, using the same purchase price, number born alive/litter with an average price per head of \$28 for segregated early weaned (SEW) pigs, and marketing 9 pigs per litter, the net present value per sow would increase by \$45.59 if

a sow would have four parities instead of three (Stalder *et al.*, 2003). Therefore, an increase of one tenth (i.e. 0.1 more litters) in average parity farrowed per sow would raise the profit in the U.S. alone by approximately \$15,000,000 per year.

PigChamp records from 2001, 2002 and 2003 indicate that the average parity of farrowed sows is 3.42, the average culling rate is 40.9%, the average death rate is 7.46%, and the average replacement rate is 66.46% (PigChamp, 2004). High replacement rates driven by involuntary culling infer that producers are required to lower their selection intensity to maintain herd size. These high replacement rates can cause a downward spiral in herd performance in systems with undersized multiplication efforts, since a heavy demand for replacement gilts may result in sub-standard gilts entering the breeding herd. Improving SPL would allow for selective culling of sows in the upper tiers of the genetic pyramid to increase rate of genetic progress.

Limited studies have been performed researching productive life in pigs. Most studies were only conducted up to either sow parity three (Rozeboom *et al.*, 1996) or four (Moeller *et al.*, 2004) allowing for some understanding as to why sows leave the herd in early parities, but never accounting for reasons why other sows can thrive well beyond four parities. These previous studies revealed significant line interactions on sow longevity and noted that further studies should be conducted to identify the genetic mechanisms associated with sows having increased numbers of parities. Scientists have begun identifying genes in model organisms that play a role in the aging process and longevity itself (Hasty *et al.*, 2003; Hekimi and Guarente, 2003; Longo and Finch, 2003; Simon *et al.*, 2003; and Tatar *et al.*, 2003). Research

has shown that yeast and *C. elegans* (nematode) share a number of homologous genes in the so called “longevity pathways” and that increased longevity is often the result of inactivation of the pathways that promote growth and a reduction in oxidative damage and other forms of stress (Longo and Finch, 2003). Similar results have also been shown in the fruit fly such as mutations in the insulin/IGF-1 pathways extending lifespan. The overriding theme gathered from studying these genes is their role in reduction of caloric intake that enables animals to live longer as well as reducing susceptibility to disease in the aging process. However, some research has indicated that leaner gilts have the tendency to be removed from the herd earlier (Stalder *et al.*, 2005).

The hypothesis that guides this comparative genomics research is that the similarity between the functions of certain genes in the various species studied suggests that the same genes may be associated with SPL in the pig. It is possible that increasing SPL might not be completely correlated with simple lifespan in model organisms. Therefore, other genes more specific to swine may need to be isolated and examined. Genes studied include those that function as antioxidants, are involved in reproduction, and components of the insulin pathway that regulate food intake. The identification of molecular markers associated with the length of a sow’s productive life would allow breeders to use marker assisted selection to select individuals, based on the animal’s genotype, at early ages that would have the best opportunity to remain in the herd far beyond the current average sow.

MATERIAL AND METHODS

Population. The commercial population used here to validate the earlier results of Mote *et al.*, 2005, consisted of 2,000 commercial crossbred females composed of two parent lines where heterosis is maximized. Commercial crossbred females were selected for this study as the problem with SPL seen in industry is focused on the sows producing the terminal offspring. Equal numbers of sows were randomly sampled from each of the two crossbred lines utilized in this study. The experimental sows were randomly sampled from three farms that contained a total of 11,400 sows in their production system. Half (1000 animals) of the sampled sows have had greater than 5 parities and serve as the selected group and the remaining half (1000 animals) are replacement gilts and served as the unselected group. Equal numbers of selected and unselected sows were sampled from each of three farms. Two of the farms utilized one sow commercial line (Line A) and the other farm utilized a second commercial line (Line B).

Data Collection. Ear tissue was sampled from all sows using the TypiFix™ ear tag from Agrobiogen. This system allows simultaneous identification and tissue collection to prevent sample misidentification. DNA was isolated from tissue samples using the Nexttec™ DNA isolation system (Nexttec GmbH Biotechnologie) adhering to the manufacture's protocol. PigChamp records were obtained for all sows at time of tissue collection. The records will be resampled approximately six months later so that gilts in the unselected group have had sufficient time to farrow their first litter and / or be culled from the breeding herd.

Targeted genes and genotyping. The first genes to be validated for components of SPL were insulin like growth factor binding protein 1 (IGFBP1) and organic cation/carnitine transporter 2 (Solute carrier family 22 member 5; SLC22A5). IGFBP1 was targeted due to its role in the insulin/IGF-1 pathways which has been implicated in model organisms for regulating feed intake and increasing lifespan. SLC22A5 was targeted for its role in transporting carnitine, a feed supplement that has been studied for effects on the number of pigs born alive per litter. Primers for the gene fragments (forward/reverse) are AAAATCAGGGTATCGGTCTTCA/TCGTTCTGTGCCATCTACA and CCTGCCCTACATTCTCATGG/CACTCTGGGGCTTTCTTCAC for IGFBP1 (403 or 393 bp) and SLC22A5 (539 bp) respectively. Genotypes were based on PCR-RFLP tests of a 10 base pair in/del in intron 2 of IGFBP1 using BstF5I and a C/G SNP in intron 9 of SLC22A5 using HaeIII. Banding patterns are 303/91, 303/160/126/91/26, and 160/126/91/26 for the 11, 12, and 22 genotypes respectively for IGFBP1. Banding patterns are 374/165, 374/304/165/70, and 304/165/70 for the 11, 12, and 22 genotypes for SLC22A5. Additional mapping information for these two genes can be found in Mote and Rothschild (2006).

Statistical analysis. Sows' genotypes were then analyzed using Fisher's exact test to identify if there was a significant effect for the genes between the select and unselected sow groups for number of parities. Contrast statements were used to identify the differences between genotypes and to determine if the gene has an

additive or dominant effect. The PROC MIXED procedure of SAS was used to determine genotype effects on the total number of pigs born alive for the sows in the select group using farm, number of parities and, average lactation length of the sow, and the sow's genotype as fixed effects. Additive and dominance effects were estimated for the total number of pigs born alive. The genotype test for total number of pigs born alive may be biased as the only animals analyzed at this time are the sows in the select group that have survived on the farm for a minimum of 5 parities.

RESULTS AND DISCUSSION

The results for Fisher's exact test concluded that there were significant genotype differences for both *IGFBP1* and *SLC22A5* between the select and unselected groups ($P = .01$ and $P < .0001$ respectively) indicating that there is genotypic effects for number of parities. Additionally, *IGFBP1* also had a significant association with the total number of pigs born alive over the sow's lifetime ($P < .04$). The effects of *IGFBP1* appear to be quite important since allele 2 is favored for both number of parities and the total number of pigs born alive over those parities. The frequency for *IGFBP1* was 0.3 for the 11 genotype and 0.18 for the 22 genotype in the select group and 0.36 for the 11 genotype and 0.14 for the 22 genotype in the unselected group. The frequency for *SLC22A5* was 0.14 for the 11 genotype and 0.38 for the 22 genotype for the select group and 0.06 for the 11 genotype and .53 for the 22 genotype in the unselected group. Both *IGFBP1* and *SLC22A5* showed dominant gene effects with allele 2 (the favorable allele) being dominant for *IGFBP1* and allele 2 being dominant for *SLC22A5* over the favorable 1 allele. Allele 2 for *IGFBP1* had

a dominant effect of an additional 2 pigs per SPL for sows in the select group after being corrected for farm, average lactation length, and parity effects. Sampling of pigs was from large synthetic lines, so it is unlikely that a founder effect exists but it cannot be completely excluded as a cause for the differences in genotypic frequencies between the select and unselected group. In addition, because sampling consisted of animals from two distinct lines, from three farms, and because a large number of sires are used in traditional multiplication systems, a discrepancy in genotypic frequency caused by a founder effect should be minimized if it exists at all.

CONCLUSION

Genetic markers associated with components of SPL have been demonstrated using parent animals recently sampled from commercial operations directly addressing the problem of SPL seen in the swine industry. Both *IGFBP1* and *SLC22A5* showed significant effects for number of parities and *IGFBP1* also was significantly associated with total number of pigs born alive among older sows. Selection for *SLC22A5* offers the most benefit as the recessive 11 genotype is preferred and only represents 6 percent in the unselected group. Both of these markers should be considered for marker assisted selection for SPL.

ACKNOWLEDGMENTS

The authors thank the members of the Rothschild lab, James Koltes, Terry Wolters and Dan Nesvold for their assistance on this project. This work was funded largely

by the National Pork Board and in part by USDA National Needs Fellowship, PIC USA, the Iowa Agriculture and Home Economics Experiment Station, State of Iowa and Hatch funding.

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CHAPTER 5. CURRENT COMMERCIAL SOWS: REPRODUCTION, CULLING AND MORTALITY

A paper submitted to the *Journal of Animal Science*

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ABSTRACT

Sow longevity is a large and often overlooked component of profitability and efficiency for commercial swine operations. Previous research has shown that a sow must remain productive through 3 parities to recover her investment costs. With current culling rates averaging near 50% and mortality rates averaging almost 9%, this leaves the responsibility of making a profit on a relatively small number of sows that can remain productive for greater than 3 parities. Published research has shown the primary reasons for culling are reproductive failure and structural soundness, but much of this work is outdated, especially when considering the genetic background of sows analyzed. Therefore, a new 20-mo study was conducted using 2,000 commercial sows with half of the females being gilts that just entered the breeding herd and the remaining half consisting of sows that produced at least 5 litters in the same breeding herd. These females were sampled in late 2005 and were from 3 different farms where 2 different genetic lines were utilized. This study revealed that the primary culling reasons for sows from current genetic lines does not greatly differ from previous research work. A large portion of sows were culled for reproductive failure and structural soundness in the present study. The predominant

reason that sows over parity 5 were culled was because of old age even though most of these sows were still producing at herd average or above. Sows from the parity 5 and older group had a greater number of pigs born alive through 3 parities ($P < 0.05$) and had a lower wean-to-first-service interval ($P < 0.05$) following their first parity when compared to the females that just entered the farm at the inception of the study. Additional comparisons only within the young group revealed that sows that dropped out of production after a single litter were inferior for number of pigs born alive ($P < 0.05$) and wean-to-first-service interval ($P < 0.05$) when compared to those sows remaining in the herd for at least 4 parities. Though sow removal reasons have not appeared to have changed over the years, this study revealed that sows could be selected for longevity without detrimental effects on reproductive performance as sows in this study that remained in production to more advanced parities outperformed their contemporaries that were removed from the breeding herd in early parities.

Key words: culling, mortality, pig, reproduction

INTRODUCTION

High culling and mortality rates in commercial sow herds can impair pork operations from maximizing returns on investments (Pla et al., 2003; Stalder et al., 2000; Stalder et al., 2003). Many sows, still in the prime productivity portion of their life, are removed from the herd after farrowing just one litter without the opportunity for an additional mating for reasons such as death caused by heart failure, structural

soundness, or failure to return to estrus (Chagnon et al., 1991; Tiranti and Morrison, 2006). Additionally, many gilts that enter the breeding herd are culled before having an attempted mating because of lameness and reproductive problems (Stone, 1981). PigCHAMP™ records (PigCHAMP, 2007) indicate that culling rates for 2005 averaged 51% with the poorest 10% of farms averaging 64%. These same PigCHAMP™ records also indicate sow mortality rates averaged almost 9% and the poorest 10% of farms averaged mortality rates greater than 13%. Culling and mortality rates of this magnitude mean that many breeding females are leaving the farm before they have produced their third parity, an age when most females recover their investment costs (Stalder et al., 2000; Stalder et al., 2003). Furthermore, these relatively high replacement rates lead to a larger than ideal portion of parity 1 females in the sow herd whose offspring are slower growing and have more health related problems (Moore, 2001).

The objective of the present study was to identify the reasons and corresponding time points in which breeding herd females drop out of production as well as understand the reproduction differences in sows that leave the breeding herd later in life compared to sows that leave before they recover their investment costs. Knowledge and understanding of culling reasons combined with corresponding reproductive performance of today's commercial sows may help to identify problems facing U.S. pork industry today and offer insights on overcoming poor sow productive life.

MATERIAL AND METHODS

This research was approved by the Iowa State University Animal Care and Use Committee.

Animal Population

A total of 2,000 breeding age females were identified for evaluation in the present study. These females were from a large Midwestern commercial swine operation with 120,000 breeding females in their system and were all under the supervision of the same veterinarians. The sows were all fed and managed as typical modern commercial females. A total of 500 females from each of two farms (Farm 1 and Farm 2) that both contained 3,200 females in production and an additional 1,000 females were selected from a third farm (Farm 3) that had 5,000 sows in production. The 11,400 sows from the three herds in the study represent 9.5% of the 120,000 breeding females in production in this specific U.S. commercial system with an average lactation length of 18 days. The females from Farms 1 and 2 were Line 42 females while the females from Farm 3 were from the Camborough 22 line. It should be noted that at the onset of the study Farm 2 was experiencing a PRRS outbreak. Both lines analyzed are commercially available lines produce by Pig Improvement Company (PIC) (Hendersonville, TN). Equal numbers of parity 0 females (replacement gilts) and females that had produced a minimum of 5 litters were selected from each farm. The parity 0 females ranged in age from approximately 7 months to those that were about to farrow and are here after termed

“young” females. The females with a minimum of 5 parities ranged from parity 5 to parity 13 and are here after termed “parity 5+” females. Other than the criteria for age group, the females were randomly selected with all “young” females being classified as acceptable replacement females by the management and workers from each of the three participating farms. The “parity 5+” females were selected as a means of acquiring a greater volume of culling information from older sows in a more timely manner when compared to the time required to identify a group of selected replacement gilts to attain the advanced parities or age examined in this study.

Data Collection

Individual sow identification numbers were obtained from each respective farm and were recorded such that general reproduction and longevity records including the number of days in the herd, total number of parities that each female produced, and removal records could be obtained by isolating individual sow records from PigCHAMPTM using the sow identification numbers. Sow removal reasons were determined and entered into the database by farm personnel as well as all performance records (number of pigs born, number of pigs born alive and wean to first service intervals). The sows were monitored for a period of 20-mo, allowing the parity 0 females sufficient time to produce 4 parities.

