NATIONAL ANIMAL GENOME RESEARCH PROGRAM - NRSP-8 Year Ending 2021

The US agricultural animal industry has and continues to benefit from the increase in knowledge and tools generated by the National Animal Genome Research Program (NRSP-8). This diverse consortium of scientists includes experts from the dairy and beef cattle, poultry, equine, sheep, goat, swine, and aquaculture sectors, as well as bioinformatics. Each NRSP-8 species group (including bioinformatics) helps to provide critical infrastructure and tools for agri-animal genomic discoveries, including genomics and bioinformatics tools and databases, genetic resource populations with economically-important phenotypes, education and training of students and scientists, and outreach to the public. NRSP-8 is also an integral and active partner in NIFA's Agricultural Genomes to Phenomes Initiative (AG2PI) "Creating a Shared vision across Crop and Livestock Communities", with 3 NRSP-8 members serving as co-PI's on this grant and others participating and contributing to the activities organized by AG2PI, including submitting and receiving seed grants.

HIGHLIGHTS OF ACCOMPLISHMENTS AND IMPACTS:
NRSP-8 community members continue to publish and speak about research results, form large collaborative research groups, lead and participate in multi-institutional grant proposals, organize workshops and conferences, and train students and post-doctoral fellows. A summary of the major accomplishments and impacts for each of the coordinator groups (aquaculture, bovine, equine, poultry, sheep/goat, swine, and bioinformatics) is included in this report.

The impacts and accomplishments of the NRSP-8 project continue to be remarkable. In just one year (2021) the NRSP-8 membership has published 224 peer-reviewed manuscripts, with a number of additional invited talks, press releases, and conference abstracts. An important contribution across three livestock species was improved functional genome annotations for comparative and agricultural research (*Nature Communications* https://doi.org/10.1038/s41467-021-22100-8. Other high-profile publications in 2021 include "Parental bias in expression and interaction of genes in the equine placenta" (*Proc Natl Acad Sci* (https://doi.org/10.1073/pnas.2006474118) and "Darwinian genomics and diversity in the tree of life" (*Proc Natl Acad Sci* https://doi.org/10.1073/pnas.2115644119). In addition, NRSP-8 bioinformatics activities and resources continue to develop and serve the greater animal science research and industry communities across species, including the trait association data base (QTLdb), trait genetic parameter data base (CorrDB), bioinformatics tools, trait ontology development, and data repositories. As an example of industry impact, the eBEEF consortium of beef cattle genetics extension faculty at 6 NRSP-8 member institutions received the 2021 Beef Improvement Federation Ambassador Award for efforts in extending the news of BIF and its principles to a larger audience. Pangenomes are being developed for several species, which will enhance our ability to capture and utilize structural variation within a genome to gain a more comprehensive view of the genetic variation driving phenotypic variation.

Funds allocated to the NRSP-8 project continue to be leveraged extensively: in 2021, the total amount of new grants that were reported to be secured was nearly $9 million. In the process of conducting research, producing manuscripts and securing funding, graduate students and postdocs are being trained in genomics and bioinformatic data processing by all species groups and in bioinformatics.

Due to the COVID19 pandemic, in-person meetings at the International Plant and Animal Genomes Conference, where the NRSP-8 general and species meetings are generally held, were again cancelled in January 2022. Instead, all species groups held virtual meetins, either at their regular PAG workshop time or separately. The NRSP-8 general meeting was held as a hybrid 1-day meeting on April 3, 2022, as a pre-conference to the inaugural AGBT-ag meeting in San Diego, in conjunction with the joint cattle, sheep and goats, and swine workshop. These meetings included 33 in-person and 31 on-line participating NRSP-8 leadership and members, along with 5 in-person and 2 on-line industry representatives. In total, 30 US institutions were represented. A major focus of the NRSP-8 general meeting was how to sustain and transition NRSP-8 activities beyond the end of the current project. This included an panel discussion with

representatives from the livestock industries about industry needs into the future. Information about NRSP-8 also continues to be made publicly available through the https://www.animalgenome.org/ website (maintained at Iowa State University) and the AnGenMap email list serve (https://www.animalgenome.org/community/angenmap/) informs over 4,685 worldwide subscribers.

The NRSP-8 community has led to significant achievements under each of the three objectives listed below for the different coordinated bioinformatic and agriculture relevant species, as outlined for 2018-2023. **Objective 1:** Advance the quality of reference genomes for all agri-animal species by providing high contiguity assemblies, deep functional annotations of these assemblies, and comparison across species to understand structure and function of animal genomes. **Objective 2:** Advance genome-to-phenome prediction by implementing strategies and tools to identify and validate genes and allelic variants predictive of biologically and economically important phenotypes and traits. **Objective 3:** Advance analysis, curation, storage, application, and reuse of heterogeneous big data to facilitate genome-to-phenome research in animal species of agricultural interest.

Aquaculture NRSP-8 Executive Summary: Annual Report 2021

Coordinator:	Benjamin J. Reading, North Carolina State University
Co-coordinators:	Steven Roberts, University of Washington
	Moh Salem, University of Maryland
	Eric Peatman, Auburn University
Species Leaders:	
Catfish:	Sylvie Quiniou, ARS Stoneville, Mississippi,
Oyster/shellfish:	Dina Proestou, ARS University of Rhode Island, Rhode Island
Salmonids:	Yniv Palti, ARS Leetown, West Virginia
Striped Bass:	Benjamin J. Reading, North Carolina State University, North Carolina

Aquaculture Workshop:
Workshop Chair-elect 2023-2024: Yniv Paltu yniv.palti@usda.gov)
Workshop Chair-elect 2022-2023: Rafet Al-Tobasei (Rafet.Al-tobasei@mtsu.edu)
Workshop Chair 2021-2022: Moh Salem (mosalem@umd.edu)
Workshop Chair 2020-2021 (*cancelled*): Moh Salem (mosalem@umd.edu)

Aquaculture Workshop Report
The Aquaculture Workshop in 2022 was cancelled for in-person activities due to the COVID-19 pandemic and travel restrictions.
Attendees: **66** (an average of 35-45 Participants usually tuning into each presentation)
Institutions Represented: **41**
Countries Represented: **11**

Leveraged Funds:
Two (2) small research projects were funded at $10,000 each in 2021 to provide preliminary data for grants: $20,000 (2022-2023). Leveraged funds from diverse grants based on previously funded small research projects totaled over $2,000,000 from federal sources in 2021. Collectively, this is almost $13 million reported since 2019 with a return on investment of 1:49 (NRSP-8, 2018-2023).

Extramural funding agencies leveraged through collaborations and seed funding opportunities provided by this NRSP-8 program include NOAA, USDA NIFA, USDA AFRI, NSF, and the FFAR. In particular, the marine finfish and shellfish aquaculture initiatives of USDA and NOAA are to be recognized (Executive Order 13921: *Promoting American Seafood Competitiveness and Economic Growth*).

Specific Major Activities Include:
There were 32 publications from the NRSP-8 Aquaculture Community in 2021, including one US Department of Homeland Security report on Threats to Food and Agricultural Resources to the US Intelligence Community (https://www.dhs.gov/sites/default/files/publications/threats_to_food_and_agriculture_resources.pdf) and 5 review papers or book chapters. Homeland Security Presidential Directive 9 (HSPD-9) directs the US Department of Homeland Security to coordinate the overall national effort to protect the Food and Agriculture Sector.

NRSP-8 Aquaculture 2021 Progress: Species Leaders indicated in **BOLD.**
Objective 1: Advance the quality of reference genomes for all agri-animal species through providing high contiguity assemblies, deep functional annotations of these assemblies, and comparison across species to understand structure and function of animal genomes.

Generally, genomic information of most aquatic species is still lacking and greatly lagging behind that of terrestrial species.

Catfish (**Quiniou**, Liu)
The complete germline repertoires of the channel catfish, *Ictalurus punctatus*, T cell receptor (TR) loci, TRAD, TRB, and TRG were obtained by analyzing genomic data from PacBio sequencing.

Oyster/Shellfish (Roberts, Gómez-Chiarri, Putnam, Puritz, Guo, Warren, **Proestou,** Eastern Oyster breeding Consortium)
Re-sequencing of an additional 179 wild and selected individuals from 16 eastern oyster populations situated along the US east Coast >20X coverage.

Improved eastern oyster (*Crassostrea virginica*) reference genome assembly using updated assembly pipeline, additional Hi-C data, and high-density genetic linkage map.

The recent availability of a high-quality reference genome for the eastern oyster, and whole-genome sequence data of samples from across the species range in the USA, provides an opportunity to explore structural variation across the genome of this species. Our analysis shows significantly greater individual-level duplications of regions across the genome than that of most model vertebrate species. The eastern oyster shows a large interindividual variation in duplications as well as particular chromosomal regions with a higher density of duplications.

Salmonids (Salem, **Palti**, Al-Tobasei)
The first *De-novo* genome assembly for the North American Atlantic salmon lineage was submit to NCBI and is in final review.

A new transcriptome assembly using PacBio long-read sequencing improved the rainbow trout genome annotation and identified alternative splicing associated with economically important phenotypes in rainbow trout.

Striped Bass (*National Breeding Program for the Hybrid Striped Bass Industry*, Fuller, Abernathy, Borski, Berlinsky, **Reading**)
The second (2.0), updated draft of the striped bass genome was uploaded to NCBI GenBank (NCSU_SB_2.0, GenBank Accession no. GCA_004916995.1). This version of the genome consists of 629 scaffolds with a final size of 598.11 Mb. Striped bass transcriptome data was used by NCBI for annotation and the fully annotated striped bass genome 2.0 is publicly available.

The Dovetail Genomics Hi-Rise pipeline was employed to create a white bass genome assembly (645.14 Mb in size), by scaffolding Illumina short read sequences with Illumina sequencing data generated by Chicago and Dovetail Hi-C pipelines.

The genome has been uploaded to NCBI GenBank (DOM_MoChry_2.0, GenBank Accession no. GCA_019097615.1) is publicly available. This genome has not yet been fully annotated.

Objective 2: Advance genome-to-phenome prediction by implementing strategies to identify and validate genes and allelic variants predictive of biologically and economically important phenotypes and traits.

Catfish (**Quiniou**, Liu)
The bacterium *Edwardsiella piscicida* causes significant losses in global aquaculture, particularly channel (*Ictalurus punctatus*) × blue (*I. furcatus*) hybrid catfish cultured in the south-eastern United States. Intraspecific genetic variability of *E. piscicida* isolates recovered from diseased channel and hybrid catfish were assessed and virulence associations among genetic variants was determined revealing 5 discrete phylogroups that correlated with virulence gene profiles.

Exogenous estrogen 17β-estradiol (E2) was shown to effectively induce feminization in catfish. Global DNA methylation and gene expression profiles of channel catfish during early sex differentiation after E2 treatment using Bisulfate sequencing and RNA-Seq. Levels of global DNA methylation after E2 treatment were not significantly different from those of controls. However, a specific set of genes were differentially demethylated, which included many sex differentiation-related pathways, such as MARK signaling, adrenergic signaling, Wnt signaling, GnRH signaling, ErbB signaling and ECM-receptor interactions. Many genes involved in these pathways were also differentially expressed after E2 treatment. Specifically, E2 treatments resulted in upregulation of female-related genes and downregulation of male-related genes in genetic males during sex reversal. However, E2-induced sex reversal did not cause sex-specific changes in methylation profiles or gene expression within the sex determination region (SDR) on chromosome 4, suggesting that E2-induced sex reversal was a downstream process independent of the sex determination process that was regulated by sex-specific methylation within the SDR.

Oyster/Shellfish (*Eastern Oyster Breeding Consortium*, Roberts, Gomez-Chiarri, Putnam, Lotterhoos, Puritz, Johnson, Eirin-Lopez, Allen, Zhang, Plough, **Proestou**)
Differences in DNA methylation patterns for 50 families of Pacific oyster (*Crassostrea gigas*) were characterized based on heat-tolerance. Hyper-methylated regions were more abundant than hypo-methylated regions among heat-phenotypes. Epigenetic marks associated to heat-resistant phenotypes occurred mainly in genes involved in catalytic activity and binding function among Pacific oyster families.

Complementary methods of metagenomics and metaproteomics were applied to more fully characterize bacterial taxa in shellfish hatchery water at high (pH 8.2) and low (pH 7.1) pH. The complementary methods of metagenomics and metaproteomics contribute to a more complete characterization of bacterial taxa that are potentially active versus truly metabolically active and thus impact water quality and inter-trophic relationships.

A seascape genomics approach was used to investigate population structure, local adaptation, and the extent to which environmental gradients influence genetic variation among natural and restored populations of Chesapeake Bay eastern oysters (*Crassostrea virginica*). Environmental factors, namely salinity, pH, and temperature, were found to play a major role in the distribution of neutral and adaptive genetic variation. This is one of a few studies to demonstrate empirically that hatchery practices have a major impact on the retention of genetic diversity.

A selectively bred Eastern oyster (*Crassostrea virginica*) family was exposed to *Perkinsus marinus* (Dermo disease) via either suspension feeding or direct injection and gene expression and GWAS was evaluated. Oysters exposed via feeding minimize infection through controlled cell death and limited inflammation responses, whereas oysters exposed via injection mount a prolonged, inflammatory immune response resulting in self harm and lower survival.

Completion of Affymetrix 60K SNP chip for U.S. East Coast eastern oyster populations. SNP array is available through ThermoFisher SKU #551377. Led by X. Guo, D. Proestou, J. Puritz of the *Eastern Oyster Breeding Consortium*.

Genomic Selection initiated in an eastern oyster breeding program. Led by J.M. Small (VIMS).

Salmonids (Salem, **Palti**, Al-Tobasei)
In a study of retrospective evaluation of genetic predictions accuracy with a training population size of 2,000 animals, genomic selection models increased the predictions accuracy compared to the traditional pedigree-based model by up to 50% for Fillet Yield and 28% for Body Weight, which suggests that using genomic selection can substantially enhance genetic improvement for the trait of fillet yield in rainbow trout aquaculture.

The genetic architecture of resistance to infectious hematopoietic necrosis virus (IHNV) was found to be oligogenic with a few moderate effects QTL and many loci with small effects in two commercial aquaculture breeding populations that were not previously exposed to the pathogen.

An improved genomic prediction for fillet yield and firmness in rainbow trout was achieved using reduced-density SNP panels.

A study revealed that sRNAs enriched in outer membrane vesicles of pathogenic *Flavobacterium psychrophilum* interact with immune genes of rainbow trout

Markers associated with adult migration timing were validated for natural and hatchery origin populations of Chinook salmon and steelhead, with genotypes explaining between 50-80% of phenotypic variation. Markers associated with age-at-maturity in steelhead were validated for natural and hatchery origin populations, with genotypes explaining 10-17% of phenotypic variation depending on sex.

Whole genome resequencing in coho salmon identified a sex determining region that was validated with amplicon assays, along with candidates for differing phenotypes of adult migration. Whole genome resequencing in ecotypes of redband trout identified a candidate gene (ceramide kinase; *cerk*) associated with thermal adaptation phenotypes. Whole genome resequening and landscape genomics analyses of Chinook salmon populations revealed candidate regions of the genome associated with environmental factors responsible for local adaptation.

Striped Bass (*National Breeding Program for the Hybrid Striped Bass Industry,* Berlinsky, Fuller, Abernathy, Woods, McGinty, Borski, **Reading**)
A machine learning pipeline was developed to analyze a dataset of 15,000 single nucleotide (SNP) markers (expressed quantitative trait loci, eQTL) that were identified among muscle transcriptome data generated from sunshine hybrid striped bass produced from striped bass males of varying geographic strains (Texas, Florida, South Carolina, Virginia, and North Carolina aquaculture domestic strain). The pipeline reduced these data to 500 unique SNPs that were considered important to the classification hybrid fish into groups based on size and sire strain (heterosis effects). When further condensed by the removal of redundant orthologs / paralogs, these 500 SNPs annotated to 33 genes, the primary functions of which were largely glucose and lipid metabolism, ATP synthesis, and structural support of muscle fibers. The novel machine

learning pipeline also enabled alleotyping of these genes. Specifically, we found that 9 of the 33 genes were inherited exclusively from the white bass (maternal) parent. All of these genes have a known role in the electron transport chain and generation of ATP. The remaining 24 genes were potentially inherited from either the striped bass or white bass parent. A deeper pathway analysis is currently being conducted to examine the relationship of these gene expressions in white muscle tissue for predictive phenomics.

Adult, male, F6 domestic striped bass (n=60) from our selective breeding program were disseminated to major aquaculture producers in the U.S. for hybrid striped bass fry and fingerling production (directly contributing to the $50 million farm gate per year industry). Additionally, fingerlings (n=40,000) and larvae (n=750,000) representing the F7 generation captive bred striped bass with 25% better growth were also disseminated to commercial aquaculture producers.

Piscidin antimicrobial peptides of the innate immune system were first described in hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). Previous work demonstrated broad antimicrobial activity of class I and II piscidins against Gram-negative and Gram-positive bacterial species. The class I and II piscidins demonstrate potent activity against *Escherichia coli* and *Flavobacterium columnare* biofilms. The class II piscidins showed more activity against *E. coli* and *F. columnare* isolates than did the class I piscidins. The piscidins in general were much less effective against inhibiting *Aeromonas hydrophila* and *A. veronii* biofilm growth; only the class I piscidins showed significant growth inhibition among the *Aeromonas* spp. examined.

Objective 3: Advance analysis, curation, storage, application, and reuse of heterogeneous big data to facilitate genome-to-phenome research in agricultural animal species of agricultural interest.

Catfish (**Quiniou**, Liu)
The rapid advances of second-generation sequencing (SGS) and third generation sequencing (TGS) is causing a paradigm shift from gene-based to genome-wide research in aquaculture; SGS and TGS could aid in establishing proper breeding strategies for sustainable aquaculture

Genomic selection plays an increasing role in selective breeding of the major aquaculture species. The potential of genomic selection to increase precision of estimated breeding value of candidates is now well established. The main challenge for further implementation of genomic selection is associated with the costs and benefits of genotyping; Methods of low-cost genotyping hold potential and will help transfer the benefits to aquaculture industries.

Oyster/Shellfish (*Eastern Oyster Breeding Consortium*, Gómez-Chiarri, Roberts, **Proestou**)
Eastern Oyster Genome Workshop, held during the *National Shellfisheries Association Annual Meeting*, March 22-25, virtual.

Salmonids (Salem, **Palti**, Al-Tobasei)
None from salmonids this year.

Striped Bass (USDA *National Breeding Program for the Hybrid Striped Bass Industry,* Abernathy, Borski, **Reading**, NOAA Sea Grant *StriperHub*)
Twelve (12) students (graduate and post doc) were trained in machine learning approaches in biological sciences including application of pattern recognition to evaluate gene and protein expression to predict traits and phenotypes in a variety of agriculturally important animals including fishes as well as poultry.

Digitization of machine learning training modules are being produced for the public and a CRISPR/Cas9 Guide to RNA design was published for student training on *CourseSource*: https://doi.org/10.24918/cs.2020.46.

Follow Striped Bass Genome Community and *StriperHub* research on Facebook: https://www.facebook.com/stripedbassgenome/

Research support mini-grants (Coordinator Small Research Project Grants)
Four (4) mini-grants ($10,000 each; $40,000 total) supported projects that fall under all three primary NRSP-8 objectives and include a variety of species as already reported in 2020-2021. Decisions regarding funding these projects were made in 2020, just prior to COVID-19 pandemic closures (in many states "shelter in place" executive orders), which made disbursement of funds challenging at best. These funds were dispersed in the 2021 calendar year due to relaxation of research and other work-related restrictions that had been implemented by various state and federal agencies across the country.
1. Shelly Trigg and Steven Roberts "Comparative Epigenomic Analyses Across Bivalve Genome Resources (CEABiGR)", University of Washington.
2. Russell Borski and Benjamin Reading "From Genotype to Phenotype: A Gene Editing Tool for Any Life History Stage using Adeno-Associated Viral Vectors for Application of CRISPR/Cas9 in Farmed Finfishes", North Carolina State University.
3. Refet Al-Tobesi and Moh Salem "FAASG Functional Annotation of the Rainbow Trout Genome: Role of DNA Methylation in Gene Expression", Middle Tennessee State.
4. Kevin Johnson, Morgan Kelly, and Jerome La Peyre "Transcriptome sequencing to describe the genomic basis for hypoxia tolerance in the Eastern oyster", Louisiana State University.

Another round of mini-grants were offered in 2021 and funded for 2022 (two grants, $10,000 each; $20,000 total):
1. Matthew George, Mackenzie Gavery, and Steven Roberts "Identifying genomic architecture features that contribute to critical phenotypes in shellfish", University of Washington.
2. Mohamed Salem "FAASG Functional Annotation of the Rainbow Trout Genome: Development of atlas of the RNA editome", University of Maryland.

Leveraged Funds and Stakeholder Use of Project Outputs
NRSP-8 2021-2022 Seed Funding: $20,000 ($40,000 in 2019-2020 delayed until 2020-2021 due to COVID-19; $30,000 in 2018-2019) Total Leveraged 2021 Funding: $ 2,218,362

Leveraged funds from diverse projects exceed $ 2,000,000 from federal sources, which is about a 1:34 return on investment of the $65,000 Aquaculture Coordinators funds for 2020-2021. Selected grants are highlighted below:
1. National Oceanic and Atmospheric Administration (NOAA), Saltonstall-Kennedy Program, Prime US Department of Commerce GRANT #573694-02785, *Collaboration with Local Fish Processing Industry to Convert Fish Trimmings and Skins into Value Added Fish Meal and Fish Oil to Promote Sustainability*. (PIs M.V. Joseph, A.V. Chouljenko, S.G. Hall, M.O. Frinsko, and B.J. Reading) $265,625 (09/01/2021 through 08/31/2023).
2. Rhode Island Sea Grant. *Does breeding disease-tolerant oysters increase disease in coastal marine ecosystems*? (PIs M. Gomez-Chiarri, D. Proestou, R. Hudson, T. Ben-Horin) $250,000 (2020 through 2023).
3. Northeast Regional Aquaculture Center (NRAC), US Department of Agriculture National Institute of Food and Agriculture (USDA NIFA). *Comparing the performance of diploid and triploid eastern oysters in the Northeast*. $250,000 (2021-2023).
4. Washington Sea Grant, GRANT#: R/SFA-11. *Enhancing sustainability of shellfish aquaculture through streamlined maturation control*. (Pis S. Roberts, A. Luckenbach, C. Trapnell, B. Vadopalas, T.-T. Wong) $175,947 (2020 through 2023).
5. Foundation for Food and Agriculture Research (FFAR). *Development of environmental conditioning practices to decrease impacts of climate change on shellfish aquaculture*. (PI S. Roberts) $753,716

(2018 through 2021).

6. National Science Foundation (NSF), GRANT #1634167. Collaborative Research: Does ocean acidification induce a methylation response that affects the fitness of the next generation in oysters? (PI S. Roberts) $429,674 (2017 through 2021).

7. Pacific States Marine Fisheries Commission. *Applying cutting-edge technology for reproductive control in emerging bivalve species*. (PI S. Roberts) $93,400 (2019 through 2022).

Other Active Research Projects and Total Leveraged Funds (Previously Reported)
Total Leveraged 2020 Funding: **$ 3,419,110** (1:53 return on investment)

Leveraged funds from diverse projects exceed $ 3.4 million from federal sources, which is about a 1:53 return on investment of the $65,000 Aquaculture Coordinators funds for 2019-2020. Selected grants are highlighted below:

1. US Department of Agriculture (USDA), USDA-NIFA special research grants program aquaculture research, GRANT # 2018-70007-28828, *Underlying mechanisms for selected disease resistance and enhanced non-specific resistance in rainbow trout*. (PI T. Welker) $309,489 (10/01/2018 through 09/30/2021).

2. US Department of Agriculture (USDA), USDA-NIFA-AFRI Foundational, Diseases of Agricultural Animals program area, GRANT # 2020-06096, *Seed Grant: Phage endolysins, Alternative antimicrobials for Streptococcus iniae*. (PI G. Ramena) $200,000 (02/01/2021 through 01/31/2022).

3. US Department of Agriculture (USDA), Agricultural and Food Research Initiative (AFRI), *FACT: AquaMine - A High Performance Genomic Data Mining System for Species of Importance to US Aquaculture*. (PI C. Elsik) $500,000 (4/1/2021-3/31/2025).

4. NOAA, *Leveraging transformative 'omics technologies to alleviate barriers to US shellfish production*. (PI S. Roberts) $233,135 (07/01/20 through 06/30/25).

5. NOAA, *Development of 'omics and bioinformatics approaches for marine organisms in support of research in aquaculture, ocean acidification, and fisheries assessments*. (PI S. Roberts) $285,153 (07/01/20 through 06/30/25).

6. NOAA, Washington Sea Grant, *Enhancing sustainability of shellfish aquaculture through streamlined maturation control*. (PI S. Roberts) $200,000 (02/01/20 through 01/31/23).

7. US Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA) GRANT # 2021-67015-33388, *Whole-Genome Analyses/Selection to Increase Muscle Yield and Reduce Fillet Downgrading In Rainbow Trout*. (PIs M. Salem, Leeds, T.I., Kumar, V.I., Smith, B.R., Cleveland, B.E., and Al-Tobesi, R.A.) $500,000 (2021 through 2025).

