NATIONAL ANIMAL GENOME RESEARCH PROGRAM - NRSP-8 Year Ending 2020

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), help 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.

HIGHLIGHTS OF ACCOMPLISHMENTS AND IMPACTS:

NRSP-8 community members 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 NRSP08 scientific group continue to be remarkable. In just one year (2020) we have published over 300 peer-reviewed manuscripts, with a number of additional invited talks, press releases, and published abstracts that have been produced. Examples of the impacts of genetic tools to deliver improved farmed fishes was featured in *Science* in November 2020 (doi:10.1126/science.abf7615) and a review was featured in *Science* magazine in May 2020 (doi:10.1126/science.abc7484). Further impactful publications include; *De novo* assembly of the cattle reference genome with single-molecule sequencing (doi: 10.1093/gigascience/giaa021), An improved pig reference genome sequence to enable pig genetics and genomics research (doi: 10.1093/gigascience/giaa051), and Haplotype-resolved genomes provide insights into structural variation and gene content in Angus and Brahman cattle (doi: 10.1038/s41467-020-15848-y), to highlight just a few.

Even more impressive is the fact that the number of publications produced by this group pales in comparison to the number of competitive federal, state, and industry funds that are leveraged by NRSP-8. In 2020, the total amount of active grants that were reported to be secured exceeds $13 Million. This includes the new "NIFA AG2PI Collaborative: Creating a Shared vision across Crop and Livestock Communities." 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.

The NRSP-8 annual meeting and community building that has historically taken place in conjunction with the International Plant and Animal Genomes Conference was cancelled this year due to the world-wide COVID19 Pandemic. However, the "Beyond NRSP-8 virtual meeting was held on September 30, 2020. This included 67 participating NRSP08 leadership and members. Importantly, information about NRSP-8 is 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 2020

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 2021-2022: Rafet Al-Tobasei (Rafet.Al-tobasei@mtsu.edu)
Workshop Chair-elect 2020-2021: Moh Salem (mosalem@umd.edu)
Workshop Chair 2019-2020: Louis Plough (lplough@umces.edu)
Workshop Chair 2018-2019: Catherine Purcell (catherine.purcell@noaa.gov)

Aquaculture Workshop Report

The Aquaculture Workshop in 2021 was cancelled due to the COVID-19 pandemic and travel restrictions imposed by state and federal governments.

Leveraged funds:

Four (4) small research projects were funded at $10,000 each in 2020 to provide preliminary data for grants: $40,000 (2019-2020). Decisions regarding funding of 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 and execution of research challenging at best. These funds will officially be 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.

Leveraged funds from diverse projects based on previously funded small research projects totaled over three million dollars from federal sources in 2020. This is in addition to over six million reported in 2019:

Total Leveraged Funding in 2020: $ 3,419,110

This is about 1:53 return on investment for the aquaculture coordinator funds ($65,000).

Total Leveraged Funding in 2019: $ 6,340,999

This is 1:98 return on investment for the aquaculture coordinator funds ($65,000).

Extramural funding agencies leveraged through collaborations and seed funding opportunities provided by this NRSP-8 program include NOAA, USDA NIFA, USDA AFRI, USDA Southern Regional Aquaculture Center, and the Ratcliffe Foundation (non-profit). In particular, the marine finfish and shellfish aquaculture initiatives of USDA and NOAA are to be recognized.

There were 30 publications from the NRSP-8 Aquaculture Community in 2020, including one research paper in the journal *Nature Scientific Reports* and two editorial features in *Science* magazine.

Specific major activities include:

Members of the NRSP-8 Aquaculture Group (among many others) played a role in contributing content matter expertise to advise NOAA and USDA on national marine aquaculture initiatives, which led to US Executive Order 13921: *Promoting American Seafood Competitiveness and Economic Growth* in May 2020. A review of this order was featured in *Science* magazine in May 2020 (doi:10.1126/science.abc7484)

and the impacts of genetic tools to deliver improved farmed fishes also was featured in *Science* in November 2020 (doi:10.1126/science.abf7615).

Catfish

Channel catfish genome assembly refined with optical mapping; blue catfish genome assembly released. For the blue catfish, 469 NGS contigs (810 Mb) were integrated into 64 chromosomal scaffolds (N50 = 25 Mb) totaling 823 Mb, and an additional 25 Mb sequence was contained in 195 unaligned scaffolds. The blue catfish chromosome assembly currently contains only 334 gaps. The channel catfish genome assembly integrated 289 NGS scaffolds (826 Mb) into 79 chromosomal scaffolds (N50 = 20 Mb) totaling 833 Mb. An additional 20 Mb sequence was contained in 376 unaligned scaffolds. The channel catfish assembly was a significant improvement over the first channel catfish genome reference assembly produced from short-read sequencing. The effect of pond- or strip-spawning on growth and carcass yield of channel catfish progeny was published along with genomic predictions of columnaris disease resistance.

Shellfish and Crustaceans

Whiteleg shrimp genome sequencing is underway. Re-sequencing of wild and selected eastern oyster populations derived from multiple geographic regions along the US east Coast and Gulf of Mexico. Proteomic profiling was published showing developmental processes and temperature-influenced physiological responses in pacific oyster. Proteomic responses of larval geoduck to ciliates was published in *Scientific Reports*. Environmentally-induced differential DNA methylation and gene expression patterns were published for the eastern oyster, including responses to ocean acidification.

Trout and Salmon

The *de novo* genome assembly of the Arlee doubled-haploid rainbow trout was accepted by NCBI and annotated in RefSeq (accession GCF_013265735.2). A new linkage map is being developed for North American Atlantic salmon using genotype data from the new HD (50K) SNP chip that was developed this year in collaboration with Mowi North America and the Center for Aquaculture Technologies. The linkage map will be used for anchoring and ordering the sequence scaffolds from the new *de-novo* assembly in chromosome sequences. PacBio iso-seq was used improve the rainbow trout genome annotation and identifies alternative splicing associated with economically important phenotypes. More than 13,000 genome structural variants from whole genome resequencing of rainbow trout from two primary aquaculture breeding programs were reported. Genome-wide association studies identified genomic loci affecting fish growth, fillet firmness and protein content, intramuscular fat, and moisture content in rainbow trout. A study identified distinct microbial assemblages associated with genetic selection for high- and low-muscle yield in rainbow trout.

Striped Bass

The second version of the striped bass genome draft was annotated through the NCBI Eukaryotic Genome Annotation Pipeline and is publicly available (GenBank accession GCA_004916995.1). A machine learning pipeline developed to analyze single nucleotide (SNP) markers (expressed quantitative trait loci, eQTL) related to growth in different strains of hybrid striped bass revealed 15,000 unique markers associated with growth traits. The white bass genome assembly was updated through the Dovetail™ Hi-C + HiRise™ scaffolding pipeline and short sequences (< 200 bp in length) are being filtered out of the assembly in preparation for submission to NCBI for annotation and public accessibility in 2021. Genotyping-by-sequencing panel was developed from diverse white bass populations for resistance to columnaris disease evaluated in striped bass, white bass, and hybrid striped bass. Genetically improved striped bass and white bass transferred to industry from *National Breeding Program for the Hybrid Striped Bass Industry*. Twenty (20) graduate and undergraduate students were trained in machine learning approaches in biological sciences including application of pattern recognition to evaluate gene and protein expression patterns related to predicting phenotypes in a variety of agriculturally important animals, including fishes and well as poultry; a CRISPR/Cas9 Guide to RNA design was published for student

training.

NRSP-8 Aquaculture 2020 Progress: Species Leaders indicated in **BOLD.**

Members of the NRSP-8 Aquaculture Group contributed content matter expertise to advise NOAA and USDA on national marine aquaculture initiatives, which led to US Executive Order 13921: *Promoting American Seafood Competitiveness and Economic Growth* in 2020. A review of this order was featured in *Science* magazine in May 2020 (doi:10.1126/science.abc7484) and the impacts of genetic tools to deliver improved farmed fishes also was featured in *Science* in November 2020 (doi:10.1126/science.abf7615).

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.

Catfish (**Quiniou**, Liu)
For the blue catfish, 469 NGS contigs (810 Mb) were integrated into 64 chromosomal scaffolds (N50 = 25 Mb) totaling 823 Mb, and an additional 25 Mb sequence was contained in 195 unaligned scaffolds. The blue catfish chromosome assembly currently contains only 334 gaps. The channel catfish genome assembly integrated 289 NGS scaffolds (826 Mb) into 79 chromosomal scaffolds (N50 = 20 Mb) totaling 833 Mb. An additional 20 Mb sequence was contained in 376 unaligned scaffolds. The channel catfish assembly was a significant improvement over the first channel catfish genome reference assembly produced from short-read sequencing of the same genome – 42 of 58 chromosome arms were assembled as single scaffolds while the remaining chromosome arms contained only two to four scaffolds each.

Shellfish and Crustaceans (Roberts, Gómez-Chiarri, Putnam, Guo, Warren, **Proestou**)
Whiteleg shrimp genome sequencing is underway. Re-sequencing of wild and selected eastern oyster populations derived from multiple geographic regions along the US east Coast and Gulf of Mexico.