Records were evaluated to guarantee that all farm identification numbers were unique and that all identification numbers had corresponding PigChampTM records. A total of 53 records were considered potentially inaccurate because duplicate farm

identification numbers existed in our data set or the recorded farm identification number did not exist in the PigChampTM database and were subsequently deleted from the “young” female group. Data from the “Parity 5+” females were also scrutinized and 27 records were removed because of potential data errors resulting in data from 973 sow being evaluated.

Removal Reasons

There were a large number of removal reasons listed for the sows that were from the pre-determined list of reasons in PigChampTM. The records were grouped into a smaller number of more general categories based on the physiological nature of the removal reason. Removal reasons such as off feed, unthrifty, and body condition were grouped into the category called “feed intake”. The reasons grouped into the “gastro-intestinal issues” included hemorrhagic bowel, prolapse, twisted gut, and ulcer. Acute heart failure and heart attack were the two removal reasons that were grouped into the “heart category” (all sows in this category had death listed as their removal type). There were several removal reasons that were included in the “structure category” and included bad legs, lameness, splay, and structure. The category of “old age” was simply those sows whose culling reason was old age. The “productivity category” included sows that were culled for farrowing productivity or lactation-wean productivity. The “reproduction category” included sows with reasons culled for aborted, bad discharge, did not conceive, difficult farrowing, retained pigs, fail to farrow, no expressed heat, and returns to service. There were also several removal reasons with a limited number of sows listed for that reason that were all

grouped into a category termed “miscellaneous”. The removal reasons grouped into the “miscellaneous” included injury, management, multiple systems failure, no removal reason listed, cannibalism, downer, heat stress, puffer sow, udder trauma, underline, or simply other.

Statistical Analysis of Reproduction Data

Proc GLM of SAS (SAS, Institute, Cary, NC) was used to estimate statistical differences for reproductive performance traits between the “young” females and the “parity 5+” group of sows as well as the reproductive differences within the “young” female group only. The traits that were analyzed were the total number of pigs born, the total number of pigs born alive, and the wean-to-first-service interval of sows. The total number of pigs weaned per litter was not analyzed as these farms utilized cross fostering and as a result data accuracy might be influenced. Initial analyses demonstrated that farm differences were not a significant source of variation between farms 1 and 2 (both possessed the same genetic line) and therefore this effect was removed from the final analysis model. Therefore, sow line and sow age group were used as fixed effects in the model used to compare dependent trait means between the “young” and “parity 5+” groups. When comparing reproductive performance traits within the “young” female group, the final model implemented included line and the total number of parities that the sows produced as fixed effects in the analysis of all dependent variables. Given that these sows were commercially available lines, parentage was unknown and therefore, the relationship among animals or even sire and dam information could not be included in the models used

for analysis in this study. Therefore a founder effect could not be accounted for in this study.

RESULTS

Reasons for removal from breeding herd

From the 947 “young” females with PigChamp™ records, 498 were removed from the farms’ breeding herds with 25 sows having to be euthanized for humane reasons, 63 dying, and 410 being culled. This means that not only did the farm lose the opportunity to obtain a salvage value on 88 (9.3% of the original “young” females) cull breeding herd females that either died or were euthanized, but they also created an added expense associated with dealing with breeding herd mortalities (i.e., rendering, composting, etc.). The predominant reason for females in the “young” group to be removed from the breeding herd was reproduction related issues accounting for 35.1% of the removals. Feet and leg structurally related reasons were responsible for 22.1% of the young females leaving the herd. The miscellaneous and feed intake categories were the next greatest incidence for removal from the herd with 14.5% and 11.3%, respectively. It was noteworthy to see that very few (7.2%) of the sows were culled for productivity reasons in early parities. These removal reasons remained relatively consistent across the early parities as shown in Table 1.

There were 66 (7.0%) “young” females that failed to produce even a single litter. Many of the gilts selected for this project were already bred before they were

identified because the supply of open gilts in breeding barns is often quite limited at any point in time. Thus, the 7.0% of females that were culled from the breeding herd before producing their first litter is likely an underestimate of the percentage of actual gilts that failed to produce 1 litter when compared to literature estimates (Moeller et al., 2004). Of the 881 females that produced a single litter, 123 of them failed before they produced their second litter. Another 119 females failed after producing a second litter while 99 females failed after producing their third litter. Thus, 42.9% of the “young” females were removed from their respective breeding herd from each farm before they produced a fourth litter. There were an additional 30 sows that had yet to farrow their fourth litter though they had a sufficient period of time as evidenced by their rather large number of nonproductive days. Additionally, 81 females dropped out after producing 4 parities while 10 were removed after producing 5 litters.

Of the 973 sows in the “parity 5+” group, there were 16 (1.6%) sows that were euthanized, 46 (4.7%) sows that died, 887 (91.1%) sows were culled and 24 (2.5%) sows remained in the breeding herd at the conclusion of the study. The predominant removal reason for the sows in the “parity 5+” group was for old age which accounted for nearly half of all the females removed. The second and third most common removal reasons were reproduction and productivity with 12.2% and 10.8% removal, respectively. As seen in Table 1, old age accounted for over half of the removals starting at parity 7 while culling for reproductive related issues dropped below 10% for the first time in the study at the eighth parity.

It is also important to understand when sows are leaving the breeding herd in relation to their last farrowing date. Knowing when breeding females tend to be removed from the breeding herd for certain failure reasons would allow producers to focus more intensely on those traits during critical time periods. Sows were grouped together by their culling date in relation to when they farrowed their final litter. The groups consisted of sows that were culled from 0 to 17 days post farrowing representing the farrowing and lactation stage of production, 18 to 30 days post farrowing representing the post weaning stage where sows should exhibit an estrus, and 31 to 60 days post farrowing when sows should either return to estrus following a failed insemination or have been checked to determine pregnancy. The farrowing interval of 61 to 140 days post farrowing should be when most bred females are considered “safe in pig.” The females that were removed from the herd over 141 days after they farrowed their final litter were most likely found “not in pig” when they were moved to the farrowing house and incurred a large amount of feed cost during these nonproductive days. The largest (33.8%) group of “young” sows exited the breeding herd from 61-140 days post farrowing with the predominant removal reason being related to reproduction (62.3%). The post farrowing intervals of 18-30 and 31-60 days represented approximately equal numbers of “young” sows leaving the farm with 20.8% and 19.8% respectively, though the reasons why the sows were removed were not identical. In the 18-30 day post farrowing group, structure related issues was the predominant reason why “young” sows were removed representing 37.7% of the removals while productivity and feed intake both accounted for 16.6%. Structure was still the most common reason for removal in “young” sows from 31 to

60 days post farrowing with 33.3%. However, in the 31 to 60 day interval, reproductive related issues represented 23.8% of the removal data for the “young” sows. The time interval for removal of the “parity 5+” group of sows was starkly different ($P < 0.001$) than that of the “young” group when compared using a Fisher’s exact analysis. The sows in the “parity 5+” group were culled in large part from 18 to 30 days post farrowing with 45% exiting the farm at this time. These sows were mainly removed due to the subjective removal reason of old age, which accounted for 65% of removals in this interval. The second largest time interval when “parity 5+” sows were removed from the breeding herd was the 31 to 60 day interval with an additional 25% of the sows being removed. Again, old age was listed as the predominant removal reason with 41.4% of the removals in this category. Feed intake also accounted for a substantial portion of the removals with 18%. It should be noted that there were substantially more females than ideal in both the “young” and “parity 5+” female groups that were removed from the herd over 141 days since they farrowed their last litter. More individual comparisons of removal reasons in relation to when the sows farrowed their final litter are reported in Table 2.

Reproduction analysis

In the “parity 5+” sow group, euthanized sows averaged 7.8 parities while the sows that died averaged 7.1 litters. The sows that were culled averaged 7.8 litters and the sows that were remaining in the breeding herd averaged 9.9 litters to date. The sows that were culled for old age ($n=457$ or 48.2%) averaged 8.0 litters, however the number of sows culled for this reason was greater than might be

expected considering that most were still producing at herd average or greater levels which would generally be considered acceptable levels. These sows averaged 11.4 live pigs born per parity, an average of 91.3 live pigs per sow over their lifetime and still produced 9.4 live pigs born in their last litter. Almost half of the sows in the “parity 5+” group that were culled for old age averaged over 10 pigs born alive throughout their lifetime while also producing at least 10 live pigs in their last litter.

Sows from the “parity 5+” group not only had the ability to remain in production until they produced at least 5 litters, but were also superior when compared to the “young” group when evaluated for reproductive performance traits at the same early parities. When comparing both age groups for their parity 1 reproductive performance records, the “parity 5+” sows had more total pigs born ($P < 0.02$) 12.3 versus 12.0, more pigs born alive ($P < 0.001$) 11.3 versus 10.8, and had a shorter wean-to-first-service interval ($P < 0.01$) 6.3 d versus 7.0 d. When making similar comparisons between the two age groups in the second parity, there was no difference ($P > 0.05$) identified for total pigs born in their second parity or wean-to-first-service interval. However, the “parity 5+” sows still maintained their superiority for the number of pigs born alive ($P < 0.03$) in the second parity with 11.5 pigs born alive versus 11.1 born alive for the “young” female group. The “parity 5+” group of sows also had more live pigs ($P < 0.05$) in their third parity than the “young” group with 12.0 and 11.7 pigs, respectively. Though not significant ($P < 0.1$), the “parity 5+” sows also had more live pigs in their fourth parity with 12.0 live pigs born

compared to 11.6 live pigs for the “young” sow group. A graphical representation of the reproductive superiority of the “parity 5+” females can be seen in figure 1.

The reproductive superiority of females that stayed in the herd longer than their contemporaries that were removed early in their life can also be observed in comparisons within the “young” group as well. When comparing the reproductive traits within the “young” female group, sows that reached parity 4 typically had greater reproductive performance traits compared to those that were removed from the breeding herd in earlier parities, even though very few sows were reported to be culled for productivity before they reached parity 4. Sows that dropped out of the breeding herd after only producing 1 litter averaged only 9.7 live pigs born in their only litter while all sows which produced at least 3 litters ($P < 0.03$) had 10.6 pigs in their first litter, a difference approaching 1 full pig. Additionally, the sows that dropped out of the breeding herd after 1 litter had a higher ($P < 0.01$) wean to first service interval (8.6 d versus 6.5 d) when compared to those breeding herd females that remained in the breeding herd until for at least 4 parities, a difference of over 2 full days.

DISCUSSION

It has been reported that producers are not completely correct when making culling decisions or are inaccurately entering the culling records into the database (Knauer et al., 2007). A possible limitation of this study is that it relies on producers to record their culling decisions into PigChampTM. However, excluding the

typographical errors, this direct information is still the most practical method of obtaining useful information for removal reasons of sows from commercial farms as validation of culling reasons and cause of death via a necropsy by an accredited veterinarian is not feasible considering the scale and duration of this study. This study has shown that breeding females from modern commercial pork production operations are still being culled from the breeding herd for similar reasons (structural soundness and reproductive failure) and percentages as those in the 1980s and the 1990s (D'Allaire et al., 1987; Lucia et al., 2000; Stone, 1981). The combination of genetic lag and low heritability (Serenius and Stalder, 2004) for sow productive life mean that it will take quite some time for selection pressure seen in the nucleus herds to filter down to the commercial operations on a scale that will be recognizable. Recent advertising by PIC (www.pic.com) states their new lines of Camborough females have greater lifetime reproductive performance and longevity, demonstrating that at a minimum this genetic company appears to be paying attention to longevity and are selecting for it as well.

It has been demonstrated time and time again that swine operations with lower replacement rates are usually more profitable than those with high replacement rates (Faust et al., 1992; Faust et al., 1993; Rodriguez-Zas et al., 2003). Extrapolating from the net present value analysis work for breed-to-wean (Stalder et al., 2003) and farrow-to-finish (Stalder et al., 2000) operations, increasing the average removal parity of breeding females by one tenth (ie., 0.1) of a parity can increase revenue by \$0.15 and \$0.23 for every weaned pig and market hog sold

from the farms, respectively. Taken as a whole for the U.S. commercial pork industry, this corresponds to an increase in profit by \$15 million. At a time when voluntary culling (culling by producers) at parities 4, 5, and 6 for reproductive performance or mothering ability are virtually nonexistent, culling sows for old age simply because they have reached a predetermined parity is haphazard to say the least. If producers would retain more of these elite older females, it would allow producers to cull low performing sows and would decrease the need for the large number of replacement gilts that enter the breeding herds. Voluntary culling of low performing sows has obvious benefits to the operation, but decreasing the number of replacement gilts entering the farm has many often overlooked benefits, such as decreasing the risk of a disease outbreak, improved average growth rate of terminal offspring, and improved herd health (both breeding herd and terminal offspring) (Moore, 2001). It has been suggested that in order to achieve an ideal parity structure that producers strive to lose no more than 5% of the replacement females before they produce a litter and 10% for every litter thereafter. The main target that producers should aim for would be to have 75% of the females that enter the herd farrow a third litter. It can be seen from figure 1 that even though many gilts in this study had already been bred previous to their random selection for entry into this study, many more females were removed than the desired target of 5%. It should be noted that producers should work with their genetic supplier to ensure that quality replacement gilts are delivered to the farm instead of making exhausting attempts to get questionable gilts to farrow a litter simply to reach the desired goal of no more than a 5% loss before parity 1. It has been shown that gilts bred at the optimal time

point of 221-240 days typically are more productive in regards to both number and size of parities than gilts that do not reach puberty in a timely manner (Babot et al., 2003). In addition to reaching puberty at an early age, getting a gilt to farrow her first litter (Goodwin, 2002) and have a short wean to first service interval following their first litter (Tantasuparuk et al., 2001) have rather large impacts on longevity and lifetime pig production.

The high incidence of removal due to reproductive and structure related reasons in the early parities is in agreement with previous studies (Chagnon et al., 1991; D'Allaire et al., 1991; Stone, 1981). Additionally, it should be noted that the removal of sows for reproduction and structure declines in later parities. This suggests that removal for reproduction and structure related issues maintain a bigger role in removals at early parities. The largest portion of sows that were culled for reproduction occurred between 61 and 140 days after they farrowed their final litter. Sows culled in this time interval include females that took an extremely long amount of time to return to estrus, sows that never expressed a full estrus, sows that didn't conceive or sows that aborted. Extra care should be taken to ensure accurate pregnancy checks occur to keep non productive days to a minimum as these sows should either be rebred or culled as soon as possible. Furthermore, as seen in this study as well as by Lucia and coworkers (2000), the females that survive to greater parities, produce more pigs per litter. Since culling for productivity represented a minimal amount of the removals in this study as well as in the swine industry today, it can be inferred the sows that thrive in today's production systems to advanced

parities are truly superior individuals in terms of both structure and fertility, reasons that typically cause many females to be removed from the breeding herd at early parities. Therefore, an integral way for producers to have more females survive to advanced parities is to focus their attention on structure during gilt selection as well as to take any feasible extra steps necessary to ensure females in early parities rebreed.