8. National Oceanic and Atmospheric Administration (NOAA), National Sea Grant Aquaculture Program, Advanced Aquaculture Collaborative Programs. *Establishing the Sea Grant Striped Bass Aquaculture Hub (StriperHub): Commercialization, Economics, and Marketing*. (PIs North Carolina Sea Grant, B.J. Reading--StriperHub Coordinator, R.J. Borski, D.L. Berlinsky) $1,191,333 (2/1/2020 through 01/30/2023).

Total Leveraged 2019 Funding: $ 6,340,999 (1:98 return on investment)

1. Southern Regional Aquaculture Center, US Department of Agriculture National Institute of Food and Agriculture (USDA NIFA). *Evaluation of Probiotics in Finfish Hatcheries to Improve Larval Production*. (PIs M.O. Frinsko, S.G. Hall, B.J. Reading) $150,000 (09/01/2018 through 08/31/2021).

2. US Department of Agriculture (USDA), Agricultural and Food Research Initiative (AFRI), *Molecular basis of sex determination and differentiation in catfish*. (PIs Z.J. Liu, R. Dunham) $500,000 (6/1/2019 through 5/31/2022).

3. NOAA, *Developing new oyster sterilization technology to avoid triploid summer mortality*. (PI L. Plough) $100,000.

4. Ratcliffe Foundation, *Shellfish Aquaculture Innovation Laboratory (SAIL): Using Science and New Technologies to Assist Shellfish Aquaculture Businesses in*

Maryland. (PI L. Plough) $391,000.
5. NOAA Regional Shellfish Aquaculture Consortium Grants, States Marine Fisheries Commissions, *From sequence to consequence: genomic selection to expand and improve selective breeding for the eastern oyster*. (PIs Eastern Oyster Breeding Consortium, X. Guo - Coordinator) $4,400,000 (8/1/2019 through 7/31/2024); similar grants were also awarded to the Gulf of Mexico and Pacific States.
6. US Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA) Special Research Grants Program Aquaculture, *Modifying microbiomes to mitigate infectious diseases in aquaculture facilities*. (PI M. Gomez-Chiarri) $299,999 (10/1/2019 through 9/30/2021).
7. US Department of Agriculture (USDA), National Institute of Food and Agriculture (NIFA) GRANT # 2018-06539, *High-Quality Reference Assembly and Annotation of the Rainbow Trout Genome*. (PIs M. Salem, Y. Palti, G. Gao, H. Zhou) $500,000 (2019 through 2022).

Publications (32 in 2021; 30 in 2020; 29 in 2019; 47 in 2018; **138 TOTAL):**
1. Johnson, K.M., Sirovy, K.A., and Kelly, M.W. 2021. Differential DNA methylation across environments has no effect on gene expression in the eastern oyster. *The Journal of Animal Ecology* 00:1-13 https://doi.org/10.1111/1365-2656.13645
2. Sirovy, K.A., Johnson, K.M., Casas, S.M., La Peyre, J.F., and Kelly, M.W. 2021. Lack of genotype-by-environment interaction suggests limited potential for evolutionary changes in plasticity in the eastern oyster, *Crassostrea virginica*. *Molecular Ecology* 30(22):5721-5734.
3. Griffiths, J.S., Johnson, K.M., and Kelly, M.W. 2021. Evolutionary Change in the Eastern Oyster, *Crassostrea Virginica*, Following Low Salinity Exposure. *Integrative and Comparative Biology* 61(5):1730-1740.
4. Trigg, S.A., Venkataraman, Y.R., Gavery, M.R., Roberts, S.B., Bhattacharya, D., Downey-Wall, A., Eirin-Lopez, J.M., Johnson, K.M., Lotterhos, K.E., Puritz, J.B., and Putnam, H.M. 2021. Invertebrate methylomes provide insight into mechanisms of environmental tolerance and reveal methodological biases. *Molecular Ecology Resources* 00:1-15 https://doi.org/10.1111/1755-0998.13542
5. Chandra Rajan, K., Meng, Y., Yu, Z., Roberts, S.B. and Vengatesen, T. 2021. Oyster biomineralization under ocean acidification: From genes to shell. *Global Change Biology* 27:3779-3797.
6. Modak, T., Litterman, R., Puritz, J., Johnson, K., Roberts, E., Proestou, D.A., Guo, X., Gomez-Chiarri, M., and Schwartz, R. 2021. Extensive genome-wide duplications in the eastern oyster (*Crassostrea virginica*). *Philosophical Transactions of the Royal Society B* 376:20200164.
7. Sullivan, M., and Proestou, D.A. 2021. Survival and transcriptomic responses to different *Perkinsus marinus* exposure methods in an Eastern oyster family. *Aquaculture* 542:736831.
8. Hornick, K.M., and Plough, L.V. 2021. Genome-wide analysis of natural and restored eastern oyster populations reveals local adaptation and positive impacts of planting frequency and broodstock number. *Evolutionary Applications* https://doi.org/10.1111/eva.13322
9. Timmins-Schiffman, E., White, S.J., Thompson, R.E., Vadopalas, B., Eudeline, B., Nunn, B.L., and Roberts, S.B. 2021. Coupled microbiome analyses highlights relative functional roles of bacteria in a bivalve hatchery. *Environmental Microbiome* 16:7 https://doi.org/10.1186/s40793-021-00376-z
10. Arredondo-Espinoza, R., Ibarra, A.M., Roberts, S.B., Sicard-Gonzalez, M.T., and Escobedo-Fregoso, C. 2021. Differentially methylated gene regions between resistant and susceptible heat-phenotypes of the Pacific oyster *Crassostrea gigas*. *Aquaculture* 543:736923.
11. Alshwairikh, Y.A., Kroeze, S.L., Olsson, J., Stephens-Cardenas, S.A., Swain, W.L., Waits, L.P., Horn, R.L., Narum, S.R., and Seaborn, T. 2021. Influence of environmental conditions at spawning sites and migration routes on adaptive variation and population connectivity in Chinook salmon. *Ecology and Evolution* https://doi.org/10.1002/ece3.8324
12. Chen, Z., Grossfurthner, L., Loxterman, J.L., Masingale, J., Richardson, B.A., Seaborn, T., Smith, B., Waits, L.P., and Narum, S.R. 2021. Applying genomics in assisted migration under climate change: Framework, empirical applications, and case studies. *Evolutionary Applications* https://doi.org/10.1111/eva.13335

13. Horn, R.L., C. Kamphaus, K. Murdoch, Narum, S.R. 2020. Detecting genomic variation underlying phenotypic characteristics of reintroduced Coho salmon. *Conservation Genetics* 21:1011-1021.

14. Waters, C.D., A. Clemento, T. Aykanat, J.C. Garza, K.A. Naish, S.R. Narum, and Primmer, C.R. 2021. Heterogeneous genetic basis of age at maturity in salmonid fishes. *Molecular Ecology* 30:1435-1456.

15. Willis, S.C., Hess, J.E., Fryer, J.K., Whiteaker, J.M., Brun, C., Gerstenberger, R. and Narum, S.R. 2020. Steelhead (*Oncorhynchus mykiss*) lineages and sexes show variable patterns of association of adult migration-timing and age-at-maturity traits with two genomic regions. *Evolutionary Applications* 13: 2836-2856.

16. Willis, S.C., J.E. Hess, J.K. Fryer, J.M. Whiteaker, and Narum, S.R. 2021. Genomic region associated with run timing has similar haplotypes and phenotypic effects across three lineages of Chinook salmon. *Evolutionary Applications* 14:2273-2285.

17. Liu, S., Gao, G., Layer, R.M., Thorgaard, G.H., Wiens, G.D., Leeds, T.D., Martin, K.E., and Palti, Y. 2021. Identification of High-Confidence Structural Variants in Domesticated Rainbow Trout Using Whole-Genome Sequencing. *Frontiers in Genetics* 12:DOI 10.3389/fgene.2021.639355.

18. Vallejo, R.L., Cheng, H., Fragomeni, B.O., Gao, G., Silva, R.M.O., Martin, K.E., Evenhuis, J.P., Wiens, G.D., Leeds, T.D., and Palti, Y. 2021. The accuracy of genomic predictions for bacterial cold water disease resistance remains higher than the pedigree-based model one generation after model training in a commercial rainbow trout breeding population. *Aquaculture* 545:737164.

19. Chapagain, P., Ali, A.R., Kidane, D.T., Farone, M., and Salem, M. 2021. sRNAs enriched in outer membrane vesicles of pathogenic *Flavobacterium psychrophilum* interact with immune genes of rainbow trout. bioRxiv 2021. https://doi.org/10.1101/2021.12.22.473952

20. Ali, A., Thorgaard, G.H., and Salem, M. 2021. PacBio Iso-Seq Improves the Rainbow Trout Genome Annotation and Identifies Alternative Splicing Associated With Economically Important Phenotypes. *Frontiers in Genetics* 12:683408.

21. Al-Tobasei, R., Ali, A., Garcia, A.L.S., Lourenco, D., Leeds, T., and Salem, M. 2021. Genomic predictions for fillet yield and firmness in rainbow trout using reduced-density SNP panels. *BMC Genomics* 22:92 https://doi.org/10.1186/s12864-021-07404-9

22. Wong, L.L., Razali, S.A., Deris, Z.M., Danish-Daniel, M., Tan, M.P., Nor, S.A.M., Ma, H., Min, W., Yantao, L., Asaduzzaman, M., Sung, Y.Y., Liu, Z., Sorgeloos, P., Van de Peer, Y., and Afiqah-Aleng, N. 2022. Application of second-generation sequencing (SGS) and third generation sequencing (TGS) in aquaculture breeding program. *Aquaculture* 548:737633.

23. Crider P., Quiniou S.M.A., Felch K.L., Showmaker K., Bengtén E. and M. Wilson. 2021. A Comprehensive Annotation of the Channel Catfish (*Ictalurus punctatus*) T Cell Receptor Alpha/Delta, Beta, and Gamma Loci. *Frontiers in Immunology* 25 November 2021 12:786402.

24. Boudry, P., Allal, F., Aslam, L., Bargelloni, L., Bean, T.P., Brard-Fudulea, S., Brieuc, M.S.O., Calboli, F.C.F., Gilbey, J., Haffray, P., Lamy, J.-B., Morvezeen, R., Purcell, C., Prodohl, P.A., Vandeputte, M., Waldbieser, G.C., Sonesson, A.K., and Houston, R.D. 2021. Current status and potential of genomic selection to improve selective breeding in the main aquaculture species of International Council for the Exploration of the Sea (ICES) member countries. *Aquaculture Reports* 20:100700.

25. López-Porras, A., Griffin, M.J., Armwood, A.R., Camus, A.C., Waldbieser, G.C., Ware, C., Richardson, B., Greenway, T.E., Rosser, T.G., Aarattuthodiyil, S., and Wise, D.J. 2021. Genetic Variability of *Edwardsiella piscicida* Isolates from Mississippi Catfish Aquaculture With An Assessment of Virulence In Channel And Channel × Blue Hybrid Catfish. *Journal of Fish Diseases* 44:1725-1751.

26. Berlinsky, D.L., Goetz, F., Kenter, L., and Reading, B.J. 2021. Regulating reproductive cycles for captive spawning. *In* Fish Physiology Volume 38: Aquaculture (Benfey, T.J., Farrell, A.P. and Brauner, C.J., Eds.).

27. Bashura, J., Burke, M., Lapitan, R., Mikulec Jr., J., Owens, T., Reading, B.J.*, Richt, J.A., Spencer, D., Valdivia-Granda, W.*, Weekes, J., Wittrock, M., and Wright, D. 2021. Threats to Food and Agricultural Resources. United States Department of Homeland Security, Office of Intelligence and Analysis, Analytic Exchange Program. 95 pp. *Authors wrote the first draft and initial revision of this

manuscript (authors are otherwise listed in alphabetical order). https://www.dhs.gov/sites/default/files/publications/threats_to_food_and_agriculture_resources.pdf

28. Prior, B.S., Lange. M.D., Salger, S.A., Reading, B.J., Peatman, E., and Beck, B.H. 2022. The effect of piscidin antimicrobial peptides on the formation of Gram-negative bacterial biofilms. *Journal of Fish Diseases* 45:99-105.

29. Andersen, L.K., Abernathy, J., Berlinsky, D.L., Bolton, G., Booker, M., Borski, R.J., Brown, T., Cerino, D., Ciaramella, Clark, R.W., M., Frinsko, M.O., Fuller, S.A., Gabel, S., Green, B.W., Herbst, E., Hodson, R.G., Hopper, M., Kenter, L.W., Lopez, F., McGinty, A.S., Nash, B., Parker, M., Pigg, S., Rawles, S., Riley, K., Turano, M.J., Webster, C.D., Weirich, C.R., Won, E., Woods III, L.C., and Reading, B.J., on behalf of StriperHub. 2021. The Status of Striped Bass, *Morone saxatilis*, as a Commercially Ready Species for U.S. Marine Aquaculture. *Journal of the World Aquaculture Society* 52(3) 710-730 (Invited Review).

30. Demi, L.M., Taylor, B.W., Reading, B.J., Tordoff, M.G., and Dunn, R.R. 2021. Understanding the evolution of nutritive taste in animals: Insights from biological stoichiometry and nutritional geometry. *Ecology and Evolution* 11:8441-8455.

31. Andersen, L.K., Clark, R.W., Hopper, M.S., Hodson, R.G., Schilling, J., Kenter, L., Daniels, H.V., Woods III, L.C., Kovach, A., McGinty, A.S., Berlinksy, D.L., and Reading, B.J. 2021. Methods of domestic striped bass (*Morone saxatilis*) spawning that do not require the use of any hormone induction. *Aquaculture* 533:736025.

32. Andersen, L.K., Kenter, L.W., Clark, R.W., McGinty, A.S., Hopper, M.S., Salger, S.A., Schilling, J., Hodson, R.G., Kovach, A., Berlinksy, D.L., and Reading, B.J. 2021. Volitional tank spawning of domestic striped bass (*Morone saxatilis*) using human chorionic gonadotropin (hCG) and gonadotropin releasing hormone analogue (GnRHa) induced 'pace-setting' females. *Aquaculture* 532:735967.

Bioinformatics NRSP-8 Coordination Program 2021 Activities
Supported by Regional Research Funds, Hatch Act

Joint Coordinators: James Reecy, Iowa State University
James Koltes, Iowa State University
Fiona McCarthy, University of Arizona

Overview: Coordination of the NIFA National Animal Genome Research Program's (NAGRP) Bioinformatics is primarily based at, and led from, Iowa State University (ISU), with additional activities at the University of Arizona (UA), and is supported by NRSP-8. The NAGRP is made up of the membership of the Animal Genome Technical Committee, including the Bioinformatic Subcommittee.

Objectives: The NRSP-8 project was renewed as of 10/01/18, with the following objectives: 1. Advance the quality of reference genomes for all agri-animal species by providing high contiguity assemblies, deep functional annotations of these assemblies, and comparison across species to understand structure and function of animal genomes; 2. Advance genome-to-phenome prediction by implementing strategies and tools to identify and validate genes and allelic variants predictive of biologically and economically important phenotypes and traits; and 3. Advance analysis, curation, storage, application, and reuse of heterogeneous big data to facilitate genome-to-phenome research in animal species of agricultural interest.

Objective 1: Advance the quality of reference genomes for all agri-animal species by providing high contiguity assemblies, deep functional annotations of these assemblies, and comparison across species to understand structure and function of animal genomes.

Objective 2: Facilitate the development and sharing of animal populations and the collection and analysis of new, unique, and interesting phenotypes.

Objective 3: Advance analysis, curation, storage, application, and reuse of heterogeneous big data to facilitate genome-to-phenome research in animal species of agricultural interest.

The following describes the project's activities over this past year.

Multi-species support

The Animal QTLdb, CorrDBMulti-species support

The Animal QTLdb, CorrDB, NAGRP Bioinformatics Tools, and the NAGRP data repository have been actively supporting the research activities for multiple species. The QTLdb has been accommodating active curation of QTL/association data for seven species (cattle, catfish, chicken, horse, pig, rainbow trout, and sheep). In 2021, a total of 24,178 new QTL/association data were curated into the database, bringing the total number of curated data to 235,970 QTL/associations. Currently, there are 34,342 curated porcine QTL, 177,199 curated bovine QTL, 16,217 curated chicken QTL, 2,605 curated horse QTL, 6,072 curated sheep QTL, and 1,413 curated rainbow trout QTL in the database (https://urldefense.com/v3/__https://www.animalgenome.org/QTLdb/__;!!JYXjzlvb!2q284OVu7UwcaM 0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJlxGccn9g$). An additional 3,068 correlations (increase by species: cattle, 1,554; chicken: 208; goat: 311; pig: 846; sheep: 149) and 1,237 heritability data (increase by species: cattle: 315; chicken: 32; goat: 2; pig: 843; sheep: 45) were curated into the Animal CorrDB in 2021. Currently there are a total of 24,104 correlation data on 874 traits and 4,319 heritability data on 1,075 traits in 6 livestock animal species (this summary includes a decrease of 1,241 data recalled as part of data quality control efforts).

A new livestock SNP ID/name matching data repository and search tool has been added to the NAGRP Bioinformatics Tools. This data collection includes 7,856,530 known SNP IDs/names and 5,055,768 SNP 'rs' to 'ss' ID matches contributed by 10 labs/research groups, and these data are not found in any other public SNP data resources. We continue to welcome such SNP data contributions to this repository.

Ontology development
We have developed a hierarchy display tool to facilitate expanding and exploring the Vertebrate Trait (VT) Ontology, Livestock Product Trait (LPT) Ontology, Clinical Measurement Ontology (CMO), and other ontology hierarchies. This tool has been implemented as part of the web portals for Animal QTLdb, VT, LPT, and CMO project websites.

This past year we continued to focus on the integration of the Animal Trait Ontology into the Vertebrate Trait Ontology (https://urldefense.com/v3/__http://bioportal.bioontology.org/ontologies/VT__;!!JYXjzlvb!2q284OVu7U wcaM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJm9osJu0w$).

Fifteen (15) dataset updates were released to the public throughout 2021. We have continued working with the Rat Genome Database to integrate ATO terms that are not applicable to the Vertebrate Trait Ontology into the Clinical Measurement Ontology (https://urldefense.com/v3/__http://bioportal.bioontology.org/ontologies/CMO__;!!JYXjzlvb!2q284OVu7 UwcaM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJlmeJ5Szw$).

Traits specific to livestock products continue to be incorporated into a Livestock Product Trait Ontology (LPT), which is available on NCBO's BioPortal

(https://urldefense.com/v3/__http://bioportal.bioontology.org/ontologies/LPT__;!!JYXjzlvb!2q284OVu7 UwcaM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJnUxIP40A$).

Three (3) LPT updates were released during 2021. Seven (7) updates of Livestock Breed Ontology (LBO; https://urldefense.com/v3/__https://www.animalgenome.org/bioinfo/projects/lbo/__;!!JYXjzlvb!2q284O Vu7UwcaM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJkuuPKz2w$) were made.

We have also continued mapping the cattle, pig, chicken, sheep, and horse QTL traits to the Vertebrate Trait Ontology (VT), LPT, and Clinical Measurement Ontology (CMO) to help standardize the trait nomenclature used in the QTLdb. A semi-automated data release pipeline was developed to minimize the manual steps involved in new data upload and version release to BioPortal.ORG and GitHub with AnimalGenome.ORG as a new data sync hub. The VT data download is available through the Github portal (https://urldefense.com/v3/__https://github.com/AnimalGenome/vertebrate-trait-ontology__;!!JYXjzlvb!2q284OVu7UwcaM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJm P9ShxIQ$) where users can automate their data updates. Anyone interested in helping to improve the ATO/VT is encouraged to contact James Reecy (jreecy@iastate.edu), Cari Park (caripark@iastate.edu), or Zhiliang Hu (zhu@iastate.edu). The VT/LPT/CMO cross-mapping has been well employed by the Animal QTLdb, CorrDB, and VCMap tools. Annotation to the VT is also available for rat QTL data in the Rat Genome Database and for mouse strain measurements in the Mouse Phenome Database. We have also continued to integrate information from multiple resources, e.g. FAO - International Domestic Livestock Resources Information, Oklahoma State University - Breeds of Livestock web site, and Wikipedia, as well as requests from community members.

Expanded Animal QTLdb functionality
We made efforts to enable the support of multiple genome builds for all livestock species by creating a pipeline lifting SNPs between different assemblies using SAMtools, BEDtools, BWA, and locally developed Perl scripts. All curated QTL/association data continue to be automatically ported to NCBI, Ensembl, UCSC genome browser, and Reuters Data Citation Index in a timely fashion. Users can fully utilize the browser and data mining tools at NCBI, Ensembl, and UCSC to explore animal QTL/association data. Efforts were continually made, working with our counterparts at these institutions, to eliminate any glitches that arose during the automated or semi-automated data porting process. In addition, we have continued to improve existing and add new QTLdb curation tools and user portal tools. Other improvements included the standardization of data links across species for external databases (db_xref) for both QTLdb and CorrDB; improved editor/curator tools to aid SNP name/ID look up and batch annotation for QTL/association data curation; and more improvements on eQTL data display and batch annotations. More improvements and developments as an on-going process are continually being carried out.

Further developments of Animal Trait Correlation Database (CorrDB)
Our efforts to overhaul and re-develop functionality within CorrDB are ongoing. We continued to strengthen the data quality control procedures to help improve data quality. The new outcome is a re-designed web interface for users to more easily access data by species (the front page). Internally, standardization of program configurations for parameters and functions will help to streamline future tool development and debugging efforts. The CorrDB efforts continue to feature co-development with the QTLdb for shared use of resources and tools, such as trait ontology development and management, literature management, breed ontology management, and bug reporting tools for improved data quality control. The improved CorrDB curator tools are available to the public for any user to register for an account to curate correlation data. As reported in earlier sections, in 2021, correlation data and heritability data continued to be curated. The public web portals continue to undergo improvement.

Facilitating research

The Data Repository for the aquaculture, cattle, chicken, horse, pig, and sheep communities to share their genome analysis data has proven to be very useful and has been actively used (https://urldefense.com/v3/__https://www.animalgenome.org/repository__;!!JYXjzlvb!2q284OVu7Uwca M0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJkhHH6Wuw$). While new data is continually being curated, we have gradually scaled down the support for hosting supplementary files for publications for more sensible use of the NRSP8 bioinformatics funds. We have redirected the community to a better data repository resource (Open Science Framework, OSF, https://urldefense.com/v3/__https://osf.io/__;!!JYXjzlvb!2q284OVu7UwcaM0zLzVS0H_HDltGOl4CGd cmSfGeqz4qt9OKdgVmjJh4LJmpJVnrKg$) for better long-term data security. In 2021, the data currently in the supplementary data repository was prepared for transfer to OSF in the coming months. Appropriate web visit forwarding will be set up on the current site to redirect to the new URL.

The data downloads from the repository generated over 2.05 TB of data traffic in 2021. Throughout the year, over 62 cases were handled through our helpdesk at AnimalGenome.ORG to help users with inquiries/requests for services affecting community research activities and the use of our services. Provided assistance ranged from data transfer and hosting, data deposition, data curation, web presentation, and data analysis, to software applications, code development, advice for tool developments, etc.

Community support and user services at AnimalGenome.ORG

We have been maintaining and actively updating the NRSP-8 species web pages for each of the six NRSP-8 species. We continue to host mailing lists/websites for various research groups in the NAGRP community (https://urldefense.com/v3/__https://www.animalgenome.org/community/__;!!JYXjzlvb!2q284OVu7Uwc aM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJkyDH-Tzg$). This includes groups like AnGenMap, FAANG international consortium working groups, and CRI-MAP users, new meetings, and user bulletin boards to facilitate these meetings, among other user forums (https://urldefense.com/v3/__https://www.animalgenome.org/community__;!!JYXjzlvb!2q284OVu7Uwc aM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJlj2OyQyg$).

The Functional Annotation of ANimal Genomes (FAANG, https://urldefense.com/v3/__https://www.faang.org/__;!!JYXjzlvb!2q284OVu7UwcaM0zLzVS0H_HDlt GOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJk-uG_w4g$) website has been continually developed and maintained to actively support the FAANG activities. The FAANG site serves not only as a FAANG-related information hub, but also as a platform for this international consortium's communication, collaboration, organization, and interaction. It serves over 760 members and 12 working groups and sub-groups, with 14 listserv mailing lists, bulletin board, database, and tools for membership and working group management. The actively hosted materials include meeting minutes, tools/protocols for FAANG activities, incorporation and use of data portal hosted at EBI, presentation slides, and video records of scientific meetings and related events, all interactively available to members through the web portal.

Site maintenance

We have further consolidated services and developmental platforms to the current Dual Quad Core Xeon Linux server. Efforts were made to improve data backup, security, and availability. This was accomplished by better use of the resources for shared workloads, better data security and network security, and improved protocols for data backup, management, and inventories.