Salmonids (Salem, **Palti**, Al-Tobasei)
The *de novo* genome assembly of the Arlee doubled-haploid line was accepted by NCBI and annotated in RefSeq (accession GCF_013265735.2); the accompanying manuscript was accepted for publication in the journal *G3*. A new linkage map is being developed for North American Atlantic salmon using genotype data from the new HD SNP chip that was developed this year for this economically important sub-species of Atlantic salmon. The linkage map will be used for anchoring and ordering the sequence scaffolds from the new *de-novo* assembly in chromosome sequences. PacBio iso-seq was used improve the rainbow trout genome annotation and identifies alternative splicing associated with economically important phenotypes. The study identified 10,640 high-confidence transcripts not previously annotated, in addition to 1,479 isoforms not mapped to the current reference genome. Intron retention and exon skipping accounted for 56% of alternative splicing (AS) events. Iso-seq and RNA-Seq data integration identified characteristic alternative splicing associated with fish growth, muscle accretion, disease resistance, stress response, and fish migration.

Striped Bass (*National Breeding Program for the Hybrid Striped Bass Industry*, Fuller, Abernathy, Borski, Berlinsky, **Reading**)
The striped bass genome assembly version 2.0 (NCSU_SB_2.0) was annotated through the NCBI Eukaryotic Genome Annotation Pipeline and is publicly available (GenBank accession GCA_004916995.1). The white bass genome assembly was updated through the Dovetail™ Hi-C + HiRise™ scaffolding pipeline and short sequences (< 200 bp in length) are being filtered out of the assembly in preparation for submitting to NCBI for annotation and public release. The newly formed *StriperHub* will also begin to disseminate public notifications of genome resource releases to the public.

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 effect of pond- or strip-spawning on growth and carcass yield of channel catfish progeny was published along with genomic predictions of columnaris disease resistance.

Oyster (*Eastern Oyster Breeding Consortium*, Roberts, Gomez-Chiarri, Putnam, Lotterhoos, Puritz, Johnson, Eirin-Lopez, Allen, Zhang, Plough, **Proestou**)
Proteomic profiling was published showing developmental processes and temperature-influenced physiological responses in pacific oyster. Proteomic responses of larval geoduck to ciliates was published in *Scientific Reports*. Environmentally-induced differential DNA methylation and gene expression patterns were published for the eastern oyster, including responses to ocean acidification.

Salmonids (Salem, **Palti**, Al-Tobasei)
The first public HD (50K) SNP chip for North American Atlantic salmon was developed by USDA-ARS in collaboration with Mowi North America and the Center for Aquaculture Technologies. It is currently being used by USDA-ARS for evaluating genomic selection for sea-lice resistance in Atlantic salmon. A discovery study identified more than 13,000 genome structural variants from whole genome resequencing of rainbow trout from two primary aquaculture breeding programs. This was the first SV discovery research in rainbow trout providing the foundation for research on the association between genome structural variants and economically relevant phenotypes in rainbow trout aquaculture. Genome-wide association studies identified genomic loci affecting fish growth, fillet firmness and protein content, intramuscular fat, and moisture content in rainbow trout. A study identified distinct microbial assemblages associated with genetic selection for high- and low- muscle yield in rainbow trout.

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 15,000 single nucleotide (SNP) markers (expressed quantitative trait loci, eQTL) that were identified among muscle transcriptome data from sunshine hybrid striped bass. The pipeline identified 500 SNPs that were considered most important to the predicting the growth phenotypes of hybrids. When orthologs and paralogs were removed, these 500 SNPs were annotated to 29 unigenes related to important growth regulatory pathways. 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=150,000) representing the first F7 generation captive bred striped bass in the United States were also disseminated to commercial aquaculture producers. Studies focused on characterizing variation related to disease resistance in white bass (most resistant), striped bass (most susceptible), and hybrid offspring were completed and published.

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)
None from catfish this year.

Oyster (*Eastern Oyster Breeding Consortium*, Gómez-Chiarri, Roberts, **Proestou**)
None from oyster this year.

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

Striped Bass (*National Breeding Program for the Hybrid Striped Bass Industry,* Abernathy, Borski, **Reading**)
Twenty (20) students (graduate and undergraduate) 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 and 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.
Follow Striped Bass Genome Community and *StriperHub* research on Facebook: https://www.facebook.com/stripedbassgenome/

Research support mini-grants (coordinator grants)
Four (4) mini-grants ($10,000 each) supported projects that fall under all three primary NRSP-8 objectives and include a variety of species. Awards listed (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 will be 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. Once awarded, the investigators will be given 12 months to complete these projects. Another round of mini-grants will be offered later in 2021-2022:

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.

Support of NRSP-8 Bioinformatics
A total of $5,000 of these Aquaculture Coordinators funds were allocated to NRSP-8 Bioinformatics support (Coordinator Jim Reecy).

Leveraged Funds and Stakeholder Use of Project Outputs
NRSP-8 Seed Funding: **$0** ($40,000 in 2019-2020 delayed until 2020-2021 due to COVID-19; $30,000 in 2018-2019)
Total Leveraged 2020 Funding: **$ 3,419,110** ($ 6,340,999 in 2019)

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:

8. 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).
7. 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).
6. 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).

5. NOAA, *Leveraging transformative 'omics technologies to alleviate barriers to US shellfish production*. (PI S. Roberts) $233,135 (07/01/20 through 06/30/25).
4. 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).
3. 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).
2. 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).
1. 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).

Publications (30 in 2020)

30. Lange, M.D., Abernathy, J., Shoemaker, C., Zhang, D., Kirby, A., Peatman, E., and Beck, B. 2020. Proteome analysis of virulent *Aeromonas hydrophila* reveals the upregulation of iron acquisition systems in the presence of a xenosiderophore. *FEMS Microbiology Letters* 367(20):fnaa169.
29. Lange, M.D., Farmer, B., and Abernathy, J. 2020. Vertebrate mucus stimulates biofilm development and upregulates iron acquisition genes in *Flavobacterium columnare*. *Journal of Fish Diseases* 43(1):101-110.
28. Trigg, S.A., Mitchell, K.R., Thompson, R.E., Eudeline, B., Vadopalas, B., Timmins-Schiffman, E.B., and Roberts, S.B. 2020. Temporal proteomic profiling reveals insight into critical developmental processes and temperature-influenced physiological response differences in a bivalve mollusc. *BMC Genomics* 21:723. doi:10.1186/s12864-020-07127-3
27. Downey-Wall, A.M., Cameron, L.P., Ford, B.M., McNally, E.M., Venkataraman, Y.R., Roberts, S.B., Ries, J.B., and Lotterhos, K.E. 2020. Ocean Acidification Induces Subtle Shifts in Gene Expression and DNA Methylation in Mantle Tissue of the Eastern Oyster (*Crassostrea virginica*). *Frontiers in Marine Science* 7:828. doi:10.3389/fmars.2020.56641
26. Timmins-Schiffman, E., Guzmán, J.M., Thompson, R.E., Vadopalas, B., Eudeline, B., and Roberts, S.B. 2020. Larval Geoduck (*Panopea generosa*) Proteomic Response to Ciliates. *Scientific Reports* 10:6042. doi:10.1038/s41598-020-63218
25. Venkataraman, Y.R., Downey-Wall, A.M., Ries, J., Westfield, I., White, S.J., Roberts, S.B., and Lotterhos, K.E. 2020. General DNA Methylation Patterns and Environmentally-Induced Differential Methylation in the Eastern Oyster (*Crassostrea virginica*). *Frontiers in Marine Science* 7:225. doi:10.3389/fmars.2020.00225.
24. Vallejo, R.L., Fragomeni, B.O., Cheng, H., Gao, G., Long, R.L., Shewbridge, K.L., Macmillan, J.R., Towner, R., and Palti, Y. 2020. Assessing Accuracy of Genomic Predictions for Resistance to Infectious Hematopoietic Necrosis Virus With Progeny Testing of Selection Candidates in a Commercial Rainbow Trout Breeding Population. *Frontiers in Veterinary Science,* 7:939. DOI 10.3389/fvets.2020.590048.
23. Ma, H., Han, Y.-C., Palti, Y., Gao, G., Liu, S., Palmquist, D.E., Wiens, G.D., and Shepherd, B.S. 2021. Structure and regulation of the NK-lysin (1–4) and NK-lysin like (a and b) antimicrobial genes in rainbow trout (*Oncorhynchus mykiss*). *Developmental & Comparative Immunology,* 116:103961. DOI https://doi.org/10.1016/j.dci.2020.103961.