These results suggest that producers analyze the tendencies of the females from their genetic supplier with regards to productivity at more advanced parities before simply culling them because they have reached a predetermined parity. This study also demonstrates that it not only behooves swine producers to select for sows that can remain in production beyond parity 5 because of lower replacement costs, disease issues and other factors, but these sows also are superior for reproductive performance traits when compared to parity 1 and parity 2 females and appear to be easier to rebreed for their next litter which correlates to greater longevity (Tantasuparuk et al., 2001).

Though the culling or removal reasons have not changed over the years for the removal of sows, this study provides additional information to the scientific literature regarding differences between sows that are culled from the breeding herd and those that remain in production well beyond the average breeding herd female. This study also revealed that sows that survived to advanced parities outperformed their contemporaries that were removed from the breeding herd early in production both in terms of reproductive performance and longevity. Since very few breeding herd

females were removed in early parities for productivity, it can be extrapolated that if selection pressure were to be placed on sows in the genetic suppliers nucleus farms for longevity that it would also benefit reproductive performance of the farm.

ACKNOWLEDGEMENTS

Comments by the anonymous reviewers are greatly appreciated. Support for B. Mote was provided in part by a USDA National Needs Fellowship, the Iowa Agriculture and Home Economics Experiment Station, State of Iowa and Hatch funding, and the National Pork Board. Data collection and assistance provided by individuals from PIC USA, Pipestone, James Koltes, Marcos Ramos, Dan Mouw, and members of the Rothschild lab is greatly appreciated.

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Table 1
Frequency (%) of removal reasons by parity at removal

Young Sow Group

Removal Category	Parity at Removal ^a						n	%
	0	1	2	3	4	5		
Reproduction	48.5	37.4	23.5	38.4	34.6	30.0	175	35.1
Structure	9.1	20.3	25.2	29.3	24.7	0.0	110	22.1
Productivity	0.0	4.1	1.7	15.1	14.8	20.0	36	7.2
Feed Intake	7.6	14.6	13.5	5.1	13.6	10.0	56	11.3
Gastro-Intestinal	0.0	6.5	5.9	4.0	1.2	30.0	23	4.6
Heart	3.0	7.3	6.7	3.0	2.5	0.0	24	4.8
Old Age	0.0	0.0	0.0	0.0	2.5	0.0	2	0.4
Miscellaneous	31.8	9.8	23.5	5.1	6.1	10.0	72	14.5
Total % ^b	13.3	24.7	23.9	19.9	16.2	2.0	498	100.0

Parity 5+ Sow Group

Removal Category	Parity at Removal ^a						n	%
	5	6	7	8	9	10+		
Reproduction	39.3	27.0	16.0	6.8	4.5	3.2	116	12.2
Structure	3.6	2.7	4.2	9.2	12.0	12.8	71	7.5
Productivity	0.0	2.7	10.8	10.9	14.9	17.0	102	10.8
Feed Intake	7.1	14.4	2.7	8.5	12.7	11.7	79	8.3
Gastro-Intestinal	0.0	1.8	5.9	1.3	0.7	1.1	25	2.6
Heart	0.0	1.8	1.4	0.7	0.7	0.0	9	0.9
Old Age	7.1	15.3	52.4	58.2	50.0	52.1	457	48.2
Miscellaneous	42.9	34.3	6.6	4.4	4.5	2.1	90	9.5
Total % ^b	3.0	11.7	30.4	31.0	14.1	9.8	949	100.0

^a Frequencies in cells sum to 100% within a column (excluding total) for each removal parity

^b Percentage of total culls for each removal parity

Table 2
Frequency (%) of removal reasons in relation to days after the final litter

Young Sow Group

Removal Category	Days After Farrowing ^a				
	0-17	18-30	31-60	61-140	141+
Reproduction	11.84	3.33	23.81	62.33	55.55
Structure	27.63	37.78	33.33	10.96	13.89
Productivity	11.84	16.67	11.91	0.68	2.78
Feed Intake	19.74	16.67	13.10	5.48	5.56
Gastro-Intestinal	10.53	8.89	3.57	2.74	0.00
Heart	7.89	4.44	7.14	2.74	5.56
Old Age	0.00	2.22	0.00	0.00	0.00
Miscellaneous	10.53	10.00	7.14	15.07	16.66
% of Total Culled ^b	17.60	20.80	19.50	33.80	8.30

Parity 5+ Sow Group

Removal Category	Days After Farrowing ^a				
	0-17	18-30	31-60	61-140	141+
Reproduction	9.22	0.23	16.32	54.17	28.21
Structure	5.67	8.75	6.28	9.37	2.56
Productivity	24.11	14.06	2.51	0.00	2.56
Feed Intake	4.26	5.53	17.99	3.13	7.70
Gastro-Intestinal	4.26	1.15	2.51	5.21	7.70
Heart	1.42	0.00	0.42	5.21	2.56
Old Age	38.30	65.67	41.42	13.54	15.38
Miscellaneous	12.76	4.61	12.55	9.37	33.33
% of Total Culled ^b	14.86	45.73	25.18	10.12	4.11

^a Frequencies in cells sum to 100% within a column (excluding total) for each post farrowing interval

^b Percentage of total culls for each post farrowing interval group

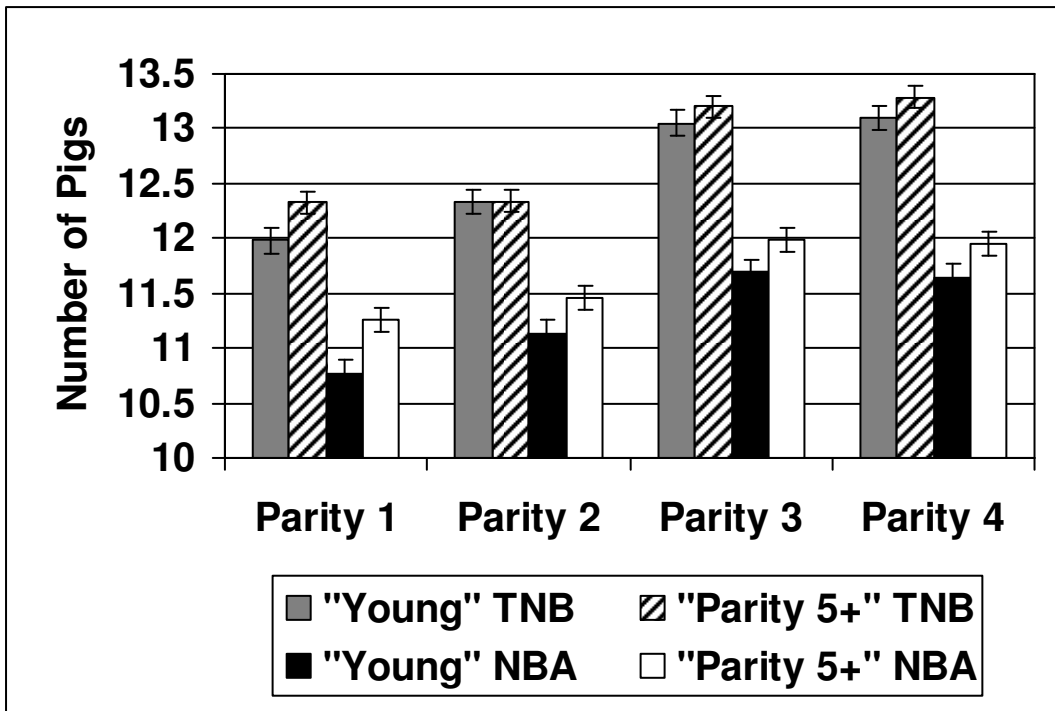


Figure 1. Total number of pigs born (TNB) and the number of pigs born alive (NBA) at each of the first 4 parities for both the “young” and “parity 5+” females.

CHAPTER 6. IDENTIFICATION OF GENETIC MARKERS FOR PRODUCTIVE LIFE IN COMMERCIAL SOWS

A paper to be submitted to the *Journal of Animal Science*

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ABSTRACT

Escalating replacement rates and production costs warrants attention on sow productive life (SPL). Increasing average SPL by one tenth of a parity would result in an annual revenue increase of over \$15 million in the United States. Research in model organisms has revealed conserved genes and gene pathways that lead to longer lifespan. The most prominent gene pathways are those involved in growth, most notably genes in the insulin pathway that serve to mimic the response of caloric restriction. The objective of this research was to test the hypothesis that these well conserved genes and gene pathways could also play a role in SPL even though the productive life of sows is both a measure of longevity and their reproductive performance. Preliminary research on three distinct populations of over 2,000 animals suggested that several genes were associated with components of SPL. Genetic markers were then analyzed against the sows' corresponding records for reproductive and longevity traits using a validation population of 2,000 commercial females. Right censored data were used to test associations of genetic markers with survival to defined time points. Three distinct models of survival analysis were implemented using nonparametric estimates of the survival distribution in a sequential order, using a parametric accelerated failure time model

with a Weibull distribution of the error term, and a Cox proportional hazards model which is a semiparametric model that uses an unspecified baseline hazard function. The genetic marker for *CCR7* was significantly associated ($P < 0.05$) with survival to early time points using the nonparametric model. The *CPT1A* gene trended towards significance ($P < 0.1$) for survival to parity 4 when all genotypes were included but was significantly associated ($P < 0.05$) with survival to parity 4 for all tests of association when the 12 and 22 genotype classes were combined. Genetic markers for *MBL2*, *IGFBP3*, and *WARS2* also tended ($P < 0.1$) towards significance for survival traits but were not consistent. Mixed model analyses were used to determine the associations of these genetic markers with reproductive traits. The genetic markers for *IGFBP1*, *MBL2*, *CPT1A*, *CCR7*, *SLC22A5*, and *ACE* were significant ($P < 0.05$) with at least one reproductive trait. These results show that molecular markers should be considered for use in marker-assisted selection to improve SPL.

Key words: longevity, pig, reproduction, sow productive life

INTRODUCTION

Sow longevity or sow productive life (SPL) has become a discussion point in the swine U.S. commercial industry. Unacceptable replacement rates occurring on commercial farms are being driven by high culling and mortality levels. High culling and mortality levels suggest that many breeding females don't produce a third litter, the point when most females recover their investment costs (Stalder et al., 2000; Stalder et al., 2003). Recent analysis of the commercial sow herd shows that 42% of the females that enter the farm wean 30 or fewer pigs before they are culled and

94% are culled before they wean 57 pigs (Anil and Deen, 2007). Current replacement rates place the burden of being profitable on a relatively small percentage of sows that remain productive beyond the average sow (Pla et al., 2003; Stalder et al., 2000; Stalder et al., 2003). Additionally, having a high replacement rate leads to having a greater than ideal proportion of parity one females in the herd whose offspring are typically slower growing and endure more health related problems when compared to offspring from older sows (Moore, 2001). Researchers working with model organisms such as mice, nematode, yeast, and the fruit fly have identified genes and gene pathways that are conserved between the species that lead to longer lifespan of these organisms (Hasty et al., 2003; Hekimi and Guarente, 2003; Longo and Finch, 2003; Simon et al., 2003; Tatar et al., 2003). The objective of this research was to test the hypothesis that these well conserved genes and gene pathways could also play a role in SPL even though the productive life of sows is both a measure of longevity and their reproductive performance during that time frame. Therefore, genes involved in the insulin pathway along with genes more specific to reproductive traits were targeted for marker development and association analyses in the evaluation of length of productive life among commercial breeding females.

MATERIALS AND METHODS

This research was approved by Iowa State University Animal Care and Use Committee.

Animal Population and DNA Isolation

Three populations were initially used to test the associations of the identified genetic markers with either the number of litters that sows produced or the number of pigs that sows produced in early parities. The first population totaled approximately 1,000 commercial females consisting of mid 1990s genetics, where half of the sows produced fewer than 4 parities and the remaining half produced greater than six parities. The second population, also mid 1990s genetics, consisted of 200 purebred sires with production records on at least 10 daughters. The third population was composed of 1,100 purebred (both Large White and Landrace) females that had reproduction records available for early parities. These populations were useful to screen genetic markers, but a more current dataset with both reproduction and culling reasons was needed to identify markers that are associated with sow productive life.

The population used to validate previous association results was chosen and consisted of a total of 2,000 breeding age females, representing the most current genetic female available. All analysis presented herein is from this validation population. These females were from a large Midwestern commercial swine operation with 120,000 breeding females in their system. Five hundred females from each of two farms (Farm 1 and Farm 2) that both possessed 3,200 females in production were randomly selected and an additional 1,000 females were randomly selected from a third farm (Farm 3) that had 5,000 sows in production. The 11,400 sows from the three herds in the study represent 9.5% of the 120,000 breeding females in production in this commercial system. The females from Farms 1 and 2

were Line 42 females (Large White x Landrace F1) while the females from Farm 3 were from the Camborough 22 line (Large White, Landrace, and Duroc composite). Both lines analyzed are commercially available lines produced by Pig Improvement Company (PIC) (Hendersonville, TN). Equal numbers of parity 0 females (replacement gilts) and females that had produced a minimum of 5 litters were selected from each farm. The parity 0 females ranged in age from approximately 7 months to those that were about to farrow and are here after termed “young” females. The females with a minimum of 5 parities ranged from parity 5 to parity 13 and are here after termed “parity 5+” females. Other than the criteria for age group, the females were randomly selected with all “young” females being classified as acceptable replacement females by the management and workers from each of the three participating farms. The “parity 5+” females were randomly selected as a means of acquiring a greater volume of culling information from older sows in a more timely manner when compared to the time required to identify a group of selected replacement gilts to attain the advanced parities or age examined in this study. Ear tissue was isolated on the 2,000 commercial females described above using the TypiFix™ ear tag from IDnostics (Switzerland). This system allows for simultaneous identification and tissue collection to prevent sample misidentification. The DNA was then isolated from the tissue samples using the Nexttec™ DNA isolation system (Germany) adhering to the manufactures protocol.

