ANNUAL PROGRESS REPORT
NATIONAL RESEARCH SUPPORT PROJECT – NRSP-8
Year Ending 2021
Preliminary Information-Not for Publication

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I. Project: NRSP-8: Swine Genome Committee

II. Cooperating Agencies and Principal Investigators

A. Agencies and Departments Cooperating: Iowa Agriculture Experiment Station, Department of Animal Science, Iowa State University

B. Co-Leaders of the Project: Christopher K. Tuggle, Jack C.M. Dekkers, Nick Serao, Max F. Rothschild and James Koltes

C. Cooperating Investigators:, Elizabeth Huff-Lonergan, James Koltes, Steven Lonergan, Zhiliang Hu, Ken Stalder, Nick Gabler, John Patience, James Reecy, Francesca Bertolini, Iowa State University; Many International Swine Genome Sequence committee and Functional Annotation of Animal Genomes collaborators as mentioned below.

III. Objectives

Objective 1: Advance the status of reference genomes for all species, including basic annotation of worldwide genetic variation, by broad sequencing among different lines and breeds of animals.

Objective 2: Develop strategies to identify and exploit genes and allelic variation that contribute to economically relevant phenotypes and traits, in part through improving functional annotation of the genomes of our species.

Objective 3: Facilitate analysis, curation, storage, distribution and application of the enormous datasets now being generated by next-generation sequencing and related "omics" technologies with regard to animal species of agricultural interest.

IV. General Project Plan

A. Use SNP chip to identify and better understand genetic control of traits (Objective 1).

B. Participate in on-going pig genome functional annotation efforts (Objective 2).

C. Development and analysis of populations measured for response to PRRSV (Objective 2)

D. Development and analysis of populations selected for increased feed efficiency (Objective 2)

E. Participate in further database and other bioinformatic resource development (Objective 3)

V. Work Progress

A. Use SNP chip and share results to better understand genetic control of traits (Objective 1).

The Dekkers group had continued work on genetic analyses and genome-wide associations were conducted for traits related to disease resilience in pigs and poultry, in collaboration with the Lamont and Tuggle groups.

Cheng, J., Putz, A.M., Harding, J., Dyck, M.K., Fortin, F., Plastow, G.S. and Dekkers, J.C.M., 2021. Genetic parameters of drinking and feeding traits of wean-to-finish pigs under a polymicrobial natural disease challenge. Journal of animal science and biotechnology, 12(1), pp.1-19. https://doi.org/10.1186/s40104-021-00622-x

Background The pork industry faces unprecedented challenges from disease, which increases cost of production and use of antibiotics, and reduces production efficiency, carcass quality, and animal wellbeing. One solution is to improve the overall resilience of pigs to a broad array of common diseases through genetic selection. Behavioral changes in feeding and drinking are usually the very first clinical signs when animals are exposed to stressors such as disease. Changes in feeding and drinking behaviors in diseased pigs may reflect the way they cope with the challenge and, thus, could be used as indicator traits to select for disease resilience. The objectives of this study were to estimate genetic parameters of feeding and drinking traits for wean-to-finish pigs in a natural polymicrobial disease challenge model, to estimate genetic correlations of feeding and drinking traits with growth rate and clinical disease traits, and to develop indicator traits to select for disease resilience.

Results In general, drinking traits had moderate to high estimates of heritability, especially average daily water dispensed, duration, and number of visits (0.44 to 0.58). Similar estimates were observed for corresponding feeding traits (0.35 to 0.51). Most genetic correlation estimates among drinking traits were moderate to high (0.30 to 0.92) and higher than among feeding traits (0 to 0.11). Compared to other drinking traits, water intake duration and number of visits had relatively stronger negative genetic correlation estimates with treatment rate and mortality, especially across the challenge nursery and finisher (− 0.39 and − 0.45 for treatment rate; − 0.20 and − 0.19 for mortality).

Conclusion Most of the recorded drinking and feeding traits under a severe disease challenge had moderate to high estimates of heritability, especially for feed or water intake duration and number of visits. Phenotypic and genetic correlations among the recorded feeding traits under disease were generally low but drinking traits showed high correlations with each other. Water intake duration and number of visits are potential indicator traits to select for disease resilience because of their high heritability and had moderate genetic correlations with treatment and mortality rates under severe disease.

Jeon, R.L., Cheng, J., Putz, A.M., Dong, Q., Harding, J.C.S., Dyck, M.K., Plastow, G.S., Fortin, F., Lunney, J., Rowland, R., PigGen Canada, and Dekkers, J.C.M., 2021. Effect of a genetic marker for the GBP5 gene on resilience to a polymicrobial natural disease challenge in pigs. Livestock Science, 244, p.104399. doi.org/10.1016/j.livsci.2021.104399

A genomic region on chromosome 4 that is tagged by single nucleotide polymorphism (SNP) WUR0000125 (WUR) was previously found to be associated with host response to porcine reproductive and respiratory syndrome (PRRS) virus infection. The objectives of this study were to 1) determine whether genotype at the WUR SNP is also associated with resilience to a natural polymicrobial disease challenge, 2) investigate the relationship of genotype at the WUR SNP with genotype at its putative causative mutation in the *GBP5* gene, and 3) compare the association of the WUR and *GBP5* SNPs with host response to PRRS virus

infection. Data from two studies were used: 1) Eight trials of the PRRS Host Genetic Consortium, in which ~200 naïve crossbred nursery pigs per trial were infected with the NVSL-97-7895 strain of PRRS to study the effects of genotype at the *GBP5* and WUR SNPs on viral load and weight gain post-infection; 2) a natural disease challenge, where 3139 naïve crossbred nursery barrows were entered into a grow-finish facility that was seeded with multiple pathogens to maximize expression of disease resilience. Results from the PRRS trials showed that the WUR and *GBP5* SNPs are in high but not complete linkage disequilibrium ($r^2 = 0.94$). A haplotype analysis showed that discordant genotypes between the WUR and *GBP5* SNPs were due to genetic recombination and not the result of genotyping errors. We had insufficient statistical power to determine whether the *GBP5* or WUR SNP had a stronger effect on phenotype. Results from the natural disease challenge indicated that the favorable allele for the WUR SNP was significantly associated with greater average daily gain ($p = 0.02$) and with lower numbers of treatments in the challenge nursery ($p = 0.05$) and across the nursery and finisher ($p = 0.01$). Therefore, swine breeders can continue to use the WUR SNP not only as a marker for resilience to PRRS but also as a marker for disease resilience to a polymicrobial disease challenge, although its linkage disequilibrium with the putative causative mutation in the *GBP5* gene must continue to be monitored.