Reaching out

We have been sending periodic updates to more than 3,000 users worldwide (https://urldefense.com/v3/__https://www.animalgenome.org/community/angenmap/__;!!JYXjzlvb!2q28 4OVu7UwcaM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJk7a4fJLw$) to inform the animal genomics research community of the news and updates regarding AnimalGenome.org. "What's New

on AnimalGenome.ORG web site" emails were sent out 3 times in 2021, consistent with the pace/pattern of the past 17 years (https://urldefense.com/v3/__https://www.animalgenome.org/bioinfo/updates/__;!!JYXjzlvb!2q284OVu7 UwcaM0zLzVS0H_HDltGOl4CGdcmSfGeqz4qt9OKdgVmjJh4LJmemFCuqA$).

<u>Plans For the Future</u>
Objective 1. Advance the quality of reference genomes for all agri-animal species by providing high contiguity assemblies, deep functional annotations of these assemblies, and comparison across species to understand structure and function of animal genomes.

We will continue to analyze "omics" data to help better annotate livestock genomes.

Objective 2. Advance genome-to-phenome prediction by implementing strategies and tools to identify and validate genes and allelic variants predictive of biologically and economically important phenotypes and traits.

Objective 3. Advance analysis, curation, storage, application, and reuse of heterogeneous big data to facilitate genome-to-phenome research in animal species of agricultural interest.

We will continue to work with bovine, mouse, rat, and human QTL database curators to develop minimal information for publication standards. We will also work with these same database groups to improve phenotype and measurement ontologies, which will facilitate transfer of QTL information across species. We will continue working with U.S. and European colleagues to develop a Bioinformatics Blueprint, similar to the Animal Genomics Blueprint recently published by USDA-NIFA, to help direct future livestock-oriented bioinformatic/database efforts.

Publications:
Hu, Zhi-Liang, Carissa A. Park, and James M. Reecy (2022). Bringing the Animal QTLdb and CorrDB into the future: meeting new challenges and providing updated services. Nucleic Acids Research, Volume 50, Issue D1, Pages D956â€"D961. DOI: 10.1093/nar/gkab1116

<u>Cattle NRSP-8 Annual Report of Cooperative Regional Projects 2021</u>
Supported by Allotments of the Regional Research Funds, Hatch Act
January 1 to December 31, 2021

Coordinator: Alison Van Eenennaam, University of California, Davis (<u>alvaneenennaam@ucdavis.edu</u>)
Co-cordinators: Bob Schnabel, University of Missouri-Columbia (<u>schnabelr@missouri.edu</u>)
                Clare Gill, Texas A&M University (<u>clare-gill@tamu.edu</u>)
                Ben Rosen, USDA ARS, Beltsville (<u>Ben.Rosen@ars.usda.gov</u>)
                Zhihua Jiang, Washington State University (<u>jiangz@wsu.edu</u>)

Professional Meetings:
Because of the Corona virus pandemic, PAG 2022 was not held at its normal time in January, 2022. Darren Hagen and Erdogan Memili organized morning session at the NRSP8 meeting in April, 2022. Coordinator funds were used to support the attendance of Darren Hagen and Joao Reboucas Dorea at AGBT 2022.

Four Ph.D. students were selected for the Jorgenson Travel awards

1. Gabriel Antonio Zayas Santiago, University of Florida (plans to attend PAG 2023) "Identification of Breed of Origin Runs-of-Homozygosity and their Effect on Meat Traits in the Multibreed Angus-Brahman Herd"
2. Emory Pacht, University of Vermont (plans to attend PAG 2023) "Identification of 5-hydroxymethylcytosine markers across four tissues in the cattle brain"
3. Lihe Liu, University of Wisconsin (plans to attend PAG 2023) "Maternal diet induces persistent epigenetic changes in the muscle of beef calves"
4. Emmanuel André Lozada-Soto, North Carolina State University (plans to attend AGBT 2022) "Current State of Inbreeding, Genetic Diversity, and Selection History in All Major Breeds of U.S. Dairy Cattle"

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.

Cattle coordinator funds were allocated to Darren Hagen at Oklahoma State to collect semen via electroejaculation from a Bos javanicus (Banteng) bull. The collection of semen from the banteng bull will be used for development of F1 hybrid fetuses and supports the cattle community's efforts to sequence extant Bos species and create a pan-genome. A private bovine reproduction company was contracted to sedate and ejaculate the bull, collect semen and blood, run health assays from the collected blood, assess semen quality, and place semen in straws and freeze for future use in in vitro fertilization. Sixty straws of usable quality semen were generated and are currently being stored in liquid nitrogen tanks. Current discussions between Oklahoma State University, collaborating scientists with USDA, and breed associations (Ayrshire, Shorthorn, and Senepol) are underway to identify donor cows for oocyte retrieval and recipient cattle for embryo transfer. The use of the cattle coordinator funds allocated to Darren Hagen for banteng semen collection allowed a unique training experience for a current Oklahoma State PhD student will generate sequence data for contribution to the pan-genome efforts. (Daren Hagen, OK)

Cattle coordinator funds were allocated to Ben Rosen to do trip sequencing. Money will support Illumina sequencing of 6 trios (30x coverage for each parent and 60x for offspring) for the Bovine Pangenome Consortium. Breeds to be sequenced are Yakut, Welsh Black, Butana, Maine-Anjou, Guzerat_x_Gelbvieh, and Charolais_x_Retinto. Sequence will be used for haplotype resolution of long-read assemblies and polishing. (Ben Rosen, USDA ARS).

As a member of the Cattle Pangenome Consortium, Brenda Murdoch (University of Idaho) and Holly Neibergs (Washington State University), worked together to generate an F1 cross from a UI Charolais cow and semen from a WSU wagyu bull. The goal of this project is to generate haploid genome assemblies for both Charolais and Wagyu cattle. Tissues from the FI fetus and the cow as well as semen from the bull were sent to Dr. Timothy Smith at USDA ARS, Clay Center, NE for sequencing. (Brenda Murdoch, ID)

Three projects are ongoing in the Neibergs lab. One is to validate loci associated with bovine respiratory disease (BRD) in cattle and to facilitate accurate prediction of cattle that are less likely to become ill with BRD. A second project is focused on validating loci and integrating functional information associated with fertility in cattle. The third project is aimed at education and establishment of genomic selection as a financial risk management tool. (Holly Neibergs, WA)

The Jiang lab has mainly focused on issues related to ovary function, oocyte quality and embryo development for reduced reproduction loss. We have collected 228 RNA-seq datasets released by twelve laboratories involving corpus lutea (16 samples), embryos (61 samples), granulosa cells (72 samples), oocytes (12 samples), ovary (34 samples) and theca cells (33 samples). Among approximately 6,575 million reads, 5,109 million were mapped to the current bovine reference genome. Such large datasets certainly help us obtain information on transcriptome progression and switches from ovary and their

associated cells to oocytes to embryos. The mapping process also assembles a large number of alternative transcripts, which would serve as references to annotate alternative polyadenylation sites executed in the bovine genome (Zhihua Jiang, WA).

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.

The Bovine FAANG Project is based on collaborative work organized by many NRSP8 cattle genomics members. The research performed FAANG assays on tissues from the bovine reference genome to create a world-class annotation to genome assembly. This project has contributed to the core activities of FAANG by providing transcriptome, ChIP seq, ATAC seq, and methylation data to enhance the annotation of the reference genome. Furthermore, physiological relevant tissues like mammary gland, fetal tissues and primary cell lines have been generated and are being analyzed to augment the annotation knowledge of the bovine genome.

NRSP8 cattle coordinator funds were used to purchase supplies to perform Chromatin Immunoprecipitation (ChIP) for several histones. ChIP was performed on fives tissues (heart, spleen, muscle, brain, liver, kidney) collected from four L1 line fetuses using antibodies for H3K4me3, H327me3, H3K4me1, H3K9me3, H3K36me3 histones and CTCF. After the ChIP pull downs the DNA was purified DNA and along with the input controls and were sent to the Meat Animal Research Center in Clay Center Nebraska for library sequencing preparation. The 147 libraries were sent to Zoetis for sequencing. The sequence data were returned to UC Davis and are currently being analyzed. These data will be included with the other tissues and assay analyses for eventual publication. (Brenda Murdoch, ID)

More in-depth sequences on a few of ChIP-seq assays including H3K4me1, H3K27me3, H3K27ac, CTCF in a few key tissues (colon, omasum, rumen, abomasum and testis) from bovine FAANG Annotation project sample archive were performed, in order to better annotate chromatin states in each of these important tissues (Huaijun Zhou, CA).

Wansheng Liu, (PA) continued to work on the USDA-NIFA supported cattle FAANG project, and to analyze the RNA-seq and small RNA-seq sequence data with Dr. James Reecy's lab at Iowa State University and other collaborators in the project. Here is a summary presented by Dr. Hamid Beiki (at ISU) in ISAG meeting on behalf of all researchers in the project. The extent of RNA transcript/miRNA diversity among 47 different bovine tissues/cell-types was assessed using poly(A) selected RNA-seq and miRNA-seq data from Hereford cattle closely related to Dominette L1, the individual from which the reference bovine genome was sequenced. A total of approximately 4.1 trillion RNA-seq reads and 1.9 billion miRNA-seq reads were collected, with a minimum of 27.5 million (M) RNA-seq and 4.2 M miRNA-seq reads from each tissue (average 87.8 M ± 49.7 M and 26.6 M ± 13.3 M, respectively). A total number of 171,985 unique transcripts (50% protein-coding) and 35,150 unique genes (64% protein-coding) were identified across tissues. A total of 159,033 transcripts (92% of predicted transcripts) were structurally validated by independent datasets such as Pacific Biosciences single-molecule long-read isoform sequencing, Oxford Nanopore Technologies sequencing, denovo assembled transcripts from RNA-seq, Ensembl and NCBI gene sets. In addition, all transcripts were extensively supported by independent data from different technologies such as Transcriptome Termini Site Sequencing (WTTS), RAMPAGE (RNA Annotation and Mapping of Promoters for the Analysis of Gene Expression), different type of histone modification data (H3K4me3, H3K4me1, H3K27ac and CTFC) and ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing). A large proportion of transcripts (69%) were novel, which were mostly produced by known protein-coding genes (87%), while 13% corresponded to novel genes. The median number of transcripts per known gene (tpg) was four, which was higher than that was observed in either the Ensembl (1.5 tpg) or NCBI (2.3 tpg) annotated gene sets. Our new bovine genome annotation extended

more than 11,000 known gene borders (5'-end extension, 3'-end extension, or both) compared to EBI or NCBI annotations. Furthermore, we detected 12,698 novel genes (80% non-coding), which are not reported in current bovine genome annotations. A majority of these genes were structurally validated by independent data (85%) or were replicated in multiple tissues (96%). These validated results show significant improvement over current bovine genome annotations (Wansheng Liu, PA).

The Neibergs lab has conducted whole genome sequencing in different breeds of cattle with detailed phenotypes on fertility to associate functional information through ChIP-seq, and ATAC-seq with the genomic variation identified through sequencing. This information is also being compared with RNA-seq information on phenotyped animals. This work is being done to identify possible regulatory regions for loci associated with fertility and to identify putative causal variants (Holly Neibergs, WA).

Work towards this objective at WI involves studies characterizing a major gene (Trio allele) for bovine ovulation rate in cattle. A *de novo* sequence assembly of a homozygote for the allele has been created and unique variants identified. Notable among the variants is a 2 Mb inversion immediately adjacent to the *SMAD6* gene which is approximately 9-fold overexpressed in conjunction with the high ovulation rate phenotype. *SMAD6* is an inhibitor of the bone morphogenetic/transforming growth factor-ß signaling pathway which is also negatively affected by known ligand and receptor variants causing high ovulation rate and high litter size in sheep. Hypotheses of this being the Trio allele and being unique to the Trio family was tested by genotyping (1) Trio descendants with ovulation rate phenotypes, (2) Hereford cattle and (3) USMARC-Twinner cattle with a PCR-based marker for the inversion. The latter two groups were chosen because (1) the Trio allele arose in the background of a Hereford haplotype, and (2) the USMARC-Twinner population had as its foundation cattle with records of high twinning rate. The two population were considered the most likely to harbor the Trio allele, if found outside the Trio family. Analysis of genotype data in Trio descendants (n=100) indicated that the inversion was perfectly concordant with Trio allele genotype (the latter inferred from average ovulation rate over four estrous cycles). All Hereford samples (n=101) were homozygous for the normal (non-inversion) allele. However, two of 80 USMARC-Twinner animals were carriers for the inversion, suggesting both that the inversion is not limited to the Trio family and that it does not cause the extreme ovulation rate observed for the Trio allele. The two carrier USMARC-Twinner animals were sires which had been progeny tested for ovulation rate and twinning rate, and their daughter averages for these traits are not consistent with the Trio allele phenotype. Fortuitously, both bulls had been previously sequenced at UW-Madison as part of the 1000 Bull Genomes project enabling further effort to identify variants unique to the Trio allele. The effort to examine those variants is ongoing. (Brian Kirkpatrick, WI)

Studied genetic markers in genomic regions related with the immune system. Used RNAseq data to identify variants in that region. To this end we wrote IVDP (Integrated Variant Discovery Pipeline). IVDP is a collection of Bash and R scripts developed to run on local server machines or HPC systems that use the slurm scheduler. The program is a fully automated system for variant calling of SNPs (single-nucleotide polymorphisms) and Indels (insertions and deletions) from Whole Genome Sequence (WGS) and RNA Sequence (RNAseq) data and a pipeline to obatin gene counts from RNAseq data. It is publicly available at https://github.com/rodrigopsav/IVDP. Developed epinetr, an R package that facilitates forward-time genomics simulation of complex epistatic networks in populations with user defined heritability parameters. epinetr is designed to enable research on the performance of additive genomic models in the presence of an underlying genetic architecture of epistasis and test epistatic genomic prediction models (Cedric Gondro, MI).

Three projects are ongoing in the Neibergs lab. One is to validate loci associated with bovine respiratory disease (BRD) in cattle and to facilitate accurate prediction of cattle that are less likely to become ill with BRD. A second project is focused on validating loci and integrating functional information associated with

fertility in cattle. The third project is aimed at education and establishment of genomic selection as a financial risk management tool. (Holly Neibergs, WA)

The Neibergs lab has re-initiated sample collection and pathogen diagnostics for bovine respiratory disease in pre-weaned dairy calves in Ohio to validate previous studies and to improve prediction of cattle that are more resistant to disease (Holly Neibergs, WA). Additionally, a project to specifically facilitate communication and training with the dairy community to utilize genomic selection to mitigate financial risk was intiated. This project is in year one and six demonstration herds are participating in Idaho and Washington. Educational workshops at the industry and college level have begun (Holly Neibergs, WA).

The Jiang lab has mainly focused on issues related to ovary function, oocyte quality and embryo development for reduced reproduction loss. We have collected 228 RNA-seq datasets released by twelve laboratories involving corpus lutea (16 samples), embryos (61 samples), granulosa cells (72 samples), oocytes (12 samples), ovary (34 samples) and theca cells (33 samples). Among approximately 6,575 million reads, 5,109 million were mapped to the current bovine reference genome. Such large datasets certainly help us obtain information on transcriptome progression and switches from ovary and their associated cells to oocytes to embryos. The mapping process also assembles a large number of alternative transcripts, which would serve as references to annotate alternative polyadenylation sites executed in the bovine genome (Zhihua Jiang, WA).

Wansheng Liu, (PA) continued to work on the PRAMEY protein in sperm function during in vitro fertilization (IVF) Our previous work revealed a decrease in the bovine PRAMEY protein in capacitated spermatozoa, and the release of the PRAMEY protein from the acrosome during the acrosome reaction, and therefore prompted the current study with the objective to examine the function of PRAMEY during fertilization. IVF was performed in multiple rounds using bovine matured oocytes and caudal epididymal sperm. Prior to IVF, spermatozoa were treated with anti-PRAMEY antibody to determine the consequence of PRAMEY protein inhibition during fertilization. Normal rabbit IgG or Dulbecco's Phosphate-Buffered Saline (DPBS) were used as controls. For sperm-ZP binding analysis, oocytes were examined at 6 h post-IVF. A total of 59, 57, and 42 oocytes were evaluated for the PRAMEY antibody, rabbit IgG and DPBS treatments, respectively. The PRAMEY treatment group (34.44 sperm/oocyte) had nearly a 2-fold increase in the number of sperm bound to the zona when compared to both the rabbit IgG (17.57) and DPBS (18.07) controls (P<0.01). For the polyspermy evaluation, 148 and 134 fertilized eggs were examined at 45 h post-IVF for the PRAMEY antibody and rabbit IgG treatments, respectively. The polyspermy rate was 18.91% in the PRAMEY antibody-treated group, which was significantly greater than 5.97% observed in the rabbit IgG control (P<0.01). To examine the effect of anti-PRAMEY antibody on early embryo development, embryos were harvested at 45 h post-IVF, and the number of 1-, 2-, 4-, and 8-cell embryos were counted. Our initial IVF experiments revealed that the percentage of 1-, 2-, and 4-cell embryos were similar between the PRAMEY antibody, rabbit IgG and DPBS treatments. However, 8-cell embryos were only observed in the PRAMEY antibody-treated group (2.7%) but not in the rabbit IgG group. To further confirm the involvement of PRAMEY in early embryo development, we repeated the IVF experiments with 103 and 89 embryos in PRAMEY antibody and rabbit IgG treatments, respectively. We found that there were significantly fewer zygotes in the PRAMEY antibody-treated group (36.89%) when compared to the rabbit IgG group (57.30%) (P<0.05). No difference was observed in the percentage of embryos reaching the 2-cell stage (P>0.05). As expected, the percentage of 4-cell embryos was significantly greater in the PRAMEY antibody-treated (26.21%) compared to the rabbit IgG group (7.87%) (P<0.05). In summary, our results indicated PRAMEY's potential involvement in anti-polyspermy defense and early embryo cleavage. This research provides the initial evidence for the involvement of the PRAME protein family in sperm function, fertilization, and early embryo development

Pramel1 KO mouse model to study the functional role of the PRAME gene family during spermatogenesis. We found that the juvenal KO males (P35-P41) were superfertile with a 38.40% increase in sperm count

and a 42.5% larger litter size compared to the WT controls, whereas the mature KO males (≥P60) were subfertile with a significant decrease in sperm count (-43.02%) and a reduction of litter size (-17.45%). Sertoli-cell-only (SCO) phenotype was observed in 7% of seminiferous tubules in the KO testis at P7-P35, but not in the mature KO testes. To determine the molecular basis behind these phenotypes, we performed a time-course study (n≥3) with a focus on germ cell development during the first round of spermatogenesis. In neonatal mice (P2-P7), the percentage of prospermatogonia that responded to the first RA pulse and became differentiated A1 spermatogonia, were significantly increased (36.24%) in the KO mice over the WT (P<0.01). RT-PCR confirmed that genes, Thy1, Id4, and Plzf, associated with undifferentiated spermatogonia were significantly downregulated, while c-Kit and Stra8 genes, associated with differentiating spermatogonia were upregulated in KO testes compared to WT. This data suggests that deletion of Pramel1 affected the balance between prospermatogonia and A1 spermatogonia by regulating cell transcription in responding to RA signaling and explains why increased sperm production and little size were observed in juvenal KO males. Immunofluorescent staining with different combinations of germ cell- and Sertoli cell-specific markers on P2-P35 testis sections further indicated that Pramel1 may affect germ cell "seating" position during homing at P3-P5, resulting a germ cell-free region during the initial establishment of spermatogenic wave that leads to the SCO phenotype. To determine the reason for subfertility observed in the mature KO males, heat-stress experiments were performed on P60 mice and germ cells were examined at day 5, 14 and 38 post-stress-treatments (n≥3). A significant decrease in sperm count (-20.27%) was observed in KO mice at day 38 (P<0.05), suggesting a significant loss of spermatogonia stem cells (SSCs) in KO testes. In summary, our data indicates that Pramel1 is required to maintain normal spermatogenesis by regulating germ cell differentiation in response to RA signaling (Wansheng Liu, PA).

A major focus of the Van Eenennaam laboratory is exploring the use of genome editing in cattle breeding programs. In the past year we have used gene editing (GnEd) to explore the hornless (polled) phenotype in cattle. Horns can be dangerous, and their physical removal is undertaken to protect animals and their handlers. Dehorning is increasingly associated with animal welfare concerns. A naturally occurring dominant genetic mutation (Pc allele) comprised of a 212 bp duplicated DNA sequence replacing a 10-bp sequence at the POLLED locus, results in the hornless phenotype. This Pc allele is intergenic, and has been hypothesized to possibly affect regulation of nearby long non-coding RNAs. We hypothesized that either the 10 bp sequence missing in the Pc allele, or alternatively the expression of a long non-coding RNA (lincRNA#1) sequence results in the polled phenotype. GnEd of bovine embryos with CRISPR-Cas9 and dual guide RNAs was used to generate targeted, homozygous deletions at both loci independently. The resulting animals were identical to controls, signifying that the absence of these two genomic regions does not result in the polled phenotype (Van Eenennaam, CA).

Additionally, multiyear genomic and phenotypic analyses of six offspring of a dairy bull GnEd to be homozygous for the Pc allele were undertaken. The offspring were heterozygous and polled as expected, and did not differ in their growth, health or development, nor the nutritional composition of their meat and milk, when evaluated against contemporary comparator controls. We further modelled how GnEd could be used to introgress the Pc allele in the US dairy cattle population by GnEd of elite artificial insemination bulls. Collectively, this research shows how the advent of targeted GnEd tools opens the way for hypothesis testing of putative gene function, and the rapid introgression of useful alleles into livestock breeding programs. We also worked on approaches to accelerate the delivery of genome editing reagents into livestock embryos using electroporation as an alternative to microinjection (Van Eenennaam, CA). .

In contrast to GnEd crops, genomic alterations introduced by GnEd in food animals including targeted deletions and intraspecies allele introgressions (e.g. Pc) that could have been produced using conventional breeding, are currently considered unapproved new animal drugs by the US Food and Drug Administration. Drug approval is a lengthy and expensive process, limiting access of US researchers and small companies to employ GnEd in livestock improvement programs. We documented the economic costs resulting from

delaying the adoption of genetic engineering and genome editing in livestock breeding programs (Van Eenennaam, CA).

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.

Development of genome imputation framework and publicly accessible server. Evaluated and identified best imputation strategies for cattle and pigs with various genotyping methods (SNP array vs Whole Genome Sequencing at different coverage depth), breed composition of haplotype reference panels (single-breed vs multi-breed), and software combinations for phasing and imputation. Developed an in-house pipeline called ImputeGen for automated phasing and imputation available at https://github.com/rodrigopsav/ImputeGen. (Cedric Gondro, MI)

Developed algorithm to optimize hyper-parameters for deep learning methods. Developed machine learning (ML) methods for genomic prediction based on genetic programming, differential evolution and a deterministic feature selection method. Evaluating various ML methods and comparing with GBLUP and Bayesian approaches. (Cedric Gondro, MI)

Advancements in genome-to-phenome prediction were made by implementing strategies and tools to identify and validate genes and allelic variants predictive of biologically and economically important phenotypes and traits. Progress toward achieving these objectives is best described by our publications. There are 8 proceedings papers published by trainees at CSU in Translational Animal Science, which were part of the Western Section of American Society of Animal Science Meeting in 2021. Merging multiple sources of genomic information, which includes gene networks, are useful to understand genes and the SNP underlying QTL. Advancing this knowledge allows improved genetic evaluation for difficult to measure traits. (Milt Thomas, Stephen Coleman, Mark Enns, Kaysie Jennings, Scott Speidel, Tim Holt, Harvey Blackburn and Ted Manahan, CO)

Impact / Usefulness of Findings:
Merging multiple sources of genomic information, which includes gene networks, are useful to understand genes and the SNP underlying QTL. Advancing this knowledge allows improved genetic evaluation for difficult to measure traits. The public good of the genetic improvement of cattle is the clear reduction in the emissions intensity of animal-source foods that is an outcome of improved efficiencies. Improvements in livestock's input-use efficiency of grains will reduce the amount of land and other inputs needed to produce animal products. Milk production per cow in the U.S. has more than quadrupled over the last 75 years, allowing farmers to meet growing demand without a proportional increase in farm inputs. In fact, the U.S. dairy cattle herd has declined by more than half, from 25 million cows in 1944 to 9 million today, even as milk production has increased by 60%. As a result, the carbon footprint of a glass of milk today is one-third of what it was in 1944. In fact, the main beneficiary of genetic improvement programs in livestock is the consumer in terms of decreased cost of animal source foods. Additionally, using genome editing to avoid the need for dehorning or disbudding horned animals, addresses an animal welfare concern

Peer Reviewed Publications (38 in 2021):
1. Kern, C., Y. Wang, X. Xu, Z. Pan, M. Halstead, K. Chanthavixay, P. Saelao, S. Waters, R. Xiang, A. Chamberlain, I. Korf, M. E. Delany, H. H. Cheng, J. F. Medrano, A. L. Van Eenennaam, C. K. Tuggle, C. Ernst, P. Flicek, G. Quon, P. J. Ross, and H. Zhou. 2021. Functional genome annotations of three livestock species provide a vital resource for comparative and agricultural research. Nat Commun 12, 1821 https://doi.org/10.1038/s41467-021-22100-8

2. Van Eenennaam, A. L., F. De Figueiredo Silva, J. F. Trott, and D. Zilberman. 2021. Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay. Annual Review of Animal Biosciences. 9:10.1–10.26 https://doi.org/10.1146/annurev-animal-061220-023052

3. Mueller, M.L., Cole, J.B., Connors, N.K., Johnston, D.J., Randhawa. I.A.S. and Van Eenennaam, A.L. 2021. Comparison of gene editing versus conventional breeding to introgress the POLLED allele into the tropically adapted Australian beef cattle population. Frontiers in Genetics. 12:593154 https://doi.org/10.3389/fgene.2021.593154

4. Owen, J.R., Hennig, S.L., McNabb, B., Mansour, T.A., Smith, J.M., Lin, J.C., Young, A.E., J. F. Trott, Murray, J.D., Delany, M.E., Ross, P.J., & Van Eenennaam, A.L. 2021. One-step generation of a targeted knock-in calf using the CRISPR-Cas9 system in bovine zygotes. BMC Genomics. 22, 118 https://doi.org/10.1186/s12864-021-07418-3

5. Lin, J.C. and A. L. Van Eenennaam. 2021. Electroporation-mediated genome editing of mammalian zygotes. Frontiers in Genetics.12:56 https://doi.org/10.3389/fgene.2021.648482

6. Van Eenennaam, A.L. and S. J. Werth. 2021. Animal Board Invited Review: Animal Agriculture and Alternative Meats: Learning from Past Science Communication Failures. Animal. 15: 100360. https://doi.org/10.1016/j.animal.2021.100360

7. Deepak, S. S. Aly, W. J. Love, P. C. Blanchard, B. Crossley, A. L. Van Eenennaam, and T. W. Lehenbauer. 2021. Etiology and risk factors to bovine respiratory disease in pre-weaned calves on California dairies and calf ranches. Preventive Veterinary Medicine:105506. https://doi.org/10.1016/j.prevetmed.2021.105506

8. Zilberman, David, Alison L. Van Eenennaam, Felipe De Figueiredo Silva, and Josephine F. Trott. 2021. "The Costs of Overregulating Animal and Plant Biotechnology: Lessons from COVID-19." ARE Update24(4): 1–4. University of California Giannini Foundation of Agricultural Economics. https://s.giannini.ucop.edu/uploads/pub/2021/04/22/v24n4_1.pdf

9. Kirkpatrick, B.W., M.E. Cooke, M. Frie, K.R.B. Sporer, B. Lett, S.J. Wells and P.M. Coussens. 2022. Genome-wide association analysis for susceptibility to infection by *Mycobacterium avium* subspecies *paratuberculosis* in US Holsteins. J Dairy Sci. 2022 Mar 17:S0022-0302(22)00170-9. doi: 10.3168/jds.2021-21276. Online ahead of print. PMID: 35307176.