22. Magadan, S., Mondot, S., Palti, Y., Gao, G., Lefranc, M.P., and Boudinot, P. 2021. Genomic analysis of a second rainbow trout line (Arlee) leads to an extended description of the IGH VDJ gene repertoire. *Developmental & Comparative Immunology* 118:103998. DOI https://doi.org/10.1016/j.dci.2021.103998.
21. Cleveland, B.M., Gao, G., and Leeds, T.D. 2020. Transcriptomic response to selective breeding for fast growth in rainbow trout (*Oncorhynchus mykiss*). *Marine Biotechnology,* 22**:**539-550.
20. Cleveland, B.M., Gao, G., Radler, L.M., and Picklo, M.J. 2020. Hepatic Fatty Acid and Transcriptome Profiles during the Transition from Vegetable-to Fish Oil-Based Diets in Rainbow Trout (*Oncorhynchus mykiss*). *Lipids*. DOI https://doi.org/10.1002/lipd.12287.
19. Gao, G., Magadan, S., Waldbieser, G.C., Youngblood, R.C., Wheeler, P.A., Scheffler, B.E., Thorgaard, G.H., and Palti, Y. 2021. A long reads-based *de-novo* assembly of the genome of the Arlee homozygous line reveals chromosomal rearrangements in rainbow trout. *G3 Genes|Genomes|Genetics* jkab052. DOI 10.1093/g3journal/jkab052.
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17. Ali, A., Al-Tobasei, R., Lourenco, D., Leeds, T., Kenney, B., Salem, M. 2020. Genome-wide identification of loci associated with growth in rainbow trout. *BMC Genomics* 21(1):209.
16. Chapagain, P., Walker, D., Leeds, T., Cleveland, B.M., Salem, M. 2020. Distinct microbial assemblages associated with genetic selection for high- and low- muscle yield in rainbow trout. *BMC Genomics* 21(1):820.
15. Banczyk, W., Salem, M. 2020. Die Rolle der DNA-Extraktion bei der Mikrobiomforschung. *BIOspektrum* 26(5):518-519 DOI: 10.1007/s12268-020-1439-6
14. Samsa, L.A., Andersen, L.K., Groth, A.M., and Goller, C.C. 2020. CRISPR/Cas9 Guide RNA Design *In Silico* Activity. *CourseSource*. https://doi.org/10.24918/cs.2020.46.
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12. Gibbens, S. 2020. Toxic 'forever chemicals' flow freely through this river—and now its fish. *National Geographic*, March 24, 2020 (*striped bass aquaculture* featuring S. Belcher from Guillette et al., 2020 below).https://www.nationalgeographic.com/science/article/toxic-chemical-pfas-found-in-north-carolina-striped-bass
11. Stokstad, E. 2020. New genetic tools will deliver improved farmed fish, oysters, and shrimp. Here's what to expect. *Science*, November 19, 2020 (*editorial* featuring B.J. Reading). doi:10.1126/science.abf7615
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9. 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.
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5. Phillips, C.A., Reading, B.J., Livingston, M., Livingston, K. and Ashwell, C.M. 2020. Evaluation via Supervised Machine Learning of the Broiler Pectoralis Major and Liver Transcriptome in Association with the Muscle Myopathy Wooden Breast. *Frontiers in Physiology* 11:1010.
4. Bosworth, B., Waldbieser, G., Garcia, A., Lourenco, D. 2020. Effect of pond- or strip-spawning on growth and carcass yield of channel catfish progeny, *Ictalurus punctatus*. *J World Aquacult Soc.* 51:407-417.
3. Proestou, D.A. and Sullivan, M.E. 2020. Variation in global transcriptomic response to *Perkinsus marinus* infection among eastern oyster families highlights potential mechanisms of disease resistance. *Fish and Shellfish Immunology* 96:141-151.
2. Guillette, T.C., McCord, J., Guillette, M., Polera, M., Rachels, K.T., Morgeson, C., Kotlarz, N., Strynar, M., Knappe, D., Reading, B.J., and Belcher, S.M. 2020. Per and Polyfluoroalkyl Substance Exposure in Striped Bass (*Morone saxatilis*) of Cape Fear River is Associated with Biomarkers of Altered Immune and Liver Function. *Environmental Science and Technology*, 136:105358.
1. Zhang, Y., Liu, Z.J., and Li, H. 2020. Genomic prediction of columnaris disease resistance in catfish. *Marine Biotechnology* 22:145-151.

Bovine NRSP-8 Supported by Allotments of the Regional Research Funds, Hatch Act
January 1 to December 31, 2020

PROJECT: NRSP-8 Cattle Genome Coordinators

COOPERATING AGENCY AND PRINCIPAL LEADERS (starting 10/1/2018):
University of California, Davis: Alison Van Eenennaam, alvaneenennaam@ucdavis.edu
University of Missouri-Columbia: Bob Schnabel, Co-coordinator, schnabelr@missouri.edu
Texas A&M University, Clare Gill, Co-coordinator, clare-gill@tamu.edu
USDA ARS, Beltsville, Ben Rosen, Co-coordinator, Ben.Rosen@ars.usda.gov
Washington State University, Zhihua Jiang, Co-coordinator jiangz@wsu.edu

PROFESSIONAL MEETINGS:
Because of the Corona virus pandemic, PAG 2021 was not held at its normal time in January, 2021.
Brenda Murdoch chaired and organized the Beyond NRSP-8 virtual meeting – September 30, 2020. Participants include 67 NRSP-8 leadership and members.

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."

Brenda Murdoch (ID), as a member of the Cattle Pangenome, worked to test method for the isolation of the bovine Y chromosome. The goal of this project is to isolate the bovine Y chromosome for sequencing. Sequencing a sample containing only (or mainly) Y chromosome has the potential to allow more complete resolution of repeat sequences and provide a more complete mapping of this chromosome than has previously been reported. Our lab has previously worked with FACS methods to troubleshoot protocols for the isolation and collection of the bovine Y chromosome. In October of 2019 we began applying a different approach to this project, and began trials using a streptavidin-biotin magnetic particle-based capture methodology for Y chromosome capture.

Thirteen bead capture trials were conducted from October 2019 through May 2020, and three sets of samples were provided to Dr. Timothy Smith at USDA ARS, Clay Center, NE for sequencing. Sequencing

results suggest that methods attempted thus far have not successfully isolated sufficient amounts of Y chromosome for sequencing. There are a number of possible explanations for these results, and further work is necessary to understand what components of the protocol are responsible for this outcome. This work was interrupted by the COVID 19 pandemic and has not been resumed. The research funding provided for this project, from the cattle coordinator, went towards cell culture supplies (including lipopolysaccharides, centrifuge tubes, serological pipets, and pipet tips), streptavidin-biotic bead capture supplies (including Y chromosome probes, Qubit assay tubes, and streptavidin Dynabeads), and sequencing supplies.

Cattle coordinator funds were allocated to Darren Hagen at Oklahoma State (OK). These funds were used to generate long-read sequencing data from a *Bos javanicus* (Banteng) bull. The generation of sequencing data and subsequent assembly of a banteng genome supports the cattle community's efforts to sequence extant Bos species and create a pan-genome. High-molecular weight DNA was extracted from leukocytes of an adult bull by Brenda Murdoch's lab at the University of Idaho. DNA was sent to the genomics core at UC-Davis and sequenced on two lanes of Oxford Nanopore Technology's PromethION sequencing platform. This resulted in 127Gb of raw read data with a read length N50 of 32Kb. Early assembly efforts of the long-read sequencing data produced a genome assembly of >2.6Gb (expected size = 3.2Gb) in 1,575 contigs. The longest contig is 0.135Gb. Our current genome assembly contains >80% of the expected single copy orthologs by BUSCO ortholog analysis. The assembly is still missing ~15% of the expected orthologs, meaning additional work must be done. To improve this assembly we expect to generate high coverage short-read sequencing data to supplement and correct for sequencing errors inherit to ONT sequencing reads.

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.

Brenda Murdoch (ID) contributions to this project include collecting fetal tissues from four Line one breed heifers.

Wansheng Liu (PA) oversaw the bovine tissue RNA extraction and RNA-seq and small RNA-seq library construction and sequencing. His group constructed and sequenced 123 RNA-seq libraries, and 123 small RNA-seq libraries, and generated a total of 6.4 billion expressed sequence reads (at Zoetis). The sequence data are being analyzed in Dr. James Reecy's lab at Iowa State University. At the end of 2020, they finished the initial transcriptional annotation of the bovine genome across over 40 different tissues and cell lines. A manuscript is under preparation for this part of the project.

Wansheng Liu (PA) recovered two lost Holstein Y lineages, namely ZIMMERMAN ALSTAR PILOT (born in 1954) and ROSAFE CALIBAN (born in 1953 and use to produce 3 and 5 male offspring, respectively. Genomic DNA extracted from one bull of each recovered Y-lineage has been sequenced for a 100X coverage (Illunima PE 100bp/read). De novo assembly has been performed for each Y chromosome individually. The draft Y chromosome assembly is 10.3Mb with 4200 contigs, and the N50 is 2831bp, which will be compared with the current Holstein Y lineages to identify lineage-specific sequences/markers.