Pasternak, J.A., MacPhee, D.J., Lunney, J.K., Rowland, R.R., Dyck, M.K., Fortin, F., Dekkers, J.C., Plastow, G.S., Harding, J.C. and PigGen Canada, 2021. Thyroid hormone suppression in feeder pigs following polymicrobial or porcine reproductive and respiratory syndrome virus-2 challenge. Journal of Animal Science, 99(11), p.skab325. *https://doi.org/10.1093/jas/skab325*

Thyroid hormones are powerful regulators of growth, development, and basal metabolic rate and can be dysregulated under conditions of severe stress or illness. To understand the role of these hormones in porcine disease response, serum samples were obtained from three batches of nursery-aged pigs ($n = 208$) exposed to a natural polymicrobial disease challenge with an array of bacterial and viral pathogens. Levels of total thyroxin (T4) and triiodothyronine (T3) assessed in sera by radioimmunoassay, decreased significantly by 14 days post-exposure (DPE). Levels of T3 partially rebounded by 48 DPE, while T4 levels remain depressed. Post-exposure T3 and T4 levels were positively correlated with acute and long-term average daily gain (ADG). Cross-sectional sampling of animals maintained at the high health source farms, showed no equivalent change in either hormone when managed under standard industrial conditions. To further elucidate the effect of porcine reproductive and respiratory syndrome virus (PRRSV)-infection on thyroid hormone levels, archived sera over 42 days post inoculation (DPI) from nursery pigs ($N = 190$) challenged with one of two PRRSV2 strains by the PRRS Host Genetics Consortium were similarly assessed, with animals selected in a two-by-two design, to investigate biological extremes in ADG and viral load (VL). All animals showed a similar decrease in both thyroid hormones reaching a minimum at 7 DPI and returning to near pre-challenge levels by 42 DPI. Post-challenge T3 and T4 levels were significantly greater in high ADG groups, with no significant association with VL or strain. The results of this study demonstrate porcine susceptibility to thyroid disruption in response to disease challenge and demonstrate a relationship between this response and growth performance.

Lim K. S., J. Cheng, A. Putz, Q. Dong, X. Bai, C. K. Tuggle, M. K. Dyck, PigGen Canada, F. Fortin, J. C. S. Harding, G. S. Plastow, and J. C. M. Dekkers. 2021. Quantitative analysis of the blood transcriptome of young healthy pigs and its relationship with subsequent disease resilience. BMC Genomics. 22(1), pp.1-18 *https://doi.org/10.1186/s12864-021-07912-8*

Background Disease resilience, which is the ability of an animal to maintain performance under disease, is important for pigs in commercial herds, where they are exposed to various pathogens. Our objective was to investigate population-level gene expression profiles in the blood of 912 healthy F1 barrows at ~ 27 days of age for associations with performance and health before and after their exposure to a natural polymicrobial disease challenge at ~ 43 days of age.
Results Most significant ($q < 0.20$) associations of the level of expression of individual genes in blood of young healthy pigs were identified for concurrent growth rate and subjective health scores prior to the challenge, and for mortality, a combined mortality-treatment trait, and feed conversion rate after the challenge. Gene set enrichment analyses revealed three groups of gene ontology biological process terms that were related to disease resilience: 1) immune and stress response-related terms were enriched among genes whose increased expression was unfavorably associated with both pre- and post-challenge traits, 2) heme-related terms were enriched among genes that had favorable associations with both pre- and post-challenge traits, and 3) terms related to protein localization and viral gene expression were enriched among genes that were associated with reduced performance and health traits after but not before the challenge.
Conclusions Gene expression profiles in blood from young healthy piglets provide insight into their performance when exposed to disease and other stressors. The expression of genes involved in stress response, heme metabolism, and baseline expression of host genes related to virus propagation were found to be associated with host response to disease.

Dervishi, E., Yang, T., Dyck, M.K., Harding, J.C.S., Fortin, F., Cheng, J., Dekkers, J.C.M. and Plastow, G., 2021. Heritability and genetic correlations of plasma metabolites of pigs with production, resilience and carcass traits under natural polymicrobial disease challenge. Scientific reports, 11(1), pp.1-13. https://doi.org/10.1038/s41598-021-99778-9
Metabolites in plasma of healthy nursery pigs were quantified using nuclear magnetic resonance. Heritabilities of metabolite concentration were estimated along with their phenotypic and genetic correlations with performance, resilience, and carcass traits in growing pigs exposed to a natural polymicrobial disease challenge. Variance components were estimated by GBLUP. Heritability estimates were low to moderate (0.11 ± 0.08 to 0.19 ± 0.08) for 14 metabolites, moderate to high (0.22 ± 0.09 to 0.39 ± 0.08) for 17 metabolites, and highest for l-glutamic acid (0.41 ± 0.09) and hypoxanthine (0.42 ± 0.08). Phenotypic correlation estimates of plasma metabolites with performance and carcass traits were generally very low. Significant genetic correlation estimates with performance and carcass traits were found for several measures of growth and feed intake. Interestingly the plasma concentration of oxoglutarate was genetically negatively correlated with treatments received across the challenge nursery and finisher (-0.49 ± 0.28; $P < 0.05$) and creatinine was positively correlated with mortality in the challenge nursery (0.85 ± 0.76; $P < 0.05$). These results suggest that some plasma metabolite phenotypes collected from healthy nursery pigs are moderately heritable and genetic correlations with measures of performance and resilience after disease challenge suggest they may be potential genetic indicators of disease resilience.

Bai, X., Yang, T., Putz, A.M., Wang, Z., Li, C., Fortin, F., Harding, J.C., Dyck, M.K., Dekkers, J.C., Field, C.J. and Plastow, G.S., 2021. Investigating the genetic architecture of disease resilience in pigs by genome-wide association studies of complete blood count traits collected from a natural disease challenge model. BMC genomics, 22(1), pp.1-15.
https://doi.org/10.1186/s12864-021-07835-4

Background Genetic improvement for disease resilience is anticipated to be a practical method to improve efficiency and profitability of the pig industry, as resilient pigs maintain a relatively undepressed level of performance in the face of infection. However, multiple biological functions are known to be involved in disease resilience and this complexity means that the genetic architecture of disease resilience remains largely unknown. Here, we conducted genome-wide association studies (GWAS) of 465,910 autosomal SNPs for complete blood count (CBC) traits that are important in an animal's disease response. The aim was to identify the genetic control of disease resilience.
Results Univariate and multivariate single-step GWAS were performed on 15 CBC traits measured from the blood samples of 2743 crossbred (Landrace × Yorkshire) barrows drawn at 2-weeks before, and at 2 and 6-weeks after exposure to a polymicrobial infectious challenge. Overall, at a genome-wise false discovery rate of 0.05, five genomic regions located on *Sus scrofa* chromosome (SSC) 2, SSC4, SSC9, SSC10, and SSC12, were significantly associated with white blood cell traits in response to the polymicrobial challenge, and nine genomic regions on multiple chromosomes (SSC1, SSC4, SSC5, SSC6, SSC8, SSC9, SSC11, SSC12, SSC17) were significantly associated with red blood cell and platelet traits collected before and after exposure to the challenge. By functional enrichment analyses using Ingenuity Pathway Analysis (IPA) and literature review of previous CBC studies, candidate genes located nearby significant single-nucleotide polymorphisms were found to be involved in immune response, hematopoiesis, red blood cell morphology, and platelet aggregation.
Conclusions This study helps to improve our understanding of the genetic basis of CBC traits collected before and after exposure to a polymicrobial infectious challenge and provides a step forward to improve disease resilience.