10. Lett, Beth M. and Brian W. Kirkpatrick. 2022. Identifying genetic variants and pathways influencing twinning rate in North American Holstein cattle. Journal of Dairy Science (accepted).

11. Patchy E, Murdoch B, McKay S. Examining the extent of environmental contributions toward DNA methylation and phenotypic variation. *Animal Frontiers* (2021) DOI: 10.1093/af/vfab056

12. Stegemiller M., Ellison M.J., Hal J.B., Sprinkle J.E. Murdoch B.M. Identifying genetic variants affecting cattle grazing behavior experiencing mild heat load. *Translational Animal Science* (2021) DOI: 10.1093/tas/txab151.

13. Stegemiller MR, Murdoch GK, Rowan TN, Davenport KM, Becker GM, Hall J.B, Murdoch BM. Genome-wide Association Analyses of Fertility Traits in Beef Heifers. *Genes* (2021) Feb 2:12(2):217. DOI: 10.3390/genes12020217.

14. Castro Dias Cuyabano, B., G. Rovere, D. Lim, T.H. Kim, H.K. Lee, S.H. Lee and C. Gondro (2021). GPS coordinates for modelling correlated herd effects in genomic prediction models applied to Hanwoo beef cattle. Animals 11:2050.

15. Kelly, S., T. Voegerl, W. Banzhaf, C. Gondro. Evolving hierarchical memory-prediction machines in multi-task reinforcement learning (2021). Genetic Programming and Evolvable Machines (in press).

16. Singh A., A. Kumar, C. Gondro, A.R. da Silva Romero, A. Karthikeyan, A. Mehrotra, A.K. Pandey, T. Dutt, B.P. Mishra (2021). Identification of genes affecting milk fat and fatty acid composition in Vrindavani crossbred cattle using 50K SNP-Chip. Tropical Animal Health Production 53(3):347

17. Tahir, M.S., L.R. Porto-Neto, C. Gondro, O.B. Sithu, K. Wockner, A.W.L. Tan, H.R. Smith, G.C. Gouveia, J. Kour, M.R.S. Fortes (2021). Meta-analysis of heifer traits identified reproductive pathways in Bos indicus cattle. Genes 12:768.

18. Han, J., C. Gondro, K. Reid, J.P. Steibel (2021). Heuristic hyperparameter optimization of deep learning models for genomic prediction. G3 jkab032.

19. Fernandes G.M., R.P. Savegnago, L.A. Freitas L. El Faro, V.M. Roso, C.C.P. Paz (2021). Multi-trait selection index and cluster analyses in Angus cattle. The Journal of Agricultural Science, 159, 455-462.

20. C. Zhai, L.C. Li Puma, A.J. Chicco, A. Omar, R.J. Delmore, I. Geonaras, S.E. Speidel, T.N. Holt, M.G. Thomas, R.M. Enns, and M.N. Nair. 2022. Pulmonary arterial pressure in fattened Angus steers at moderate altitude influences early postmortem mitochondria functionality and meat color during retail display. J. Anim. Sci. JAS-2021-5955 (accepted 12/27/21).

21. A.D. Del Rio-Aviles, A. Correa-Calderon, L. Avenado-Reyes, U. Macias-Cruz, M.A. Sanchez-Castro, M.G. Thomas, R.M. Enns, S.E. Speidel, R. Zamorano-Algandar, J.C. Leyva-Corona, C. Garcia-Benitez, and P. Luna-Nevarez. 2021. Effects of an injectable mineral supplementation on physiological responses and milk production of heat-stressed Holstein cows. J. Anim. Behav. Biometero. 9:2116. doi:10.31893/jab.21016.

22. J.F. Torres-Simental, C. Pena-Calderon, L. Avendano-Reyes, A. Correa-Calderon, U. Macias-Cruz, A. Rodriguez-Borbon, J.R. Reyna-Granados, F. Rivera-Acuna, M.G. Thomas, and P. Luna-Nevarez. 2021. Predictive markers for superovulation response and embryo production in beef cattle managed in NorthWest Mexico are influenced by climate. Livest. Sci 250:105490. https://doi.org/10.1016/j.livsci.2021.104590.

23. N.I. Angulo-Valenzuela, M.G. Thomas, D.G. Riley, J.F. Medrano, and P. Luna-Nevarez. 2021. A SNP within the PMCH gene as molecular marker associated to fertility traits in Angus and Brangus heifers raised in desert environment (short communication). Trop. Anim. Health Prod. 53:355.

24. R. Zamorano-Algandar, M.A. Sanchez-Castro, A.I. Hernandez-Cordero, R.M. Enns, S.E. Speidel, M.G. Thomas, J.F. Medrano, G. Rincon, J.C. Leyva-Corona, J.R. Reyna-Granados, P. Luna-Nevarez. 2021. Molecular breeding value estimation from candidate genes associated with reproductive traits in lactating Holstein cows managed in warm environmental conditions. Livst. Sci. 250:104536.

25. D.W. Bailey, M.G. Trotter, C. Tobin, and M.G. Thomas. 2021. Opportunities to apply precision livestock management on rangelands. Front. Sustain. Food Sys: Agroecology and Ecosystems Services. E-pub. 611915.

26. Goldkamp AK, Li Y, Rivera RM, Hagen DE. 2021. Characterization of tRNA Expression Profiles in Large Offspring Syndrome. BMC Genomics (in press).

27. J.N. Kiser, H.L. Neibergs. 2021. Identifying loci associated with bovine corona virus in dairy and beef cattle. Frontiers in Veterinary Science - Veterinary Infectious Diseases. https://doi.org/10.3389/fvets.2021.679074

28. A.T. Massa, M.R. Mousel, C. Durfee, M.K. Herndon, K.M. Hemmerling J.B. Taylor, H.L. Neibergs, S.N. White. 2021. A DNA regulatory element haplotype at Zinc Finger genes is associated with host resilience to ovine lentivirus in multiple populations. Animals. 11(7). 1907 https://doi.org/10.3390/ani11071907

29. J.E. Williams, M.K. McGuire, C.L. Meehan, M.A. McGuire, S.L. Brooker, E.W. Kamau-Mbuthia, E.W. Kaumundi, S. Mbugua, S.E. Moore, A.M. Prentice, G.E. Otoo, J.M. Rodriguez, R.G. Pareja, J.A. Foster, D.W. Sellen, D.G. Kiat, H.L. Neibergs, B.M. Murdoch. 2021. Key Genetic Variants Associated with Variation of Milk Oligosaccharides from Diverse Human Populations. Genomics. 113(4):1867-1875 https://doi.org/10.1016/j.ygeno.2021.04.004

30. J.N. Kiser, Z. Wang, R. Zanella, E. Scraggs, M. Neupane, B. Cantrell, C.P. Van Tassell, S.N. White, J.F. Taylor, H.L. Neibergs. 2021. Functional variants surrounding EDN2 are associated with Mycobacterium avium subspecies paratuberculosis infection. Frontiers in Veterinary Science; Advances in the Diagnosis and Control of Johne's Disease. https://doi.org/10.3389/fvets.2021.625323

31. Phelps MP, Yablonka-Reuveni Zipora. 2021. Female outperformance in voluntary running persists in dystrophin-null and klotho-overexpressing mice. Journal of Neuromuscular Diseases, 8:S5271-S281. PMID: 34275905, DOI: 10.3233/JND-210703

32. Phelps MP. In Press. Genome Editing and Conservation. Encyclopedia of Biodiversity, Third Edition.

33. Jiang Z. Peer Review Report For: The genome sequence of the Norway rat, Rattus norvegicus Berkenhout 1769 [version 1; peer review: 1 approved]. Wellcome Open Res 2021, 6:118 (https://doi.org/10.21956/wellcomeopenres.18591.r46050).

34. Zhu T, Qi X, Chen Y, Wang L, Lv X, Yang W, Zhang J, Li K, Ning Z, Jiang Z, Qu L. Positive selection of skeleton-related genes during duck domestication revealed by whole genome sequencing. BMC Ecol Evol. 2021 Sep 6;21(1):165.
35. Nie C, Qu L, Li X, Jiang Z, Wang K, Li H, Wang H, Qu C, Qu L, Ning Z. 2021. Genomic Regions Related to White/Black Tail Feather Color in Dwarf Chickens Identified Using a Genome-Wide Association Study. Front Genet. 30;12:566047.
36. Wen J, Shao P, Chen Y, Wang L, Lv X, Yang W, Jia Y, Jiang Z, Zhu B, Qu L. 2021. Genomic scan revealed KIT gene underlying white/gray plumage color in Chinese domestic geese. Anim Genet. 52(3):356-360.
37. Zhu J, Zhang Q, Huang Y, Jiang Y, Li J, Michal JJ, Jiang Z, Xu Y, Lan W. 2021. Long-term trends of microplastics in seawater and farmed oysters in the Maowei Sea, China. Environ Pollut. 273:116450.
38. Zinski AL, Carrion S, Michal JJ, Gartstein MA, Quock RM, Davis JF, Jiang Z. 2021. Genome-to-phenome research in rats: progress and perspectives. Int J Biol Sci. 17(1):119-133.

Proceedings Papers (16 in 2021):
1. K.L. Duggan, T.N. Holt, M.G. Thomas, S.E. Speidel, R.M. Enns. 2021. Effect of pulmonary arterial pressure and annual precipitation on reproductive performance of Angus heifers in south central Wyoming. Trans. Anim. Sci. 5:S175-S179. Suppl. S1.
2. L.K. Giess, M.G. Thomas, S.E. Speidel, M.M. Culbertson, W.R. Schafer, S.C. McGuire, R.M. Enns. 2021. Whole herd reporting data from the American Simmental Association as a data source for heifer pregnancy phenotypes. Trans. Anim. Sci. 5:S199-S203 Suppl. S1.
3. R.A. González-Murray, P.G. Martínez, V. Vigíl, H. Yazar-Gunes, M.A. Sánchez-Castro, R.M. Enns, S.E. Speidel, M.G. Thomas. 2021. Heterosis effects on age at first calving in a multibreed beef cattle herd in Panama. Trans. Anim. Sci. 5:S185-S188. Suppl. S1.
4. K.R. Heffernan, R.M. Enns, H.D. Blackburn, S.E. Speidel, C.S. Wilson, M.G. Thomas. 2021. Case study of inbreeding within Japanese Black cattle using resources of the American Wagyu Association, National Animal Germplasm Program, and a cooperator breeding program in Wyoming. Trans. Anim. Sci. 5:S170-S174. Suppl. S1.
5. I.M. Kukor, M.G. Thomas, R.M. Enns, T.N. Holt, S.E. Speidel, M.A. Cleveland, B.P. Holland, A.B. Word, G.B. Ellis. 2021. Sire differences within heart and heart fat score in beef cattle, Trans. Anim. Sci. 5:S149–S153. Suppl. S1.
6. H.M. Saad, R.M. Enns, M.G. Thomas, L.L. Leachman, S.E. Speidel. 2021. Foot scores genetic parameters estimation in beef cattle. Trans. Anim. Sci. 5:S180-S184. Suppl. S1.
7. H. Yazar-Gunes, R.A. González-Murray, M.A. Sánchez-Castro, T.L. Passafaro, Y.L. Bernal-Rubio, K.J. Andersen, S.E. Speidel, M.G. Thomas, R.M. Enns. 2021. New approach to sustained cow fertility trait. Trans. Anim. Sci. 5:S195-S198. Suppl. S1.
8. T.R. Zimprich, S.E. Speidel, D.W. Schafer, B.A. Lashell, T.N. Holt, R.M. Enns, S.F. Cunningham, M.G. Thomas. 2021. Yearling pulmonary arterial pressure at moderate elevations as a varied indicator for pulmonary arterial pressure at high elevations in beef bulls in Colorado, Trans. Anim. Sci. 5:S204-S208. Suppl. S1.
9. Van Eenennaam, A. L. 2021. Genome editing approaches to augment cattle breeding programs. Proceedings paper in Digest 76 of the British Cattle Breeders Club. pages 22-26.
10. Van Eenennaam, A. L. 2021. Gene editing: Today and in the Future. 2021 Proceedings of the 53rd Beef Improvement Federation Symposium and Convention. Des Moines, IA June 22-25, 2021. Pages 33-38.
11. Castro Dias Cuyabano, B., G. Rovere, D. Lim, T.H. Kim, H.K. Lee, S.H. Lee and C. Gondro (2021). GPS coordinates for modelling correlated herd effects in genomic prediction models applied to Hanwoo beef cattle. Animals 11:2050.
12. Kelly, S., T. Voegerl, W. Banzhaf, C. Gondro. Evolving hierarchical memory-prediction machines in multi-task reinforcement learning (2021). Genetic Programming and Evolvable Machines (in press).

13. Singh A., A. Kumar, C. Gondro, A.R. da Silva Romero, A. Karthikeyan, A. Mehrotra, A.K. Pandey, T. Dutt, B.P. Mishra (2021). Identification of genes affecting milk fat and fatty acid composition in Vrindavani crossbred cattle using 50K SNP-Chip. Tropical Animal Health Production 53(3):347

14. Tahir, M.S., L.R. Porto-Neto, C. Gondro, O.B. Sithu, K. Wockner, A.W.L. Tan, H.R. Smith, G.C. Gouveia, J. Kour, M.R.S. Fortes (2021). Meta-analysis of heifer traits identified reproductive pathways in Bos indicus cattle. Genes 12:768.

15. Han, J., C. Gondro, K. Reid, J.P. Steibel (2021). Heuristic hyperparameter optimization of deep learning models for genomic prediction. G3 jkab032.

16. Fernandes G.M., R.P. Savegnago, L.A. Freitas L. El Faro, V.M. Roso, C.C.P. Paz (2021). Multi-trait selection index and cluster analyses in Angus cattle. The Journal of Agricultural Science, 159, 455-462.

Abstracts:

1. Van Eenennaam, A. L. 2021. Regulation of Gene Editing in Livestock. The Society for In Vitro Biology virtual meeting. June 5-9, 2021, In Vitro Cellular & Developmental Biology - Animal, 57(SUPPL 1): S6-S6. https://link.springer.com/content/pdf/10.1007/s11626-021-00573-7.pdf

2. Trott, J.F., A. E. Young, B. R. McNabb, X. Yang, T. F. Bishop and A L. Van Eenennaam. 2021. Comparative Evaluation of Human-Edible Animal Products Derived From Offspring of Genome Edited And Control Cattle. Journal of Animal Science. 99: Issue Supplement_3, 11-12 https://doi.org/10.1093/jas/skab235.020

3. J.C. Lin. T.A. Hua & A.L. Van Eenennaam. (2021) Optimization of electroporation parameters for the introduction of genome editing reagents into early sheep embryos, 13th Transgenic Animal Research Conference. August 9-12, 2021. https://ucanr.edu/sites/TARC/files/355976.pdf

4. Hennig, S.L., B.R. McNabb, A.L.Van Eenennaam, and J.D. Murray. 2021. An Efficient CRISPR/Cas9 Dual-Guide Approach for Large Knockouts in Bovine Embryos. 13th Transgenic Animal Research Conference. August 9-12, 2021. https://ucanr.edu/sites/TARC/files/355977.pdf

5. Lett, B., B.W. Kirkpatrick. 2021. Identifying genetic variants and pathways influencing twinning rate in North American Holstein cattle. American Dairy Science Association.

6. Beiki H., Gill C.A., Jiang H., Jiang Z., Liu W., McKay S.D., Michal J.J., Murdoch B.M., Rincon G., Rijnkels M., Smith T.P.L., Ross P.J., Zhou H., Reecy J.M. Bovine transcriptome annotation using integration of multi-omics data. 38th International Society of Animal Genetic Virtual Conference, July 26-30, 2021.

7. Walker K, Murdoch B, Colacchio A., Davenport K, Colle M, Bass P, Murdoch GK. Single nucleotide Polymorphisms Associated with Advanced Skeletal Maturity in Finished Beef Heifer. American Society of Animal Science – Canadian Society of Animal Science, Kentucky July 14-18, 2021.

8. Stegemiller M, Ellison MJ, Hall JB, Sprinkle JE, Murdoch BM. Identifying Genetic Variants Associated with Grazing, Walking, and Slope use of cattle Experiencing Mild Heat Load. American Society of Animal Science – Canadian Society of Animal Science, Kentucky July 14-18, 2021.

9. C. Gondro (2021). Genomic prediction and epistasis: disentangling the relationship between epistatic variance, linear models and genetic architectures. SBMA Brazil (invited).

10. C. Gondro (2021). Practical (and not yet quite practical) applications of genomic technologies to improve meat quality. American Meat Science Association 2021 RMC (invited).

11. H. Ostrovski, R.P. Savegnago, W. Huang and C. Gondro (2021). Real-Time, On-Site Whole Genome Sequencing with Oxford Nanopore Technologies' MinION. American Society of Animal Science.

12. M.Y. Nawaz, R.P. Savegnago and C. Gondro (2021). Identifying Genomic Signatures of Selection in Hanwoo and Angus Beef Cattle Using Imputed Whole Genome Sequence. American Society of Animal Science.

13. Characterization of tRNA expression profiles in large offspring syndrome. American Society of Animal Science Annual 2021 Meeting Abstracts, Louisville, Kentucky, July 15, 2021.

14. Insights into translation through tRNA seqeuencing and ribosome profiling. Proceedings of the 38th International Conference on Animal Genetics, Virtual, July 28, 2021.

Graduate Student Education: (7 Ph.D., 1 M.S. in 2021)

- Sadie Hennig, Doctorate of Philosophy candidate at UC Davis, contributed to the genome editing research. She is funded by a USDA Predoctoral GEEAP National Needs Fellowship.
- Maci Mueller, Doctorate of Philosophy candidate at UC Davis, contributed to the genome editing research. She is funded by a USDA Predoctoral GEEAP National Needs Fellowship.
- Carly Guiltinan, Doctorate of Philosophy student at UC Davis, contributed to the Single-cell transcriptome analysis of bovine fetal gonads to trace germline specification events. She is funded by a USDA Predoctoral GEEAP National Needs Fellowship.
- Jason Lin, Master of Science student at UC Davis, contributed to the genome editing research.
- Kimberly Davenport, Doctorate of Philosophy, research training has contributed to the helping with reference genomes and the Cattle FAANG project.
- Gabrielle Becker, Doctorate of Philosophy candidate, is working on Cattle Pangenome.
- Morgan Stegemiller, Doctorate of Philosophy candidate, research has included genome-wide association studies of U of I heifers and fertility traits.
- Anna Goldkamp, Doctorate of Philosophy candidate, research has included small RNA analysis, tRNA gene expression, transcriptional analysis, and ribosome profiling to describe active translation in tissues.

Presentations:

1. C. Gondro (2021). Genomic prediction and epistasis: disentangling the relationship between epistatic variance, linear models and genetic architectures. SBMA Brazil (invited).
2. C. Gondro (2021). Practical (and not yet quite practical) applications of genomic technologies to improve meat quality. American Meat Science Association 2021 RMC (invited).
3. H. Ostrovski, R.P. Savegnago, W. Huang and C. Gondro (2021). Real-Time, On-Site Whole Genome Sequencing with Oxford Nanopore Technologies' MinION. American Society of Animal Science.
4. M.Y. Nawaz, R.P. Savegnago and C. Gondro (2021). Identifying Genomic Signatures of Selection in Hanwoo and Angus Beef Cattle Using Imputed Whole Genome Sequence. American Society of Animal Science.
5. Z. Jiang. Biodiversity: does RNA diversity matter (8th International Symposium of Innovative Bioproduction Indonesia on Biotechnology and Bioengineering 2021)
6. Z. Jiang. Advancing Genome to Phenome Research in Swine: Progress and Perspectives (9th International Congress: Veterinary Science and Profession. University of Zagreb)
7. Z. Jiang. Mapping of alternative polyadenylation sites to Rattus norvegicus mRatBN7.2 (Rat Genome Annotation Consortium Monthly Update).
8. Van Eenennaam, A.L. "Emerging Technologies: Regulatory Oversight of Intentional Genomic Alterations in Animals", Food Drug and Law Institute (FDLI) Online Conference 10/7/2020
9. Van Eenennaam, A.L. "Genome Editing Applications in Animals" CRISPR in Agriculture Research, Syntego World CRISPR Day symposium (virtual) 10/20/2020
10. Van Eenennaam, A.L. "Gene editing in livestock: promise, prospects and policy" Iowa State seminar (virtual) 10/21/2020
11. Van Eenennaam, A.L. "Does a plant-based diet mean improved health for the climate, agriculture, and the population?" Grains and Health Symposia, Calgary Online Conference (virtual) 10/26/2020
12. Van Eenennaam, A.L. "Agricultural animal transgenesis for food applications" Transgenic Technology, Israel, (virtual) 10/27/2020
13. Van Eenennaam, A.L. "Using genome editing for livestock health", ASAS-Southern Section Genetics and Genomics Webinar Series (virtual) 11/4/2020
14. Van Eenennaam, A.L. "Genome editing applications in animals" Virtual Workshop in Genome Editing Technologies in Kenya,11/10/2020
15. Van Eenennaam, A.L. "The importance of innovation to the future of beef production", Wagyu Virtual International Conference, South Africa (virtual) 11/11/2020