Genetic Markers

SNPs were identified in 20 genes (*insulin-like growth factor binding protein 1 (IGFBP1)*, *insulin-like growth factor binding protein 2 (IGFBP2)*, *insulin-like growth*

factor binding protein 3 (IGFBP3), insulin-like growth factor binding protein 5 (IGFBP5), insulin-like growth factor binding protein 7 (IGFBP7), carnitine O-palmitoyltransferase 1 (CPT1A), organic cation/carnitine transporter 2 (Solute carrier family 22 member 5; SLC22A5), angiotensin I converting enzyme (ACE), and C-C chemokine receptor 7 (CCR7), tryptophanyl tRNA synthetase 2 (mitochondrial) (WARS2), tryptophanyl tRNA synthetase (cytoplasmic) (WARS), cyclooxygenase 2 (COX2), tryptophan/serine protease (UNQ9391), Vitamin D Receptor (VDR), calmodulin (CALM1), superoxide dismutase 1 (SOD1), mannose-binding lectin 2 (MBL2), copper chaperone for superoxide dismutase (CCS), insulin-like growth factor 2 receptor (IGF2R), and beta 2 adrenergic receptor (B2AR) that could be classified into one or more of the following groups: insulin/growth, reproduction, nutrition, health, anti-inflammatory, or longevity. All genetic markers that were tested for this project are listed in Tables 1 and 2.

Data Collection

PigCHAMPTM production records were obtained throughout the research trial (20 months) by downloading the farm's database with the sow's farm identification number and correlating it to the TypiFixTM ear tag. The data that was collected regarding the productive life of the sows included the date the sow entered the herd, their first service date, their removal date, their removal parity, the removal type (cull, mortality, or euthanized), the removal reason, lifetime nonproductive days, and the total days in the herd. The reproductive data collected included farrowing date, gestation length, total born, number born alive, stillborn, mummies, total pigs weaned, lactation length, and wean-to-first-service interval for each parity that the

sow produced. Means, maximum data points, and minimum data points were obtained using the Univariate procedures in SAS (SAS Inst., Cary, NC). The maximum and minimum data points were identified as possible outliers and data were subsequently verified to ensure that they were within realistic bounds for a given trait.

Statistical Analysis

To determine if the genetic markers were associated with the survival aspect of SPL instead of strictly reproduction, several types of data analyses were used. The initial method employed was the use of a Fisher's exact test to identify if there was a significant difference between the genotypic frequencies of the sows that had produced at least 5 parities at the onset of the research project (Parity 5+) and the gilts that had just entered the sows farm (Young) that serve to represent the typical unselected females in a commercial herd. A Fisher's exact test was also employed to determine if there was a significant difference in the genotypic frequencies of the "Young" sows that dropped out of production before they produced a fourth parity and those "Young" sows that produced at least four parities. Survival analysis was also performed on the "Young" sow group using the LIFETEST, LIFEREG, and PHREG procedures of SAS. The LIFETEST procedure computes nonparametric estimates of the survival distribution in a sequential order and simultaneously computes a Log-Rank statistic that places more weight on differences between groups that occur at later points in times and a Wilcoxon statistic that gives more weight to differences between groups that occur at earlier time points. The LIFETEST procedure is useful for screening large numbers of quantitative variables,

but it is not adequate for testing the effects of variables controlling other covariates. Therefore, such data require the regression models of the LIFEREG and PHREG procedures to be used (Allison, 1995) to simultaneously account for the fixed effects of genotype of the sow and the farm in which she was housed. The LIFEREG procedure fits a parametric accelerated failure time model that in this case is right censored and uses a Weibull distribution of the error term. Right censored data are commonly used among survival analysis of life data. Sows that were still in the breeding herd the last time the data were sampled are considered right censored as their failure (date when they are removed from the breeding herd) would occur at some time point after we sampled the data. The PHREG procedure fits a Cox proportional hazards model, which is a semiparametric model that uses an unspecified baseline hazard function. The fixed effects that were included into the final models for the LIFETEST, LIFEREG, and PHREG procedures were genotype of the sow and the farm on which the sows was housed. For the three survival analysis tests, survival to parity 1, parity 2, parity 3, parity 4, 250 days post first service, 300 days post first service, and 500 days post service were tested to determine if a significant genotypic effect on survival existed. Data were right censored at the aforementioned time points for each sow that survived beyond said time point.

The PROC GLM procedure of SAS was used to determine genotype effects on the reproductive traits that were analyzed. The statistical model included genotype, farm, and age group (when the trait was analyzed using both the “Parity 5+” and “Young” sow groups). Both the “Parity 5+” and the “Young” groups were analyzed

individually as well as in a combined analysis of the two groups. The reproductive traits that were focused on were the total number of pigs born and the number of pigs born alive for each litter as well as lifetime records for both traits. The sire and dam information for these sows was unknown as is the usual case in commercial herds using pooled semen from several sires and therefore neither sire nor dam could be included as random effects. The genetic markers were tested for significant associations for each parity as well as for combined lifetime productivity.

RESULTS

Initial analyses (data not shown) of the first 3 distinct populations totaling more than 2,300 breeding animals showed that several of these genetic markers were associated with components of SPL (either survival to parity six or for reproductive traits) and warranted further research (Mote et al., 2006). Those markers showing no tendency for association with any trait included in SPL were dropped from further analyses. These three populations were not ideally suited for longevity studies as they only contained data on either longevity or reproduction, but not both.

Therefore, a fourth commercial population was identified to serve as our validation population that contained both longevity and reproduction information.

Initial analysis of the validation population using Fisher's exact test demonstrated significant differences ($P < 0.05$) in the genotypic frequencies indicative that the marker could be involved in the sows' ability to survive to parity 5. Seven genes showed a significant difference ($P < 0.05$) between the genotypic frequencies of the superior sows and the young gilts. These seven genes were *insulin-like growth factor binding protein 1 (IGFBP1)*, *insulin-like growth factor binding protein 3*

(*IGFBP3*), carnitine *O*-palmitoyltransferase 1 (*CPT1A*), organic cation/carnitine transporter 2 (Solute carrier family 22 member 5; *SLC22A5*), angiotensin I converting enzyme (*ACE*), and C-C chemokine receptor 7 (*CCR7*), tryptophanyl *tRNA* synthetase 2 (mitochondrial) (*WARS2*).

At the conclusion of the 20 month trial when all of the “Young” sows had had the opportunity to farrow 4 litters, data were again obtained from PigCHAMP™ software and they were considered to be the final data set. The records from only the “Young” sow group were then analyzed using a Fisher’s exact test to determine if a significant difference in genotypic frequencies existed between the “Young” sows that were able to produce 4 parities and those that did not. The only genetic marker that showed any significant difference ($P < 0.05$) between the genotypic frequencies of the “Young” sows that produced 4 litters and those that did not was *CPT1A* ($P < .05$) though *MBL2* trended ($P < 0.1$) towards significance.

When the LIFETEST procedure of SAS was used for data analyses on the “Young” females, *CCR7* showed a significant association ($P < 0.05$) with survival to 250 days after first service, 300 days after first service, and survival to parity 1. A graph of the survival curve for *CCR7* can be seen in Figure 1. The marker for *CPT1A* showed a trend towards significance ($P < 0.1$) when all genotypes were included in the analysis of survival to parity 4, but was significantly associated ($P < 0.05$) with survival to parity 4 when the animals in the 22 genotype class were combined with the animals in the 12 genotype class. The two classes were combined since the initial analysis showed that there was not a significant difference between the 12 and 22 genotypes and that the 22 genotype class consisted of less than 10% of the data.

A graph of the survival curve for *CPT1A* can be seen in Figure 2. Additional genetic markers showing a tendency for association ($P < 0.1$) were *IGFBP3* with survival to 250 days after first service, *MBL2* with survival to parity 4, and *WARS2* with survival to 250 days after first service. Data analyses using the PHREG or LIFEREG procedures revealed the same outcome for the association tests of *CCR7* and *CPT1A*. The *CCR7* genetic marker showed a tendency ($P < 0.1$) for association with survival to 250 days after first service. The *CPT1A* gene once again showed a trend towards association ($P < 0.1$) for survival to parity 4 when all genotypes were analyzed independently, but was again significantly associated ($P < 0.05$) with survival to parity 4 when animals in the 22 homozygous genotype class were included in with the 12 heterozygous genotypes. A compilation of all the genetic markers that were significantly associated with sow survival is shown in Table 3.

The reproduction analyses of these genes also proved to be beneficial to understanding the different roles these genes play in SPL. The genetic marker for *IGFBP1* was significantly associated with several reproductive traits for the different sow groups. The marker was significantly associated with the number of pigs born alive in parity 1 in the “Young” sows with the favored genotype (22) having 1.2 and 1.0 more pigs born alive than the 11 and 12 genotypes respectfully. In the “Parity5+” group, 12 and 22 genotypes were significantly different from the 11 genotype for both the number of pigs born and the number of pigs born alive. In the “Parity 5+” group, the sows with either the 12 or 22 genotypes had an advantage of at least 2.4 pigs born over the sow’s lifetime compared with the sows that possessed the 11 genotype. After dropping the 11 genotype class from further analysis (which

represented less than ten percent of the data), *MBL2* was significantly associated with early reproductive traits. It was significantly associated with the total number of pigs born and with the number of pigs born alive in parities 1 and 2 when all sows were analyzed together with the beneficial genotype class having an additional 0.35 pigs per litter for all traits. Furthermore, *CPT1A* was significantly associated with reproductive traits as well, especially in the later parities. The favored genotype class (22) was associated with at least a 0.4 advantage in total number of pigs born and number of pigs born alive for all sows in parities 3 and 4. Additionally, the same genotype class had an advantage of 0.00325 more pigs per day of herd life for the “Young” sows and had an advantage of 0.002 more pigs per day of herd life for all sows combined. When extrapolated out on a pigs per year basis, this represents 1.18 and 0.7 more pigs per year per sow for “Young” sows and all sows, respectively. The genetic marker for *VDR* was significantly associated with total born in the “Young” sows in parity 1 and in the lifetime number of pigs born alive in the “Young” group of sows as well. Other markers such as *SLC22A5*, *ACE*, and *CCR7* also were associated with some reproductive traits, though their effects were not as consistent across sow groups or parities. Complete results of all genetic markers that were significantly associated with reproductive traits are shown in Table 4.

DISCUSSION

In model organisms, the alleles associated with leaner phenotypes or associated with reduced caloric intake are often the preferred allele for longevity (Tatar et al., 2003). It has been shown that gilts that are leaner have the tendency to be removed

from the herd sooner (Stalder et al., 2005). Furthermore in swine production, one of the most critical points for sow survival is maximized feed intake during lactation.

Sows that do not meet energy requirements during lactation often are in an energy deficit situation for several days during a typical 21-day lactation period and are subsequently in poorer body condition at weaning which further contributes to delayed wean to estrus intervals and culling from the breeding herd. Though for model organisms, reduced caloric intake is preferred for longevity and sows need to maximize feed intake during lactation, these same genes that are important for longevity in model organisms could still prove beneficial to SPL.

Several different methods to analyze sow survival for SPL were presented herein. The limitation with using the Fisher's exact test between the "Parity 5+" group and the "Young" group was that it did not account for a founder effect or initial environmental conditions. Additionally, the beneficial genotype suggested by using this method for CCR7 was the 11 genotype which was the worst of the three genotypes when using any of the survival analysis methods. However, using a Fisher's exact test when analyzing just the sows in the "Young" group that either produced 4 parities or failed to produce 4 parities produced very similar results to the survival analysis results from the LIFEREG and PHREG analyses. Therefore, longevity analysis of an older group with a younger group should likely be avoided. SPL is a complicated trait to analyze since it is a combination of several different traits, all of which have a relatively large environmental component. Reproductive traits are notoriously lowly heritable (Roehe and Kennedy, 1995; Holl and Robison, 2003) with a low repeatability though some managers still cull sows for poor

reproductive performance based on a single record. Additionally, there is the possibility of large human error in the culling process itself as the culling reason listed for many sows can be inaccurate as the culling reasons listed by farmers did not always match postmortem veterinarian analysis (Knauer et al., 2007). This leads to SPL having a low heritability (Serenius and Stalder, 2004). Therefore, it is not surprising that we did not find large gene effects, especially for the survival to later parities component of SPL.

The genetic markers for *CCR7* and *CPT1A* show the greatest promise for their use as genetic markers for sow survival. The marker for *CCR7* was associated with early survival time points, such as survival to 250 days after first insemination, 300 days after first insemination, and survival to parity 1. Survival to parity 1 is often over looked, but should be the first critical time point that producers use as a benchmark to identify if they have problem with sow survival. Additionally, the consistency demonstrated by *CPT1A* regardless of the type of analysis performed, leaves little doubt that it is associated with survival to parity 4. Survival to parity 4 is also a critical time point as sows typically need to produce at least 3 parities to recover their investment costs (Stalder et al., 2000; Stalder et al., 2003). Sows that have either the 12 or 22 genotypes for *CPT1A* have a lower hazard rate (0.37 lower) than the 11 genotype class when analyzed at survival to parity 4. If we were to extrapolate the data out to get the mean survival times for each genotype class, the mean survival of the 11 genotype class would be 4.58 parities and the mean survival for either the 12 or 22 genotypes would be 6.61 parities. This large effect is most likely over inflated as roughly half the sows were still in production at the conclusion

of the study and also this doesn't account for producers who start to cull some sows at parity 6 for "old age." However, it still stands that the sows with 12 and 22 genotypes had a greater chance of being profitable for the operation when compared to animals having the 11 genotype. Both *IGFBP1* and *CPT1A* showed the clearest and most consistent associations with both the total number of pigs born and more importantly the number of pigs born alive. For *CPT1A*, the beneficial allele for reproductive traits is also the preferred allele for sow survival.

In summary, several markers were significantly associated with either the sow survival portion or the reproductive portion of SPL. *CCR7* should be considered in marker-assisted selection schemes for improved sow survival and *IGFBP1* should be considered if selection pressure is warranted on reproductive traits. The inclusion of *CPT1A* in a marker-assisted selection scheme should improve both the sow survival and reproductive components of SPL and should therefore be strongly considered for improvement of sow productive life in commercial females.