Jeon, R.L., C. Gilbert, J. Cheng, A. M. Putz, M. K. Dyck, G. S. Plastow, F. Fortin, PigGen Canada, J. C. M. Dekkers, and J. C. S. Harding. 2021. Proliferation of peripheral blood mononuclear cells from healthy piglets after mitogen stimulation as indicators of disease resilience. J. Anim. Sci. 99(8), p.skab084 https://doi.org/10.1093/jas/skab084

Disease resilience refers to the productivity of an animal under disease. Given the high biosecurity of pig nucleus herds, traits that can be measured on healthy pigs and that are genetically correlated with disease resilience, that is, genetic indicator traits, offer a strategy to select for disease resilience. Our objective was to evaluate mitogen stimulation assays (MSAs) on peripheral blood mononuclear cells (PBMCs) from young healthy pigs as genetic indicators for disease resilience. Data were from a natural disease challenge in which batches of 60 or 75 naïve Yorkshire × Landrace piglets were introduced every 3 wk into a continuous flow barn that was seeded with multiple diseases. In this environment, disease resilience traits, including growth, treatment, and mortality rates, were recorded on 3,136 pigs that were genotyped with a high-density marker panel. PBMCs from 882 of these pigs from 19 batches were isolated from whole blood collected prior to the disease challenge and stimulated with five mitogens: concanavalin A (ConA), phytohemagglutinin (PHA), pokeweed mitogen (PWM), lipopolysaccharide (LPS), and phorbol myristate acetate (PMA). The proliferation of cells was evaluated at 48, 72, and 96 h and compared with unstimulated samples (rest count). Heritabilities of cell proliferation were estimated using a model with batch as a fixed effect and covariates of entry age; rest count; complete blood count proportions of lymphocytes, monocytes, eosinophils, and basophils; and pen, litter, and animal genetics as random effects. Heritability estimates were highest for response to ConA (0.30 ± 0.09, 0.28 ± 0.10, 0.17 ± 0.10, and 0.25 ±0.10 at 48, 72, and 96 h after stimulation and for area under the curve across the three time points, respectively). Estimates were in a similar range for response to PHA and

PMA but low for PWM and LPS. Responses to ConA, PHA, and PMA were moderately genetically correlated with several disease resilience traits and in the expected direction, but individual estimates were not significantly different from zero due to large SEs. In conclusion, although validation is needed, MSAss, in particular based on ConA, show promise as genetic indicator traits for disease resilience.

Lake, J.A., Dekkers, J.C. and Abasht, B., 2021. Genetic basis and identification of candidate genes for wooden breast and white striping in commercial broiler chickens. Scientific Reports, 11(1), pp.1-13. https://doi.org/10.1038/s41598-021-86176-4
Wooden breast (WB) and white striping (WS) are highly prevalent and economically damaging muscle disorders of modern commercial broiler chickens characterized respectively by palpable firmness and fatty white striations running parallel to the muscle fiber. High feed efficiency and rapid growth, especially of the breast muscle, are believed to contribute to development of such muscle defects; however, their etiology remains poorly understood. To gain insight into the genetic basis of these myopathies, a genome-wide association study was conducted using a commercial crossbred broiler population (n = 1193). Heritability was estimated at 0.5 for WB and WS with high genetic correlation between them (0.88). GWAS revealed 28 quantitative trait loci (QTL) on five chromosomes for WB and 6 QTL on one chromosome for WS, with the majority of QTL for both myopathies located in a ~ 8 Mb region of chromosome 5. This region has highly conserved synteny with a portion of human chromosome 11 containing a cluster of imprinted genes associated with growth and metabolic disorders such as type 2 diabetes and Beckwith-Wiedemann syndrome. Candidate genes include *potassium voltage-gated channel subfamily Q member 1* (*KCNQ1*), involved in insulin secretion and cardiac electrical activity, *lymphocyte-specific protein 1* (*LSP1*), involved in inflammation and immune response.

Mushi, J.R., Chiwanga, G.H., Mollel, E.L., Walugembe, M., Max, R.A., Msoffe, P.M., Gallardo, R., Kelly, T., Lamont, S., Dekkers, J. and Zhou, H., 2021. Antibody response, viral load, viral clearance and growth rate in Tanzanian free-range local chickens infected with lentogenic Newcastle disease virus. Journal of Veterinary Medicine and Animal Health, 13(2), pp.98-105. https://doi.org/10.5897/JVMAH2021.0912
This study is aimed at evaluating antibody responses, viral loads, viral clearance and growth rate of Tanzanian free-range local chicken (FRLC) challenged with LaSota strain of Newcastle disease virus (NDV) as indicator traits for selection of chickens for breeding with enhanced resistance to the disease and economic value. Three popular free-range local chicken ecotypes: Kuchi, Ching'wekwe and Morogoro-medium from three ecological zones of Tanzania were used for the experiments. Progenies from the breeder chickens were challenged with 107 titer of 50% egg infectious dose (EID50) of the virus at 28 days of age. The viral loads and viral clearance rates evaluated by qRT-PCR from tear samples collected at 2- and 6-days post infection (dpi) showed that Kuchi could clear NDV better than Morogoro-medium and Ching'wekwe. Anti-NDV antibody levels determined from blood samples collected at 10 dpi using ELISA showed that Kuchi ecotype expressed higher mean anti-NDV antibodies compared to Morogoro-medium and Ching'wekwe. Growth rates determined from body weights collected for 38 days from day of hatch (D0) to 10 dpi showed higher growth rate for Kuchi than Morogoro-medium and Ching'wekwe chickens. Kuchi chickens were potentially more resistant to ND compared to Morogoro-medium and Ching'wekwe.

Saelao, P., Wang, Y., Chanthavixay, G., Yu, V., Gallardo, R.A., Dekkers, J.C., Lamont, S.J., Kelly, T. and Zhou, H., 2021. Distinct transcriptomic response to Newcastle disease virus

infection during heat stress in chicken tracheal epithelial tissue. Scientific reports, 11(1), pp.1-9. https://doi.org/10.1038/s41598-021-86795-x
Newcastle disease (ND) has a great impact on poultry health and welfare with its most virulent (velogenic) strain. In addition, issues exacerbated by the increase in global temperatures necessitates a greater understanding of the host immune response when facing a combination of biotic and abiotic stress factors in poultry production. Previous investigations have revealed that the host immune response is tissue-specific. The goal of this study was to identify genes and/or signaling pathways associated with immune response to NDV (Newcastle disease virus) in the trachea, an essential organ where NDV replicate after the infection, by profiling the tissue specific transcriptome response in two genetically distinct inbred chicken lines when exposed to both abiotic and biotic stressors. Fayoumis appear to be able to respond more effectively (lower viral titer, higher antibody levels, immune gene up-regulation) and earlier than Leghorns. Our results suggest NDV infection in Fayoumis appears to elicit proinflammatory processes, and pathways such as the inhibition of cell viability, cell proliferation of lymphocytes, and transactivation of RNA, more rapidly than in Leghorns. These differences in immune response converge at later timepoints which may indicate that Leghorns eventually regulate its immune response to infection. The profiling of the gene expression response in the trachea adds to our understanding of the chicken host response to NDV infection and heat stress on a whole genome level and provides potential candidate genes and signaling pathways for further investigation into the characterization of the time-specific and pathway specific responses in Fayoumis and Leghorns.