16. <u>Van Eenennaam, A.L.</u> "One-step generation of a targeted gene knock-in calf using the CRISPR-Cas9 system in bovine zygotes", Centre for Genetic Improvement of Livestock (CGIL) Seminar, Department of Animal Biosciences, University of Guelph, Canada (virtual) 11/13/2020
17. <u>Van Eenennaam, A.L.</u> "Genome editing in livestock", National Center for genome editing in agriculture, Israel, (virtual) 1/14/2021
18. <u>Van Eenennaam, A.L.</u> "Genome editing approaches to augment cattle breeding programs", British Cattle Breeders Club Virtual Conference, England (virtual) 26/1/2021
19. <u>Van Eenennaam, A.L.</u> How Can We Design a Cow to Better Meet Human Needs?" Virtual presentation to two high school classes in Minnesota at request of their science teacher; 2/3/2021 and 2/4/2021
20. <u>Van Eenennaam, A.L.</u> "Addressing Climate Change and Sustainability Through Innovation", 2021 USDA Agricultural Outlook Forum, Virtual presentation 2/18/2021
21. <u>Van Eenennaam, A.L.</u> "Current Status of Genome editing to Augment Cattle Breeding Programs", Virtual presentation to the Board of the American Angus Association, 2/23/2021
22. <u>Van Eenennaam, A.L.</u> "How Genes Advance Progress: Genomics' Role in Beef Sustainability?" Global Roundtable for Sustainable Beef virtual webinar, 2/26/2021
23. <u>Van Eenennaam, A.L.</u> "Biotechnology, Genomics, and Reproduction: The Ultimate Ménage à Trois" Spring 2021 RPBO Seminar Series (Warnick Lecture), University of Florida, FL (virtual) 3/10/2021
24. <u>Van Eenennaam, A.L.</u> "Polled genetics- ready for prime time?" Golden State Dairy Management Virtual webinar, 3/25/2021
25. <u>Van Eenennaam, A.L.</u> "Animal Agriculture and Alternative Meats: Learning from Past Science Communication Failures" British Society of Animal Science, Virtual meeting, 4/13/2021
26. <u>Van Eenennaam, A.L.</u> "Animal Agriculture and Alternative Meats: Learning from Past Science Communication Failures" University of California ANR Livestock and Dairy Program Team meeting, (virtual) 4/16/2021
27. <u>Van Eenennaam, A.L.</u> "Gene Editing in Livestock: What is Gene Editing?" UC Davis Virtual Picnic Day, 4/17/2021
28. <u>Van Eenennaam, A.L.</u> "Animal Agriculture and Alternative Meats: Learning from Past Science Communication Failures, Animal Agriculture Alliance Virtual meeting, (virtual) 4/28/2021
29. <u>Van Eenennaam, A.L.</u> "Animal Agriculture and Alternative Meats: Learning from Past Science Communication Failures" Internet Lecture to University of Wyoming, (virtual) 4/29/2021
30. <u>Van Eenennaam, A.L.</u> "Genome Editing in Animals", Invited virtual lecture to "GMO & Biosafety" course, Brac University, Bangladesh (virtual) 5/5/2021
31. <u>Van Eenennaam, A.L.</u> "Genetic Engineering of Livestock: The Opportunity Cost of Regulatory Delay", Invited virtual lecture to Dalhousie University, Faculty of Agriculture Truro, NS, Canada (virtual) 5/6/2021
32. <u>Van Eenennaam, A.L.</u> "Advanced Breeding Techniques Using Genomics, Reproduction, & Biotechnology" Invited virtual lecture to Cornell University, Faculty of Animal Science, Ithaca, NY (virtual) 5/11/2021
33. <u>Van Eenennaam, A.L.</u> "Regulation of Gene Editing in Livestock", Society for In Vitro Biology's Virtual Annual Meeting, SIVB 2021: In Vitro OnLine 6/9/2021
34. <u>Van Eenennaam, A.L.</u> "Gene Editing Today and in the Future", Beef Improvement Federation Annual Research Symposium and Convention, Des Moines, Iowa 6/24/2021
35. <u>Van Eenennaam, A.L.</u> "Comparative Evaluation of Human-edible Animal Products Derived from Offspring of Genome Edited and Control Cattle" 2021 ASAS-CSAS-SSASAS Annual Meeting and Trade Show, Virtual Presentation, 7/15/2021
36. <u>Van Eenennaam, A.L.</u> "Gene editing in livestock: Science and policy" Academy of Veterinary Consultants (AVC), Amarillo, TX 8/6/2021
37. <u>Van Eenennaam, A.L.</u> "Alternative meats: What are they and where are we now?" Academy of Veterinary Consultants (AVC), Amarillo, TX 8/6/2021
38. <u>Van Eenennaam, A.L.</u> "Gene Editing in Livestock: What is Gene Editing?" Idaho State University Virtual Event, 8/21/2021

39. <u>Van Eenennaam, A.L.</u> "Effective Science Communication to Raise Awareness on Animal Biotechnology", The International Service for the Acquisition of Agri-biotech Applications (ISAAA) Impact of Gene Technology in Animal Agriculture and Food Production Virtual Workshop, 8/31/2021

40. <u>Van Eenennaam, A.L.</u> "Genome Editing Opportunities in Livestock", Royan Virtual Congress 2021, Iran (virtual) 9/3/2021

41. <u>Van Eenennaam, A.L.</u> "The Interdependence of Sustainability, Innovation, & Science Communication Around Animal Agriculture" Agricultural Science Association (ASA) Ireland, (virtual) 9/10/2021

42. <u>Zhou H</u>, Beiki H, Corum S, Gill C, Hu R, Jiang H, Jiang J, Kern C, Kern C, Liu W, Lyu P, Ma W, McKay S, Medrano J, Michal JJ, Murdoch BM, Reecy JM, Rincon G, Rijnkels M, Smith TPL, Thomas M, Wang H, Xu X, Zhang X, Zhang Y, Ross PJ. Functional Annotation of the Bovine Genome. 38th International Society of Animal Genetic Virtual Conference, July 26-30, 2021.

43. <u>Rosen BD</u>, Bickhart DM, Smith TPL, Boichard D, Brockmann GA, Chamberlain AJ, Couldrey C, Daetwyler HD, Djikeng A, Droegemueller C, Gandham RK, Hagen DE, Hanotte O, Jiang Y, Jiang Z, Larkin D, Liu G, Low WY, Marsan PA, Murdoch BM, Muchadeyi FC, Mwacharo J, Neibergs HL, Pausch H, Demyda-Peyrás S, Prendergast J, Ross PJ, Schnabel RD, Solkner J, Soudre A, Tijani A, Williams JL, and the Bovine Pangenome Consortium. The Bovine Pangenome Consortium. 38th International Society of Animal Genetic Virtual Conference, July 26-30, 2021.

44. <u>Murdoch B</u>. Ovine and Bovine pangenome CyVers workshop, April 8, 2021

Reductions in beef genetics Extension specialists and outreach funding in the US led a group of beef genetics Extension faculty in 6 states (California, Kansas, Kentucky, Missouri, Nebraska, and Tennessee) to coordinate their efforts and develop a national Extension program to meet beef producers and Extension educators' needs in beef genetics programming. This effort, collectively called eBEEF, has utilized four platforms to provide these outreach efforts: Beef Improvement Federation, National Beef Cattle Evaluation Consortium, eBEEF.org website and the National Cattlemen's Beef Association education program. Materials provided through eBEEF include the publication of an updated version of the 2021 Beef Cattle Sire Selection Manual, major contributions to the Beef Improvement Federation Guidelines for Uniform Beef Improvement Programs, 32 factsheets, 165 videos, over 18 train-the-trainer webinar series, 5 direct producer education webinars, and numerous in person educational programs. This collaboration has proved to be an effective model to provide beef genetics outreach programming to a national audience. eBEEF was awarded the **2021 Beef Improvement Federation BIF Ambassador Award** for efforts in extending the news of BIF and its principles to a larger audience.

Active Grants and Contracts Leveraged Through NRSP-8 Research: (Total $7,934,295)
1. Myokines an Avenue for Improved Growth (2021) AFRI NIFA **2021-67016-33718** PI: G. Murdoch Co-PI's: B Murdoch, K. Thornton-Kurth, G. Chibisa. Awarded $200,000.
2. NIFA AG2PI Collaborative: Creating a Shared Vision Across Crops and Livestock Communities. (2020) AFRI NIFA **2020-70412-32615** PI: P. Schnable Co-PI's: B. Murdoch, J. Dekkers, C. Tuggle, C. Lawrence-Dill, J. Clark, E Lyons. Award $960,000.
3. RNA methylation as a mechanistic link between genotype and phenotype (2020) AFRI NIFA **2020-67016-31577** PI: S McKay, Co PI: B Murdoch. Awarded $200,000.
4. Social Interaction and Consumer Acceptance of Genome Editing in Domestic Livestock USDA NIFA Competitive Grant. **2020-67023-31637** $445,000 Jill McClusky, Washington State (PD), Co-PI's: J. Winfree, A. Van Eenennaam, P. Glazebrook, S. Badruddoza UC Davis subcontract $85,445 Key personnel B Murdoch. $62,987. 9/1/2020 – 8/31/2022
5. High throughput multiparametric phenotyping of domestic livestock cells, organoids and embryos USDA NIFA Competitive Grant. **2020-70410-32899** $498,416. A. L. Van Eenennaam (PD). E. Maga, J. D. Murray, A. Denicol, and P. Berger (Co-PDs). 9/1/2020 – 8/31/2021
6. Developing a Platform for Efficient Genome Editing in Livestock, USDA NIFA Competitive Grant **2020-67015-31536**. $300,000. A. L. Van Eenennaam (PD). P. Ross, E. Maga and T. Berger (Co-PDs). 7/1/2020 – 6/30/2022

7. Multiplexed Gene Editing in Livestock Embryonic Stem Cells, USDA NIFA Competitive Grant **2020-67015-31538**. $300,000. E. A. Maga (PD). 7/1/2020 – 6/30/2022
8. Tools and Resources for Cattle Pangenomes. USDA National Institute of Food and Agriculture Competitive Grant **2020-67015-31675**. $500,000 C.T. Brown (PD) 7/1/2020-6/30/2023.
9. The Functional Annotation of the Bovine Animal Genome. (2017- 2021) AFRI NIFA **2018-67015-27500** PI: P. Ross Co-PI's: J. Medrano, Z. Huajun, J. Honglin, M. Rijnkels, C. Gill, J. Reecy, J. Zhihua, L. Wansheng, B. Murdoch, S. McKay, M. Thomas, T. Smith. Awarded $2,500,000.1/15/2018-1/14/2022
10. Phenotype, genome and animal products derived from offspring of a genome edited, hornless bull and controls. USDA Biotechnology Risk Assessment Competitive Grant **2017-33522-27097** $500,000 A.L. Van Eenennaam (PD) 9/1/2017 – 8/31/2021.
11. USDA National Need Fellowship: "Genome Editing for Enhanced Animal Production" (GEEAP) UC Davis; Project # **2017-38420-26790**; $238,500 P. Ross (PD) 6/1/2017-5/31/2022.
12. The cattle Pangenome - non funded cattle community project. PI: Timothy P.L. Smith, Co-PI's: Benjamin Rosen, Derick Bickhart, member B Murdoch.
13. Genomes to phenomes of cattle and sheep in Idaho. (2019) National Animal Genome Research Program – NRSP008 - Hatch Grant PI: Murdoch B.
14. Optimizing and characterizing sustainable beef cattle production in forage base systems on Western rangelands. Hatch Multistate Research project PI - Sprinkle J, Co-PI: Hall J, Jensen SJ, Ellison MJ, Sager JK, Murdoch BM, Glaze BJ.
15. Reproductive performance in domestic ruminants. Hatch PI – Hall J, Co-PI: Ahmadzadeh A, Murdoch B, Sprinkle J.
16. Advanced carcass maturity: developing an understanding, screening method and possible solution. Idaho Beef Council. PI: G. Murdoch Co-PI: B. Murdoch. Awarded $34,385.
17. Functional importance of microbiota on sensory attributes of whole-muscle dry aged beef. Idaho Beef Council PI: Bass P, Collaborators: Colle M, Murdoch G, Murdoch B, Williams J, Rezamand P, McGuire M, McGuire M, Mitchell T. awarded $42,550.
18. Ribosome-Bound Transcriptomics Linking Gene Expression To Translation. USDA-NIFA Competitive Grant **2021-67016-33417**; $200,000 D. Hagen (PD) 1/1/2021-12/31/2022.
19. Establishing a Pan-Epigenome for Cattle and Sheep. USDA-NIFA Competitive Grant **2022-67016-36216**; $1,300,000. S. McKay (PD), D. Hagen and B. Murdoch (co-PDs).
20. Impact of Breed on Beef Production and Sustainability (2021) AFRI NIFA PI: K Thornton, Co-PI's: Murdoch G, Murdoch B, Matarneh S. Award $74,999.

Equine NRSP-8 Annual Report (Coordinator and Workshop) 2021

Coordinators: Ernest Bailey, University of Kentucky
                        Samantha Brooks, University of Florida
                        Molly McCue, University of Minnesota

NRSP8 Workshop:
Chair: Mike Mienaltowski, University of California, Davis
Co-chair: Felipe de Avila, University of California, Davis

2021 Equine Workshop Report
Due to the Covid19 pandemic, the January 2021 Plant and Animal Genome Conference and the USDA-NSRP8 meeting in San Diego was cancelled. Mike Mienaltowski and Felipe de Avila organized a virtual workshop meeting held in placed of the physical meeting based on the program planned for the in-person meeting

NRSP-8 Progress:

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.

Whole genome sequences of diverse horses have been added to the Sequence Read Archive (SRA) of NCBI in connection with research projects conducted and funded in member laboratories using samples from modern horse breeds plus ancient DNA samples.

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.

For the Functional Annotation of Animal Genomes (FAANG) initiative, sampling and preservation of 86 tissues, 2 cell lines, and 5 fluids from two Thoroughbred mares was completed in 2016 (Burns, et. al 2018) and data continues to be added to the community databases. During 2020 and 2021, tissues were added from two stallions to this project they these were tested for histone marks, RNA seq and whole genome sequencing, just as for the two mares.

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.

Datasets are the two mares and two stallions (https://www.ebi.ac.uk/ena/data/view/PRJEB26698), mRNA-seq (https://www.ebi.ac.uk/ena/data/view/ERA1487553). In addition, a resource for view the data in Integrated Genome Viewer (https://equinegenomics.uky.edu/) was created by Dr. Ted Kalbfleisch of the University of Kentucky.

Communication: The coordinators maintain an email list and use it to broadcast information for USDA-NRSP8, the USDA, the Havemeyer Foundation and other information relevant to the workshop. In addition to the PAG conference, workshops are usually held once every two years at a Dorothy Russell Havemeyer Workshop and at a conference of the International Society for Animal Genetics. Many of the NRSP8 members also participant in the biennial Equine Science Society Conferences. Due to the pandemic, the Havemeyer conference and the Equine Science Society meeting planned for 2020 were cancelled.

Website: The website for the International Horse Genome program was maintained, including reports from the different meetings, identification of participants and tools. The website can be found at: https://horsegenomeworkshop.com/

July 2021 Havemeyer International Equine Genome Workshop Cancelled
A workshop was planned for July 2020 in Ithaca, NY but was postponed to July 2021 due to the pandemic. Ultimately, that meeting had to be postponed to July 2022. Details can be found at the following website: https://havemeyergenome2020.com/. However, beginning in February 2021 a series of 4 virtual meetings were held (February, April, June, August) Details can be found at the following website: https://havemeyergenome2020.com/

Coordinator Funds:
Initially, coordinator funds were budgeted for student support to attend the NRSP8/PAG conference, invited speakers at that conference support of a species workshop in Ithaca during July 2021 and support of the FAANG project. Because of the pandemic, meetings were cancelled, and the funds were reallocated to

support tests of the FAANG tissues and collection of tissues from two stallions. In addition, $5000 was allocated for support of development of the bioinformatics site to visualize FAANG horse data on IGV.

Publications for 2021 by workshop participants downloaded from Pubmed

1. Brooks SA. Genomics in the Horse Industry: Discovering New Questions at Every Turn. J Equine Vet Sci. 2021 May;100:103456. doi: 10.1016/j.jevs.2021.103456. Epub 2021 Mar 26. PMID: 34030792.
2. Librado P, Orlando L. Genomics and the Evolutionary History of Equids. Annu Rev Anim Biosci. 2021 Feb 16;9:81-101. doi: 10.1146/annurev- animal-061220-023118. Epub 2020 Nov 16. PMID: 33197207.
3. Bugno-Poniewierska M, Raudsepp T. Horse Clinical Cytogenetics: Recurrent Themes and Novel Findings. Animals (Basel). 2021 Mar 16;11(3):831. doi: 10.3390/ani11030831. PMID: 33809432; PMCID: PMC8001954.
4. Lee W, Mun S, Choi SY, Oh DY, Park YS, Han K. Comparative Analysis for Genetic Characterization in Korean Native Jeju Horse. Animals (Basel). 2021 Jun 28;11(7):1924. doi: 10.3390/ani11071924. PMID: 34203473; PMCID: PMC8300358.
5. Durward-Akhurst SA, Schaefer RJ, Grantham B, Carey WK, Mickelson JR, McCue ME. Genetic Variation and the Distribution of Variant Types in the Horse. Front Genet. 2021 Dec 2;12:758366. doi: 10.3389/fgene.2021.758366. PMID: 34925451; PMCID: PMC8676274.
6. Esdaile E, Kallenberg A, Avila F, Bellone RR. Identification of W13 in the American Miniature Horse and Shetland Pony Populations. Genes (Basel). 2021 Dec 14;12(12):1985. doi: 10.3390/genes12121985. PMID: 34946933; PMCID: PMC8702037.
7. Vershinina AO, Heintzman PD, Froese DG, Zazula G, Cassatt-Johnstone M, Dalén L, Der Sarkissian C, Dunn SG, Ermini L, Gamba C, Groves P, Kapp JD, Mann DH, Seguin-Orlando A, Southon J, Stiller M, Wooller MJ, Baryshnikov G, Gimranov D, Scott E, Hall E, Hewitson S, Kirillova I, Kosintsev P, Shidlovsky F, Tong HW, Tiunov MP, Vartanyan S, Orlando L, Corbett-Detig R, MacPhee RD, Shapiro B. Ancient horse genomes reveal the timing and extent of dispersals across the Bering Land Bridge. Mol Ecol. 2021 Dec;30(23):6144-6161. doi: 10.1111/mec.15977. Epub 2021 May 27. PMID: 33971056.
8. Peng S, Petersen JL, Bellone RR, Kalbfleisch T, Kingsley NB, Barber AM, Cappelletti E, Giulotto E, Finno CJ. Decoding the Equine Genome: Lessons from ENCODE. Genes (Basel). 2021 Oct 27;12(11):1707. doi: 10.3390/genes12111707. PMID: 34828313; PMCID: PMC8625040.
9. Martinez MM, Costa M, Corva PM. Analysis of Genetic Variability in the Argentine Polo Horse With a Panel of Microsatellite Markers. J Equine Vet Sci. 2021 Jan;96:103320. doi:10.1016/j.jevs.2020.103320. Epub 2020 Nov 10. PMID: 33349401.
10. Yousefi-Mashouf N, Mehrabani-Yeganeh H, Nejati-Javaremi A, Bailey E, Petersen JL. Genomic comparisons of Persian Kurdish, Persian Arabian and American Thoroughbred horse populations. PLoS One. 2021 Feb 16;16(2):e0247123. doi: 10.1371/journal.pone.0247123. PMID: 33592064; PMCID: PMC7886144.
11. Cieslak J, Brooks SA, Wodas L, Mantaj W, Borowska A, Sliwowska JH, Ziarniak K, Mackowski M. Genetic Background of the Polish Primitive Horse (Konik) Coat Color Variation-New Insight into Dun Dilution Phenotypic Effect. J Hered. 2021 Aug 25;112(5):436-442. doi: 10.1093/jhered/esab034. PMID: 34432873.
12. Tozaki T, Ohnuma A, Kikuchi M, Ishige T, Kakoi H, Hirota K, Kusano K, Nagata S. Simulated validation of intron-less transgene detection using DELLY for gene- doping control in horse sports. Anim Genet. 2021 Oct;52(5):759-761. doi: 10.1111/age.13127. Epub 2021 Aug 2. PMID: 34339052.
13. Pirosanto Y, Laseca N, Valera M, Molina A, Moreno-Millán M, Bugno- Poniewierska M, Ross P, Azor P, Demyda-Peyrás S. Screening and detection of chromosomal copy number alterations in the domestic horse using SNP-array genotyping data. Anim Genet. 2021 Aug;52(4):431-439. doi: 10.1111/age.13077. Epub 2021 May 19. PMID: 34013628.
14. Fornal A, Kowalska K, Zabek T, Piestrzynska-Kajtoch A, Musiał AD, Ropka- Molik K. Genetic Variability and Population Structure of Polish Konik Horse Maternal Lines Based on Microsatellite

Markers. Genes (Basel). 2021 Apr 9;12(4):546. doi: 10.3390/genes12040546. PMID: 33918718; PMCID: PMC8069725.

15. Patterson Rosa L, Staiger EA, Martin K, Vierra M, Foster G, Lundquist E, Brooks SA, Lafayette C. Stock-type equine disciplines Hunter, Reining and Roping are associated with the A allele at the DMRT3 locus for gait phenotypes in the horse. Anim Genet. 2021 Oct;52(5):773-774. doi: 10.1111/age.13110. Epub 2021 Jul 12. PMID: 34250625.

16. Peng S, Bellone R, Petersen JL, Kalbfleisch TS, Finno CJ. Successful ATAC- Seq From Snap-Frozen Equine Tissues. Front Genet. 2021 Jun 16;12:641788. doi: 10.3389/fgene.2021.641788. PMID: 34220931; PMCID: PMC8242358.

17. Vostry L, Vostra-Vydrova H, Citek J, Gorjanc G, Curik I. Association of inbreeding and regional equine leucocyte antigen homozygosity with the prevalence of insect bite hypersensitivity in Old Kladruber horse. Anim Genet. 2021 Aug;52(4):422-430. doi: 10.1111/age.13075. Epub 2021 May 10. PMID: 33970495; PMCID: PMC8360196.

18. Ayad A, Almarzook S, Besseboua O, Aissanou S, Piórkowska K, Musiał AD, Stefaniuk-Szmukier M, Ropka-Molik K. Investigation of Cerebellar Abiotrophy (CA), Lavender Foal Syndrome (LFS), and Severe Combined Immunodeficiency (SCID) Variants in a Cohort of Three MENA Region Horse Breeds. Genes (Basel). 2021 Nov 26;12(12):1893. doi: 10.3390/genes12121893. PMID: 34946842; PMCID: PMC8701198.

19. Nogueira PPO, Amorim GBAG, Oliveira OM, Demyda-Peyrás S, Santos BM, Mota LSLSD. Sex Reversal Syndrome in an Egyptian Arabian Horse Detected Using Genomic Data - A case report. J Equine Vet Sci. 2021 Sep;104:103692. doi: 10.1016/j.jevs.2021.103692. Epub 2021 Jun 21. PMID: 34416985.

20. Dahlgren AR, Knych HK, Arthur RM, Durbin-Johnson BP, Finno CJ. Transcriptomic Markers of Recombinant Human Erythropoietin Micro-Dosing in Thoroughbred Horses. Genes (Basel). 2021 Nov 24;12(12):1874. doi: 10.3390/genes12121874. PMID: 34946824; PMCID: PMC8702184.

21. Hales EN, Habib H, Favro G, Katzman S, Sakai RR, Marquardt S, Bordbari MH, Ming-Whitfield B, Peterson J, Dahlgren AR, Rivas V, Ramirez CA, Peng S, Donnelly CG, Dizmang BS, Kallenberg A, Grahn R, Miller AD, Woolard K, Moeller B, Puschner B, Finno CJ. Increased α-tocopherol metabolism in horses with equine neuroaxonal dystrophy. J Vet Intern Med. 2021 Sep;35(5):2473-2485. doi: 10.1111/jvim.16233. Epub 2021 Jul 31. PMID: 34331715; PMCID: PMC8478026.

22. Patterson Rosa L, Martin K, Vierra M, Foster G, Lundquist E, Brooks SA, Lafayette C. Two Variants of KIT Causing White Patterning in Stock-Type Horses. J Hered. 2021 Aug 25;112(5):447-451. doi: 10.1093/jhered/esab033. PMID: 34223905.

23. Tozaki T, Ohnuma A, Takasu M, Nakamura K, Kikuchi M, Ishige T, Kakoi H, Hirora KI, Tamura N, Kusano K, Nagata SI. Detection of non-targeted transgenes by whole-genome resequencing for gene-doping control. Gene Ther. 2021 Apr;28(3-4):199-205. doi: 10.1038/s41434-020-00185-y. Epub 2020 Aug 7. PMID: 32770095.

24. Hack YL, Crabtree EE, Avila F, Sutton RB, Grahn R, Oh A, Gilger B, Bellone RR. Whole-genome sequencing identifies missense mutation in GRM6 as the likely cause of congenital stationary night blindness in a Tennessee Walking Horse. Equine Vet J. 2021 Mar;53(2):316-323. doi: 10.1111/evj.13318. Epub 2020 Aug 3. PMID: 32654228.