ACKNOWLEDGEMENTS

Support for B. Mote was provided in part by a USDA National Needs Fellowship. Funding was provided in part by the Iowa Agriculture and Home Economics Experiment Station, State of Iowa and Hatch funding and the National Pork Board. Data collection and assistance provided by individuals from PIC USA, Pipestone, Drs. James Koltes, Marcos Ramos, and Timo Serenius, and Mr. Dan Mouw and members of the Rothschild Lab is greatly appreciated.

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Table 1. Genetic markers associated with at least one component of SPL and their related SNP information from a study of candidate genes on sow productive life

Gene	Forward/Reverse Primer (5'-3')	PCR Size	Location ¹	SNP ² : Postion ³	Enzyme	Allele 1	Allele 2
ACE	TCATCATCCAGTTCCAGTTCC/ GTTTCGGCGTCCAGTTGTACT	540	Intron 12	C/T: 95	AluI	369, 102, 36, 33	276, 102, 93, 36, 33
CCR7	AAGTCCTGGGTCTTCGGAGT/ GGATGATGACGAGGTAGCAGA	385	Exon 3	C/T: 147	HpyCH4III	385	240, 145
CPT1A	AGCTCTAGTTGGTTGTGGAATC / ACCTACGGGTAAGCGGGAAC	350	Intron 11	T/C:87	BstNI	299, 51	212, 87, 51
IGFBP1	AAAATCAGGGTATCGGTCTTCA / TCGTTCCTGTGCCATCTACA	403/393	Intron 2	CATCCCCAGG ⁴ : 252	BtsCI	302,91	160, 125, 91, 26
IGFBP3	CAAGTCTCAAGCACGGACAC/ GCCAGGGGCTCTCTCTTTT	438	Intron 2	A/G: 114	BsaHI	438	326, 112
MBL2	ACCTGCCTTGTGATTTCTTG/ GAATGCCAGAGAGTCAGAGC	251	Exon 1	T/C: 63	Bsp1286I	251	189, 62
SLC22A 5	CCTGCCCTACATTCTCATGG/ CACTCTGGGGCTTTCTTCAC	539	Intron 9	C/G: 235	HaeIII	374, 165	304, 165, 70
VDR	ACCAGATCGTGCTGCTGAAG/ GGGAGACGATGCAGATGG	404	Intron 8	T/C: 220	HpyCH4IV	279, 125	185, 125, 94
WARS2	CAATTACCTGGGAGCCATTG/ CTTCTCTGGGTTTATGCCACA	175	Exon 2	G/A: 142	PstI	175	142, 33

¹The SNP's position within the gene.

²The first base by convention is allele 1 and the second SNP is allele 2.

³The position of the SNP from the beginning of the PCR fragment.

⁴The CATCCCCAGG is a ten base pair insertion/deletion.

Table 2. Genetic markers that were not associated with one component of SPL and their related SNP information from a study of candidate genes on sow productive life

Gene	Forward/Reverse Primer (5'-3')	PCR Size	Location ¹	SNP ² : Position ³	Enzyme	Allele 1	Allele 2
B2AR	GTCTTCCCTGAAGGCCTATG/ CTCCCCTGTGAATCAGTGCT	195	Exon 1	T/C: 25	BsaXI	195	151, 30,14
CALM	ATTGCAGACTCTCCTTAAATATG/ TACATGCAAGGCAGCCAAC	253	Intron 4	G/C: 141	DdeI	162, 91	112, 91, 50
CCS	TTTCCTAACAGGTCCACTG/ AGAGCGCTCACCTCTCTCC	160	Exon 7	A/C: 132	NciI	103, 57	103, 29, 28
COX2	TCAATCGACCAGAGCAGAGA/ CGAGCTGTGGATCTTGAACA	555	Intron 9	A/G: 172	BsrBI	555	386, 169
IGF2R	GTCCGGCCATTAGGAAGAAG/ TTCTTTCTCTTTCTCTGGGTGC	491	Intron 16	C/T: 359	BsII	341, 150	215, 150, 126
IGFBP2	GGAACCTTGCTCACCTTTGTC/ CAGGAAGAAGCCCAGGTATG	361	Intron 2	A/T: 135	MboII	346, 15	200, 146, 15
IGFBP5	CGCCTGAGATGAGACAGGA/ GGACAGGAGGGGTGAGAGG	312	Intron 2	C/A: 107	AvaI	252, 60	147, 105, 60
IGFBP7	GCCCAGAAAAGCATGAAGTG/ CTTGTCCTCCCACTGTCCCT	398	Intron 3	A/G: 117	MspI	216, 182	216, 115, 67
SOD1	GATTTGGTTTTGTAGCATTT/ GGCCTCTGATAAAAAGGAAGG	249	Intron 3	A/G: 94	NheI	249	154, 95
UNQ9391	TGTAGGGTCGCGTATGGACT/ GGGGTTGGTGGAGTGAGTAA	552	Intron 3	T/G: 194	BsmAI	504, 48	336, 138, 48
WARS	CCCTTGTTCTTGGTGTCACT/ GGCCACCACCACTGATTAAC	363	Intron 5	C/T: 214	MseI	345, 18	213, 132, 18

¹The SNP's position within the gene.

²The first base by convention is allele 1 and the second SNP is allele 2.

³The position of the SNP from the beginning of the PCR fragment

Table 3. Association results of genetic markers with survival in sows in a study of candidate genes for sow productive life

Gene	Trait	Fisher's Exact ⁷	PROC LIFETEST ⁸	PROC LIFEREG ⁹	PROC PHREG ¹⁰
CCR7 ¹	250 days	$P < 0.05$	$P < 0.01$	$P < 0.07$	$P < 0.06$
CCR7 ¹	300 days	NS	$P < 0.03$	NS	NS
CCR7 ¹	Parity 1	NS	$P < 0.04$	NS	NS
CPT1A ²	Parity 4	$P < 0.04$	$P < 0.1$	$P < 0.06$	$P < 0.06$
CPT1A ^{2,3}	Parity 4	$P < 0.02$	$P < 0.03$	$P < 0.02$	$P < 0.02$
IGFBP3 ⁴	250 days	NS	$P < 0.1$	NS	NS
MBL2 ⁵	Parity 4	$P < 0.1$	$P < 0.06$	$P < 0.1$	NS
WARS2 ⁶	250 days	NS	$P < 0.1$	NS	NS

¹ C-C chemokine receptor 7 (CCR7)

² Carnitine O-palmitoyltransferase I (CPT1A)

³ Refers to analysis of CPT1A when the 12 and 22 genotypes were combined together because of the low number of 22 individuals.

⁴ Insulin-like growth factor binding protein 3 (IGFBP3)

⁵ Mannose-binding lectin 2 (MBL2)

⁶ Tryptophanyl tRNA synthetase 2 (mitochondrial) (WARS2)

⁷ Fisher's Exact test between the "Young" sows that survived to defined time point and those that did not.

⁸ The LIFETEST procedure computes nonparametric estimates of the survival distribution in a sequential order.

⁹ The LIFEREG procedure used fits a parametric accelerated failure time model with right censored data with a Weibull distribution of the error term.

¹⁰ The PHREG procedure used fits a Cox proportional hazards model, a semiparametric model, that uses an unspecified baseline hazard function.

Table 4. Genetic markers that were significantly associated with reproductive traits and the corresponding LS means of the genotypes from a study on candidate genes for sow productive life

Gene	Group	Trait	Parity	Pr > F	11 Genotype	12 Genotype	22 Genotype
ACE ¹	All Sows	Total NBA	Lifetime	0.01	61.92 ± 0.76	63.11 ± 0.38	64.77 ± 0.44
CCR7 ²	Parity 5+	NBA	4	0.05	12.46 ± 0.15	12.11 ± 0.16	11.34 ± 0.48
					0.068 ±	0.071 ±	0.073 ±
CPT1A ³	Young	Pig per day	Lifetime	0.05	0.001	0.001	0.002
CPT1A ³	All Sows	Total born	3	0.03	13.01 ± 0.14	13.47 ± 0.13	13.67 ± 0.31
CPT1A ³	Parity 5+	Total born	3	0.03	13.17 ± 0.18	13.30 ± 0.14	14.05 ± 0.28
CPT1A ³	All Sows	NBA	4	0.04	11.75 ± 0.14	12.25 ± 0.13	12.21 ± 0.30
CPT1A ³	Parity 5+	NBA	4	0.01	11.87 ± 0.17	12.52 ± 0.13	12.31 ± 0.27
CPT1A ³	All Sows	Total born	4	0.03	13.02 ± 0.15	13.58 ± 0.13	13.48 ± 0.32
CPT1A ³	Parity 5+	Total born	4	0.02	13.10 ± 0.17	13.71 ± 0.14	13.78 ± 0.28
IGFBP1 ⁴	Young	NBA	1	0.04	10.64 ± 0.20	10.90 ± 0.19	11.86 ± 0.42
IGFBP1 ⁴	Parity 5+	NBA	2	0.04	11.24 ± 0.19	11.88 ± 0.16	11.80 ± 0.27
IGFBP1 ⁴	Parity 5+	Total born	2	0.02	12.05 ± 0.20	12.79 ± 0.16	12.61 ± 0.28
IGFBP1 ⁴	All Sows	Total born	4	0.02	13.05 ± 0.16	13.64 ± 0.14	13.04 ± 0.27
IGFBP1 ⁴	Young	Total born	4	0.05	12.98 ± 0.30	13.70 ± 0.28	12.19 ± 0.63
IGFBP1 ⁴	Parity 5+	NBA	Lifetime	0.04	86.69 ± 0.78	89.09 ± 0.64	89.17 ± 1.12
MBL2 ⁵	All Sows	Total born	1	0.03	11.86 ± 0.24	12.24 ± 0.10	12.58 ± 0.14
SLC22A5 ⁶	All Sows	NBA	4	0.04	12.15 ± 0.36	11.73 ± 0.13	12.19 ± 0.12
SLC22A5 ⁶	Young	NBA	4	0.04	12.55 ± 0.94	11.34 ± 0.26	12.18 ± 0.22
SLC22A5 ⁶	All Sows	Total born	4	0.03	13.28 ± 0.37	13.06 ± 0.14	13.59 ± 0.13
VDR ⁷	Young	Total born	2	0.04	NA	13.00 ± 0.26	12.36 ± 0.16
VDR ⁷	Young	Total NBA	Lifetime	0.05	NA	37.55 ± 0.52	36.34 ± 0.31

¹Angiotensin I converting enzyme (ACE)

²C-C chemokine receptor 7 (CCR7)

³Carnitine O-palmitoyltransferase I (CPT1A)

⁴Insulin-like growth factor binding protein 1 (IGFBP1)

⁵Mannose-binding lectin 2 (MBL2)

⁶Organic cation/carnitine transporter 2 (Solute carrier family 22 member 5) (SLC22A5)

⁷Vitamin D Receptor (VDR)

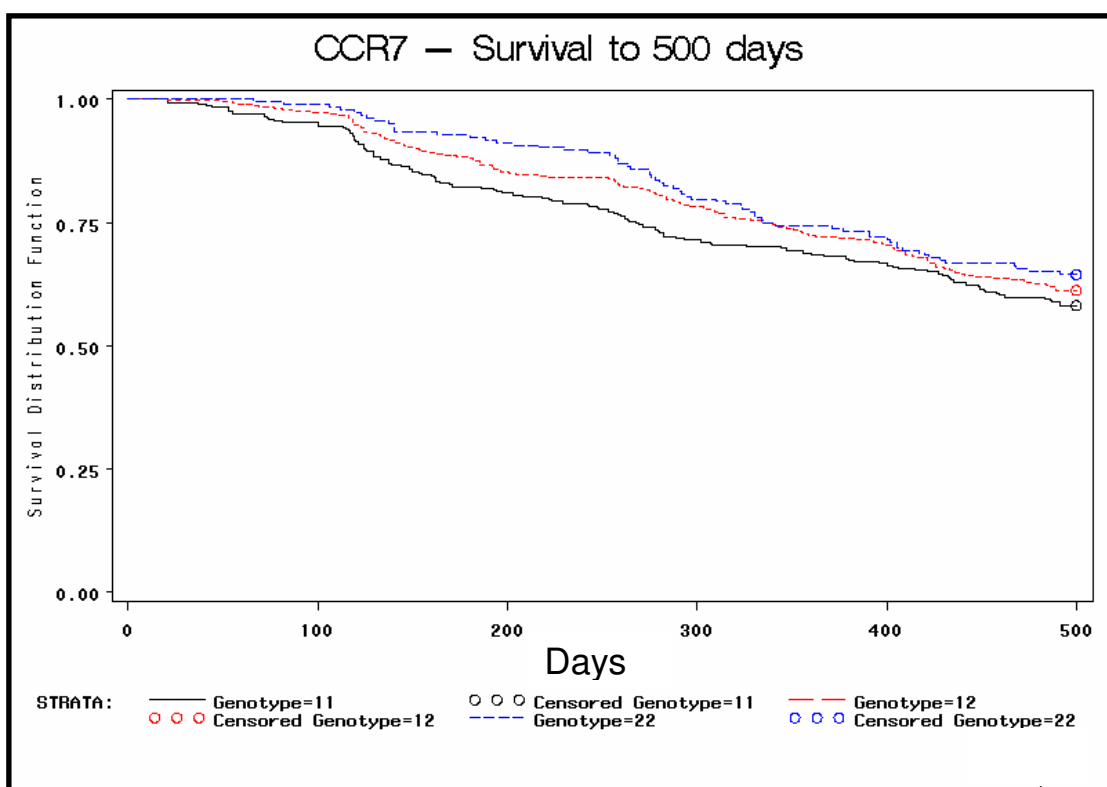


Figure 1. Survival curves for the three genotypes of CCR7 for sows up to 500 days in the herd. A significant difference was seen between the genotype classes at 250 days and 300 days, but was not significant at later dates.