Kramer, L.M., Wolc, A., Esfandyari, H., Thekkoot, D.M., Zhang, C., Kemp, R.A., Plastow, G. and Dekkers, J.C.M., 2021. Purebred-crossbred genetic parameters for reproductive traits in swine. Journal of Animal Science, 99(10), p.skab270. https://doi.org/10.1093/jas/skab270
For swine breeding programs, testing and selection programs are usually within purebred (PB) populations located in nucleus units that are generally managed differently and tend to have a higher health level than the commercial herds in which the crossbred (CB) descendants of these nucleus animals are expected to perform. This approach assumes that PB animals selected in the nucleus herd will have CB progeny that have superior performance at the commercial level. There is clear evidence that this may not be the case for all traits of economic importance and, thus, including data collected at the commercial herd level may increase the accuracy of selection for commercial CB performance at the nucleus level. The goal for this study was to estimate genetic parameters for five maternal reproductive traits between two PB maternal nucleus populations (Landrace and Yorkshire) and their CB offspring: Total Number Born (TNB), Number Born Alive (NBA), Number Born Alive > 1 kg (NBA > 1 kg), Total Number Weaned (TNW), and Litter Weight at Weaning (LWW). Estimates were based on single-step GBLUP by analyzing any two combinations of a PB and the CB population, and by analyzing all three populations jointly. The genomic relationship matrix between the three populations was generated by using within-population allele frequencies for relationships within a population, and across-population allele frequencies for relationships of the CB with the PB animals. Utilization of metafounders for the two PB populations had no effect on parameter estimates, so the two PB populations were assumed to be genetically unrelated. Joint analysis of two (one PB plus CB) vs. three (both PB and CB) populations did not impact estimates of heritability, additive genetic variance, and genetic correlations. Heritabilities were generally similar between the PB and CB populations, except for LWW and TNW, for which PB populations had about four times larger estimates than CB. Purebred-crossbred genetic correlations (*rpc*) were larger for Landrace than for Yorkshire,

except for NBA > 1 kg. These estimates of *rpc* indicate that there is potential to improve selection of PB animals for CB performance by including CB information for all traits in the Yorkshire population, but that noticeable additional gains may only occur for NBA > 1 kg and TNW in the Landrace population.

Wolc, A., Settar, P., Fulton, J.E., Arango, J., Rowland, K., Lubritz, D. and Dekkers, J.C., 2021. Heritability of perching behavior and its genetic relationship with incidence of floor eggs in Rhode Island Red chickens. Genetics Selection Evolution, 53(1), pp.1-9. https://doi.org/10.1186/s12711-021-00630-5

Background As cage-free production systems become increasingly popular, behavioral traits such as nesting behavior and temperament have become more important. The objective of this study was to estimate heritabilities for frequency of perching and proportion of floor eggs and their genetic correlation in two Rhode Island Red lines.

Results The percent of hens observed perching tended to increase and the proportion of eggs laid on the floor tended to decrease as the test progressed. This suggests the ability of hens to learn to use nests and perches. Under the bivariate repeatability model, estimates of heritability in the two lines were 0.22 ± 0.04 and 0.07 ± 0.05 for the percent of hens perching, and 0.52 ± 0.05 and 0.45 ± 0.05 for the percent of floor eggs. Estimates of the genetic correlation between perching and floor eggs were -0.26 ± 0.14 and -0.19 ± 0.27 for the two lines, suggesting that, genetically, there was some tendency for hens that better use perches to also use nests; but the phenotypic correlation was close to zero. Random regression models indicated the presence of a genetic component for learning ability.

Conclusions In conclusion, perching and tendency to lay floor eggs were shown to be a learned behavior, which stresses the importance of proper management and training of pullets and young hens. A significant genetic component was found, confirming the possibility to improve nesting behavior for cage-free systems through genetic selection.

Dekkers, J.C.M. Frederic Fortin, Michael Dyck, John Harding, Graham Plastow, 56 Awardee Talk: Genetic Improvement of Disease Resilience, Journal of Animal Science, Volume 99, Issue Supplement_3, November 2021, Page 31, https://doi.org/10.1093/jas/skab235.053

Infectious disease represents one of the largest cost components to the swine industry, incurring veterinary costs, loss of pigs due to mortality, reduced performance, and reduced animal welfare. Strategies to reduce the incidence and impact of infectious disease include biosecurity, vaccination, veterinary treatment, and selection for genetic resistance. However, biosecurity protocols that keep most infectious pathogens out are not feasible at the commercial level in hog-dense regions, effective vaccines are only available and/or efficacious for some pathogens, and complete genetic resistance is also limited to only a few pathogens (e.g. F18 E.coli) or is only possible to achieve by gene editing (e.g. the PRRS-resistant pig created by editing the CD163 gene). Given these limitations, commercial pigs will continue to be exposed to and infected by pathogens for the foreseeable future. In such a scenario, the ability of an animal to clear the infection while maintaining performance is an important characteristic, which is referred to as disease resilience and is a useful target for inclusion in breeding programs. However, collection of data on disease resilience for genetic improvement requires animals to be exposed to disease, which is not possible in the nucleus herds of breeding programs that most selection is practiced in. To study the genetic basis of disease resilience and develop phenotypes, genetic tests, or indicator traits that could be used to select for disease resilience, a polymicrobial natural disease challenge model was established in grow-finish pigs at the Center de Développement du Porc du Québec, in collaboration with PigGen Canada. The purpose of this presentation is to present results on

phenotypes that are relevant to disease resilience, including estimates of genetic parameters, and on potential indicator traits for disease resilience that could be collected in nucleus herds.

Dekkers, J.C.M., 2021. Multiple trait breeding programs with genotype-by-environment interactions based on reaction norms, with application to genetic improvement of disease resilience. Genetics Selection Evolution, 53(1), pp.1-12. https://doi.org/10.1186/s12711-021-00687-2

Background Genotype-by-environment interactions for a trait can be modeled using multiple-trait, i.e. character-state, models, that consider the phenotype as a different trait in each environment, or using reaction norm models based on a functional relationship, usually linear, between phenotype and a quantitative measure of the quality of the environment. The equivalence between character-state and reaction norm models has been demonstrated for a single trait. The objectives of this study were to extend the equivalence of the reaction norm and character-state models to a multiple-trait setting and to both genetic and environmental effects, and to illustrate the application of this equivalence to the design and optimization of breeding programs for disease resilience.

Methods Equivalencies between reaction norm and character-state models for multiple-trait phenotypes were derived at the genetic and environmental levels, which demonstrates how multiple-trait reaction norm parameters can be derived from multiple-trait character state parameters. Methods were applied to optimize selection for a multiple-trait breeding goal in a target environment based on phenotypes collected in a healthy and disease-challenged environment, and to optimize the environment in which disease-challenge phenotypes should be collected.

Results and conclusions The equivalence between multiple-trait reaction norm and multiple-trait character-state parameters allow genetic improvement for a multiple-trait breeding goal in a target environment to be optimized without recording phenotypes and estimating parameters for the target environment.