Determine the role of the bovine PRAMEY during sperm capacitation and acrosome reaction. The goal of this work was to determine whether the PRAMEY protein plays a role during bovine sperm capacitation and acrosome reaction (AR). Freshly ejaculated sperm was collected from normal Holstein AI bulls (n=5) at 3 different time points for biological and technical replicates. PRAMEY localization was determined by western blot (WB) and immunofluorescent (IF) staining on sperm samples with the following treatments: A. 0 hr. control, B. 5 hr. control, C. capacitated and AR, D. capacitated and non-AR, E. capacitated and AR with PRAMEY antibody, F. capacitated and AR with Rabbit IgG, G. capacitated and non- AR with PRAMEY antibody, and H. capacitated and non-AR with Rabbit IgG. Sperm ($1x10^8$) were incubated at 37°C with 5% CO_2 and humidity to induce capacitation and the acrosome reaction for 4 hrs. and 1 hr., respectively. Treatment A had no incubation time, treatment B was incubated for 5 hrs. in PBS, and treatments C-H were treated to induce capacitation using an SP-TALP buffer and heparin. Treatments C, E, and F were then incubated with an SP-TALP buffer and Lysophosphatidylcholine (LPC) to induce the AR, while treatments D, G, and H were incubated in SP-TALP only during that time. WB analysis indicated that three PRAMEY isoforms (58, 30, and 13 kDa) were detected in the current experiments. The 30 kDa isoform was moderately to highly expressed in all treatments except in the AR sperm. The 13 kDa isoform was detected in the 5 hr. control and non-AR samples, but not in the 0 hr. control, suggesting that the 13 kDa isoform appears after sperm have went through the hyperactivation process of capacitation, and that the 13 kDa isoform could be the active PRAMEY isoform for sperm motility. Furthermore, the PRAMEY isoforms (58, 30, and 13 kDa) were not detected in the AR sperm (C, E, and F treatments) except for treatment (E) where the 58 kDa isoform is rescued due to the addition of PRAMEY antibody during the AR process. These results prompt us to hypothesize that PRAMEY is released during the acrosome reaction. To test our hypothesis, IF staining with PRAMEY-specific antibody was performed on fixed sperm from all eight treatments. A typical acrosome-enriched PRAMEY staining pattern was observed in sperm from all non-AR treatments (A, B, D, G, H), whereas little to no PRAMEY staining was observed in the acrosome region of the AR sperm (C, E, F treatments), supporting our hypothesis. In conclusion, our preliminary data demonstrated that the 13 kDa PRAMEY isoform may play a role in sperm motility during capacitation, and the 58 and 30 kDa PRAMEY is involved in the acrosome reaction.

Cattle Coordinator funds were used to augment USDA NIFA grant number 2020-67015-30829 titled "Identification of Expression QTL Associated With Feed Efficiency in Beef Cattle" by Robert Schnabel (MO). This project was designed to generate transcriptome (RNA-seq) data for up to 150 animals for each of three tissues (liver, hypothalamus, small intestine) in order to identity genetic variants associated with differences in gene expression levels, commonly referred to as "bulk RNA-seq". Single cell transcriptome profiling is a relatively new technology that measures abundance of transcripts at the level of individual cells or nuclei, as opposed to bulk collections of cells from tissues. The single cell transcriptome work was not budgeted, nor envisioned, when the grant was awarded. For a subset of these animals, three individuals with high feed efficiency and three individuals with low feed efficiency phenotypes were selected to perform 10X Genomics single cell transcription profiles. The libraries and sequence data have been generated but not analyzed as of this report. These data will provide information as to which cell types are present within the liver tissue examined, provide preliminary data as to which genes and/or cell types are associated with differences in feed efficiency and provide resources that can be used by the community to deconvolute bulk transcriptomes into representative cell types present."

Cattle Coordinator funds were used to perform single cell sequencing on four separate bovine ovaries by Dr. Anna Denicol, UC Davis (CA). Two samples were obtained from pregnant cows (45-50 days of gestation) and two were from cows of unknown pregnancy status. Upon arrival to the laboratory, the outer 2-4 mm of the cortical region of each ovary was dissected and subjected to tissue dissociation. Briefly, dissociation included mincing the tissue into small fragments no bigger than 2x2mm, followed by enzymatic digestion by gentle agitation in 1 mg/ml collagenase for 15 min at 37 °C. This step was repeated twice. After each agitation, the tissue was mechanically dissociated by repeated pipetting. After the second pipetting, the solution was sequentially strained through a 70 and 40 µm cell strainers to remove oocytes

and cell clumps, and cell viability was tested using Trypan Blue. The single cell suspensions were submitted for single cell RNA sequencing at the UC Davis DNA Technologies Core Facility. Single cell RNA sequencing uses a microfluidics platform (developed by 10x Genomics, Pleasanton, CA) where each cell within the sample is lysed inside an individual droplet, followed by cDNA synthesis, barcoding, and sequencing. The raw data obtained from sequencing of the four ovarian samples was run through the cell ranger pipeline (10x Genomics) for read alignment and barcode-feature matrix generation. Each sample was then imported into R studio for further quality control analysis, including the proportion of mitochondrial RNA in the sample, proportion of genome mapping, and the total amount of features. After passing the quality control steps, the data were normalized and clustered according to differential gene expression using R studio. Sample anchoring was used to ensure that similar patterns were seen in each sample. The resulting clustering analysis revealed 11 cell clusters, represented in all four samples. Based on the patterns of gene expression, our preliminary analysis identified the following cell populations: stromal cells, identified by expression of the transcripts *COL1A2, COL16A1*, *DCN*, *LUM*, *MMP2* and *TCF21* (clusters 2 and 3); follicular cells, identified by expression of the transcripts *AMH*, *INHA*, *FST*, and *FSHR* (primarily seen in cluster 0). The transcript for the surface marker *CD44*, which has been described as a marker of multipotent stem cells, was localized to cluster 5, together with *CD69, CXCR4* and *ITGB5*, markers commonly associated with immune cells. Cluster 5 cells also showed high expression levels of *PRDM1*, a marker of germ cells. Another important marker of the germ cell lineage, *SOX17*, was identified strongly in clusters 1 and 9. Finally, *BMP4*, one of the transcripts associated with specification of primordial germ cells during fetal development, was identified in cluster 3. Of note, the canonical oocyte markers *GDF9* and *ZP3* were not found in the data. These preliminary analyses demonstrate distinct patterns of gene expression that can be associated with specific cell types in the adult bovine ovary. We identified expression of early germ cell markers, but not of fully-grown oocytes. The absence of oocytes was expected as exclusion of these cells was intended by the serial filtration and size exclusion. Future steps will include confirmation of co-localization of *CD44* and *PRDM1* at the protein level by flow cytometry analysis, and further characterization of the stromal cell populations. We also plan to expand the single cell sequencing analysis of the adult bovine ovary to include the different stages of the estrus cycle.

Heifer fertility research performed genome-wide association analyses with antral follicle counts (AFC) and reproductive tract score (RTS) from approximately 290 U of I heifers collected over two years (ID). The research project used 50K GGP genotyping data, which was subsequently imputed to approximately 900K SNP data by collaborator Troy Rowen, to identify marker associated with these measures of fertility. The resulting manuscript has been submitted to gene and is currently under review. Importantly, these data and the expertise stemming from the NRSP08 objectives and supported work was leverage and contributed to two other hatch projects.

Risk Assessment, welfare analysis, and extension education for dairy calf respiratory disease management (Van Eenennaam, UC Davis, CA) Over 13,000 calves were enrolled including 1,096 cases and 3,061 control Holstein heifers from a single dairy where all of the calves have been 50K genotyped. Two papers on this project were published this year.

Comparative evaluation of the phenotype, genome, and animal products derived from offspring of a genome edited, hornless bull and controls. (Van Eenennaam, UC Davis, CA) A paper documenting the offspring of the genome edited polled offspring was published in Nature Biotechnology. This paper sparked a lot of media attention and was carried on 122 news outlets, and has an Altmetric score of 1006! We continued to phenotype the offspring and collected meat data on them at slaughter. We also bred the heifer and collected milk from her post-calving. We now have all the data collected to write up a paper documenting the characteristics of these offspring of a genome edited, hornless bulls, and their controls. This is very relevant to the regulatory discussions around the use of genome editing in livestock for agricultural purposes.

Genetic containment of livestock via CRISPR-mediated gene knock-in" (Van Eenennaam, UC Davis, CA) The overall goals of this project were to advance current knowledge of gene editing tools to develop a cisgenic sterility method for genetic containment, and to concomitantly develop an approach to improve the efficiency of beef production. Two papers documenting an improved rate of targeted gene knock-in of in-vitro fertilized bovine embryos were published. A one-step method to produce a SRY gene knock-in calf resulted in a bull born in April of 2020 DNA was extracted from placenta, blood and a fibroblast line derived from the calf and analyzed for SRY-GFP knock-in, as well as genotypic sex. PCR and Sanger sequencing revealed a non-mosaic biallelic edit with the insertion of the SRY-GFP construct into the target location on one chromosome 17, and a 26 base pair insertion into the other, in addition to an XY genotype. An abstract documenting the arrival of this bull was presented at the American Society of Animal Science meeting in June, 2020.Additionally, we produced a 30 minute educational video entitled "Making a CRISPR Cow" on the making of a gene knock-in calf which is available on YouTube https://youtu.be/fYBWDNt8rTo. This elicited a lot of media interest and this research was featured in stories in Wired, and Grist. Additionally, a 10 minute segment in a NOVA episode on germline genome editing featured this bull and the work being done in livestock genome editing at UC Davis (https://www.pbs.org/wgbh/nova/video/gene-editing-reality-check/) .