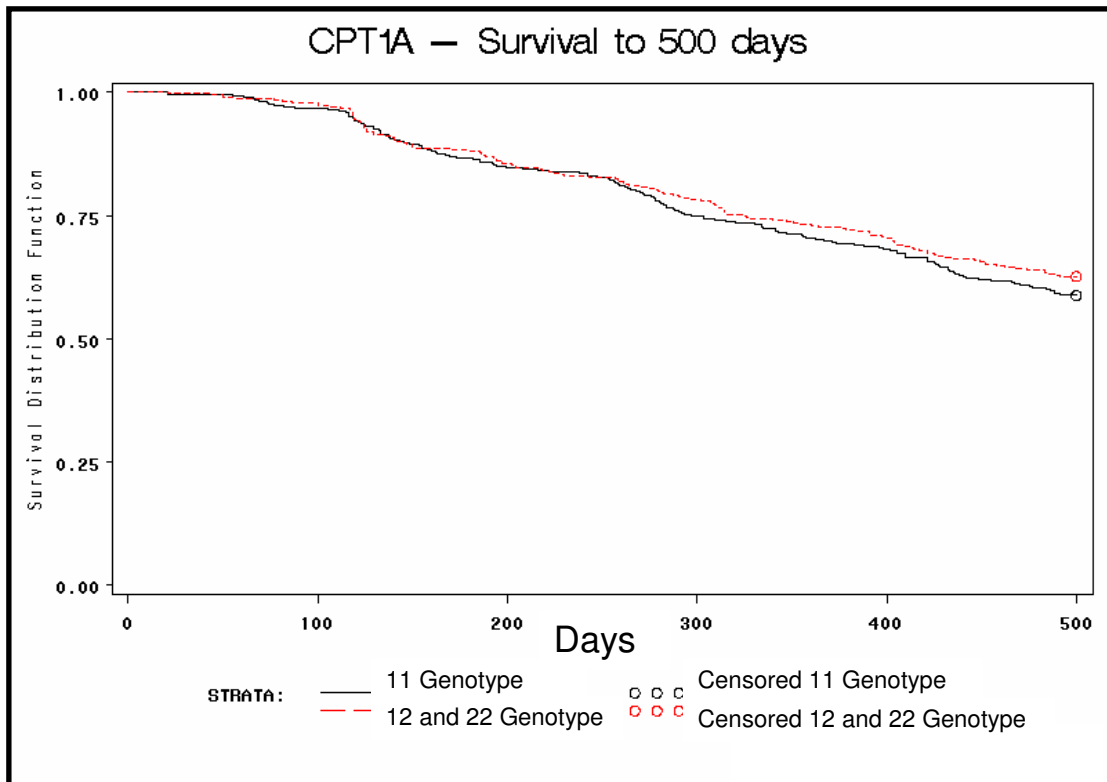


Figure 2. Survival curves for the genotype classes of *CPT1A* for sows up to 500 days in the herd. The 12 and 22 genotype classes were combined as the 22 genotype represented less than 10% of the data. *CPT1A* was significant for survival to parity 4.

CHAPTER 7. POLYDACTYL INHERITANCE IN THE PIG

A paper to be submitted to *Journal of Heredity*

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ABSTRACT

Two pigs were identified having “extra feet” known as preaxial polydactyly within a purebred population of Yorkshire pigs. Polydactyly is a commonly inherited disorder in many species that may be controlled by either recessive or dominance forms of inheritance. The Hedgehog and WNT morphogens are key regulators in many of the known polydactyl phenotypes. Additionally, genes known to be regulators or modifiers of these morphogens are also possibly implicated in causing polydactyl phenotypes. Pedigree analysis revealed a common ancestor providing the possibility for a recessive mode of inheritance. Additional matings were carried out with the parents and siblings of the affected pigs producing a total of 14 pigs expressing a polydactyl phenotype, though only 7 were born alive. A limited genome scan utilizing microsatellites on all chromosomes with the exclusion of chromosome 18 was conducted. Using comparative genomics, SNPs were identified on chromosome 18 in candidate genes and tested with the use of an Elston-Stewart algorithm but no regions showed a significant LOD score indicating that the mutation causing this unique phenotype did not exist on this chromosome. With limited data, it appears that the mode of inheritance that best fits this data is a recessive phenotype with 50% penetrance.

INTRODUCTION

The polydactyly condition in pigs is more common than was initially thought. The first report of a polydactyl pig was in 1931 (Curson 1931), with additional reports in 1938 (Hughes 1938), 1959 (Gaedtke 1959), and 1963 (Ptak 1963). Additionally, the National Swine Registry (the governing body of the Yorkshire breed of pigs) states in their requirements for registration that a Yorkshire pig with an extra dewclaw is not allowed to be registered (National Swine Registry 2007).

Other vertebrates have also been known to express different polydactyl phenotypes that are observed either simply by themselves or as one phenotype of a syndrome. Some autosomal recessive congenital anomalies known to cause polydactyly in humans include Bardet-Biedl syndrome (Davis et al. 2007), Ellis van Creveld syndrome (Chakraborty et al. 2007), Meckel syndrome (Baala et al. 2007), Pallister-Hall syndrome (Kang et al. 1997), and Joubert syndrome (Chance et al. 1999). In humans, the most occurring form of polydactylism is postaxial polydactyly where the extra digit is typically that of an extra little finger or little toe. This form of polydactylism is seen in the Amish population of Eastern Pennsylvania and is due to Ellis van Creveld syndrome (Chakraborty et al. 2007). Polydactylism, when not in conjunction with a congenital health disorder, is typically dominant in nature in the human population with one affected child in every 400-500 births. Various forms of polydactylism have been found in humans, horses (Carstanjen et al. 2007), baboons (Moore et al. 2007), cats such as the ones Ernest Hemingway had (Ernest Hemingway Home and Museum 2002), mice (both naturally occurring and those

made during mutagenesis) (Lettice et al. 2002), and chickens (recessive) (Huang et al. 2006).

Many morphogens and genes involved in their regulation are crucial in the developmental processes of vertebrates. The *Hedgehog* and *WNT* gene families have been implicated in many of the observed polydactyl phenotypes (Sheth et al. 2007; Zeller and Zuniga 2007; Yang et al. 1998), especially the Hedgehog gene member *Sonic Hedgehog* (*SHH*). A cis-acting regulator of *SHH* known as *LMBR1*, especially the sequence in intron 5 that is highly conserved in tetrapod animals, has also been shown to be involved in producing a polydactyl phenotype (Sagai et al. 2004; Huang et al. 2006; Wang et al. 2007). Additionally, genes such as *engrailed* (Lawrence et al. 1999), *TWIST1* (Firulli et al. 2007), *GLI3* (Fujioka et al. 2005), and the *HOX* genes (Sheth et al. 2007; Zeller and Zuniga 2007; Tarchini et al. 2006) have shown to be involved in developmental processes and have shown polydactyl phenotypes when mutations appear in these genes. All of these genes reside on porcine chromosome 18.

Therefore, additional matings were conducted to positively identify if the phenotype was in fact genetic in nature and not due to environment influences. Additional pigs with various polydactyl phenotypes were produced and genetic markers were utilized in an attempt to identify the mode of inheritance and the gene/genes causing the observed phenotype in our population of pigs.

MATERIALS AND METHODS

Population

Polydactyl pigs were identified in a purebred Yorkshire pig breeding population located at the Iowa State University swine breeding farm (Madrid, Iowa). All animals were raised under approved animal care regulations. Pig 137-06 was a male pig (Fig. 1A and 1B) having an “extra foot” on the medial side of both of his front feet. Unfortunately, he had already been castrated when he was identified. He was one of eight piglets in a litter resulting from a mating between the 18-1 (sire) and 52-11 (dam) animals. Pig 156-08 was a female pig (Fig. 1C) possessing an “extra foot” on the medial side of only one front foot (left) and was from a litter of nine piglets that resulted from the mating of the 95-04 (sire) and 102-11 (dam) animals. All future matings involved at least one animal derived from the above four parents. Pictures of live pigs were taken at the farm and X-rays were taken on the pig that was euthanized because he was unable to be used in breeding experiments. Pedigrees are provided in figures 3 and 4.

DNA

For adult or mature animals, blood samples were collected and used for DNA isolation. For planned matings, tail tissue samples were obtained at birth from each pig, placed into a labeled 1.7 ml tube, and stored at -80° C. For each animal, a 25 mg of the tail sample was used for DNA isolation using the DNeasy kit from Qiagen (Valencia, CA) following the manufacturer's protocol.

Initial Microsatellite Screen

DNA from the founder parents, the initial 2 affected individuals, and 4 unaffected siblings were sent to GeneSeek (Lincoln, NE) to be screened using a small microsatellite marker panel. There were 2 markers (*S00008* and *SW1430*) on SSC1, 2 markers (*SW2683* and *S0226*) on SSC2, 5 markers (*SW72*, *SW1443*, *SW902*, *SW102*, and *S0002*) on SSC3, 3 markers (*S0227*, *SW2409*, and *S0217*) on SSC4, 2 markers (*SW2* and *SW967*) on SSC5, 2 markers (*SW122* and *SW2419*) on SSC6, 2 markers (*S0025* and *S0101*) on SSC7, 2 markers (*S0086* and *S0225*) on SSC8, 4 markers (*SW911*, *SW2093*, *SW174*, and *SW1349*) on SSC9, 2 markers (*SW830* and *SW951*) on SSC10, 1 marker (*SW2413*) on SSC11, 3 markers (*S0143*, *SW874*, and *S0147*) on SSC12, 1 marker (*SW769*) on SSC13, 3 markers (*SW857*, *SW295*, and *SW1557*) on SSC14, 2 markers (*S0355* and *SW1119*) on SSC15, 2 markers (*SW2411* and *SW2517*) on SSC16, and 1 marker (*SW24*) on SSC17.

There were no markers in this panel on either SSC18 or the X chromosome.

Genotypes on all markers were assigned using standard protocols by the molecular biology staff at GeneSeek.

Statistical Analysis

An extension of the Elston-Stewart algorithm was used in a model-based linkage analysis to map the genomic location most likely to contain the locus causing the polydactyl phenotype. The implementation of the Elston-Stewart algorithm used here is described in Elston and Stewart (1971) and Fernandez et al. (2001, 2002), while use of linkage mapping has been previously described (Ott 1974). Likelihood ratios were calculated for each marker interval, both with complete penetrance and with

fifty percent penetrance, assuming that the polydactyl mutation is at the center of this interval (L1), or that the polydactyl mutation is at another chromosomal location (L2). The log base 10 of this likelihood ratio (L1/L2) resulted in the LOD score where a LOD score greater than 3 being classified as significant. Likelihood (L) can be expressed as

$$L \propto \Pr(y) = \sum_{\underline{G}} \Pr(y | \underline{G}) * \Pr(\underline{G}),$$

where y is a vector of polydactyl phenotypes, and \underline{G} is a vector of genotypes at the markers flanking the interval in question.

RESULTS

Pedigree Analysis

The 95-04 boar was mated to several females that produced piglets at the same time as the 156-08 pig was born, with none of them having an observed polydactyl phenotype. Additionally, the 18-01 boar and the 52-11 sow had several previous litters (with other mates) with none of their previous offspring possessing a polydactyl phenotype. Pedigree analysis of the initial two animals exhibiting the polydactyl phenotype showed that a common ancestor (a Yorkshire boar named Crank High) was found on both sides of each animal's pedigree. None of the parents had expressed a polydactyl phenotype, none of the additional litters born prior to our planned matings resulted in a pig having the polydactyl condition, and the fact that there is a shared common ancestor all suggested that the polydactyl phenotype is likely recessive in nature. Given these results all future matings and analyses were carried out to examine that hypothesis.

Matings

Both dams of the first affected animals, one of the sires (95-04), and all of the full siblings to each of the original affected pigs remained on the breeding farm when the first pigs were identified as having extra digits. The 95-04 boar was then mated to both the 52-11 and the 102-11 dams resulting in two additional litters totaling 21 head. The 192 litter (10 piglets) were full sibs to the 156-08 gilt while the 207 litter (11 pigs) were half sibs to both the 137-06 and the 156-08 pigs. Two of the eleven piglets in the 207 litter possessed what appeared to be extra dewclaws (Fig. 2A) on their front feet (one piglet had one extra dewclaw while the other possessed an extra dewclaw on both front feet) though both piglets were stillborn. The 95-04 boar was then mated to several of his daughters and one unrelated female that was a full sibling to the 137-06 barrow. The 95-04 boar produced two litters with 156-06 with the first litter only having 2 piglets with one pig having an “extra foot” without a normal dewclaw (Fig. 2B) while the second litter produced a total of 8 piglets born with one piglet having an extra dewclaw on one front foot. This was interesting in that this was the first time that a repeat mating resulted in affected pigs with different polydactyl phenotypes. In the two litters produced by mating 95-04 to 156-05, there were a total of 5 (2 in one litter and 3 in the other) affected pigs out of 29 total piglets born with 15 of those being born dead. The 2 affected piglets in the first litter from 156-05 had extra dewclaws on their front feet while the 3 affected piglets in the second litter had “extra feet” on their front feet. This is the second observation of a repeat mating where the affected piglets had different phenotypes. There was one affected piglet out of 12 born in the litter from mating 95-04 to 137-09. The sire 95-

04 was mated to three of his daughters (156-07, 192-05 and 207-03) where there were no observed affected piglets out of 21 born in the three litters, though all of the pigs that were born dead (n=5) were unfortunately discarded by farm personnel before a phenotype could be assigned. Additionally, the full brother by sister mating of the boar 156-03 with 192-07 also resulted in no affected piglets being born from a total of 11 piglets in that litter. To view the pedigree of the informative matings within the polydactyl family and their outcome, see Fig. 3. In total, there were 12 affected pigs (half were born dead) out of 140 pigs that were born in this project using pigs originating from the founder animals of this population. An interesting note was that of the 140 pigs born in this project, there were 40 pigs born dead and 13 mummified fetuses, much more than normally expected, suggesting some type of possible lethal expression also.