Dekkers, J., Su, H. and Cheng, J., 2021. Predicting the accuracy of genomic predictions. Genetics Selection Evolution, 53(1), pp.1-23. https://doi.org/10.1186/s12711-021-00647-w

Background Mathematical models are needed for the design of breeding programs using genomic prediction. While deterministic models for selection on pedigree-based estimates of breeding values (PEBV) are available, these have not been fully developed for genomic selection, with a key missing component being the accuracy of genomic EBV (GEBV) of selection candidates. Here, a deterministic method was developed to predict this accuracy within a closed breeding population based on the accuracy of GEBV and PEBV in the reference population and the distance of selection candidates from their closest ancestors in the reference population.

Methods The accuracy of GEBV was modeled as a combination of the accuracy of PEBV and of EBV based on genomic relationships deviated from pedigree (DEBV). Loss of the accuracy of DEBV from the reference to the target population was modeled based on the effective number of independent chromosome segments in the reference population (M_e). Measures of M_e derived from the inverse of the variance of relationships and from the accuracies of GEBV and PEBV in the reference population, derived using either a Fisher information or a selection index approach, were compared by simulation.

Results Using simulation, both the Fisher and the selection index approach correctly predicted accuracy in the target population over time, both with and without selection. The index approach, however, resulted in estimates of M_e that were less affected by heritability, reference size, and selection, and which are, therefore, more appropriate as a population

parameter. The variance of relationships underpredicted M_e and was greatly affected by selection. A leave-one-out cross-validation approach was proposed to estimate required accuracies of EBV in the reference population. Aspects of the methods were validated using real data.
Conclusions A deterministic method was developed to predict the accuracy of GEBV in selection candidates in a closed breeding population. The population parameter M_e that is required for these predictions can be derived from an available reference data set, and applied to other reference data sets and traits for that population. This method can be used to evaluate the benefit of genomic prediction and to optimize genomic selection breeding programs.

Rothschild's group has been continuing to analyze the role of genetics in phenotypes in a variety of livestock and aquaculture species:

a) His group has examined methods to estimate breed composition for pigs using a case study focused on Mangalitsa pigs and two different methods:

Chinchilla-Vargas, J. , F. Bertolni, K. J. Stalder, J. P. Steibel, and M. F. Rothschild ***2021.*** *Livestock Sci. (in Press)*

Breed associations and registries maintain breed purity by enforcing certain conformational characteristics defining the breed along with cataloguing the pedigree of every animal that is approved for registry within that breed. Furthermore, developing niche markets is often based on specialized products using heritage breeds have worked to ensure breed purity. Genomic technology and the progressively lower costs of genotyping can be helpful when assessing of breed purity by estimating breed composition. In this research, genotypes from 648 pigs and 11 breeds were used to develop marker panels to estimate breed composition with special emphasis on Mangalitsa pigs as a heritage breed. Two sets of panels were created. The first set was based on Fst scores that were calculated individually for ~31,000 available markers across the pig genome. Here, panels composed of the 10, 50, 100, 500 and 1000 markers with the highest F_{st} scores were generated. The second set was composed by randomly selected markers and had the same number of markers as the F_{st}-derived panels. Two statistical methods, linear regression and random forest were then used on the marker panels to estimate breed composition, of 107 pigs including 47 individuals known to have Mangalitsa background. F_{st} appeared to be better at identifying Mangalitsa individuals when compared to random markers regardless of the method used to estimate breed composition. However, random markers were more accurate at estimating breed composition for non-Mangalitsa individuals. When the results were compared across methods for estimating breed composition, linear regression produced more accurate estimates of breed composition than random forest. Here, F_{st}-selected markers estimated purebred Mangalitsa to be 87% Mangalitsa while random forest estimated 78%. However, both methods lacked accuracy when estimating breed composition for crossbred individuals. The results presented in this study allow us to conclude that: 1) While random forest proved to be effective at classifying individuals into breeds, it was less accurate at estimating breed composition when compared to the linear regression method. 2) Markers filtered using F_{st} scores are more effective at identifying Mangalitsa breed composition while not as effective at identifying other breeds. 3) If F_{st}-filtered markers that are effective at identifying Mangalitsa from other breeds are being used to estimate breed composition for individuals of other breeds, a greater number of markers is needed so that markers that are useful at differentiating between non-Mangalitsa breeds can be included.

b) A second project in collaboration with James Koltes and others analyzed the genetic basis of blood-based traits and their relationship with performance and environment in beef cattle at weaning:

Josue Chinchilla-Vargas, Luke M. Kramer, John D. Tucker, Donald S. Hubbell III, Jeremy G. Powell, Toby D. Lester, Elizabeth A. Backes, Karen Anschutz, Jared E. Decker, Kenneth J. Stalder, Max F. Rothschild and James E. Koltes

The objectives of this study were to explore the usefulness of blood-based traits as indicators of health and performance in beef cattle at weaning and identify the genetic basis underlying the different blood parameters obtained from complete blood counts (CBCs). Disease costs represent one of the main factors determining profitability in animal production. Previous research has observed associations between blood cell counts and an animal's health status in some species. CBC were recorded from approximately 570 Angus based, crossbred beef calves at weaning born between 2015 and 2016 and raised on toxic or novel tall fescue. The calves (N = ~600) were genotyped at a density of 50k SNPs and the genotypes (N = 1160) were imputed to a density of 270k SNPs. Genetic parameters were estimated for 15 blood and 4 production. Finally, with the objective of identifying the genetic basis underlying the different blood-based traits, genome-wide association studies (GWAS) were performed for all traits. Heritability estimates ranged from 0.11 to 0.60, and generally weak phenotypic correlations and strong genetic correlations were observed among blood- based traits only. Genome-wide association study identified ninety-one 1-Mb windows that accounted for 0.5% or more of the estimated genetic variance for at least 1 trait with 21 windows overlapping across two or more traits (explaining more than 0.5% of estimated genetic variance for two or more traits). Five candidate genes have been identified in the most interesting overlapping regions related to blood-based traits. Overall, this study represents one of the first efforts represented in scientific literature to identify the genetic basis of blood cell traits in beef cattle. The results presented in this study allow us to conclude that: (1) blood-based traits have weak phenotypic correlations but strong genetic correlations among themselves. (2) Blood-based traits have moderate to high heritability. (3) There is evidence of an important overlap of genetic control among similar blood-based traits which will allow for their use in improvement programs in beef cattle.

c) Finally, his group analyzed signatures of selection and genomic diversity of Muskellunge (Esox masquinongy) from two populations in North America

Josue Chinchilla-Vargas, Jonathan R. Meerbeek, Max F. Rothschild and Francesca Bertolini Genes 12(7):1021.