IMPACT / USEFULNESS OF FINDINGS:

Peer Reviewed Publications

1. Rosen BD, Bickhart DM, Schnabel RD, Koren S, Elsik CG, Tseng E, Rowan TN, Wai Y. Low WY, Zimin A, Couldrey C, Hall R, Li W, Rhie A, Ghurye J, McKay SD, Thibaud-Nissen F, Hoffman J, Murdoch BM, Snelling WM, McDaneld TG, Hammond JA, Schwartz JC, Nandolo W, Hagen DE, Dreischer C, Schultheiss SJ, Schroeder SG, Phillippy AM, Cole JB, Van Tassell CP, Liu G, Smith TPL, Medrano JF. 2020 De novo assembly of the cattle reference genome with single-molecule sequencing. *Gigascience*. 1;9(3).
2. Lu, C., Yang, M., Rossi, R.M., Wang, A., Feitosa, W.B., Diaz, F. J., Liu, W.-S. 2020 Deletion of the mouse X-linked Prame gene causes germ cell reduction in spermatogenesis. *Mol. Reprod. Dev.* 87(8), 666-679. DOI: 10.1002/mrd.23324
3. Yao, Y., Zhang, Y., Liu, W.-S., Deng, X. 2020. Highly efficient synchronization of sheep skin fibroblasts at G2/M phase and isolation of sheep Y chromosomes by flow cytometric sorting. Scientific Reports 10, 9933. DOI:10.1038/s41598-020-66905-x
4. Chen, X., Zheng, Y., Lei, A., Zhang, H., Niu, H., Li, X., Zhang, P., Liao, M., Lv, Y., Zhu, Z., Pan, C., Dong, W., Chen, H., Wu, D., Liu, W.-S., Hamer, G., Zeng, S., Zeng, W. 2020. Early cleavage of preimplantation embryos is regulated by tRNAGln-TTG-derived small RNAs present in mature spermatozoa. J. Biol. Chem. 295(32), 10885-10900. DOI: 10.1074/jbc.RA120.013003.
5. Dechow, C.D., Liu, W.-S., Specht, L.W., Blackburn, H. 2020. Reconstitution and modernization of lost Holstein male lineages using samples from a gene bank. J. Dairy Sci. 103, 4510-4516. DOI: https://doi.org/10.3168/jds.2019-17753. This article was selected by the Editor-in-Chief as the "Editor's Choice".
6. Young, A.E., T.A. Mansour, B.R. McNabb, J.R. Owen, J.F.Trott, C.T. Brown, and A.L. Van Eenennaam, 2020. Comparative evaluation of the phenotype and genome from offspring of a genome edited, hornless bull and controls. Nature Biotechnology 38. 225-232.
7. Dubrovsky, S., Van Eenennaam, A. L., Aly, S., Karle, B. M., Rossitto, P., Overton, M., Lehenbauer, T. and Fadel, J. 2020. Cost of Bovine Respiratory Disease (BRD) and cost-benefit of implementation of preventative measures in preweaned calves on California dairies: The BRD 10K study. Journal of Dairy Science. 103: 1583-1597.
8. Bishop, T.F., and Van Eenennaam, A.L. 2020 Genome editing approaches to augment livestock breeding programs.J Exp Biol. 2020 Feb 7;223(Pt Suppl 1). pii: jeb207159.
9. Maier, G., Love, W., Karle, B., Dubrovsky, S., Williams, D., Champagne, J., Anderson, R., Rowe, J., Lehenbauer, T., Van Eenennaam, A.L. and Aly, S.S. 2020 A novel risk assessment tool for bovine respiratory disease in preweaned dairy calves. Journal of Dairy Science. 103:1-17.

10. Camargo, L.A., J. R. Owen, A. L. Van Eenennaam, and P.J. Ross. 2020. Efficient one-step knockout by electroporation of ribonucleoproteins into zona-intact bovine embryos. Frontiers in Genetics. 11:570069.
11. Hennig, S. L., Owen, J. R., Lin, J. C., Young, A. E., Ross, P. J., Van Eenennaam, A. L., and Murray, J. D. 2020. Evaluation of Mutation Rates, Mosaicism and Off Target Mutations when injecting Cas9 mRNA or Protein for Genome Editing of Bovine Embryos. Scientific Reports 10, 22309
12. Owen, J.R., Hennig, S.L., McNabb, B., Lin, J.C., Young, A.E., Murray, J.D., Ross, P.J., and Van Eenennaam, A.L.2020. Harnessing endogenous repair mechanisms for targeted gene knock-in of bovine embryos. Scientific Reports. 10, 16031.
13. Halstead, M.M., C. Kern, P. Saelao, Y.Wang, G. Chanthavixay, J. F. Medrano, A. L. Van Eenennaam, I. Korf, C. K. Tuggle, C. W. Ernst, H. Zhou, and P. J. Ross. 2020. A comparative analysis of chromatin accessibility in cattle, pig, and mouse tissues. BMC Genomics. 21(1):698.

Peer Reviewed Abstracts

1. Stegemiller MR, Davenport KM, Reynolds MK. Rowan TN, Hall JB, Murdoch BM. Genome Wide Analysis of Antral follicle Counts in Crossbred Heifer. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
2. Bhattarai S, Cantrell B, Murdoch B, Funston R, Weaber R, McKay S. DNA Methylation in the Regions of Structural Variation in the Limbic System of Cattle. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
3. Stassen E, Bhattarai S, Buttolph T, Murdoch B, Woo J, Yampara-Iquise H, Schnabel RD, Taylor JF, White S, McKay S. A survey of RNA Methylation within the Bovine AMPK Gene Family. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
4. Quigley S, Murdoch B, Funston R, Weaber R, McKay S. Identification of 5-Hydroxymethyhlcytosine Markers in the Cattle Brain. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
5. Rosen R, Davenport KM, Bhattarai S, Basnayake V, Kalbfleisch T, Murdoch B, Smith TPL, Murdoch J, Heaton MP, McKay S. Estimating Genetic diversity in Vermont Moose using SNP. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
6. Ross P, Hamid B, Canovas A, Corum S, Gill CA, Hu R, Jiang H, Jiang Z, Kern C, Kern C, Liu W, Lyu P, Ma W, McKay S, Medrano JF, Michal JJ, Murdoch BM, Reecy JM, Rijnkel M, Rincon G, Smith TPL, Thomas MG, Wang G, Wang H, Xing Y, Xu X, Zhang Y, Zhou H. Functional Annotation of the Bovine Genome. The FAANG Workshop. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 10th, 2020.
7. Kern, C., Wang, Y., Xu, X., Pan, Z., Halstead, M.M., Chanthavixay, G., Saelao, P., Waters, S.M., Trakooljul, N., Wimmers, K., Korf, I., Delany, M.E., Cheng, H.H., Medrano, J.F., Van Eenennaam, A.L., Tuggle, C.K., Ernst, C.W., Ross, P.J. and Zhou, H. 2020. Genome-Wide Identification and Annotation of Functional Regulatory Regions in the Chicken, Cattle, and Pig Genomes. Plant and Animal Genome XXVII Conference Abstract PE0450
8. Hennig, S.L., et al. 2020. Improving dairy cattle welfare standards through genetic dehorning by using CRISPR/Cas9 [abstract]. Transgenic Research, 29:484.
9. Owen, J.R., Hennig, S.L., et al. 2020. Harnessing endogenous repair mechanisms for targeted gene knock-in during early bovine embryonic development [abstract]. Transgenic Research. 29:470.
10. Mueller, M. L., et al. 2020. Comparison of gene editing versus conventional breeding to introgress the POLLED allele into the Australian Brahman population [abstract]. Transgenic Research. 29:484
11. Joseph R Owen, Sadie L Henning, Bret R McNabb, Jason C Lin, Amy E Young, James D Murray, Pablo J Ross, Alison L Van Eenennaam, PSX-32 Late-Breaking Abstract: Production of a Gene Knock-In Bull Calf by Embryo-Mediated Genome Editing, Journal of Animal Science, Volume 98:Supplement_4, November 2020, Pages 358–359,
12. Kern, C, and Liu, W.-S. (2020) Role of the Bovine PRAMEY Protein in Sperm Function. SSR, July 9-12, 2020. Virtual meeting.

13. Yang, Y., Oatley, J., and Liu, W.-S. (2020) Deletion of the *Pramel1* gene leads to germ cell reduction and subfertility in male mice. SSR, July 9-12, 2020. Virtual meeting.

Graduate Student Education:

1. Kimberly Davenport, Doctorate of Philosophy candidate, research training has contributed to the helping with reference genome and the FAANG project.
2. Gabrielle Becker, Master of Science student, is working on the isolation of Y chromosome for sequencing.
3. Morgan Stegemiller, Master of Science student, research has included genome-wide association studies of U of I heifers and fertility traits.
4. The data generated by the cattle coordinator funds allocated to Darren Hagen allowed novel training experiences for a current Oklahoma State PhD student and pre-doctoral student now pursuing a PhD at University of Arizona
5. 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.
6. 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.
7. 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.
8. Jason Lin, Master of Science student at UC Davis, contributed to the genome editing research.