Additional matings were carried out to test further test the inheritance of this phenotype (Fig. 4). Before the sire 95-04 became sterile, he had been mated to 4 Duroc (unrelated) females producing 50 piglets and 8 additional Yorkshire (unrelated) females that produced 79 piglets with none of these 129 piglets being affected (data not shown). Excluding the 95-04 boar, there were other boars outside of this population that have had reports of offspring with a polydactyl phenotype. Swine Genetics International (Cambridge, Iowa) possessed frozen semen on two boars named First Rate and Rebound that had reports of offspring with extra dewclaws. Pedigree analysis of both First Rate and Rebound show that they also had a common relative (Crank High) in their pedigree. First Rate was mated to the female 137-09 who had already produced an affected pig when mated to the 95-04

boar. The resulting litter produced 9 live piglets at birth with two individuals that possessed an extra dewclaw. One of the affected piglets had other birth defects such that he was unable to stand and had to be euthanized. This clearly suggested that this phenotype, in some form, also existed outside the breeding population at Iowa State University. The boar Rebound was mated to the female 156-06 the litter which resulted in 6 piglets born with no affected pigs. Since the 156-05 had 5 affected piglets in her two litters, she was mated to a Duroc boar that had sired multiple litters with no affected pigs observed. The resulting litter produced 14 piglets with no affected pigs. An additional litter was also produced using a purebred Yorkshire boar (Admiral) and the 207-02 female with 12 piglets produced and no affected pigs. A complete analysis of all matings (both within the polydactyl family and test matings) and the resulting total number of litters farrowed, total pigs born, and the number of affected animals born can be seen in Table 1. Due to many complications such as fertility problems, male pigs being castrated inadvertently by farm personnel, or pigs accidentally being sent to market, no affected pig in this population ever produced any offspring. Personal reports from other breeders that had sows with extra dewclaws as well as the report from Hughes (Hughes 1938) suggest that there is not full penetrance with this trait as the affected by affected matings produced both affected and unaffected pigs.

Results of the Microsatellite Genome Scan

Analysis of the 39 microsatellite markers positioned on 17 chromosomes was undertaken with the hypothesis that the phenotype was a simple recessive disorder where all parents of the original affected individual would be heterozygous at the

many loci, the affected offspring would be homozygous, and the unaffected siblings would be the opposite homozygote and/or heterozygous. No marker or specific chromosomal region showed the expected results (data not shown). Given the coverage of the markers, only SSC3 in its entirety could be ruled out while many of the other chromosomes could have regions excluded from further analysis. Though no markers were included from the X chromosome, this chromosome can also be eliminated from further research as we have had roughly equal numbers of affected individual from each sex.

SNP Marker Analysis

Since no microsatellite markers identified a region of interest and no markers were located on SSC18, SNPs were identified and genotyped in candidate genes as well as other genes located throughout SSC18. The candidate genes analyzed on SSC18 included *LMBR1*, *SHH*, *EN2*, *HOXA10-13*, *GLI3*, *WNT2*, and *WNT16*. Additionally, *SHH* has been shown to affect digit development in a gradient/time dependent manner (Hill 2007) and only a slight modification to the amount/time that *SHH* is expressed in the zone of polarizing activity can cause the observed phenotypes. When *SHH* is not expressed, mice only develop one digit and an over expression of this gene is known to cause preaxial polydactyly (Hill 2007). Therefore, it is believed that this phenotype is caused by an up regulation of *SHH*. The A/G SNP identified in *LMBR1* was 59 bp 5' of the highly conserved sequence noted in figure 6 of Sagai et al. (2004), though genotyping results of the polydactyl population showed that the genotypes did not fit the expected results if it were to cause the polydactyl phenotype. No mutations were identified in this population in

SHH, *EN2*, *GLI3*, or any of the *HOX* genes. The C/T SNP identified in *WNT16* was located 102 bp into intron 1 and the C/T SNP found in *WNT2* was located in intron 4 (bp 96885 in AC153102) also were not in agreement with the genotypes anticipated if they controlled the polydactyl phenotype in a recessive manner.

SNPs utilized in the Elston-Stewart algorithm were in the following genes: *LMBR1*, *LEP*, *GPR37*, *SPAM1*, *WNT16*, *WNT2*, *PACAPR* (Kollers et al. 2006), *MPP6*, and *IGFBP1* (Mote and Rothschild 2006) spanning 83 of 91 cM of SSC18. No marker interval showed a significant association with the polydactyl phenotype in question.

DISCUSSION

Of additional interest is the fact that there was a high level of mummies and stillborns in this population suggesting that the causative mutation behind this phenotype could in fact be partially lethal. It is also noteworthy that whenever two or more affected pigs are born in a litter they always possessed the same phenotype in that the pigs either had what appeared to be an “extra foot” or an extra dewclaw. This observation of affected pigs within a litter having the same phenotype did not extend to whether or not the pigs within a litter had the same number of feet affected as some pigs had only one front foot that expressed an extra digit while others had an extra digit on both front feet. The different polydactyl phenotypes (extra “foot” with the normal number of dewclaws, extra “foot” without the normal number of dewclaws, and extra dewclaws) on one or both front feet of affected individuals suggested that this phenotype is due to variable expressivity. This makes identifying the causative mutation difficult as not all animals with the causative mutation would have a single observable phenotype.

The inheritance of this polydactyl phenotype in our pig population does not fit typical Mendelian inheritance patterns nor did the polydactyl phenotype in the Duroc Jersey swine noted by Hughes (Hughes 1938). For the observed number of polydactyl animals to match a dominant inheritance pattern in our population, there would need to be several concessions. First, the effect must have been inherited through the dams of the original affected pigs in this population. The sire 95-04 can comfortably be excluded as carrying a dominant gene with virtually any level of penetrance since the test matings outside of this population produced 129 offspring with all being unaffected with an additional 11 pigs being born resulting from two father daughter matings that did not produce any affected offspring (data not shown). Second, if the trait was dominant and inherited through the dams, the penetrance level of this phenotype would have to be 13.2% to obtain the observed results. Additionally, the founder animals were derived from at least the third generation of a closed breeding population without any previous animals being identified as polydactyl, therefore further casting doubt on the possibility of dominance inheritance with any level of penetrance. The number of affected offspring ($n=14$ of 106 total born) that resulted from predicted carrier by carrier matings ($n=11$) also did not fit a simple recessive inheritance pattern. A simple recessive inheritance would have predicted 26.5 affected pigs. Chi square analysis showed that the observed results did not fit a single gene recessive pattern ($P < 0.01$). In our population, where there was a high level of stillborn and mummified fetuses, (not all mummified fetuses were developed sufficiently to classify in them into specific polydactyl or normal phenotype groups), it is also possible that some affected pigs could have been absorbed in the womb due

to developmental difficulties arising from embryonic lethality. However, given that there is variable expressivity of this disorder, there could also be a reduced penetrance before a phenotype is observable. A recessive phenotype with a penetrance level of 50% could not be ruled out as a possible explanation as this actually fits our data quite well (Chi square $P > 0.8$) and also that of Hughes (Hughes 1938) who actually was able to produce offspring from affected by affected parents. It was disheartening that in our population we were never able to make matings with an affected individual as a parent due to various circumstances that were beyond the control of both ourselves and the 7 affected pigs that were born alive. Matings of an affected pig with known carriers or other affected pigs would have greatly enhanced our ability to predict not only the inheritance, but also the location of the causative mutation. Combined, these results suggest that the polydactyl phenotype in this population is not due to a single gene dominant mode of inheritance with or without reduced penetrance and is suggested to be recessive in nature.

ACKNOWLEDGEMENTS

Support for B. Mote was provided in part by a USDA National Needs Fellowship. Funding was provided in part by the Iowa Agriculture and Home Economics Experiment Station, State of Iowa and Hatch funding. Data collection and assistance provided by individuals from the farm crew at the Lauren Christian Swine Research Center, PIC USA, Dr. James Koltes, Mr. Dusty Loy, Ms. Penny Fang, and Mr. Dan Mouw and members of the Rothschild Lab is greatly appreciated. Contribution of semen from Swine Genetics International, Cambridge IA is appreciated.

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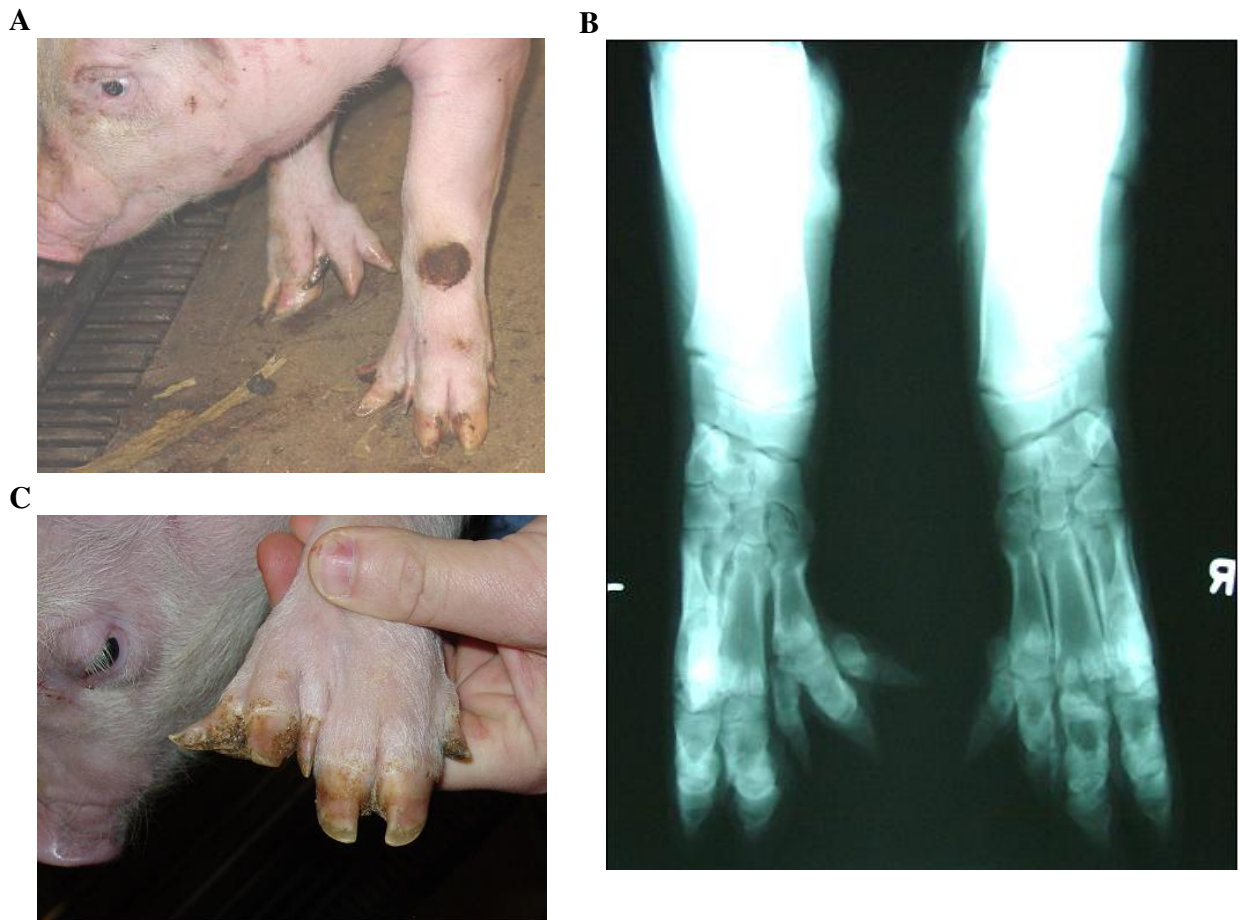


Fig. 1
Original pigs affected with preaxial polydactyly in a breeding population of Yorkshire swine. **A** Yorkshire male (ID 137-06) expressing a preaxial polydactyl phenotype on both front feet. **B** Radiograph showing both front feet of the Yorkshire male (137-06) with preaxial polydactyly. **C** Yorkshire female (ID 156-08) expressing a preaxial polydactyl phenotype on one (left) front foot.

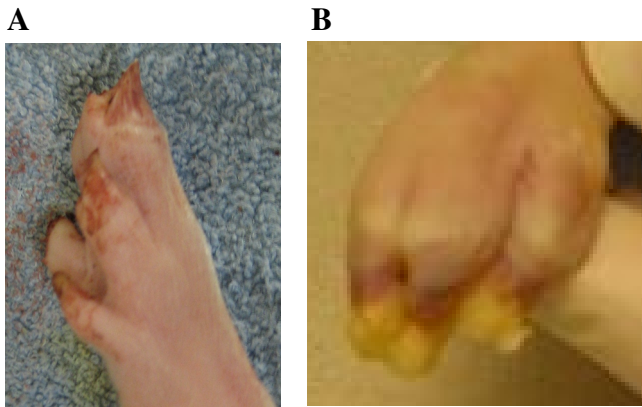


Fig. 2
Variations of the preaxial polydactyl phenotype seen in Yorkshire pigs. **A** Pigs expressed a normal looking foot with an additional dewclaw. **B** This pig was missing one dewclaw and had what appeared to be an “extra foot” where a dewclaw normally would be.

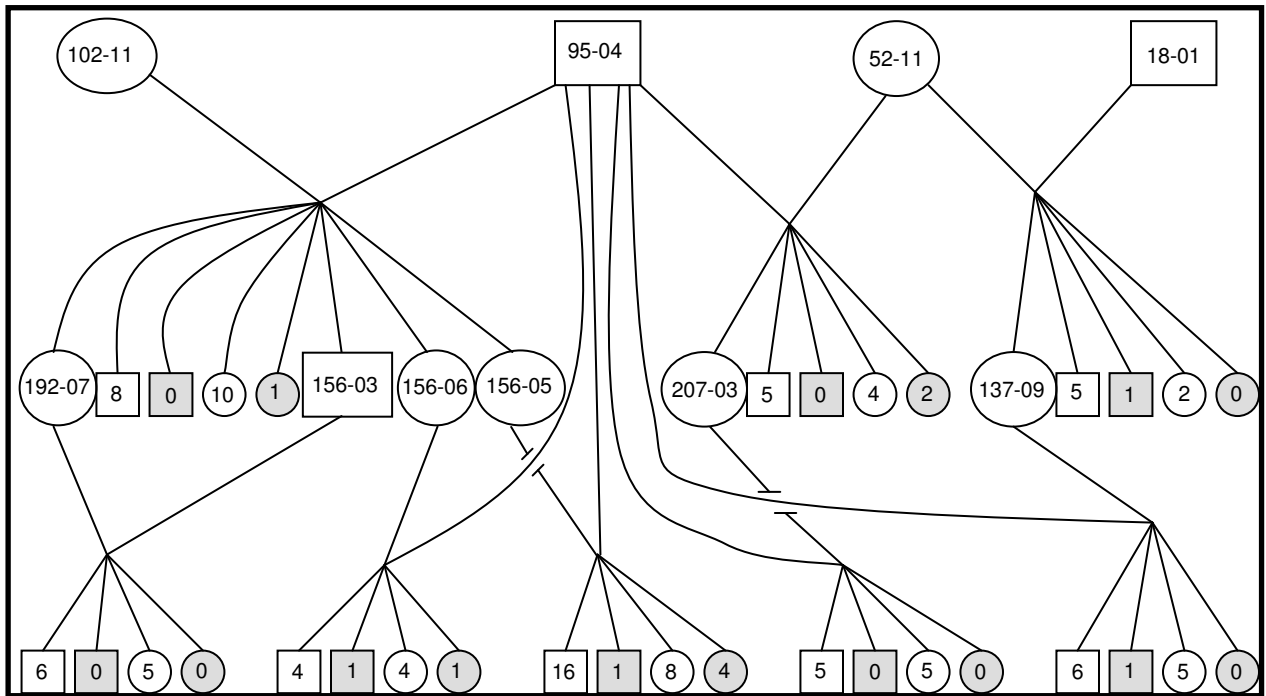


Fig. 3
Pedigree of Yorkshire animals where polydactyl animals existed. Circles represent females. Squares represent males. Shaded figures represent polydactyl animals. Large circles and squares are parents while smaller circles and squares represent the number of offspring from the mating.