25. Librado P, Khan N, Fages A, Kusliy MA, Suchan T, Tonasso-Calvière L, Schiavinato S, Alioglu D, Fromentier A, Perdereau A, Aury JM, Gaunitz C, Chauvey L, Seguin-Orlando A, Der Sarkissian C, Southon J, Shapiro B, Tishkin AA, Kovalev AA, Alquraishi S, Alfarhan AH, Al-Rasheid KAS, Seregély T, Klassen L, Iversen R, Bignon-Lau O, Bodu P, Olive M, Castel JC, Boudadi-Maligne M, Alvarez N, Germonpré M, Moskal-Del Hoyo M, Wilczyński J, Pospuła S, Lasota-Kuś A, Tunia K, Nowak M, Rannamäe E, Saarma U, Boeskorov G, Lõugas L, Kyselý R, Peške L, Bălăşescu A, Dumitraşcu V, Dobrescu R, Gerber D, Kiss V, Szécsényi-Nagy A, Mende BG, Gallina Z, Somogyi K, Kulcsár G, Gál E, Bendrey R, Allentoft ME, Sirbu G, Dergachev V, Shephard H, Tomadini N, Grouard S, Kasparov A, Basilyan AE, Anisimov MA, Nikolskiy PA, Pavlova EY, Pitulko V, Brem G, Wallner B, Schwall C, Keller M, Kitagawa K, Bessudnov AN, Bessudnov A, Taylor W, Magail J, Gantulga JO,

Bayarsaikhan J, Erdenebaatar D, Tabaldiev K, Mijiddorj E, Boldgiv B, Tsagaan T, Pruvost M, Olsen S, Makarewicz CA, Valenzuela Lamas S, Albizuri Canadell S, Nieto Espinet A, Iborra MP, Lira Garrido J, Rodríguez González E, Celestino S, Olària C, Arsuaga JL, Kotova N, Pryor A, Crabtree P, Zhumatayev R, Toleubaev A, Morgunova NL, Kuznetsova T, Lordkipanize D, Marzullo M, Prato O, Bagnasco Gianni G, Tecchiati U, Clavel B, Lepetz S, Davoudi H, Mashkour M, Berezina NY, Stockhammer PW, Krause J, Haak W, Morales-Muñiz A, Benecke N, Hofreiter M, Ludwig A, Graphodatsky AS, Peters J, Kiryushin KY, Iderkhangai TO, Bokovenko NA, Vasiliev SK, Seregin NN, Chugunov KV, Plasteeva NA, Baryshnikov GF, Petrova E, Sablin M, Ananyevskaya E, Logvin A, Shevnina I, Logvin V, Kalieva S, Loman V, Kukushkin I, Merz I, Merz V, Sakenov S, Varfolomeyev V, Usmanova E, Zaibert V, Arbuckle B, Belinskiy AB, Kalmykov A, Reinhold S, Hansen S, Yudin AI, Vybornov AA, Epimakhov A, Berezina NS, Roslyakova N, Kosintsev PA, Kuznetsov PF, Anthony D, Kroonen GJ, Kristiansen K, Wincker P, Outram A, Orlando L. The origins and spread of domestic horses from the Western Eurasian steppes. Nature. 2021 Oct;598(7882):634-640. doi: 10.1038/s41586-021-04018-9. Epub 2021 Oct 20. PMID:34671162; PMCID: PMC8550961.

26. Ricard A, Duluard A. Genomic analysis of gaits and racing performance of the French trotter. J Anim Breed Genet. 2021 Mar;138(2):204-222. doi: 10.1111/jbg.12526. Epub 2020 Nov 29. PMID: 33249655; PMCID: PMC7898598.

27. Dini P, Kalbfleisch T, Uribe-Salazar JM, Carossino M, Ali HE, Loux SC, Esteller-Vico A, Norris JK, Anand L, Scoggin KE, Rodriguez Lopez CM, Breen J, Bailey E, Daels P, Ball BA. Parental bias in expression and interaction of genes in the equine placenta. Proc Natl Acad Sci U S A. 2021 Apr 20;118(16):e2006474118. doi: 10.1073/pnas.2006474118. PMID: 33853939; PMCID: PMC8072238.

28. Bailey E, Petersen JL, Kalbfleisch TS. Genetics of Thoroughbred Racehorse Performance. Annu Rev Anim Biosci. 2022 Feb 15;10:131-150. doi: 10.1146/annurev-animal-020420-035235. Epub 2021 Nov 15. PMID: 34780248.

29. Tozaki T, Ohnuma A, Kikuchi M, Ishige T, Kakoi H, Hirota KI, Kusano K, Nagata SI. Rare and common variant discovery by whole-genome sequencing of 101 Thoroughbred racehorses. Sci Rep. 2021 Aug 6;11(1):16057. doi: 10.1038/s41598-021-95669-1. PMID: 34362995; PMCID: PMC8346562.

30. Kingsley NB, Hamilton NA, Lindgren G, Orlando L, Bailey E, Brooks S, McCue M, Kalbfleisch TS, MacLeod JN, Petersen JL, Finno CJ, Bellone RR. "Adopt-a- Tissue" Initiative Advances Efforts to Identify Tissue-Specific Histone Marks in the Mare. Front Genet. 2021 Mar 26;12:649959. doi: 10.3389/fgene.2021.649959. PMID: 33841506; PMCID: PMC8033197.

31. Nowacka-Woszuk J, Mackowski M, Mantaj W, Stefaniuk-Szmukier M, Cieslak J. Equine STX17 intronic triplication confirmed by droplet digital PCR analysis of its breakpoints. Anim Genet. 2021 Aug;52(4):567-568. doi: 10.1111/age.13073. Epub 2021 May 3. PMID: 33939848.

32. Stefanetti V, Pascucci L, Wilsher S, Cappelli K, Capomaccio S, Reale L, Passamonti F, Coletti M, Crociati M, Monaci M, Marenzoni ML. Differential Expression Pattern of Retroviral Envelope Gene in the Equine Placenta. Front Vet Sci. 2021 Jul 9;8:693416. doi: 10.3389/fvets.2021.693416. PMID: 34307531; PMCID: PMC8298818.

33. Patterson Rosa L, Walker N, Mallicote M, MacKay RJ, Brooks SA. Genomic Association of Chronic Idiopathic Anhidrosis to a Potassium Channel Subunit in a Large Animal Model. J Invest Dermatol. 2021 Nov;141(11):2639-2645.e3. doi: 10.1016/j.jid.2021.05.014. Epub 2021 May 31. PMID: 34081968.

34. Williams ZJ, Velez-Irizarry D, Petersen JL, Ochala J, Finno CJ, Valberg SJ. Candidate gene expression and coding sequence variants in Warmblood horses with myofibrillar myopathy. Equine Vet J. 2021 Mar;53(2):306-315. doi: 10.1111/evj.13286. Epub 2020 Jun 25. PMID: 32453872; PMCID: PMC7864122.

35. Shang S, Jiang R, Luo R, Jia S, Irwin DM, Wang Z, Zhang S. Development of a 19-plex short tandem repeat typing system for individual identification and parentage testing of horses (Equus caballus). Anim Genet. 2021 Oct;52(5):754-758. doi: 10.1111/age.13119. Epub 2021 Jul 15. PMID: 34268790.

36. Rosengren MK, Sigurðardóttir H, Eriksson S, Naboulsi R, Jouni A, Novoa-Bravo M, Albertsdóttir E, Kristjánsson Þ, Rhodin M, Viklund Å, Velie BD, Negro JJ, Solé M, Lindgren G. A QTL for conformation of back and croup influences lateral gait quality in Icelandic horses. BMC Genomics. 2021 Apr 14;22(1):267. doi: 10.1186/s12864-021-07454-z. PMID: 33853519; PMCID: PMC8048352.

37. Al Abri MA, Brooks SA, Al-Saqri N, Alkharousi K, Johnson EH, Alqaisi O, Al- Rawahi A, Al Marzooqi W. Investigating the population structure and genetic diversity of Arabian horses in Oman using SNP markers. Anim Genet. 2021 Jun;52(3):304-310. doi: 10.1111/age.13056. Epub 2021 Mar 17. PMID: 33730759.

38. Dugué M, Dumont Saint Priest B, Crichan H, Danvy S, Ricard A. Genomic Correlations Between the Gaits of Young Horses Measured by Accelerometry and Functional Longevity in Jumping Competition. Front Genet. 2021 Jan 29;12:619947. doi: 10.3389/fgene.2021.619947. PMID: 33584826; PMCID: PMC7879571.

39. Polak G, Gurgul A, Jasielczuk I, Szmatoła T, Krupiński J, Bugno-Poniewierska M. Suitability of Pedigree Information and Genomic Methods for Analyzing Inbreeding of Polish Cold-Blooded Horses Covered by Conservation Programs. Genes (Basel). 2021 Mar 17;12(3):429. doi: 10.3390/genes12030429. PMID: 33802830; PMCID: PMC8002693.

40. Gmel AI, Burger D, Neuditschko M. A Novel QTL and a Candidate Gene Are Associated with the Progressive Motility of Franches-Montagnes Stallion Spermatozoa after Thaw. Genes (Basel). 2021 Sep 25;12(10):1501. doi: 10.3390/genes12101501. PMID: 34680896; PMCID: PMC8536120.

41. Hammons V, Ribeiro L, Munyard K, Sadeghi R, Miller D, Antczak D, Brooks SA. GWAS Identifies a Region Containing the SALL1 Gene in Variation of Pigmentation Intensity Within the Chestnut Coat Color of Horses. J Hered. 2021 Aug 25;112(5):443-446. doi: 10.1093/jhered/esab037. PMID: 34343312; PMCID: PMC8386761.

42. Metzger J, Kreft O, Sieme H, Martinsson G, Reineking W, Hewicker-Trautwein M, Distl O. Hanoverian F/W-line contributes to segregation of Warmblood fragile foal syndrome type 1 variant PLOD1:c.2032G>A in Warmblood horses. Equine Vet J. 2021 Jan;53(1):51-59. doi: 10.1111/evj.13271. Epub 2020 May 19. PMID: 32323341.

43. El-Sheikh Ali H, Loux SC, Kennedy L, Scoggin KE, Dini P, Fedorka CE, Kalbfleisch TS, Esteller-Vico A, Horohov DW, Erol E, Carter CN, Smith JL, Ball BA. Transcriptomic analysis of equine chorioallantois reveals immune networks and molecular mechanisms involved in nocardioform placentitis. Vet Res. 2021 Jul 8;52(1):103. doi: 10.1186/s13567-021-00972-4. PMID: 34238364; PMCID: PMC8268225.

44. Tozaki T, Ohnuma A, Kikuchi M, Ishige T, Kakoi H, Hirota KI, Kusano K, Nagata SI. Robustness of digital PCR and real-time PCR against inhibitors in transgene detection for gene doping control in equestrian sports. Drug Test Anal. 2021 Oct;13(10):1768-1775. doi: 10.1002/dta.3131. Epub 2021 Jul 21. PMID: 34270866.

45. Williams ZJ, Velez-Irizarry D, Gardner K, Valberg SJ. Integrated proteomic and transcriptomic profiling identifies aberrant gene and protein expression in the sarcomere, mitochondrial complex I, and the extracellular matrix in Warmblood horses with myofibrillar myopathy. BMC Genomics. 2021 Jun 11;22(1):438. doi: 10.1186/s12864-021-07758-0. PMID: 34112090; PMCID: PMC8194174.

46. Gorssen W, Meyermans R, Janssens S, Buys N. A publicly available repository of ROH islands reveals signatures of selection in different livestock and pet species. Genet Sel Evol. 2021 Jan 4;53(1):2. doi: 10.1186/s12711-020-00599-7. PMID: 33397285; PMCID: PMC7784028.

47. Donnelly CG, Bellone RR, Hales EN, Nguyen A, Katzman SA, Dujovne GA, Knickelbein KE, Avila F, Kalbfleisch TS, Giulotto E, Kingsley NB, Tanaka J, Esdaile E, Peng S, Dahlgren A, Fuller A, Mienaltowski MJ, Raudsepp T, Affolter VK, Petersen JL, Finno CJ. Generation of a Biobank From Two Adult Thoroughbred Stallions for the Functional Annotation of Animal Genomes Initiative. Front Genet. 2021 Mar 8;12:650305. doi: 10.3389/fgene.2021.650305. PMID: 33763124; PMCID: PMC7982670.

48. Tozaki T, Ohnuma A, Kikuchi M, Ishige T, Kakoi H, Hirota KI, Kusano K, Nagata SI. Design and storage stability of reference materials for microfluidic quantitative PCR-based equine gene doping

tests. J Equine Sci. 2021 Dec;32(4):125-134. doi: 10.1294/jes.32.125. Epub 2021 Dec 28. PMID: 35023990; PMCID: PMC8731687.

49. Druml T, Brem G, Velie B, Lindgren G, Horna M, Ricard A, Grilz-Seger G. Equine vitiligo-like depigmentation in grey horses is related to genes involved in immune response and tumor metastasis. BMC Vet Res. 2021 Oct 25;17(1):336. doi: 10.1186/s12917-021-03046-x. PMID: 34696794; PMCID: PMC8543801.

50. Nowacka-Woszuk J, Mackowski M, Stefaniuk-Szmukier M, Cieslak J. The equine graying with age mutation of the STX17 gene: A copy number study using droplet digital PCR reveals a new pattern. Anim Genet. 2021 Apr;52(2):223-227. doi: 10.1111/age.13044. Epub 2021 Feb 7. PMID: 33550611.

51. Grillos AS, Roach JM, de Mestre AM, Foote AK, Kinglsey NB, Mienaltowski MJ, Bellone RR. First reported case of fragile foal syndrome type 1 in the Thoroughbred caused by PLOD1 c.2032G>A. Equine Vet J. 2021 Dec 22. doi: 10.1111/evj.13547. Epub ahead of print. PMID: 34939209.

52. Druml T, Brem G, Horna M, Ricard A, Grilz-Seger G. DPF3, A Putative Candidate Gene For Melanoma Etiopathogenesis in Gray Horses. J Equine Vet Sci. 2022 Jan;108:103797. doi: 10.1016/j.jevs.2021.103797. Epub 2021 Oct 21. PMID: 34801788.

53. Tozaki T, Ohnuma A, Hamilton NA, Kikuchi M, Ishige T, Kakoi H, Hirota KI, Kusano K, Nagata SI. Low-copy transgene detection using nested digital polymerase chain reaction for gene-doping control. Drug Test Anal. 2022 Feb;14(2):382-387. doi: 10.1002/dta.3173. Epub 2021 Oct 18. PMID: 34608764.

Sheep/ Goat NRSP-8 Annual Report of Multi-State Research Activity

Project Title: NRSP-8 Sheep/Goats Species Committee
Period Covered: January 1 to December 31, 2021

Accomplishments And Impacts:

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.

The sheep reference genome, Oar_rambouillet_v1.0, has been updated in collaboration with Ben Rosen and Derek Bickhart at the USDA ARS. Oxford Nanopore PromethION long reads data generated by Tim Smith at the USDA ARS U.S. Meat Animal Research Center was added to PacBio sequence generated by Kim Worley at Baylor College of Medicine to improve the contiguity of the assembly. The latest release of the Canu genome assembler, was implemented to improve the reference genome and increase the consensus quality for the Oar_rambouillet_v1.0 assembly. The ARS-UI_Ramb v2.0 assembly improved the previous reference assembly from a contig N50 (Mb) of 2.57, LG50 (contigs) of 313, and 7,486 contigs to a contig N50 (Mb) of 42.39, LG50 (contigs) of 25, and 1,344. The new version of the Rambouillet genome has been submitted to NCBI and the manuscript for this improved assembly has just been accepted in in Gigascience 2022. (Brenda Murdoch, UI and Ben Rosen, USDA ARS)

The Development of the ovine pangenome will enhance our ability to capture and utilize structural variation within a genome affords to gain a more comprehensive view of the genetic variation driving phenotypic variation. A detailed understanding of how different genomes and gene products from diverse breeds of sheep that have evolved to result in a variety of important biological traits will facilitate the development of new breeding strategies and approaches designed for the sustainability of food and fiber production. One of the objectives of this project is to deliver high-quality genomes, using the recently describe trio binning approach, for eight breeds/species of sheep selected for their divergent economically important traits which will serve as the basis of the ovine pangenome. To date we have collected and have begun the sequencing of three F1 representing six different breeds of sheep. (Brenda Murdoch, UI and Ben Rosen, USDA ARS)

The study of runs of homozygosity (ROH) is crucial in understanding genetic diversity and providing a comprehensive picture of the population demography in domesticated animals and humans. The length of ROH segments is associated with the age of inbreeding, with longer ROH runs emanating from more recent common ancestors and shorter ones deriving from older common ancestors. The ROH distribution reveals information about population history in terms of selection and other major historical events. (Solkner et al. 2010, Nandolo et al. 2019, Nandolo et al., 2018, Purfield et al. 2012). Analysis of ROHs was conducted in four different goat breeds to determine the age of the ROH segments, the frequency of the ROHs by size, and gene discovery in these ROH areas. This study used SNP data from Boer (n=34), Myotonic (n=14), Kiko (n=12) and Spanish (n=20) breeds. The RZooROH package in R was used for the primary analysis, but additional analysis used Plink, R, and GREP. Multiple K values were tested for the RZooROH analysis with K=12 as the final choice. The results indicate that all breeds were under selection pressure for the last 150 years, which is consistent with increase of animal husbandry practices after 1900. Additionally, the Spanish, Kiko and Myotonic breeds have some older selection pressure data around the 800-1100 period. For the Spanish and Kiko which have a common ancestry based on PCA analysis, this time period may indicate the movement of goats with the Moor occupation of Spanish regions during that time period. The PCR analysis indicates that the Boer and Myotonic breeds each have different ancestry from the Spanish and Kiko breeds. The regions of the genome with high homozygosity were isolated for analysis of genes in those potential conserved regions. This analysis is still ongoing. The study of ROH segments is vital because it helps with conservation, breeding management of goat populations, and the identification of significant genes associated with crucial economic traits. (Brian Sayre, VSU)

Studies have been conducted to develop a greater understanding of the functional significance of galectin gene expression and how it can be modulated to ensure ruminant (cattle, sheep and goats) health and welfare for sustainable food production and public health. To expand our basic understanding of Galectin genetics and function in ruminant blood we evaluated 1.the expression of Galectin variants in blood from cattle sheep and goats. (Mulumebet Worku, NCA&TSU)

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.

Gastrointestinal nematode infections present a serious threat to the sheep industry therefore understanding parasite resistance in sheep is very imperative. This project aims to understand the mechanisms of gastrointestinal nematode resistance in Katahdin sheep and contribute to the understanding of this genotype-phenotype relationship. To date this project has collected fecal samples, performed FEC and collected blood samples from approximately 5000 Katahdins over four years for genotyping. The GWAS analyses of these samples has just been published in 2022. (Brenda Murdoch, UI)

Flock54 is a new genotyping by sequencing panel of 1000 markers that was developed for the benefit of the U.S. sheep industry. This panel includes causative markers previously identified and published (OMIA), parentage markers (Heaton et al) as well as to yet to be validated marker associated economic traits of interest to the U.S. sheep industry. Providing low cost genotyping tools, options and information to the U.S. sheep industry will allow greater uptake and adoption of genomic tools to enhance genetic progress, improved profitability and therefore sustainability of the U.S. sheep industry. (Brenda Murdoch, UI)

Artificial Neural Network as an Alternative Genome-Wide Association Method. Identification of genes or causative mutations in multi-factorial economically important traits or diseases has been a difficult problem. A number of limitations to traditional genome-wide association (GWA) analysis have been identified. Recently, many new approaches have been developed to expand the information included in the analysis with improved outcomes. Artificial neural networks are an expansion of machine learning that develop weighted outcomes without input bias. We have developed an artificial neural network method to analyze

single nucleotide polymorphism (SNP) data for GWA. Dairy cattle SNP data (n=20,405 cows; SNPs=60,720/cow) were analyzed for 5 phenotypes (milk yield, fat yield, protein yield, fat percentage and protein percentage). The artificial neural network developed for SNP analysis (NN-SNP) was a feed-forward design with an input for each SNP, three hidden layers and a regression output layer. The NN-SNP was trained on 75% of the data with the remaining 25% used for testing. Comparisons were between traditional GWA methods and NN-SNP methods for accuracy and correlation of predicted phenotypes. Weights and predicted phenotype data from NN-SNP analyses were well correlated with traditional GWA analyses. As expected from traditional GWA, NN-SNP strongly identified the region around DGAT (BTA14) for fat percentage and similar SNP data patterns for all other phenotypes. Additionally, a strong response for fat percentage was identified on BTA5 that was not as evident with traditional GWA. Further analysis is on-going to refine the NN-SNP method. The development of an artificial neural network approach to GWA has great potential for expanded analyses that can include many different data types and approaches. The overall goal of this research is to create a novel method for candidate gene discovery using multiple data sets and merging through a neural network model. (Brian Sayre, VSU)

We determined the effect of dietary galectin antagonists (Ginger in St Croix sheep and Gum Arabica extracts in goats) on nematode infection and immune gene expression in small ruminants. Preliminary results indicate GA impacts global transcription, translation and fecal microbial diversity in goats. Immunomodulation may result from differential gene expression and changes in gut microbiota in response to GA. Specific mechanisms and pathways involved and their relation to animal health are understudy. (Mulumebet Worku, NCA&TSU)

Impact / Usefulness of Findings:

Invited Seminars: This list of seminars to provide information and education among scientists, Extension educators, graduate students and the general public to benefit society.
1. Murdoch BM, Davenport KM, Salavati M, Clark E, Archibald A, Massa AT, Mousel MR, Herndon MK, White SN, Worley KC, Bhattarai S, McKay SD, Dalrymple B, Kijas J, Caulton A, Clarke S, Brauning R, Hadfield T, Smith TPL, Cockett NE. The Ovine Functional Annotation of Animal Genome Project. 38th International Society of Animal Genetic Virtual Conference, July 26-30, 2021.
2. Davenport KM, Bickhart DM, Worley KC, Murali SC, Cockett N, Heaton MP, Smith TPL, Murdoch BM[*], Rosen B[*]. An improved, high-quality ovine reference genome to facilitate functional annotation of gene regulatory elements. 38th International Society of Animal Genetic Virtual Conference, July 26-30, 2021.
3. Davenport KM, Bickhart DM, Worley KC, Murali SC, Cockett N, Heaton MP, Smith TPL, Murdoch BM[*], Rosen BD[*]. An improved, high-quality ovine reference genome assembly. American Society of Animal Science – Canadian Society of Animal Science, Kentucky July 17, 2021.
4. Davenport KM, Massa AT, Mousel MR, Herndon MK, White SN, Salavati M, Clark E, Archibald A, Bhattarai S, McKay SD, Worley KC, Dalrymple B, Kijas J, Caulton A, Clarke S, Brauning R, Hadfield T, Cockett NE, Smith TPL, Murdoch BM on behalf of The Ovine FAANG Project Consortium. Functional annotation of the reference Rambouillet sheep genome International Sheep Genome Consortium. June 8-10, 2021
5. Davenport KM*, Loux SC*, Bickhart DM, Worley KC, Freking BA, Vander Ley BL, Murali SC, Schroeder SG, Cockett N, Heaton MP, Clarke S, Murphy TW, Kalbfleisch TS, Murdoch BM, Smith TPL, Rosen BD. High-quality ovine reference genome assemblies. International Sheep Genome Consortium. June 8-10, 2021
6. Murdoch BM, Cockett N, Hadfield T, Mousel M, White S, Clark E, Salavati M, Clarke S, Hess A, Brauning R, Hagen D, Kalbfleisch TS, Heaton M, Bickhart D, Rosen B, Smith TPL. Constructing the Ovine Pan-genome. International Sheep Genome Consortium. June 8-10, 2021.
7. Murdoch B. Ovine and Bovine pangenome CyVers workshop, April 8, 2021

Peer Reviewed Journal Articles (Total 10)

1. Thorne JW, Murdoch BM, Freking BA, Redden RR, Murphy TW, Taylor JB, et al. Evolution of the sheep industry and genetic research in the United States: opportunities for convergence in the twenty-first century. *Anim Genet*. (2021) Aug;52(4):395–408. DOI: 10.1111/age.13067

2. Davenport KM, Taylor JB, Henslee D, Southerland C, Yelich J, Ellison MJ, Murdoch BM. Variation in type two taste receptor genes is associated with bitter tasting phenylthiocarbamide consumption in mature Targhee and Rambouillet rams. *Translational Animal Science*. (2021) Jul 1;5(3):txab142. DOI:10.1093/tas/txab142

3. Mousel MR, White S., Herndon MK, Herndon DR, Taylor JB, Becker GM, Murdoch BM. Genes involved in immune, gene translation and chromatin organization pathways associated with Mycoplasma ovipneumoniae presence in nasal secretions of domestic sheep. *PLoS One* (2021) July 12;16(7). DOI 10.1371/journal.pone.0247209.

4. Davenport KM, Massa AT, Bhattarai S, McKay SD, Mousel MR, Herndon MK, White SN, Cockett NE, Smith TPL, Murdoch BM. Characterizing Genetic Regulatory Elements in Ovine Tissues. *Frontiers in Genetics* (2021) May 20;12. DOI: 10.3389/fgene.2021.628849.

5. Massa AT, Mousel MR, Herndon MK, Herndon DR, Murdoch BM, White SN. Genome-wide histone modifications and CTCF enrichment predict gene expression in sheep macrophages. *Frontiers in Genetics* (2021) Jan 07.DOI: 10.3389/fgene.2020.612031.

6. Worku M, Rehrah D, Ismail HD, Asiamah E, Adjei-Fremah S. A Review of the Neutrophil Extracellular Traps (NETs) from Cow, Sheep and Goat Models. Int J Mol Sci. 2021 Jul 28;22(15):8046. doi: 10.3390/ijms22158046. PMID: 34360812; PMCID: PMC8347029.

7. National Science Foundation (NSF) Convergence Accelerator: Digital and Precision Agriculture. Workshop Report Virtual workshop held May 10, 12, 14, & 21, 2021. Report Authors: James M. Reecy, Iowa State University, Carissa A. Park, Iowa State University, Gregory Goins, North Carolina A&T State University, Mahmut Kandemir, Penn State University, Nadilia Gomez, Iowa State University, Brian Aldridge, University of Illinois, Porche' Spence, North Carolina A&T State University, Millie Worku, North Carolina A&T University, Catherine Keske, University of California, Merced, Pedro Sanchez, University of Arizona, U. Sunday Tim, Iowa State University, Hongwei Zhang, Iowa State University

8. Sarilda Kilimanjaro. 2021. Evaluation of The Effect of Ginger Extracts on Galectin gene expression and secretion in blood from St. Croix Sheep MSC Thesis.