Active Grants and Contracts Leveraged Through NRSP-8 Research:

Despite the difficulties of the pandemic, over $3.4 million of new grants related to animal and bovine genomics were secured by members of NRSP-8 in 2020.

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. Huaijun, 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.

USEFULNESS OF FINDINGS:
Invited Seminars: This list of seminars (presenter underlined) highlight the transfer of information to provide information and education among scientists, Extension educators, graduate students and the general public to benefit society.

1. Murdoch BM The functional annotation of the sheep and cattle genomes. Lecture in ANSC691 – Current Topics in Genomics, Instructor Luiz Brito Nov 10th, 2020.
2. Van Eenennaam AL "How Regulatory Factors Influence the Application of Genomics in Animals", Plant and Animal Genome Conference , San Diego, CA 1/13/2020
3. Van Eenennaam AL "Embryo gene editing: techniques, uses and future perspectives", Narrowing the gaps between embryo gene editing and ethics" International Embryo Technology Society (IETS), New York, NY 1/18/2020
4. Van Eenennaam AL "Gene Editing in Livestock: Prospects and Policy", UC Santa Cruz, 1/27/2020
5. Van Eenennaam AL "Beef Cattle Genetics" UC Davis Farm Club, UC Davis 2/22/2020
6. Van Eenennaam AL "The Interdependence of Sustainability, Innovation, and Science Communication Around Animal Agriculture" Oregon Dairy Farmers Annual Convention, Salem, OR 2/24/2020
7. Van Eenennaam AL "Making the best use of current genetic selection tools", National Cattlemen's Beef Association (NCBA) Cattlemen's Webinar, 2/27/2020
8. Van Eenennaam AL "The future of genome editing in food animal species", 2020 ASAS-ADSA Midwest Meeting, Omaha, NE 3/3/2020
9. Van Eenennaam AL "Genome Editing in Livestock", UC Berkeley University Extension, Virtual conference 3/21/2020
10. Van Eenennaam AL "Where are we and where are we going with genomic selection?", Zoetis Dairyman's Roundtable, ~~Sioux Falls, SD~~ Switched to video conference 3/24/2020
11. Van Eenennaam AL "Alternative meats and alternative statistics: What do the data say?", Montana Nutrition Conference, ~~Bozeman, MT~~ Switched to video conference 4/14/2020
12. Van Eenennaam AL "Insights on Agriculture, Environmental Sustainability and Climate Change", International Food Information Council Webinar Series, 6/25/2020
13. Van Eenennaam AL "Environmental Impacts of Protein Production", International Food Technology Institute Virtual Conference ~~Chicago, IL~~ Switched to video conference 7/13/2020

14. Van Eenennaam AL "Genome Engineering for Agricultural Applications" ~~Rochester Minnesota~~ Switched to video conference Genome Writer's Guild, 7/23/2020
15. Van Eenennaam AL "Production of a Gene Knock-In Bull Calf by Embryo-Mediated Genome Editing", Revive and Restore virtual meeting 7/21/2020
16. Van Eenennaam AL "Potential of CRISPR in Livestock" International Consortium on Applied Bioeconomy Research (ICABR) Annual Program Online 7/24/2020
17. Van Eenennaam AL "Gene editing in livestock: promise, prospects and policy" Arizona State University Animal Science Departmental Seminar Online 9/21/2020
18. Van Eenennaam AL "Advanced Genetic Technologies" Reaching Out While Locked In! Beef Management Webinar Series, University of Kentucky Online 9/22/2020
19. Van Eenennaam AL "Genome Editing Approaches for Livestock —Advancing Animal Health and Welfare" Davis Sr High women in Science, Technology, Engineering, and Mathematics (WiSTEM) Online 9/29/2020
20. Van Eenennaam AL "Emerging Technologies: Regulatory Oversight of Intentional Genomic Alterations in Animals", Food Drug and Law Institute (FDLI) Online Conference 10/7/2020
21. Van Eenennaam AL "Genome Editing Applications in Animals"CRISPR in Agriculture Research, Syntego World CRISPR Day symposium 10/20/2020
22. Van Eenennaam AL "Gene editing in livestock: promise, prospects and policy" Iowa State seminar 10/22/2020
23. Van Eenennaam AL "Agricultural animal transgenesis for food applications" Transgenic Technology , Israel, 10/27/2020
24. Van Eenennaam AL "CAST Past Borlaug Communication Awardee Session", CAST Annnual Meeting online meeting 10/28/2020
25. Van Eenennaam AL "Using genome editing for livestock health", ASAS-Southern Section Genetics and Genomics Webinar Series 11/4/2020
26. Van Eenennaam AL "Genome editing applications in animals" Virtual Workshop in Genome Editing Technologies in Kenya,11/10/2020
27. Van Eenennaam AL "The importance of innovation to the future of beef production", Wagyu Virtual International Conference, South Africa 11/11/2020
28. Van Eenennaam AL "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 11/13/2020

Equine NRSP-8 2020Annual Report (Coordinator and Workshop)

Leadership:
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

2020 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 will organize the next workshop meeting in January 2022.

Station Reports: Meeting to report on 2020 was cancelled

Progress on the Workshop Objectives:

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.

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. This biobank has been instrumental in the development of epigenetic assays and data collection for the horse, including RNA-seq, ChIP-seq, and CTCF-binding assays. In 2020, data was published for 4 histone modification in the 8 prioritized tissues (adipose, brain, heat, lamina, liver, lung, muscle and ovary) (Kingsley et al., 2020). This sequencing was completed and uploaded to EMBL-ENA (https://www.ebi.ac.uk/ena/data/view/PRJEB26698). Scientists were invited to "adopt a tissue" and fund annotation for additional tissues. During 2020 annotation was complete for 4 additional tissues, namely spleen, skin, metacarpal and sesmoid bone.

Also during 2020, tissues were added from two stallions to this project and will be tested during 2021.

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 from whole-genome sequencing of the two mares (https://www.ebi.ac.uk/ena/data/view/PRJEB26698), mRNA-seq (https://www.ebi.ac.uk/ena/data/view/ERA1487553) and smRNA-seq (in submission) across 47 tissues from the two mares and reduced read bisulfate sequencing (RRBS) across 8 tissues (in submission) on the two mares continue to be publicly available at EMBL-ENA. As noted above, whole genome sequence and transcriptomic information has been added to the SRA in connection with research projects by individual scientists.

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/

September 2020 Havemeyer International Equine Genome Workshop Cancelled
A workshop was planned for July 2020 in Ithaca, NY but was cancelled due to the pandemic. Beginning in February 2021 a series of virtual meetings were planned (February, April, June) The meeting originally planned for 2020 will be held in Ithaca in July 2022. 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 2020 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 bioinformatics activities related to NRSP8 at Iowa State University.

Total Leveraged Funding Summary for 2020

Federal Funding:	$	1,048,171
Internal :	$	1,322,621
Industry:	$	1,313,792
Total	**$**	**3,684,584**

Publications for 2020 by workshop participants downloaded from Pubmed

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9. Librado P, Orlando L. Genomics and the Evolutionary History of Equids. Annu Rev Anim Biosci. 2021 Feb 16;9:81-101. doi: 10.1146/annurevanimal-061220-023118. Epub 2020 Nov 16. PMID: 33197207.
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Sheep/ Goat NRSP-8 Annual Report of Multi-State Research Activity

PROJECT: NRSP-8
PROJECT TITLE: NRSP-8 Sheep/Goats Species Committee
PERIOD COVERED: January 1 to December 31, 2020
DATE OF THIS REPORT: February 15, 2021

PARTICIPANTS: *Voting member.
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Louisiana State University: James E. Miller*
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BRIEF SUMMARY OF MINUTES OF ANNUAL MEETING:

The Plant and Animal Genomes XXIV conference was repeatedly delayed in 2021 due to the COVID-19 pandemic. It has recently been announced that this conference will be held in 2022 instead of 2021. A separate annual meeting of NRSP8 under development for early 2021.

ACCOMPLISHMENTS AND IMPACTS:

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.

The Ovine FAANG Project was funded in 2017 based on collaborative work organized by many NRSP8 Sheep and Goat genomics members. The research performed FAANG assays on tissues from the reference genome Rambouillet to create a world-class coordinated genome assembly and annotation project. This project has contributed to the core activities of FAANG by providing transcriptome data for 60 tissues. Furthermore, Cap gene expression (CAGE) data for 56 tissues has been generated and analyzed to identify transcription start site in the sheep genome. A ChIP-seq protocol and bioinformatics pipeline was preparation for 47 tissues for the Ovine FAANG Project. The histone modifications H3K4me3, H3K27ac, H3K4me1, and H3K27me3 were used for ChIP-seq to determine regions of active promoter and enhancer. Additionally, ATAC seq has been performed on these tissues. Whole genome bisulfite sequencing for eight tissues and reduced representation bisulfite sequencing from the same sheep are in progress. Differentially methylated regions are being identified and methylation regions are being overlaid with chromatins states characterized with ChIP-seq and ATAC seq.

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 is awaiting final quality confirmation and annotation. The manuscript for this improved assembly is underway.