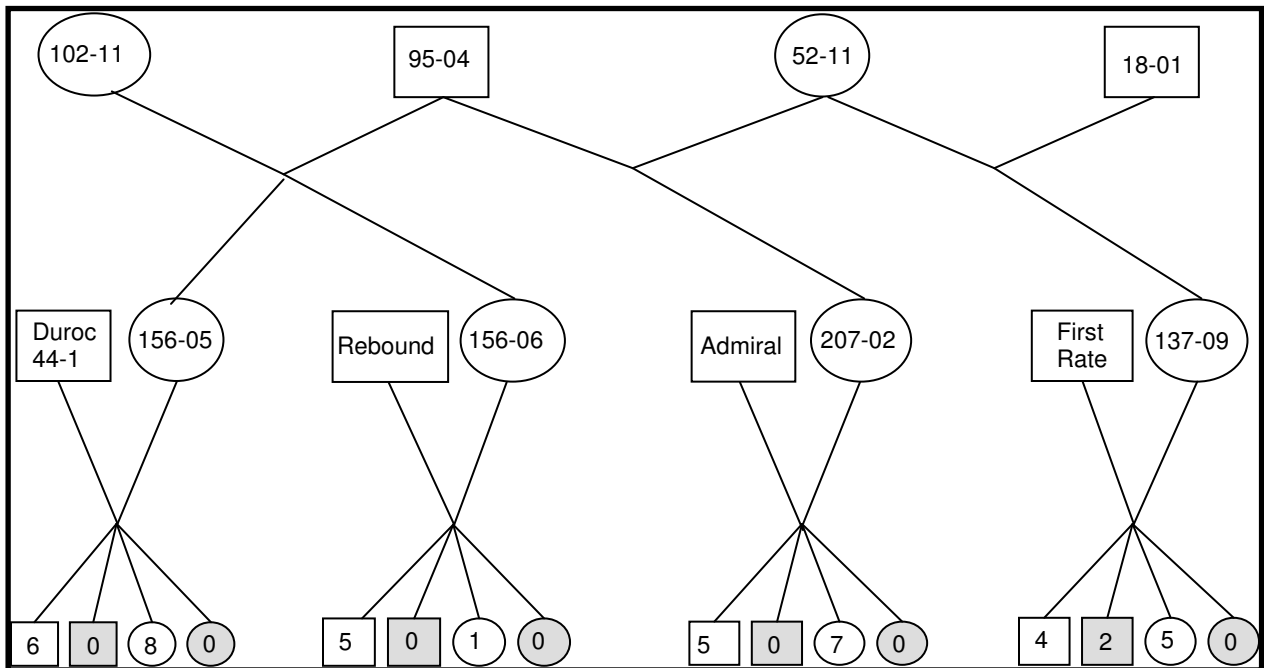


Fig. 4

Pedigree structure of Yorkshire population where polydactyl pigs appeared when females were mated to males outside the breeding population to test inheritance. Circles represent females. Squares represent males. Shaded figures represent polydactyl animals. Large circles and squares are parents while smaller circles and squares represent the number of offspring from the mating.

Table 1 Phenotypic results of all matings by status of parents to produce polydactyl offspring

Mating ¹	Litters	Total Pigs Born	Number Affected	No Phenotype ²
Carrier X Affected	NA ³	NA	NA	NA
Carrier X Carrier	11	113	14	4
Carrier X Normal	17	178	0	14
Normal X Normal	1	11	0	0

¹Status of sires and dam predicted by phenotype of offspring

²No phenotype could be assigned for some mummified or discarded animals

³Affected sow died before having litter

CHAPTER 8. GENERAL CONCLUSIONS

The small margins seen in the U. S. swine industry today due to high petroleum costs increasing transportation costs, high corn prices caused in part by increased demands to produce ethanol, and record prices for soybeans will have producers looking for additional ways to decrease operational costs and improve production efficiency. Additionally, the recent PETA (People for the Ethical Treatment of Animals) sponsored and voter passed laws in Florida and Arizona that ban the use of gestation crates as well as the self adoption of this law in Colorado has shown the swine industry that others (i.e. the customers) are watching what the industry does. Though producers are beginning to realize the animal well-being and economic ramifications of poor sow productive life, the current state of the swine industry demands that changes be made if swine operations are going to continue to be in business.

The knowledge obtained from model organisms, cattle, and even humans has revealed QTL regions that are associated with longevity and productive life. Further, candidate gene research has revealed genes and gene pathways that are conserved across many species that all contribute to longevity in a like manner. These results have suggested that these genes and gene pathways could also be targeted to identify genetic markers that are associated with sow productive life.

Genetic abnormalities are also an additional drain on the revenues of commercial livestock farms. The economic burdens of these genetic abnormalities, though often truly unknown, are considerable. Several deformities in cattle (DUMPS and dwarfism), sheep (dwarfism), and pigs (scrotal ruptures, splayleg, and

cryptorchidism) have been shown to have genetic causes. Genetic markers have been identified for some of these traits, most recently the genetic mutation causing dwarfism in the American Angus cattle, through the use of combined linkage and candidate gene studies. These results give promise that a genetic marker associated with the polydactyl phenotype can be identified in swine.

Summary of the current state of the swine genome

The efforts from the swine genome community have pushed the reality of having a completed genome sequence to near reality, though it won't quite be out in the year of the pig as hoped. An additional benefit that will transpire from the sequencing project is the identification of SNPs that can be used in SNP chips which can be used in large scale fine mapping of traits of interest, even in commercial populations. Continued use of microarrays will persist in uncovering gene pathways that act to regulate traits. These efforts mean that molecular swine geneticists won't have to rely on the homology of the sequences of humans, mice, and cattle to that of the pig to identify genetic markers for use in marker-assisted selection schemes for economic traits of interest. These efforts should facilitate swine producers in their efforts to continue to provide the growing world with a wholesome source of protein.

Summary of SNP identification and mapping of growth related genes

Genes that are involved in growth or are in growth related pathways serve a fundamental role in multiple genetic traits of interest to the swine community. We described a few SNPs that were identified and their genomic location within the swine genome. The genes that were targeted were *insulin-like growth factor binding protein 1 (IGFBP1)*, *insulin-like growth factor binding protein 2 (IGFBP2)*, *insulin-like*

growth factor II receptor (IGF2R), beta-2 adrenergic receptor (ADRB2), carnitine O-palmitoyltransferase I (CPT1A), and organic cation/carnitine transporter 2 (Solute carrier family 22 member 5; SLC22A5). The SNPs identified in these genes were analyzed to identify their associations with sow productive life.

Summary of the current reproduction rates, removal reasons and mortality in commercial swine

A current sampling of 2,000 commercial sows was undertaken and monitored for 20 months to identify if the culling and mortality reasons have changed over recent years and as well as to examine the reproduction rates of the sows over their productive lives. Reproduction failures and lameness have previously been listed as the predominant removal reasons for sows in early parities. The present research showed that these traits are still the main culling reasons seen in today's commercial sow. Almost half of sows that produced at least 5 parities were removed from the breeding herd simply because of the apparent preconceived notion that they were "old", even though these sows were producing at or above herd averages. Sows that produced at least 3 parities had larger numbers of pigs born alive during early parities compared to sows that were removed from the breeding herd before their third parity. These sows also were quicker to return to estrus following their first litter, which has been shown to be associated with sow longevity in previous research. Although sow removal reasons have not appeared to have changed markedly over the years, this study revealed that sows could be selected for longevity without detrimental effects on reproductive performance as sows in this study that remained in production to more advanced parities outperformed their

contemporaries that were removed from the breeding herd in early parities.

However, further studies should be carried out with more comprehensive data to analyze the genetic correlations between the two traits.

Summary of candidate genes associations for sow productive life

Research from model organisms identified several genes and gene pathways that are associated with longevity. The main “longevity pathway” identified involved the insulin/insulin-like growth factor pathway that plays a critical role in growth and caloric restriction. Genetic markers identified in predominantly growth related genes were tested for their association with sow productive life. The genetic markers for CCR7 and CPT1A demonstrated consistent associations, regardless of analyses performed, with sow survival to early time points and to parity 4, respectively. Genetic markers for MBL2, IGFBP3, and WARS2 also illustrated tendencies for being associated with survival though they were not as consistent. A mixed model analysis determined associations of IGFBP1, MBL2, CPT1A, CCR7, SLC22A5, and ACE with various reproductive traits. One candidate gene of particular interest, CPT1A, demonstrated that the beneficial allele for longevity is also the beneficial allele for reproduction. These results demonstrate that genetic markers can be first identified using lessons learned from model organisms and second used in helping to improve sow productive life.

Summary of work to elucidate the causative mutation creating polydactyl pigs

Purebred Yorkshire swine have been identified that have a preaxial polydactyl phenotype. Affected offspring from test matings proved that this phenotype was an inherited disorder and existed in swine outside of the research population. Several

species have this genetic mutation as well. Candidate genes were analyzed to identify SNPs that might be associated with this disorder with limited success. A limited genome scan utilizing microsatellite markers was conducted on 17 chromosomes with SNP markers used on chromosome 18. No region or candidate gene showed association with the observed polydactyl phenotypes. This may be complicated by the variable expressivity and the possibility of incomplete penetrance.

FUTURE RESEARCH

Future research in sow productive life will include the resampling of data from the commercial sows at a time in which all sows have been removed from the breeding herd. This data will give complete reproduction records for all the 2,000 sows that were sampled as well as give the actual reproductive life of these sows. With the actual productive life of these sows, no statistical censoring will be required and the associations of genetic markers can be tested against the true productive life of these sows. Additional research should also be focused on the additional genes in the so called longevity pathway as well as more genes that focus on reducing oxidative stressors.

Future research regarding polydactyl pigs should include producing offspring from affected individuals to truly test the mode of inheritance. In addition, candidate genes in developmental pathways should be sequenced to identify SNPs that could be responsible for the observed polydactyl phenotype in pigs.

CONCLUSIONS

In conclusion, no genetic markers were identified that were clearly associated with the polydactyl phenotype in pigs. However, genetic markers such as those for CCR7 and CPT1A were identified that were significantly ($P < 0.05$) associated with sow productive life. Several genetic markers (IGFBP1, MBL2, CPT1A, CCR7, SLC22A5, and ACE) were significantly ($P < 0.05$) associated with various reproductive traits. The genetic marker, from this work, that holds the most promise to the swine industry is the marker for CPT1A as the beneficial allele for reproduction is also the beneficial allele for sow productive life. Including this marker in selection indexes should help to increase the profit margin for commercial swine operations from an increase in offspring produced per litter and an increase in sow productive life.

ACKNOWLEDGEMENTS

The first acknowledgement must go out to the very people that brought me into this world, my parents Darryl and Barbara Mote. Thank you for always believing in me and standing behind me no matter what crazy things I decided to do like joining the Army or going to Nebraska for my undergraduate degree. Knowing that you both were behind me made things like basic training easy. The values of hard work and dedication that you both instilled in me from an early age are two of the biggest reasons for the success that I have had thus far in my life. To my sisters and brothers, thank you for the encouragement to always keep going. Ronnie, you will never know what it meant to me to have family with me when I made the jump to graduate school.

They say being a teacher is a thankless job, but I must take time and say thank you to the teachers that helped me along the way. Mrs. Leslie Harmon, even though I never memorized the periodic table like you wanted me to, I must thank you for sharing your love of science with me. Mr. Tonny Hamby, thank you for the countless trips you made away from your family hauling us kids to every livestock show and judging contest this side of the Mississippi. You are more than a teacher, you are a great friend. To Drs. Johnson and Nielsen, thank you for your guidance while I was at Nebraska. You gave me a great foundation of knowledge to build the rest of my career on. To Drs. Freking and Gross, thank you for the great learning opportunities you both gave me while I was a summer intern for each of you. I feel those summers taught me as much about genetics as any of my classes. To my committee members, thank you for all always being there to answer my many

questions and for the guidance in my research and career choices. Last but certainly not least in the list of great teachers in my life is my major professor and mentor Dr. Max Rothschild. Thank you for taking me into your family; anyone who has ever worked for you knows that you don't just take someone into the lab, they become a part of your family. The guidance both in research and in life choices are both cherished and will never be forgotten.

Lastly, I must thank all the friends that have been with me through thick and thin. To my best friend and love of my life Brandi, thanks for putting up with me and making me the happiest man alive. Bernabe, Ben, Bryce, James, Jason, Oliver and Jamie thanks for always listening to me when things weren't going my way and celebrating with me when things did. Jeremiah thanks for all your help getting through grad school. It's not everyday that two country boys from Happy, Texas that have been friends for 20 years both head to Iowa State to get their PhDs. To the members of the Rothschild lab, both past and present, I must thank you for your friendship. Thanks Kim for always being willing to drive to La Fuentes for lunch all the time. You are a great friend even though we didn't always see eye to eye on world politics. Vicki, thanks for being a great friend and a landlord. Letting me keep my pigs at your places goes way above and beyond a simple friendship. Having those pigs around gave me a great way to relieve my tension after those stressful days in the lab. To the countless others that have been with me and helped me along the way, I apologize for not specifically mentioning you, but please know that your friendship, help and guidance is just as greatly appreciated and for that I must say thank you.