9. Yaser M Ahmed, Hamid Ismail, Djaafar M Rehrah, Mulumebet Worku, Immunomodulatory Effects of Gum Arabica in Goat Blood, Journal of Animal Science, Volume 99, Issue Supplement_2, May 2021, Page 42, https://doi.org/10.1093/jas/skab096.077

10. Yaser M Ahmed, Hamid Ismail, Djaafar M Rehrah, Mulumebet Worku, Effect of Gum Arabica (GA) Drench on Indicators of Anemia in Goats, Journal of Animal Science, Volume 99, Issue Supplement_2, May 2021, Pages 37–38, https://doi.org/10.1093/jas/skab096.068

Peer Reviewed Abstracts

1. Thorne J, Redden R, Murdoch B. Resolving the Disparity in Genomic Advancement of Livestock. Vision III Star Gazing the Galaxy of Animal Genetics and Genomics. Ames Iowa Nov 3-4, 2021. Becker G.M., Burke J.M., Lewis R.M., Miller J.E., Morgan J.L.M., Notter D.R., Murdoch B.M. DEL-1 Gene is Associated with Increased Weaning Fecal Egg Counts in Katahdin Sheep, 38th International Society of Animal Genetic Virtual Conference, July 26-30, 2021.

2. Davenport K.M., Bickhart D.M., Worley K.C., Murali S.C., Cockett N., Heaton M.P., Smith T.P.L., Murdoch B.M., Rosen B.D. An improved, high-quality ovine reference genome to facilitate functional annotation of gene regulatory elements. 38th International Society of Animal Genetic Virtual Conference, July 26-30, 2021.

3. Bhattarai S., Davenport K.M., Massa A.T., Mousel M.R., Herndon M.K., White S.N., Cockett N., Smith T.P.L., Murdoch B.M., McKay S.D. on behalf of The Ovine FAANG Project Consortium. Whole

genome bisulfite sequencing reveals tissue specific DNA methylation profiles in Sheep. International Sheep Genome Consortium. June 8-10, 2021.

4. Davenport K.M., Massa A.T., Mousel M.R., Herndon M.K., White S.N., Salavati M., Clark E., Archibald A., Bhattarai S., McKay S.D., Worley K.C., Dalrymple B., Kijas J., Caulton A., Clarke S., Brauning R., Hadfield T., Cockett N.E., Smith T.P.L., Murdoch B.M. on behalf of The Ovine FAANG Project Consortium. Functional annotation of the reference Rambouillet sheep genome. International Sheep Genome Consortium. June 8-10, 2021.

5. Davenport K.M.*, Loux S.C.*, Bickhart D.M., Worley K.C., Freking B.A., Vander Ley B.L., Murali S.C., Schroeder S.G., Cockett N., Heaton M.P., Clarke S., Murphy T.W., Kalbfleisch T.S., Murdoch B.M., Smith T.P.L., Rosen B.D. High-quality ovine reference genome assemblies. International Sheep Genome Consortium. June 8-10, 2021.

6. Murdoch B.M., Cockett N., Hadfield T., Mousel M., White S., Clark E., Salavati M., Clarke S., Hess A., Brauning R., Hagen D., Kalbfleisch T.S., Heaton M., Bickhart D., Rosen B., Smith T.P.L. Constructing the Ovine Pan-genome. International Sheep Genome Consortium. June 8-10, 2021.

7. Becker G, Stegemiller M, Schauer C, Stewart W, Murdoch B. Genome-wide Association Study of Rambouillet Rams with Angular Limb Deformities. American Society of Animal Science – Canadian Society of Animal Science, Kentucky July 14-18, 2021.

8. Thorne T, Bowdridge SA, Murdoch BM, Redden R. Sire Fecal Egg Count EBV are Predictive of Offspring Response to an Artificial Gastrointestinal Nematode Infection in Rambouillet Sheep. American Society of Animal Science – Canadian Society of Animal Science, Kentucky July 14-18, 2021.

9. Davenport K, Bickhart DM, Worley K, Murali, Shwetha, Cockett N, Heaton MP, Smith TPL, Murdoch BM*, Rosen BD*. An Improved, High-quality Ovine Reference Genome Assembly. American Society of Animal Science – Canadian Society of Animal Science, Kentucky July 14-18, 2021.

Graduate Student Education: In total four graduate students have benefited through research and training opportunities by this hatch program.

1. Kimberly Davenport, Doctorate of Philosophy candidate, research training has contributed to the new reference genome and the FAANG project.

2. Jacob Thorne, Doctorate of Philosophy student, research training has contributed researching fertility traits in sheep.

3. Gabrielle Becker Doctorate of Philosophy student, is working on the GWAS of FEC in Katadhin sheep and the improvement of Flock54 genotyping by sequencing project.

4. Morgan Stegemiller, Doctorate of Philosophy student, research has included population structure analysis for Flock54 genotyping and using whole genome sequencing for genotyping in 50 individuals from five breeds of sheep.

Active Grants and Contract Leveraged Through NRSP08 Research:

1. Establishing a Pan-Epigenome for Cattle and Sheep. (2022) AFRI NIFA PI: S. McKay Co-PI's: B. Murdoch, D. Hagen. Award $1,300,000. Flock54℠ a new genomic selection tool that enhances the U.S. sheep industry. (2021) IGEM PI: Murdoch B. Awarded $96,866.

2. Developing the Ovine Pangenome. (2021) AFRI NIFA PI: B Murdoch Co-PI's: B. Rosen, T. PL Smith, S. White, M. Mousel, E. Clark, R. Brauning, S. Clarke, N. Cockett. Awarded $500,000.

3. USDA ARS Host Genomics (2020) USDA PI: B Murdoch Awarded $156, 360.

4. Assessment of the resilience of local Baladi goat in Lebanon: a viable sustainable solution to a changing climate in a transhumant system. (2020) Partnerships for Enhanced Engagement in Research (PEER) Program. PI P Aad, Co-PI's J Burke, B Murdoch Awarded $155,050.

5. NIFA AG2PI Collaborative: Creating a Shared Vision Across Crops and Livestock Communities. (2020) AFRI NIFA PI: P. Schnable Co-PI's: B. Murdoch, J. Dekkers, C. Tuggle, C. Lawrence-Dill, J. Clark, E Lyons. Award $960,000.

6. RNA methylation as a mechanistic link between genotype and phenotype (2020) AFRI NIFA PI: S McKay, Co PI: B Murdoch. Awarded $200,000.
7. Flock54℠ a new genomic selection tool that enhances the U.S. sheep industry. (2020) IGEM PI: Murdoch B. Awarded $251,500.
8. Social Interaction and Consumer Acceptance of Genome Editing in Domestic Livestock-PI: McCluskey J. Co-PI's: J. Winfree, A. Van Eenannaam, P. Glazebrook, S. Badruddoza, Key personnel B Murdoch. Awarded $62,987.
9. A foundation for genomics: building a repository of genotypes in Rambouillet sheep. PI- Ronald Lewis, Co-PI's: L. Brito. J. Peterson, T. Murphy B. Murdoch. Awarded $51,892.
10. Genomes to phenomes of cattle and sheep in Idaho. (2019) National Animal Genome Research Program – NRSP008 - Hatch Grant PI: Murdoch B.

NRSP-8 Poultry Annual Report October 1, 2020 – September 30, 2021

Coordinators: Huaijun Zhou (UC Davis); Hans Cheng (USDA-ARS)
Chair: **Bindu Nanduri (Mississippi State University)**
Secretary: Byungwhi Kong (University of Arkansas)

Poultry Workshop: Held January 8 - 9, 2022 in conjunction with NC1170 Poultry Workshop at the virtual meeting and attendance overview. Attendance during the 1.5 day workshop averaged n=37 with peak attendance in excess of 65.
- Two keynote speakers presented isoseq and immune cell atlas, respectively.
- Three speakers presented at special session of a mini symposium on chicken genome and annotation: Next Steps
- Representatives of 16 agricultural experiment stations attended from across the US including the membership of NRSP-8 Poultry group: Iowa State, University of Arizona, University of Arkansas, Western University of Health Sciences, Mississippi State University, Univ of Delaware, University of California Davis, University of Minnesota, USDA-ARS-ADOL, California State University Fresno, University of Maryland, and City of Hope Beckman Research Institute.
- Attendees also included members of the poultry layer and broiler breeding companies, and scientists from the US, Europe, South America, Asia, and Africa.

Grants: (Total $24,120,006)
Wayne Kuenzel, Douglas Rhoads, Adnan Alrubaye, Byung whi Kong, University of Arkansas: (Total $10,346,797)
- Validation of a SNP panel for breeding against ascites in broilers. NIFA-AFRI; 3/2018-2/2021; $500,000; PI Rhoads, CoI Orlowski. No cost extension through 4/2022.
- Empowering US Broiler Production for Transformation and Sustainability. USDA-NIFA Sustainable Agriculture Systems; 5/2019 - 4/2024; $9,191,300; PD Bottje CoIs Kuenzel, Kong, Dridi, Alrubaye, Rhoads
- Gene Editing and Transgenic Poultry Production. UA Chancellor Grant. 7/1/2019 - 6/30/2022; $115,665; PI Kong CoI Kuenzel
- Placement of 2D and 3D neuroanatomical visual data into a virtual environment to facilitate research and learning. UA Chancellor Grant. 2020-2023; $84,661; PI Kuenzel CoIs Fredrick, Gignac
- A newly discovered function of a septal brain structure in poultry. Arkansas Biosciences Institute; 2020- 2021; $25,000; PI Kuenzel CoI Jurkevich,
- Evaluation of feed additives on the Incidence of BCO lameness in broilers when using the wire-

flooring as an induction model. BIOMIN America Inc; 9/2020-1/2021, $49,933; PI Alrubaye CoI Rhoads

- High resolution mapping of genetic determinants for resistance to Infectious bronchitis virus in broilers. Arkansas Biosciences Institute; 7/2020-6/2022; $74,521; PI Rhoads CoIs Toro, Alrubaye, Dridi, Pummill
- Plasma biomarker discoveries for early diagnosis of woody breast in broilers. Adisseo; 4/1/2020-3/31/2021; $50,400; PI Kong CoIs Owens, Kuenzel
- Validation of plasma biomarkers discovered for early diagnosis of woody breast in broilers. Adisseo; 8/1/2021-3/15/2022; $83,741; PI Kong CoI Owens, Rochell
- Evaluation of feeding the Availa-ZMC to a selected parent breeder line and identifying the incidence of BCO lameness in broiler offspring. UA Chancellor Grant. 10/2021-5/2022; $83,546; PI Alrubaye, CoI Rhoads
- An updated stereotaxic atlas of the chick brain. Arkansas Biosciences Institute; 2021-2022; $39,300; PI Kuenzel CoI Jurkevich
- Evaluation of selected Nuproxa feed additives for protection against lameness and improving the well- being of broilers in a lameness challenge model. Nuproxa; 9/21-4/22; $48,730; PI Alrubaye; CoI Rhoads

Huaijun Zhou, University of California, Davis: (Total $6,000,000 chicken)
- NIFA: The project titled "Functional Annotation of the Bovine Genome" H. Zhou (PI), P. Ross, J. Mendaro et al. 1/15/2018-1/14/2023. $2,500,000
- NIFA: The project titled "Functional annotation and validation of regulatory elements in the chicken genome." H. Zhou (PI) 4/1/2020 – 3/31/2023 $500,000
- NIFA: The project titled "Functional Annotation of the Swine Genome" C. Tuggle (PI), H. Zhou, et al. 1/15/2018-1/14/2023 $2,500,000
- NIFA: The project titled "Epigenetic regulatory mechanisms affecting growth and feed efficiency by comparing modern broilers with control meat-type genetic line" H. Zhou (PI), 1/1/2021 – 12/31/2023 $500,000
- USAID: The project titled "Improving food security in Africa by enhancing resistance to disease and heat in chickens; Feed the future innovation lab for genomics to improve poultry", H. Zhou, S. J. Lamont, J. Dekkers etc. 09/23/2018-09/22/2023 $5,000,000

Susan Lamont and Jack Dekkers, Iowa State University
- USAID grant "Improving Food Security of Africa by Enhancing Resistance to Disease and Heat in Chickens." Zhou et al. (Subaward to ISU).
- USDA-NIFA Postdoctoral Fellowship to Dr. Melissa Monson. "Chicken Innate Gene Expression Responses to Avian Influenza Infection: Research and Investigator Development"
- USDA Hatch-Multistate and Animal Health funds to Iowa State Universit

Kent Reed, Univ of Minnesota: (Total $338,014)
- Prolonging Fertility in Turkey Breeders as a Sustainable Model for Poultry Production: The Role of the Immune System. Minnesota Rapid Agricultural Response Fund. 2021, Co-PI w/ Kahina Ghanem (UMN Project Lead), Budget $113,014.
- Exploring the Determinants of Susceptibility of Minnesota Turkeys to Avian Influenza Virus. USDA- Minnesota Agricultural Experiment Station, 2021-2024, co-Investigator w/ Hinh Ly (PI), and Sunil Mor (co-I), Budget $225,000.

Hans. H. Cheng, USDA-ARS: (Total $3,365,195)

* Experiments for this NIFA-supported project are enhanced by financial support from ARS, competitive grants, and collaborations within and external to this project. A list of public support is given below:

- ARS CRIS Project, Employing Genomics, Epigenetics, and Immunogenetics to Control Diseases Induced by Avian Tumor Viruses.
- ARS CRIS Project, Genetic and Biological Determinants of Avian Herpesviruses Pathogenicity, Transmission, and Evolution to Inform the Development of Effective Control Strategies.
- USDA, AFRI, award no. 2018-67015-28308, Genomic screens to identify regulatory elements with causative polymorphisms accounting for Marek's disease genetic resistance in chicken. PI, Cheng; co-PIs, Erez Lieberman Aiden (Baylor) and Bill Muir (Geneysis Bioinformatic Services). $498,116.
- USDA, AFRI, award no. 2020-67015-31574, A chicken pan-genome reference panel and single cell atlas to broadly study environmental challenges. PI, W. Warren (U. of Missouri); co-PIs, H. Cheng and C. Elsik (U. of Missouri). $500,000.
- USDA, AFRI, award no. 2021-67015-34560, MDV pathogenesis: the role of somatic cell integration on host chromosome architecture. PI, M. Campbell (UC Davis); co-PIs, Yoshi Izumiya and Clifford Tepper (UC Davis) and H. Cheng. $650,000.
- NSF, NIH, USDA EEID grant, proposal no. 2011057, US-UK Collab: Combined influence of imperfect vaccines, host genetics, and non-genetic drivers on virus transmission and virulence evolution. PI, J. Dunn; co-PIs, H. Cheng and C. Hearn, A Doeschl-Wilson and S. Lycett (Roslin Institute), N. Osterrieder (Freie U. Berlin), K, Rich (ILRI), E. Karcher and D. Karcher (Purdue U.), and J. Fulton (Hy-Line). $1,717,079.

Behanm Abasht, University of Delaware (Total $580,000)
- US Poultry and Egg Association ($80,000) 09/01/2019 - 08/30/2021 (+ 6-month NCE): Feed additive for reducing incidence of Wooden Breast in commercial broiler chickens, PI Abasht
- USDA-National Institute of Food and Agriculture 2021-67015-34543: Targeting Genomic Regions Identified Through Genome-Wide Association Study of Wooden Breast, 2021-2024, $500,000, PI Abasht

Tom Porter, University of Maryland (Total $500,000)
- USDA, AFRI, award no. 2017-67015-26572, Mitigation of heat stress in broiler chickens through early-life thermal conditioning. PI, T. E. Porter. 05/15/2017 – 05/14/2021, $500,000.

Fiona McCarthy, University of Arizona (Total $490,000)
- USDA, AFRI, award no. 2018-67015-28126, Developing functional annotation workflows for animal genomics research. PI, F. McCarthy. 05/01/2017 – 04/30/2022, $490,000.

Yvonne Drechsler, Western University of Health Sciences (Total $2,500,000)
- USDA, AFRI, award no. 2018-67015-27499, Genome-wide annotation of cis-regulatory elements in the chicken genome. PI, Y. Drechsler. 03/01/2018 – 02/28/2023, $2,500,000.

Impacts:

Our members are highly focused on fundamental, translational and applied research to benefit U.S. Agriculture and through genomics improve poultry health and contribute to the productivity of the relevant industries. Below are listed some of the highlights from 2020-21 research. Many of the efforts are focused on projects that directly impact poultry health and production.

- Genetic variation was characterized in commercial and research lines of chickens, as well as in local African chicken populations.

- Genes, pathways and genomic regions associated with important biological traits in chickens were identified.
- Our efforts are focused on projects that directly impact poultry health and production. Extreme temperature variations threaten the quality of poultry muscle as a healthy, high quality food product. Identification of molecular mechanisms associated with altered muscle development will result in development of mitigation strategies based on improved genetic selection, nutritional intervention, and other strategies to improve poultry muscle food quality and quantity.
- Development of low-density panel associated with resistance to heat stress and Newcastle disease virus infection can be used to breeding more resilient chickens in adaption to hot climate in developing countries.
- Knowledge of genes associated with enhanced immune response may inform further information on vaccine efficacy in poultry production.
- Functional annotation of regulatory elements will be important for animal genome community for identifying potential causative variants for economically important traits.
- The eQTL across tissues identified in chicken provide valuable sources for deciphering the genetic and biological basis of complex traits in chickens.
- Utilizing pre-harvest farm practices and soil composition data we were able to identify factors that can promote Salmonella, Listeria and Campylobacter prevalence in pastured poultry meat. Based on the results we made recommendations for micro and macro nutrient levels in the soil that do not support growth of these harmful bacteria that contaminate poultry products.
- We validated two genes and demonstrated that we can breed for resistance to ascites without reducing production traits (manuscript submitted and tentatively accepted pending revisions)
- We worked with industry to validate three systems for inducing lameness and evaluation of commercial probiotics to reduce lameness.
- We used Whole Genome Resequencing to investigate the genetic basis for resistance to Infectious Bronchitis Virus in Leghorns.
- Blood metabolite biomarkers may be suitable candidate targets in the prediction of WB onset at early ages.
- Detailed studies of two distinct stressors has shown that two brain regions [septum (S) and hypothalamus (H)] and a consistent sequence of gene expression within 3 distinct sets of neurons occurs that contain: 1) CRH in S, 2) CRH in H and 3) AVT in H. The 3 sets of peptides stimulate the anterior pituitary (P) followed by the adrenal (A) to release corticosterone, a stress hormone, thereby activating the SHPA axis in poultry, a system perhaps also functioning in other avian species.
- SNPs causing non-synonymous amino acid changes found in HS and LS lines of quail could be used for genetic markers as stress resistant phenotypes in poultry.
- We maintained large number of chicken lines representing a number of traits, especially those associated with viral diseases, unique research populations and maintained under specific pathogen free (SPF) conditions.
- We developed and updated databases including Chickspress (http://geneatlas.arl.arizona.edu), AgBase (https://agbase.arizona.edu), the Chicken Gene Nomenclature Committee (CGNC: http://birdgenenames.org), and iMicrobe (https://www.imicrobe.us) to provide resources of genetic information of poultry.
- Characterization of gene regulatory elements in the chicken genome will aid in the selection of markers for disease resistance in breeding.
- Establishing immunoglobulin-like receptors as valuable contributors to cellular immunity will provide confidence as disease indicator and their use in programs selectively breeding for disease resistance.
- We investigated the role of the host epigenome in immune evasion of viruses and disease resistance

and susceptibility to develop a deeper understanding of the genetic processes involved.
- We investigated the role of CHIR in disease resistance, improving the existing nomenclature.

2019-2020 Poultry Genome Committee publications

Book chapters:
1. Lamont, S.J., Dekkers, J.C.M., Wolc, A. and Zhou, H. 2022. Immunogenetics and the mapping of immunological functions. Pp. 277-297. In: Avian Immunology, 3rd ed. B. Kaspers, K.A. Schat, T. Gobel, L. Vervelde, Eds., Elsevier, London, San Diego, Cambridge, Oxford
2. Cheng, H.H., Warren, W.C., and Zhou, H. 2021. Avian genomics. In Sturkie's Avian Physiology, 7th ed. C.G. Scanes and S. Dridi, eds. (San Diego, CA: Academic Press), pp. 7-16.
3. Kuenzel, W.J. and A. Jurkevich. 2021. The avian subpallium and autonomic nervoussystem. In: Sturkie's Avian Physiology. (Eds. C.G. Scanes and S. Dridi) Elsevier. 7th Ed., SanDiego, CA, Chapter 16:257-290, Academic Press/ Elsevier, Oxford, UK.

Refereed journal articles: (Total 41)
1. Abasht B, Papah MB, Qiu J. Evidence of vascular endothelial dysfunction in Wooden Breast disorder in chickens: Insights through gene expression analysis, ultra-structural evaluation and supervised machine learning methods. PLoS One. 2021;16(1):e0243983. doi: 10.1371/journal.pone.0243983. eCollection 2021. PubMed PMID: 33395447; PubMed Central PMCID: PMC7781381.
2. Aston E.J., Wang, Y., Tracy, K.E., Gallardo, R.A., Lamont, S.J., Zhou, H. 2021. Comparison of cellular immune responses to avian influenza virus in two genetically distinct, highly inbred chicken lines. Vet. Immunol. Immunopathol. 235: 110233. https://doi.org/10.1016/j.vetimm.2021.110233
3. Bottje WG, Lassiter KR, Kuttappan VA, Hudson NJ, Owens CM, Abasht B, Dridi S, Kong BC. Upstream Regulator Analysis of Wooden Breast Myopathy Proteomics in Commercial Broilers and Comparison to Feed Efficiency Proteomics in Pedigree Male Broilers. Foods. 2021;10(1). doi: 10.3390/foods10010104. PubMed PMID: 33419207; PubMed Central PMCID: PMC7825620.
4. Bowman, A., C. Fitzgerald, J.F. Pummill, D. Rhoads, T. Yamashita (2021) Reduced toxicity of *Centruroides vittatus* (Say, 1821) scorpion may result from lowered sodium toxin gene expression and toxin protein production. Toxins (in press).
5. Del Vesco, A.P., Jang, H.J., Monson, M.S., and Lamont, S.J. 2021. Role of chicken oligoadenylate synthase like gene during *in vitro* Newcastle disease virus infection. Poultry Sci. doi.org/10.1016/j.psj.2021.101067
6. Ekesi, N.S., A. Hasan, A. Parveen, A. Shwani, D. Rhoads (2021). Embryo lethality assay for evaluating virulence of isolates from bacterial chondronecrosis with osteomyelitis in broilers. Poultry Science 100:101455.
7. Ekesi, N.S., B. Dolka, A. Alrubaye, D. Rhoads (2021). Analysis of genomes of bacterial isolates from lameness outbreaks in broilers. Poultry Science: 100(7): 101148
8. Glass, M.C., Smith, J.M., Cheng, H.H. and Delany, M.E. 2021. Marek's disease virus telomeric integration profiles of neoplastic host tissues reveal unbiased chromosomal selection and loss of cellular diversity during tumorigenesis. Genes 12, 1630.
9. D. E. Goszczynski, M. M. Halstead, A. D. Islas-Trejo, H. Zhou and P. J. Ross. 2021. Transcription initiation mapping in 31 bovine tissues reveals complex promoter activity, pervasive transcription, and tissue-specific promoter usage. Genome Res. 31: 732-744
10. M. M. Halstead, D. E. Goszczynski, A. D. Islas-Trejo, H. Zhou and P. J. Ross. 2021. Large-scale multiplexing permits full-length transcriptome annotation of 32 bovine tissues from a single Nanopore flow cell. Front. Genet. | doi: 10.3389/fgene.2021.664260.
11. Heidari, M., Zhang, H., Hearn, C.J., and Sunkara, L. 2021. B cells do not play a role in vaccine-mediated immunity against Marek's disease. Vaccine 10:100128.

12. Jang, H.-J., Monson, M., Kaiser., M., Lamont, S.J. 2020. Induction of chicken host defense peptides within disease-resistant and -susceptible lines. GENES 11:1195; doi:10.3390/genes11101195

13. Jehl F, Degalez F, Bernard M, [and 20 others, including Abasht B]. 2021. RNA-seq data for reliable SNP detection and genotype calling: interest for coding variant characterization and cis-regulation analysis by allele- specific expression in livestock species. *Frontiers in Genetics.* 12, p.1104.

14. Kadhim, H.J., S.W. Kang and W.J. Kuenzel. 2021. Possible roles of brain derived neurotrophic factor and corticotropin releasing hormone neurons in the nucleus of hippocampal commissure functioning within the avian neuroendocrine regulation of stress. Stress 24(5):590-601. doi.org/10.1080/10253890.2021.1929163

15. Kong B, Khatri B, Kang S, Shouse S, Kadhim H, Kidd M Jr, Lassiter K, Hiltz J, Mallmann B, Orlowski S, Anthony N, Bottje W, Kuenzel W and Owens C. 2021. Blood plasma biomarkers for woody breast disease in commercial broilers. Front. Physiol. 12:712694. doi: 10.3389/fphys.2021.712694

16. 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

17. Lee, K., N. Anthony, S. Orlowski, D. Rhoads (2021). SNP-based breeding for broiler resistance to ascites and evaluation of correlated production traits. Hereditas (accepted pending revisions)

18. Liao, L., Wu, Z., Chen, W., Zhang, H., Li, A., Yan, Y., Xie, Z., Li, H., Lin, W., Ma, J., Zhang, X., Xie, Q. 2021. Anti-CD81 antibodies block vertical transmission of avian leukosis virus subgroup J. Vet. Microbiol. 264:109293.