Sequencing the ovine Y chromosome. We reported in previous years on a collaborative research project with China Agricultural University to isolate and sequence the sheep Y chromosome. We have established a highly efficient synchronization method for G2/M phase of sheep fibroblasts, which was successfully applied to flow-sorting chromosomes of sheep, with a focus on isolation and sequencing of the ovine Y chromosome. The isolated (~80,000) Y chromosomes were verified by fluorescence quantitative real-time polymerase chain reaction, further confirmed by fluorescence in situ hybridization, and amplified by the MALBAC method before next-generation sequencing. The sequence results indicated that 68.90% of reads were Y chromosome-related sequences as they are homologous to the bovine Y chromosome. The remaining 31.1% of reads were aligned to the sheep reference genome, including 13.57% reads to chromosome X and 6.68% to chromosome 17. Importantly, the paired-end reads that are properly aligned to the bovine Y sequence assembly accounted for 46.49%, indicating the success in the ovine Y chromosome isolation and the high quality of the Y chromosome sequences. This study not only set up a foundation for future sequencing, assembly and annotation of the ovine Y chromosome, but also provide a validated approach to overcoming difficulties in sequencing Y chromosome in other mammalian species.

WC1 co-receptors belong to the scavenger receptor cysteine-rich (SRCR) superfamily and are encoded by a multi-gene family. Each type I transmembrane bovine WC1 protein contains an extracellular SRCR domain arrangement that can be characterized as [a1-(b2-c3-d4-e5-d6)-(b7-c8-d9-e10-d'11)] or [a1-(b2-c3-d4-e5-d'11). The repetitive nature of the exon duplication and gene duplication makes annotation of the WC1 locus challenging; thus techniques worked out in this project will have an impact on annotation of other gene and exon duplicated loci.

Expression of particular WC1 genes defines functional subpopulations of lymphocytes known as WC1 gamma delta T cells. WC1 genes can be grouped as WC1.1-type or WC1.2-type based on the sequence of their N-terminal a1 SRCR domain, which is 4 amino acids longer for WC1.2 type a1 SRCR domains. Reciprocal expression of either WC1.1-type or WC1.2-type proteins is correlated with gamma delta T cell responsiveness to pathogens, which explains how so many large homologous open reading frames have been maintained for millions of years.

We have previously identified complete genomic sequences for 13 different bovine WC1 genes through annotation of the bovine genome Btau_3.1 build. In 2020, we made significant progress in verifying caprine gene models obtained through annotation of the PacBio sequenced San Clemente goat genome, by comparison to the Yunan goat genome and cloning of corresponding full-length WC1 cDNA from the UMass Boer goat herd. We found evidence for up to 30 goat WC1 genes, which is more than twice that of cattle. Moreover, goats had seven different WC1 gene structures, of which 4 are unique to goats. Caprine WC1 genes also had multiple splice variants of their transcripts coding for the intracytoplasmic domains that in some cases eliminated tyrosines shown previously to be important for signal transduction. The most distal WC1 SRCR domains known as SRCR a1, based on sequence and position, were highly conserved among goat breeds but fewer were conserved between goats and cattle. In the ovine Rambouillet sheep assembly, we found 15 complete genes and 42 partial genes, with 6 different predicted structures. We were able to confirm transcription of all but five of the annotated ovine WC1 genes in the UMass Amherst Dorset sheep flock. Of the five annotated a1 domains for which we were not able to obtain cDNA evidence, three had frame shift mutations, or are truncated, indicating they may be pseudogenes. We were also able to

amplify the full-length transcript comprising the 11 SRCR-domain gene WC1-10, verifying that that gene structure as it appears in the genome assembly. Along with goat, sheep, and cattle, swine are a fellow member of order *Artiodactyla*. In swine, cDNA evidence shows that porcine WC1 is also expressed as a multigenic array consisting of 9 genes (WC1-1 to WC1-9) each encoding 6 SRCR domains. We annotated *Sscrofa11.1* for sequence derived from full-length cDNA transcripts representing the 9-hypothetical porcine WC1 genes. We were able to map 7 of the 9 genes, leaving two (WC1-5 and WC1-8) unplaced in the current assembly.

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.

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. This project was funded in the beginning of 2016 and is currently in a no cost extension with the aim understanding parasite resistance and resilience and generating estimated breeding value regarding this trait for the industry. To date this project has collected fecal samples, performed FEC and collected blood samples from approximately 5000 Katahdins over four year for genotyping. The GWAS analyses of these samples and levels of FEC are in progress.

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.

WC1-3 is required for the γδ T cell response to the spirochete *Leptospira.* This is correlated with the direct binding of WC1-3 to *Leptospira*. Because this binding can be ablated by single amino acid changes, we hypothesize that WC1 binding to pathogens and the consequent immune response can be altered by single nucleotide polymorphisms and allelic variation in populations. We are coupling genomic annotation, cDNA cloning of WC1 transcripts, and phylogenetic analysis with functional assays to determine WC1 binding affinity for pathogens such as *Leptospira*, *Mycobacteria, and PRRSV* across livestock species and breeds.

Molecular Signatures and Regulatory Checkpoints for Animal Health-Nc.X320-5-19-120-1
Evaluated the possible immunomodulatory effect of Gum arabica (GA) a well-known traditional herbal medication from *Acacia Senegal* (L.) Willdenow trees, by looking at indicators of goat health, gene expression in blood using RNA Seq and fecal microbiome.

Procedures: Clinically healthy Boer, Spanish, and Spanish-Boer cross goats (N=12) from the NCA&T Small ruminant unit were used to study the effect acacia supplement on goat's health parameters. Following initial screening for infection, goats were assigned to two groups of six (*n*=12). Goats were drenched daily with 10 mL of GA (treatment I) extract for 6 weeks. A control group of six age-matched goats received sterile water (treatment II).The Famacha score, PCV, total and White blood cell differential count and protein concentration of total plasma protein was determined. Effects of global transcriptome and fecal microbiome were assessed using RNA and DNA Seq. Undergraduate students conducted NCBI database-based reviews on goat galectin genes.

Results: Indicators of anemia and immune modulation in goat peripheral blood changed in response to GA extract administration. Differentially expressed gene and changes in microbial diversity that may be related to anemia associated health outcomes in goats have been identified and are being validated.

Impact: Increased knowledge about the impact of immunomodulation on gene expression and gut microbiome relevant to anemic conditions. One undergraduate student and PhD student submitted abstracts. A functional genomics collaboration was between ARS, University of Idaho, and University of Vermont to identify gene regulatory elements in domestic sheep. Specifically, ChIP-Seq and whole genome bisulfide sequencing was conducted with liver, spleen, and brain samples from 2 male and 2 female mixed breed year-old healthy sheep. Genomic regions were identified as associated with active promoters, active and repressed enhancers, and silencers at a level like sheep adipose and mouse liver. Identifying regulatory elements in these tissues will improve our understanding of mechanisms that control or influence economically important traits. This work was submitted to Frontiers in Genetics.

Functional genomics work with sheep macrophages. We worked to determine how the DNA of sheep macrophages is organized and identify DNA "on/off" switches that will improve our ability to define DNA changes that control macrophage response to foreign invaders. This is the first study of sheep immune cells with a core FAANG assay, called chromatin immunoprecipitation with DNA sequencing (ChIP-Seq). Over 248,000 regions of the genome were identified as regulatory elements (DNA "on/off" switches). Approximately 12% of the unclassified sheep macrophage genome was assigned putative biological function. Active regions identified in this study compared well with previously published gene expression data (Sheep Gene Expression Atlas). These data will improve the ability of scientists to identify causal mutations that affect disease outcome variability.

Improvement of *Babesia bovis* genome and identification of differentially expressed genes in two life stages. Babesia parasites alternate between a mammalian host, where they cause babesiosis, and the tick vector. A comparison of stages that occur within mammalian and tick hosts can provide insight into the adaptation of Babesia to these different environments. Here, we improved the genome assembly of Babesia bovis, a bovine hemoparasite, closing a 139 kbp gap, and used RNA-Seq datasets derived from mammalian blood and tick kinete stages to greatly improve the genome annotation. Approximately 1/3 of the originally annotated genes required structural changes and 334 new genes were identified, leading to an improved predicted proteome compared to the original annotation. RNA-Seq data was used to identify B. bovis genes that were differentially expressed in the two hosts. In blood stages, 28% of the genes were upregulated up to 300-fold, whereas, 26% of the genes in kinetes were upregulated, reaching >19,000-fold increase. We identified differentially expressed genes that may serve as suitable targets for the development of vaccines to control bovine babesiosis, one of the most economically devastating tick-borne diseases of cattle.

Identification of domestic sheep genes associated with detection of *Mycoplasma ovipneumoniae* DNA in nasal mucus. A contributor to polymicrobial pneumonia in domestic sheep is *M. ovipneumoniae*. Determining if age, breed, sampling time, and year of sample collect impact detection of *M. ovipneumoniae* will help determine if genomic studies should be conducted. Rambouillet had the lowest compared with Polypay and Suffolk and year-old sheep had the greatest detect amounts of *M. ovipneumoniae* from nasal mucus. Warmer weather may have had an impact on detection as April and September/October sampling times were higher than February. Breed differences suggested there may be DNA differences affecting *M. ovipneumoniae* detection. Indeed, testing over 500,000 markers with average *M. ovipneumoniae* DNA detected in nasal mucus from Polypay, Rambouillet, and Suffolk sheep found regions on chromosomes 4, 6, 7, 9, 10, 15, 17, and 22 were associated. Markers were within or near genes known to have immune functions and change DNA organization. Both these functions would be expected to change when sheep respond to polymicrobial pneumonia. Work is ongoing to identify the exact DNA changes that caused the difference in *M. ovipneumoniae* detection in nasal mucus in order to develop tools for use by the sheep industry to reduce polymicrobial pneumonia.