Muskellunge (Esox masquinongy) is the largest and most prized game fish in North America. However, little is known about Muskellunge genetic diversity in Iowa's propagation program. We used Whole-Genome Sequencing of 12 brooding individuals from Iowa and publicly available RAD- seq of 625 individuals from the St. Lawrence River in Canada to study the genetic differences between populations, analyze signatures of selection, and evaluate the levels of genetic diversity in both populations. Given that there is no reference genome available, reads were aligned to the genome of Pike (Esox lucius). Variant calling produced 7,886,471 biallelic variants for the Iowa population and 16,867 high-quality SNPs that overlap with the Canadian samples. Principal component analysis (PCA) and Admixture analyses showed a large genetic difference between Canadian and Iowan populations. Window-based pooled heterozygosity found 6 highly heterozygous windows in the Iowa population and Fst between populations found 14 windows with fixation statistic (Fst) values larger than 0.9. Canadian inbreeding rate (Froh = 0.32) appears to be higher due to the inbreeding of Iowa population (Froh = 0.03), presumably due to isolation of subpopulations. Although inbreeding

does not seem to be an immediate concern for Muskellunge in Iowa, the Canadian population seems to have a high rate of inbreeding. Finally, this approach can be used to assess the long-term viability of the current management practices of Muskellunge populations across North America.

B. Participate in on-going pig genome functional annotation efforts (Objective 2).

Work with colleagues to continue sequencing regions of interest and examine them for traits of interest in the pig, as well as annotate genes of interest, especially those involved in the immune system.

Tuggle's group has been participating in functional annotation of the porcine genome, with emphasis on the immune system. To improve functional annotation through developing epigenetic data across tissues and cell types, the large-scale project funded ("Functional Annotation of the Porcine Genome", 2018-67015-27501). This is a multi-Station collaborative work with MSU, USDA-ARS-MARC, USDA-ARS-NADC, and UC-Davis on functional annotation of the pig genome, especially related to ENCODE-type projects. Work at ISU has been conducted on the following components thus far:

1) Identification and expression profiling of porcine immune cell-specific genes will provide researchers with a more vivid portrait of healthy and diseased states in individual pigs, permit more accurate biomarker screening, and will improve porcine genome annotation. As an initial study to begin cataloging porcine cell-specific patterns, we separated peripheral blood mononuclear cells from triplicate blood samples into seven different cellular phenotypes using cell-surface markers and both magnetic and fluorescence-based sorting. To verify the accuracy of these cell sorts, mRNA was isolated from these sorted populations and assayed using a set of over 200 Nanostring assays in the lab of Dr. Joan Lunney (USDA-ARS-NADC). A manuscript on sorted immune cells and PBMC scRNAseq was published in 2021. The Nanostring and scRNAseq data analysis was performed by Haibo Liu, in collaboration with Drs. Kristen Byrne and Crystal Loving at USDA-ARS-NADC and Dr. Joan Lunney, USDA-ARS-BARC.
2) We are also testing the use of single-cell ATAC-seq data to identify the accessible regions of circulating immune cells (PBMC), and to compare to the scRNAseq data we have just completed and published. We believe the combined analysis will be powerful to identify the transcriptional relationships between regulatory factor and target gene, as well as active enhancers across these cell types, which is a major purpose of the FAANG project. Pengxin Yang is performing the data collection and analysis of these data from the reference FAANG animals.
3) We have also completed the tissue preparation and scRNAseq analysis of thymus, spleen, lymph node, and bone marrow tissues. The data is currently being evaluated for quality and both within-tissue and across tissue specific patterns to identify the cell types within each tissue and in comparison to circulating cells. This work is supported by USDA-ARS-NADC and USDA-NIFA-AFRI funds, and is being completed by Lance Daharsh with Crystal Loving at USDA-NADC.
4) We are assaying DNA methylation, RNA expression, and ATAC-seq analysis of chromatin accessibility, as well as histone modifications and CTCF binding in fetal tissues to expand our functional annotation data by determining the correlation between epigenetic modifications and gene expression in swine fetal tissues. In this study, we performed both RNA-seq (4 tissues: brain, liver, muscle and placenta, two developmental stages, Day 30 of gestation (D30G) and Day 70 pf gestation(D70G)), Whole-genome Bisulfite sequencing

and ChIP-seq (liver and muscle, D70G) to identify variants of allele-biased expression (ABE) and allele-biased histone modifications (ABHM), in F1 fetuses from reciprocal crosses of Meishan (MS) and White composite (WC) pigs. The sets of variants and their associated genes showing ABE and ABHM will provide a foundation for understanding gene regulation and the underlying phenotypic differences between MS and WC. The genomic and RNA sequencing data was collected by Dan Nonneman and Tim Smith at USDA-MARC, the WGBS data was collected and analyzed by Ryan Corbett in the C. Ernst lab at Michigan State, and the ChIP-seq data was collected by Buyue Niu, and the data analyzed by Dr. Haibo Liu at Iowa State with assistance from Brittany Keel at USDA-MARC. A manuscript on genome sequence analysis to identify SNPs, multi-tissue RNAseq and the relationship of these markers to DNA methylation state to measure effect on expression, and allele-specific expression of genes in these tissues is in preparation for submission in 2022 by R. Corbett and H. Liu.

5) In a collaboration between the Tuggle and Koltes labs, we are investigating neutrophil specific transcriptional regulation: Gene co-expression networks were developed to differentiate immune cell types generated from bulk RNA-seq. Within these subnetworks, neutrophil specific genes were identified based on differences in expression compared to other immune cell types (fold change expression). Within the neutrophil network, transcription factors were investigated for their cell-type specificity. In addition, the expression of candidate neutrophil specific transcription factors were compared across mouse and human data using publicly available data to identify conserved and swine specific transcriptional regulation in neutrophils. Finally, target gene expression within the cell networks were evaluated for transcription factor binding sites to verify neutrophil specificity.

James Koltes group: As part of the Pig FAANG project, Kyu-sang Lim is assembling PacBio and Illumina based de novo transcriptomes from immune cells and a host of tissues to identify tissue specific splicing, epigenetic markings associated with splice variation and DNA variants associated with these splicing events.

C. Development and analysis of populations measured for response to PRRSV (Objective 2)

The Dekkers group has ongoing collaborative efforts on genetics of host response to disease in grow-finish pigs, using experimental PRRS, and PRRS-PCV2 challenge studies in collaboration with the Tuggle and Lunney groups:

Dong, Q., Lunney, J.K., Lim, K.S., Nguyen, Y., Hess, A.S., Beiki, H., Rowland, R.R., Walker, K., Reecy, J.M., Tuggle, C.K. and Dekkers, J.C., 2021. Gene expression in tonsils in swine following infection with porcine reproductive and respiratory syndrome virus. BMC veterinary research, 17(1), pp.1-21. doi.org/10.1186/s12917-021-02785-1

Background Porcine reproductive and respiratory syndrome (PRRS) is a threat to pig production worldwide. Our objective was to understand mechanisms of persistence of PRRS virus (PRRSV) in tonsil. Transcriptome data from tonsil samples collected at 42 days post infection (dpi) were generated by RNA-seq and NanoString on 51 pigs that were selected to contrast the two PRRSV isolates used, NVSL and KS06, high and low tonsil viral level at 42 dpi, and the favorable and unfavorable genotypes at a genetic marker (WUR) for the putative PRRSV resistance gene GBP5.