19. Maharjan P, Beitia A, Weil J, Suesuttajit N, Hilton K, Caldas J, Umberson C, Martinez D, Kong B, Owens C, Coon C. 2021. Woody breast myopathy broilers show age-dependent adaptive differential gene expression in Pectoralis major and altered in-vivo triglyceride kinetics in adipogenic tissues. Poultry Science. 100(7): 101092. doi.org/10.1016/j.psj.2021.101092.

20. Mandal A, Mandal R, Yang Y, Khatri B, Kong B, and Kwon Y. 2021. In vitro characterization of chicken gut bacterial isolates for probiotic potentials. Poultry Science. 100(2):1083-1092. doi: 10.1016/j.psj.2020.11.025.

21. KKZ Mon, C Kern, G Chanthavixay, Y Wang, H Zhou. 2021. Tolerogenic Immunoregulation towards Salmonella Enteritidis Contribute to Colonization Persistence in Young Chicks. Infect Immun doi: 10.1128/IAI.00736-20.

22. Monson, M.S. and Lamont, S.J. 2021. Genetic resistance to avian pathogenic *Escherichia coli* (APEC): current status and opportunities. Avian Pathology, DOI: 10.1080/03079457.2021.1879990

23. Monson, M.S., Bearson, B.L., Sylte, M.J., Looft, T., Lamont, S.J., and Bearson, S.M.D. 2021.Transcriptional response of blood leukocytes from turkeys challenged with *Salmonella enterica* serovar Typhimurium UK1. Vet. Immunol. Immunopathol.232: doi.org/10.1016/j.vetimm.2020.110181

24. Mushi, J.R., Chiwanga, G.H., Mollel, E.L., Walugembe, M., Max, R.A., Msoffe, P.M., Gallardo, R.A., Kelly, T.R., Lamont, S., Dekkers, J., Zhou, H, and Muhairwa, A. 2021. Antibody response, viral load, viral clearance, and growth rate in Tanzanian free-range local chickens infected with lentogenic Newcastle disease virus. J Vet Med Animal Health. Vol. 13(2): 98-105, DOI: 10.5897/JVMAH2021.0912

25. Overbey EG, Ng TT, Catini P, Griggs LM, Stewart P, Tkalcic S, Hawkins RD and Drechsler Y*: Transcriptomes of an Array of ChickenOvary, Intestinal, and Immune Cells and Tissues. Front. Genet. 2021; 12:664424. doi: 10.3389/fgene.2021.664424

26. Pacheco Santana, T., Gasparino, E., de Souza Khatlab, A., Oliveira Brito, C., Teixeira Barbosa, L., Lamont, S., and Del Vesco. A. 2021. Effect of prenatal ambient temperature on the performance and oxidative metabolism of Japanese quail (*Coturnix coturnix japonica*) layers exposed to heat stress during growth. Scientific Reports 11:9809, https://doi.org/10.1038/s41598-021-89306-0

27. Reed KM, Mendoza KM, Abrahante JE, Velleman SG, and Strasburg GM. 2021. Data mining identifies circular RNAs in skeletal muscle of thermally challenged turkey poults. Frontiers in Physiology, 12:1406. doi:10.3389/fphys.2021.732208.

28. Saelao, P., Wang, Y., Chanthavixay, G., Yu, V., Gallardo, R.A., Dekkers, J.C.M., 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.11:7450, https://doi.org/10.1038/s41598-021-86795-x

29. Saha S, Cooksey AM, Childers AK, Poelchau MF, McCarthy FM. Workflows for Rapid Functional Annotation of Diverse Arthropod Genomes. Insects. 2021; 12(8):748. https://doi.org/10.3390/insects12080748

30. Ana Paula da Silva, Ruediger Hauck, Sabrina R.C. Nociti, Colin Kern, H.L. Shivaprasad, Huaijun Zhou, Rodrigo Gallardo. 2021. Molecular biology and pathological process of an infectious bronchitis virus with enteric tropism in commercial broilers. Viruses. 13,1477. https://doi.org/10.3390/v13081477

31. Shumaker S, Khatri B, Shouse S, Seo D, Kang S, Kuenzel W, Kong B. 2021. Identification of SNPs associated with stress response traits within high stress and low stress lines of Japanese quail. Genes. 12(3):405. https://doi.org/10.3390/genes12030405.

32. Stephan T., S.M. Burgess, H.H. Cheng, C.G. Danko, C.A. Gill, E.D. Jarvis, K.P. Koepfli, J.E. Koltes, E. Lyons, P. Ronald, O.A. Ryder, L.M. Schriml, P. Soltis, S. VandeWoude, H. Zhou, E.A. Ostrander, E.K. Karlsson. 2021. Darwinian genomics and diversity in the tree of life. PNAS Vol. 119, No. 00 e2115644119. Doi.org/10.1073/pnas.2115644119

33. M. Tixier-Boichard, S. FABRE, S. Dhorne-Pollet, A. Goubil, H. Acloque, S. Vincent- Naulleau, P. Ross, Y. Wang, G. Chanthavixay, H. Cheng, C. Ernst, V. Leesburg, E. Giuffra, H. Zhou. 2021. Tissue resources for the functional annotation of animal genomes" Frontiers in Genetics. https://doi.org/10.3389/fgene.2021.666265.

34. Tomlinson MJ, Polson SW, Qiu J, Lake JA, Lee W, Abasht B. Investigation of allele specific expression in various tissues of broiler chickens using the detection tool VADT. Sci Rep. 2021;11(1):3968. doi: 10.1038/s41598-021- 83459-8. PubMed PMID: 33597613.

35. ZWM Tong, AC Karawita, C Kern, H Zhou, JE Sinclair, L Yan, KY Chew, S. Lowther, L. Trinidad, A. Challagulla, KA Schat, ML Baker, KR Short. 2021. Primary Chicken and Duck Endothelial Cells Display a Differential Response to Infection with Highly Pathogenic Avian Influenza Virus. Genes 12 (6), 901

36. Velleman SG, Coy CS, Abasht B. Effect of growth selection of broilers on breast muscle satellite cell function: Response of satellite cells to NOV, COMP, MYBP-C1, and CSRP3. Comp Biochem Physiol A Mol Integr Physiol. 2021;255:110917. doi: 10.1016/j.cbpa.2021.110917. PubMed PMID: 33548540.

37. Wang, Y., Jia, X., Hsieh, J.C.F., Monson, M.S., Zhang, J., Shu, D., Nie, Q., Persia, M.E., Rothschild, M.F, and Lamont, S.J. 2021. Transcriptome response of liver and muscle in heat- stressed laying hens. 2021. Genes 12, 255. doi.org/10.3390/genes12020255

38. 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

39. Xu J, Strasburg GM, Reed KM, and Velleman SG. 2021. Effect of temperature and growth selection on intracellular lipid accumulation and adipogenic gene expression in turkey pectoralis major muscle satellite cells. Frontiers in Physiology 12:667814.doi: 10.3389/fphys.2021.667814. (also included in Avian Muscle Development and Growth Mechanisms: Association with Muscle Myopathies and Meat Quality II, Petracci M, and Velleman SG eds. Frontiers in Physiology eBook, pp 63-78.)

40. Yan, Y., Zhang, H., Gao, S., Zhang ,H., Zhang, X., Chen, W., Lin, W., and Xie, Q. 2021. Differential DNA methylation and gene expression between ALV-J-positive and ALV-J-negative chickens. Front Vet Sci 8:659840.

41. Yuan, Y., Zhang, H., Yi, G., You, Z., Zhao, C., Yuan, H., Wang, K., Li, J., Yang, N., Lian, L. 2021. Genetic diversity of MHC-BF/BL region in 21 chicken populations. Front. Genet. 12:710770.

Presentations

1. Kong B and Bottje W. Comparative pathway analyses between woody breast myopathy and feed efficiency using proteomics data. 2021. USDA Multistate Project NC1170 Poultry Workshop. Virtual Meeting. February 23 - 24.
2. Shumaker S, Khatri B, Shouse S, Seo D, Kang S, Kuenzel W, Kong B. 2021. Genome-wide SNPs regulating nervous system functions associated with stress response traits in high and low stress lines of Japanese quail. Annual Meeting of Poultry Science Association. Virtual Conference. July 19-22.
3. Fiona McCarthy and David Webb. Updating Chicken Gene Nomenclature, NC1170: Advanced Technologies for the Genetic Improvement of Poultry 2021 Meeting.
4. Xu J, Strasburg GM, Reed KM, Velleman SG. Heat stress affects proliferation and differentiation through the mTOR/S6K pathway in breast muscle satellite cells from turkeys with different growth rates. Poultry Science Association Annual Meeting, 2021.

Thesis and Dissertations
Ye Bi, M.S. Longitudinal Analysis of CD4 and CD8 T cell Receptor Repertoires Associated with Newcastle Disease Virus Infection in Hy-Line Birds

Patent:
B Kong, C. Owens. W. Kuenzel. Markers For Poultry Myopathy and Uses Thereof. US, Provisional Patent. April 30, 2021. No.: 63/181,657

Swine NRSP-8 Genome Committee Report
January 1, 2021 – December 31, 2021
Coordinators: Christopher Tuggle, Iowa State Univeristy
               Kathy Ernst Michigan State University

Chair (for 2022 Workshop): Michigan State University, East Lansing, MI, USA, huangw53@msu.edu
Secretary (for 2022 Workshop): Nick Serão, Iowa State Universtiy, Ames, IA, USA, serao@iastate.edu

The 2022 NRSP-8 Swine Workshop was held online through Zoom on February 4, 2022. The workshop included 15 presentations delivered by scientists at various stages (students, postdocs, and PIs) at five universities, USDA-NIFA national program leaders, and industry representatives. Approximately 37 participants attended the workshop. The research presentations represent a wide range of topics performed at participating stations, including functional validation of phenotype associated variants by gene editing, functional annotation of the procine genome, association between gut microbiome and feed efficiency, genetics of disease resilience, new resources and tools, among others.

Following the research presentations, Robert Godfrey, Division Director of Animal Systems at NIFA and his colleagues Frank Siewerdt and Angelica Van Goor provided updates from USDA on funding and other opportunities at NIFA. William Herring from Genus/PIC delivered an insightful talk highlighting progress and new frontiers in the swine breeding industry. Chris Tuggle, Swine NRSP-8 Co-coordinator, led discussion on NRSP-8 progress and future plans, encouraged attendees to participate in the NRSP-8 discussion by going to the AGBT-Ag meeting and voicing their thoughts and ideas.

Finally, Dan Nonneman, of USDA-MARC, was elected as the secretary for 2022. The virtual Swine NRSP-8 meeting was widely received as a successful one. Many new ideas were exchanged, exciting research is being performed, and new collaborations expected, at participating stations.

Applications of Findings:

1. At the University of Nebraska-Lincoln, Candidate genes and SNPs located in the QTL regions identified by GWAS, genome and RNA sequencing could explain some of the observed phenotypic variation in viral disease resistance. Following validation, some of these polymorphisms could be used in selection to improve general fitness of the swine herds. This information was shared with industry to assist them in genomic selection applications.

2. The project conducted at Michigan State University provides improved methods for genomic selection, GWA and eQTL analyses in pigs. It also examines difficult to record phenotypes like pork quality and novel phenotypes including pig behavior to provide tools for incorporating these phenotypes into selection programs. In addition, this project provides fundamental knowledge about transcriptional regulation and gene function that will lead to future improvements in pig production efficiency.

3. North Carolina State University scientists have a) found that microbial composition differed between production systems and was associated with growth performance and carcass quality in pigs, providing a first characterization of microbial communities' value throughout the pork production systems and information for incorporating microbial composition into the selection process in the quest for affordable and sustainable protein production in swine; b) investigated body weight in growing pigs from feeding behavior data using machine learning algorithms, connecting the dynamics of feeding behavior to body growth and providing a promising picture of the involvement of feeding behavior data in predicting the body weight of group-housed pigs; c) found evidence for recombination variability in purebred swine populations; d) estimated microbiability of meat quality and carcass composition traits in swine, indicating that better understanding of microbial composition could aid the improvement of complex traits, particularly carcass composition traits in swine by inclusion of microbiome information in the genetic evaluation process; e) explored the role of gut microbiota in host feeding behavior among breeds in swine, providing insight into the interaction between gut microbiota and feeding behavior and highlighting the genetic background and age effects in swine microbial studies; f) found that addition of epistatic genetic effects increased genomic prediction accuracy for reproduction and production traits in Duroc pigs; g) found that gut microbiota and host genetics contributed to the phenotypic variation of digestive and feed efficiency traits in growing pigs fed a conventional and a high fiber diets; h) explored methods to summarize gut microbiota composition for microbiability estimation and phenotypic prediction in swine; i) predicted swine performance across production systems using microbial information; j) used an autoencoder to compress microbiota information to predict growth traits in swine, suggesting that including all available microbial information may be the best option in predicting performance; k) developed a new software tool for using FAANG data like stratified LD score regression; l) developed a new software tool for million-scale genome- wide association studies and genomic predictions; m) investigated chromosome dosage compensation patterns of growth and carcass traits in Landrace and Yorkshire pigs.

4. At Iowa State University, scientists carried out a variety of projects aiming to advance the status of reference genomes, develop strategies to identify and exploit genes and allelic variation contributing to phenotypes and traits, and facilitate analysis, curation, storage, distribution and application of large-scale datasets. 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. The use of random forest and regression approaches and a limited number of SNPs are useful to predict breed from pigs without pedigree information. 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. The blood transcriptome, metabolome, and proteome of young healthy pigs contains potential biomarkers to select for disease resilience in pigs. They have published the first integrated analysis of RNAseq analysis of 8 different flow-cytometry sorted cell populations and scRNAseq analysis of PBMC. They identified cell types that are present in specific sorted cell populations, as well across these sorted cells. 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. They 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.

5. USDA BARC scientists analyzed effect of PRRSV infection on thyroid hormone levels in pigs, assessed effect of the WUR allele on PRRS virus genetic diversity, and evaluated gene expression in single cells. The complex interactions between thyroid hormone levels and multiple immunogenetic pathways during PRRSV infection means that there is potential to select for pleiotropic QTL to simultaneously improve host immunity for reproductive and respiratory PRRS. They Ccontinued to use samples collected and stored through the PRRS host genetics consortium (PHGC) and use data accumulated in the PHGC database facilitating phenotype/genotype association analyses. The well-validated transcriptomic data from sorted PBMC populations and the first single-cell transcriptomic data will be invaluable for annotation of pig genes controlling immunogenetic traits as part of the porcine FAANG project, as well as further study of, and development of new reagents for, porcine immunology.

Publications (published refereed journal articles only) (Total 49)

1. 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.
2. Chamberlain, A.J., H. Cheng, E. Giuffra, C. Kuehn, C. K. Tuggle, and H. Zhou. 2021. Editorial: "Functional Annotation of Animal Genomes" Research Topic. Frontiers in Genetics: Livestock Genomics. 12:768626. doi: 10.3389/fgene.2021.768626
3. 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.
4. Cheng, J., Dekkers, J.C. and Fernando, R.L., 2021. Cross-validation of best linear unbiased predictions of breeding values using an efficient leave-one-out strategy. Journal of Animal Breeding and Genetics.
5. Chinchilla-Vargas J, M. F. Rothschild, F. Bertolini. 2021. Signatures of selection and genomic diversity of Muskellunge (Esox masquinongy) from two populations in North America. Genes 12(7):1021.
6. Chinchilla-Vargas, J., Bertolini, F., Stalder, K. J., Steibel, J.P., & Rothschild, M. F. (2021). Estimating breed composition for pigs: A case study focused on Mangalitsa pigs and two methods. Livestock Science, 244, 104398.
7. Daza, K.R., D. Velez-Irizarry, S. Casiró, J.P. Steibel, N.E. Raney, R.O. Bates and C.W. Ernst. 2021. Integrated genome-wide analysis of microRNA expression quantitative trait loci in pig longissimus dorsi muscle. Front. Genet. 12:644091.
8. Dekkers, J., Su, H. and Cheng, J., 2021. Predicting the accuracy of genomic predictions. Genetics Selection Evolution, 53(1), pp.1-23.
9. 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.
10. 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.
11. 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

12. 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.

13. Fabbri, M.C., Dadousis, C., Tiezzi, F., Maltecca, C., Lozada-Soto, E., Biffani, S., Bozzi, R., 2021. Genetic diversity and population history of eight Italian beef cattle breeds using measures of autozygosity. PloS one 16, e0248087.

14. Gomez-Raya, L., Rauw, W.M. and Dekkers, J.C., 2021. Vector space algebra for scaling and centering relationship matrices under non-Hardy–Weinberg equilibrium conditions. Genetics Selection Evolution, 53(1), pp.1-18.

15. Han, J.J., Reid, K., Gondro, C., & Steibel, J.P. (2021). Heuristic hyperparameter optimization of deep learning models for genomic prediction. G3, 11, (7), jkab032.

16. He, Y., Maltecca, C., Tiezzi, F., 2021. Potential use of gut microbiota composition as a biomarker of heat stress in monogastric species: a review. Animals 11, 1833.

17. He, Y., Tiezzi, F., Howard, J., Maltecca, C., 2021. Predicting body weight in growing pigs from feeding behavior data using machine learning algorithms. Computers and Electronics in Agriculture 184, 106085.

18. Herrera-Uribe, J., J. Wiarda, S.K. Sivasankaran, L. Daharsh, H. Liu, K.A. Byrne, T.P.L . Smith, J.K. Lunney, C. L. Loving, C. K. Tuggle. 2021. Reference transcriptomes of porcine peripheral immune cells created through bulk and single-cell RNA sequencing. Frontiers in Genetics, 12:689406.

19. Hickmann, F.M., Neto, J.B., Kramer, L.M., Huang, Y., Gray, K.A., Dekkers, J.C., Sanglard, L.P. and Serão, N.V., 2021. Host genetics of response to porcine reproductive and respiratory syndrome in sows: Antibody response as an indicator trait for improved reproductive performance. Frontiers in Genetics, 12.

20. 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

21. 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

22. Johnson RK, Trenhaile-Grannemann MD, Moreno R, Ciobanu DC, Miller PS. Effects of restricting energy during the gilt development period on growth and reproduction of lines differing in lean growth rate: Responses in reproductive performance and longevity. J Anim Sci. 2021 Nov 26:skab352. doi:10.1093/jas/skab352. Epub ahead of print. PMID: 34849984.

23. Kern, C., Y. Wang, X. Xu, Z. Pan, M. Halstead, K. Chanthavixay, P. Saelao, S. Waters, R. Xiang, A. Chamberlain, I. Korf, M. E. Delany, H. H. Cheng, J. F. Medrano, A. L. Van Eenennaam, C. K. Tuggle, C. Ernst, P. Flicek, G. Quon, P. Ross, H. Zhou. 2021. Comprehensive annotation of regulatory elements in the chicken, pig, and cattle genomes provides insight for comparative genome biology: A core set of regulatory elements show conservation across species independent of evolutionary distance. Nature Communications, 12(1):1821. https://doi.org/10.1038/s41467-021-22100-8.

24. Khanal, P., Maltecca, C., Schwab, C., Fix, J., Tiezzi, F., 2021. Microbiability of meat quality and carcass composition traits in swine. Journal of Animal Breeding and Genetics 138, 223–236.

25. 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.

26. Lozada-Soto, E.A., Maltecca, C., Lu, D., Miller, S., Cole, J.B., Tiezzi, F., 2021. Trends in genetic diversity and the effect of inbreeding in American Angus cattle under genomic selection. Genetics Selection Evolution 53, 1–15.

27. 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.

28. Li, Y., M. K. Adur, W. Wang, R. B. Schultz, B. Hale, W. Wierson, S.E. Charley, M. McGrail, J. Essner, C.K. Tuggle, and J.W. Ross. 2021. Effect of ARTEMIS (DCLRE1C) deficiency and microinjection timing on editing efficiency during somatic cell nuclear transfer and in vitro fertilization using the CRISPR/Cas9 system. Theriogenology, 170:107-116.

29. Lim, K.-S., J. Cheng, A. Putz, Q. Dong, X. Bai, C.K. Tuggle, M.K. Dyck, P. 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. 22:614.

30. 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

31. Lozada-Soto, E.A., Maltecca, C., Wackel, H., Flowers, W., Gray, K., He, Y., Huang, Y., Jiang, J., Tiezzi, F., 2021. Evidence for recombination variability in purebred swine populations. Journal of Animal Breeding and Genetics 138, 259–273.

32. Lunney JK, Van Goor A, Walker K, Hailstock T, Franklin J, Dai C. 2021. Importance of the pig as a human biomedical model. Science Translational Medicine. 13: eabd5758 (2021) doi: 10.1126/scitranslmed.abd5758. PMID: 34818055.

33. Makanjuola, B.O., Maltecca, C., Miglior, F., Marras, G., Abdalla, E.A., Schenkel, F.S., Baes, C.F., 2021. Identification of unique ROH regions with unfavorable effects on production and fertility traits in Canadian Holsteins. Genetics selection evolution 53, 1–11.

34. Maltecca, C., Dunn, R., He, Y., McNulty, N.P., Schillebeeckx, C., Schwab, C., Shull, C., Fix, J., Tiezzi, F., 2021. Microbial composition differs between production systems and is associated with growth performance and carcass quality in pigs. Animal microbiome 3, 1–20.

35. Misztal, I., Aguilar, I., Lourenco, D., Ma, L., Steibel, J.P., & Toro, M. (2021). Emerging issues in genomic selection. Journal of Animal Science. 99(6), 1-14.36.

36. 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.

37. Pan, Z., Y. Yao, H. Yin, Z. Cai, Y. Wang, L. Bai, C. Kern, M. Halstead, K. Chanthavixay, N.Trakooljul, K. Wimmers, G. Sahana, G. Su, M. S. Lund, M. Fredholm, P. Karlskov-Mortensen, C. W. Ernst, P. Ross, C. K. Tuggle, L. Fang, H. Zhou. 2021. Pig genome functional annotation enhances biological interpretations of complex traits and comparative epigenomics. Nature Communications. 12:5848. doi: 10.1038/s41467-021-26153-7.

38. 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.

39. 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

40. Pérez-Enciso, M., & Steibel, J.P. (2021). Phenomes: the current frontier in animal breeding. Genetics Selection Evolution, 53(1), 1-10.

41. 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.

42. Sanglard LP, Huang Y, Gray KA, Linhares DCL, Dekkers JCM, Niederwerder MC, Fernando RL, and Serao NVL. 2021. Further host-genomic characterization of total antibody response to PRRSV

vaccination and its relationship with reproductive performance in commercial sows: genome-wide haplotype and zygosity analyses. Genetics Selection Evolution, 53:91.

43. Sanglard, L.P., Hickmann, F.M., Huang, Y., Gray, K.A., Linhares, D.C., Dekkers, J., Niederwerder, M.C., Fernando, R.L., Braccini Neto, J. and Serão, N.V., 2021. Genomics of response to PRRSV in purebred and crossbred sows: antibody response and performance following natural infection versus vaccination. Journal of Animal Science. https://doi.org/10.1093/jas/skab097

44. Shen, B., Freebern, E., Jiang, J., Maltecca, C., Cole, J.B., Liu, G.E., Ma, L., 2021. Effect of Temperature and Maternal Age on Recombination Rate in Cattle. Frontiers in Genetics 1307.

45. Spencer TE, Wells KD, Lee K, Telugu BP, Hansen PJ, Bartol FF, Blomberg L, Schook LB, Dawson H, Lunney JK, Driver JP, Davis TA, Donovan SM, Dilger RN, Saif LJ, Moeser A, McGill JL, Smith G, Ireland JJ. 2022. Future of Biomedical, Agricultural and Biological Systems Research using Domesticated Animals. Biol. of Repro:ioac019. doi: 10.1093/biolre/ioac019.

46. Sutton KM, Eaton CW, Borza T, Burkey TE, Mote BE, Loy JD, Ciobanu DC. Genetic Diversity and Detection of Atypical Porcine Pestivirus Infections1. J Anim Sci. 2021 Dec 4:skab360. doi: 10.1093/jas/skab360.

47. Tiezzi, F., Fix, J., Schwab, C., Shull, C., Maltecca, C., 2021. Gut microbiome mediates host genomic effects on phenotypes: a case study with fat deposition in pigs. Computational and structural biotechnology journal 19, 530–544.

48. Tixier-Boichard, M., S. Fabre, S. Dhorne-Pollet, A. Goubil, H. Acloque, S. Vincent-Naulleau, P. Ross, Y. Wang, G. Chanthavixay, H. Cheng, C. Ernst, V. Leesburg, E. Giuffra and H. Zhou. 2021. Tissue resources for the functional annotation of animal genomes. Front. Genet. 12:666265.

49. Usala, M., Macciotta, N.P.P., Bergamaschi, M., Maltecca, C., Fix, J., Schwab, C., Shull, C., Tiezzi, F., 2021. Genetic parameters for tolerance to heat stress in crossbred swine carcass traits. Frontiers in Genetics 1821.