Results The number of differentially expressed genes (DEGs) differed markedly between models with and without accounting for cell-type enrichments (CE) in the samples that were

predicted from the RNA-seq data. This indicates that differences in cell composition in tissues that consist of multiple cell types, such as tonsil, can have a large impact on observed differences in gene expression. Based on both the NanoString and the RNA-seq data, KS06-infected pigs showed greater activation, or less inhibition, of immune response in tonsils at 42 dpi than NVSL-infected pigs, with and without accounting for CE. This suggests that the NVSL virus may be better than the KS06 virus at evading host immune response and persists in tonsils by weakening, or preventing, host immune responses. Pigs with high viral levels showed larger CE of immune cells than low viral level pigs, potentially to trigger stronger immune responses. Presence of high tonsil virus was associated with a stronger immune response, especially innate immune response through interferon signaling, but these differences were not significant when accounting for CE. Genotype at WUR was associated with different effects on immune response in tonsils of pigs during the persistence stage, depending on viral isolate and tonsil viral level. **Conclusions** Results of this study provide insights into the effects of PRRSV isolate, tonsil viral level, and WUR genotype on host immune response and into potential mechanisms of PRRSV persistence in tonsils that could be targeted to improve strategies to reduce viral rebreaks. Finally, to understand transcriptome responses in tissues that consist of multiple cell types, it is important to consider differences in cell composition.

Dong, Q., Dunkelberger, J., Lim, K.S., Lunney, J.K., Tuggle, C.K., Rowland, R.R. and Dekkers, J.C., 2021. Associations of natural variation in the CD163 and other candidate genes on host response of nursery pigs to porcine reproductive and respiratory syndrome virus infection. Journal of Animal Science, 99(10), p.skab274. https://doi.org/10.1093/jas/skab274
Pigs with complete resistance to porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) have been produced by genetically knocking out the *CD163* gene that encodes a receptor of the PRRSV for entry into macrophages. The objectives of this study were to evaluate associations of naturally occurring single nucleotide polymorphisms (SNPs) in the *CD163* gene and in three other candidate genes (*CD169*, *RGS16*, and *TRAF1*) with host response to PRRSV-only infection and to PRRS vaccination and PRRSV/porcine circovirus 2b (PCV2b) coinfection. SNPs in the *CD163* gene were not included on SNP genotyping panels that were used for previous genome-wide association analyses of these data. An additional objective was to identify the potential genetic interaction of variants at these four candidate genes with a mutation in the *GBP5* gene that was previously identified to be associated with host response to PRRSV infection. Finally, the association of SNPs with expression level of the nearby gene was tested. Several SNPs in the *CD163*, *CD169*, and *RGS16* genes were significantly associated with host response under PRRSV-only and/or PRRSV/PCV2b coinfection. The effects of all SNPs that were significant in the PRRSV-only infection trials depend on genetic background. The effects of some SNPs in the *CD163*, *CD169*, and *RGS16* genes depend on genotype at the putative causative mutation in the *GBP5* gene, which indicates a potential biological interaction of these genes with *GBP5*. In addition, genome-wide association results for the PRRSV-only infection trials revealed that SNPs located in the *CDK5RAP2* or *MEGF9* genes, near the *TRAF1* gene, had suggestive effects on PRRS viral load, which indicates that these SNPs might contribute to PRRSV neuropathogenesis. In conclusion, natural genetic variants in the *CD163, CD169*, and *RGS16* genes are associated with resistance to PRRSV and/or PCV2b infection and appear to interact with the resistance quantitative trait locus in the *GBP5* gene. The identified SNPs can be used to select for increased natural resistance to PRRSV and/or PRRSV-PCV2b coinfection.

D. Development and analysis of populations selected for increased feed efficiency (Objective 2)

A Yorkshire population selected for RFI was developed at ISU by Dekkers and collaborators. Deep phenotypes and genomic data have been collected to address questions related to the genetic basis of feed efficiency and the impact of selection for feed efficiency on related traits.

Patterson, B.M., Outhouse, A.C., Helm, E.T., Johnson, L., Steadham, E.M., Dekkers, J.C., Schwartz, K.J., Gabler, N.K., Lonergan, S.M. and Huff-Lonergan, E., 2021. Novel observations of peroxiredoxin-2 profile and protein oxidation in skeletal muscle from pigs of differing residual feed intake and health status. Meat and Muscle Biology, 5(1). https://doi.org/10.22175/mmb.12241
This study's objective was to determine the impact of a dual respiratory and enteric bacterial health challenge on the antioxidant protein peroxiredoxin-2 (Prdx-2) profile and protein oxidation in the skeletal muscle of pigs from 2 lines that were divergently selected for residual feed intake (RFI). The hypotheses were that (1) differences exist in the Prdx-2 profile between 2 RFI lines and infection status and (2) muscle from less efficient high-RFI and health-challenged pigs have greater cellular protein oxidation. Barrows (50 ± 7 kg, N = 24) from the 11th generation of the high-RFI (n = 12) and low-RFI (n = 12) Iowa State University lines were used. Pigs (n = 6 per line) were inoculated with *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis* (MhLI) on day 0 post infection to induce a respiratory and enteric health challenge. Uninoculated pigs served as controls (n = 6 per line). Necropsy was at 21 d post infection. Sarcoplasmic protein oxidation, various forms of Prdx-2, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) content were determined. Neither RFI line nor infection status significantly affected protein carbonylation. Under nonreducing conditions, MhLI pigs had a greater amount of a slower-migrating GAPDH band (P = 0.017), indicating oxidative modification. Regardless of health status, the low-RFI pigs had less total Prdx-2 (P = 0.035), Prdx-2 decamer (P = 0.0007), and a higher ratio of hyperoxidized peroxiredoxin relative to Prdx-2 (P = 0.028) than the high-RFI pigs. The increased pool of active Prdx-2 in high-RFI pigs suggests greater oxidative stress in muscle in high- versus low-RFI pigs. The increase in oxidized GAPDH seen in muscle from MhLI pigs—particularly the high-RFI MhLI pigs—may be a response to the greater oxidative stress in the high-RFI MhLI. This work suggests that antioxidant proteins are important in growth and health-challenge situations.

E. Participate in further database and other bioinformatic resource development (Objective 3)

We continue to host a website (www.faang.org) and WIKI for use by members of FAANG (Functional Annotation of ANimal Genomes). The intent of this website is to popularize this new collaborative project to improve the annotation of all livestock genomes, and to serve as a collection space for protocols, member information, and communication to the public. For data specific to pig, this will be linked to the Pig Genome Database to facilitate analysis and dissemination of additional porcine genome data. In the past year, we have added webpages and resources for funded projects as well as new opportunities to be announced.

VI. Additions to the Project

Tuggle group: As an addition to the FAANG project, we have completed an initial analysis of Nanopore long-read data to sequence and assemble the genome of the FAANG project founder animals. The purpose of this project is to test whether an assembly of the individual animal improves the mapping rate and information obtained from sequence-based assays being used in the USDA-FAANG project.

VII. Applications of Findings

A. The genome sequence assembly of the individual FAANG project founder pigs will help inform the large resource of functional information for agricultural and biomedical researchers.

B. Use of random forest and regression approaches and a limited number of SNPs are useful to predict breed from pigs without pedigree information.

C. Several new genes that may be important QTL have been or are being mapped. These include genes associated with meat quality, disease incidence, feed efficiency, IMF and other performance traits.

D. The blood transcriptome, metabolome, and proteome of young healthy pigs contains potential biomarkers to select for disease resilience in pigs

E. We have published the first integrated analysis of RNAseq analysis of 8 different flow-cytometry sorted cell populations and scRNAseq analysis of PBMC. We identified cell types that are present in specific sorted cell populations, as well across these sorted cells.

F. Genes that can predict specific scRNAseq cell types may be very useful to direct future immunoreagent development as well as improve analysis of whole blood RNAseq data.

G. We have identified the gene expression of many cell types present in the most important immune tissues in the pig, which can be used to better annotate genes in the pig and serve as candidate genes for improving pig disease resistance.

VIII. Future Project Plans

Objective 1: Advance the status of reference genomes for all species, including basic annotation of worldwide genetic variation, by broad sequencing among different lines and breeds of animals.

We will complete Nanopore long-read sequencing and assemble the genome of the FAANG project founder animals. We will then test whether individual genome assemblies are more informative than the reference genome assembly made from a different breed.

Objective 2: Develop strategies to identify and exploit genes and allelic variation that contribute to economically relevant phenotypes and traits, in part through improving functional annotation of the genomes of our species

Add to growing FAANG effort to create and analyze functional annotation of the pig genome through producing transcriptomic data for sets of tissues and development of predictive datasets for cell types within these tissues.

Analyze the relationship between DNA methylation, histone modification, chromatin accessibility, and gene expression in isolated immune cells.

Objective 3: Facilitate analysis, curation, storage, distribution and application of the enormous datasets now being generated by next-generation sequencing and related "omics" technologies with regard to animal species of agricultural interest.

Continue development and improvement of FAANG and other general applicability databases.

IX. Publications

A. Publications during the year

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B. Publications Planned

Chen, Y., Lonergan, S., Lim, K.S., Putz, A.M., Dyck, M.K., PigGen Canada, Fortin, F., Plastow, G.S., Harding, J.C., and Dekkers, J.C. Protein levels in the plasma of young, healthy pigs as potential indicators of disease. (In preparation for submission).

Chen, Y., Lonergan, S., Lim, K.S., Dervishi, E., Putz, A.M., Dyck, M.K., PigGen Canada, Fortin, F., Plastow, G.S., Harding, J.C., and Dekkers, J.C. Prediction of disease resilience of pigs using multi-omics data. (In preparation for submission to Genet. Sel. Evol.)./

Cheng J., J. Schmied, B. Mallard, R. Fernando, S. Kachman, H. Cheng, J. C. S. Harding, M. K. Dyck, F. Fortin, G. S. Plastow, PigGen Canada, and J. C. M. Dekkers. 2021. Genome-wide association studies for high immune response traits of pigs from a natural polymicrobial disease challenge model. (In preparation for submission to BMC genomics.)

Cheng J., R. Fernando, S. Kachman, H. Cheng, K. Lim, J. C. S. Harding, M. K. Dyck, F. Fortin, G. S. Plastow, PigGen Canada, and J. C. M. Dekkers. 2022. Genome-wide association studies on disease resilience traits from a natural polymicrobial disease challenge model in pigs identify the importance of major histocompatility complex region. (Accepted for publication in G3.)

Cheng J., K. Lim, A. M. Putz, J. C. S. Harding, M. K. Dyck, F. Fortin, G. S. Plastow, PigGen Canada, and J. C. M. Dekkers. 2022. Genetic analysis of disease resilience of wean-to-finish pigs under a natural disease challenge model using reaction norm models (Submitted to Genetics Selection Evolution)

Corbett, R.J., A.M. Luttman, J. Herrera-Uribe, H. Liu, N. E. Raney, J.M. Grabowski, C.L. Loving, C.K. Tuggle, and C.W. Ernst. Assessment of DNA methylation in porcine immune cells reveals novel regulatory elements associated with cell-specific gene expression and immune capacity traits. Submitted to BMC Genomics.

de Souza, M.M., D. A. Koltes, H. Beiki, M. A. Sales, T. Tsai, C. V. Maxwell, J. Zhao, and J. E. Koltes. Early life exposure of pigs to topsoil alters miRNA and mRNA expression in peripheral blood mononuclear cells. To be submitted to a special edition on host microbiome interaction in *Frontiers in Genetics*.

Dong, Q., Jeon, H., Lunney, J.K., Lim, K.S., Walker, K., Tuggle, C.K., Rowland, R.R., and Dekkers, J.C., Blood transcriptome response to vaccination and co-infection with Porcine Reproductive and Respiratory Virus and Porcine Circo Virus Type 2B in nursery tips. (In preparation for submission to PlosOne)

Fanalli, S. L., B.P.M da Silva, B. Petry, M.H. de Almeida Santana, G.H. G. Polizel, R.C.Antunes, V.V. de Almeida, G.C.M. Moreira, A.L. Filho, L.L. Coutinho, JúJ.C.C.Balieiro, J.M. Reecy, J.E. Koltes, D. Koltes, A.S.M. Cesar. Effects of dietary fatty acids on gene expression and biological processes in different tissues of pigs: A Review. Submitted to *Animals*

Herrera-Uribe, J., Lim, K., Vella, G., Beiki, Huang, J., Byrne K, C. Loving, J.E. Koltes and C.K. Tuggle. Integrative profiling of chromatin accessibility and gene expression elucidate specific transcriptional network in porcine neutrophils. Manuscript in preparation.

Huntley*, NF, MM de Souza*, MD Schulte, H Beiki, AO de Lima, AE Jantzi, SM Lonergan, EJ Huff-Lonergan, JF Patience and JE Koltes. Dietary intake of xylose impacts the transcriptome and proteome of porcine tissues involved in xylose metabolism. To be submitted to *Frontiers in Animal Sciences*.

Lim, K.-S. J. Herrera-Uribe, C.L. Loving, K.A. Byrne, T.P.L. Smith, H. Beiki, J.M. Reecy, C.K. Tuggle, and J.E. Koltes. 2022. Diversity of alterative splicing in porcine peripheral blood immune cells. Proc. of Plant and Animal Genome XXIX. San Diego, CA, USA.

Mauch, E.D., K.L. Bunter, A. Wolc, N.V.L. Serão, and J.C.M. Dekkers. Correlated responses to selection for residual feed intake on nursery pig growth, feed efficiency, and juvenile Insulin-like Growth Factor I concentration (In preparation for submission to J. Anim. Sci.)

Mauch, E.D., A. Wolc, N.V.L. Serão, C.K. Tuggle, and J.C.M. Dekkers. Genome wide association and genomic prediction in lines of pigs divergently selected for residual feed intake and fed diets differing in energy and fiber. (In preparation for submission to J. Anim. Sci.)

Mauch, E.D., H. Gilbert, B. Servin, and J.C.M. Dekkers. Detection of selection signatures for residual feed intake in two independent divergent selection experiments in swine. (In preparation for submission to Genetics Selection Evolution)

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