Major challenges facing the animal industry include climate change and consumer concerns regarding quality and nutritional value. Research focused on thermotolerance, nutritional and health value of *Bos indicus* influenced cattle, resilience to climatic stressors and resistance to internal parasites in small ruminants – requires large populations with high precision phenotypes coupled with high-density genotyping, new molecular technologies and bioinformatics techniques. The improvement of these traits is conditional on understanding their genetic structure and, subsequently, the development of population-specific genomic tools for selection, management, and marketing.

We assessed the natural variation present in these traits and quantified the magnitude of the genetic component contributing to this variation. We identified critical genomic regions associated with these phenotypes to be explored via next generation sequencing and fine mapping. Current efforts are aimed at identifying an optimal set of genetic markers to use in marker assisted selection and management programs for ensuring the economic viability and sustainability of Florida producers and the U.S. animal agriculture. A multidisciplinary approach allowed me to identify gene networks and key drivers for fatty acid composition and meat quality (marbling and tenderness) in beef cattle and we are now and targeting candidate genes to discover the mechanism causing phenotypic differences.

a. **Increasing Annual Lamb Productivity through the Identification of Genes and Diagnostic for Selection of Out of Season Breeding:** Several approaches were taken to identify regions of the genome contributing to out of season breeding. GWAS was performed using HD SNP chip data on 257 ewes of various breeds. Analysis across breed and within the Dorset and Polypay breeds identified several QTLs that are biologically plausible in genetic control of out of season lambing. In particular, identified pathways involved eye development and known hormones involved in reproductive capability. This manuscript has been published.

b. Identification of genes associated with mature body size and growth:
615 ewes, across 22 breed, were characterized for mature body size using 28 measures of various body parts to get an accurate representation of skeletal size. Principal component analysis was performed on the measurements to represent overall body size (PC1) and body thickness (PC2). 184 of these ewes are genotyped on the HD SNP chip. We plan to perform GWAS for mature body size and body thickness both across and within breed. We have birth weight and weaning weight data on 104 individuals we plan to analyze for growth trait associations along with mature body size. Analysis has been completed and identified multiple regions of genomic association to body size. This manuscript has been published.

c. Characterizing genetic variants responsible for coat color changes in United States sheep breeds: The genetic basis for brown coat color has been identified in several US sheep breeds. Experiment.com/moorit-sheep was used to raise funds to perform Sanger sequencing of *TYRP1* in several U.S. sheep breeds known to have color variation. Two mutations were associated with brown versus black. This project was presented at the 11th World Congress on Genetics Applied to Livestock Production and included in the conference proceedings. More recently, a genetic variant was identified for lilac dilution in Jacob sheep. This manuscript has been published.

d. Genetic diversity of Ethiopian short fat-tailed and Awassi sheep breeds: This study evaluated genetic diversity and population structure in these two Ethiopian sheep breeds using genome-wide 50K SNP markers. It looked for signatures of selection and adaptation. This manuscript has been published.

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.

Data from numerous projects including many published works (see below) and genome assembly and annotation project inputs have been and are being deposited into public databases to facilitate genome-to-phenome research.

IMPACT / USEFULNESS OF FINDINGS:
The characterization of WC1 genes in ruminants and swine has utility in several areas: improved vaccine design against multiple important pathogens targeted to recruit gamma delta T cells, potential selective breeding based on SNPs in WC1 genes linked to resistance to pathogens, and the possible involvement of WC1 as a receptor in PRRSV infection of swine. Because WC1 genes are present in most mammalian and avian species, elucidation of WC1 genes in agriculturally important ruminants and swine has global application potential in improving food security and in containing zoonotic diseases in non-human animal reservoirs. This project also helps support undergraduate and graduate agricultural research training.

The increased productivity and sustainability possible through genomic selection is directly proportional to the rate of adoption of these genomic tools by the industry. To promote and facilitate the adoption of new technology, we work closely with beef producers and their associations and give invited talks at many producer-oriented events. These efforts to inform and educate the stakeholder on the application and the expected benefits associated with the adoption of genomic technology is one vehicle used to enhance the impact of this research and keep the research relevant to stakeholders and society at large.

Total Leveraged Funding Summary for 2020

Federal Funding:	$	3,051,296
State/Local/Institutional:	$	50,000
Industry:	$	70,408
Total	**$**	**3,717,704**

PUBLICATIONS:
Refereed manuscripts and book chapters: 36

1. Salavati M, Caulton A, Clark R, Gazova I, Smith TPL. Worley KC, Cockett NE, Archablad AL, Clarke SM, Murdoch BM, Clark EL. on behalf of the Ovine FAANG Project Consortium. Global analysis of transcription start sites in the new ovine reference genome (Oar rambouillet v1.0). *Frontiers in Genetics* (2020).
2. Henslee D, Murdoch B, Yelich, Taylor BJ and Ellison M. Comparative genomics of sheep Tas2r repertoire in cattle, goat, human, dog and mice. *Animal Gene* (2020) Oct 22.
3. Davenport KM, Hiemke C, McKay SD, Thorne JW, Lewis RM, Taylor T, Murdoch BM. Genetic structure and admixture from terminal breeds in the United States. *Animal Genetics* (2020) Jan 23.
4. Becker GM, Job RJ, Davenport KM, Burke JM, Lewis RM, Miller JE, Morgan JL, Notter DR, and Murdoch BM. Genome-wide association study to identify loci associated with gastrointestinal nematode resistance in Katahdin. *Animal Genetics* (2020) Jan 3.
5. Lu, C., Yang, M., Rossi, R.M., Wang, A., Feitosa, W.B., Diaz, F. J., Liu, W.-S. (2020) Deletion of the mouse X-linked Prame gene causes germ cell reduction in spermatogenesis. *Mol. Reprod. Dev.* 87(8), 666-679. DOI: 10.1002/mrd.23324
6. Yao, Y., Zhang, Y., Liu, W.-S., Deng, X. (2020) Highly efficient synchronization of sheep skin fibroblasts at G2/M phase and isolation of sheep Y chromosomes by flow cytometric sorting. Scientific Reports 10, 9933. DOI:10.1038/s41598-020-66905-x
7. Chen, X., Zheng, Y., Lei, A., Zhang, H., Niu, H., Li, X., Zhang, P., Liao, M., Lv, Y., Zhu, Z., Pan, C., Dong, W., Chen, H., Wu, D., Liu, W.-S., Hamer, G., Zeng, S., Zeng, W. (2020) Early cleavage of preimplantation embryos is regulated by tRNAGln-TTG-derived small RNAs present in mature spermatozoa. J. Biol. Chem. 295(32), 10885-10900. DOI: 10.1074/jbc.RA120.013003.
8. Dechow, C.D., Liu, W.-S., Specht, L.W., Blackburn, H. (2020) Reconstitution and modernization of lost Holstein male lineages using samples from a gene bank. J. Dairy Sci. 103, 4510-4516. DOI:

https://doi.org/10.3168/jds.2019-17753. This article was selected by the Editor-in-Chief as the "Editor's Choice".
9. Baldwin, C.L., A. Yirsaw, A. Gillespie, L. LePage, F. Zhang, P. Damani-Yokota, and J.C. Telfer. 2020. γδ T cells in livestock: Responses to pathogens and vaccine potential. *Transboundary and Emerging Diseases* 67(Suppl2) 119-1128.
10. Gillespie, A.E., A. Yirsaw, S. Kim, K. Wilson, J. McLaughlin, M. Madigan, K. Loonie, E. Britton, F. Zhang, P. Damani-Yokota, K.P. Gunasekaran, J. Telfer, and C.L. Baldwin. 2020. Gene characterization and expression of the γδ T cell co-receptor WC1 in sheep. *Comparative and Developmental Immunology*, in press.
11. Yirsaw, A., A. Gillespie, F. Zhang, T.P.L. Smith, D Bickhart, K.P. Gunasekaran, M. Amir, H. Park, J. Telfer, and C.L. Baldwin. Defining the caprine γδ T cell WC1 multigenic array and evaluation of its expressed sequences and gene structure conservation among goat breeds and relative to cattle. BMC Genetics, in press.
12. Yirsaw, A.W., A. Gillespie, E. Britton, A. Doerle, L. Johnson, S. Marston, J. Telfer and C.L. Baldwin. Goat γδ T cell subpopulations defined by WC1 expression, responses to pathogens and cytokine production. *Comparative and Developmental Immunology*, in press.
13. Gillespie, A.E., A. Yirsaw, K.P. Gunasekaran, T.P. Smith, D. Bickhart, M. Turley, T. Connelley, J.C. Telfer and C.L. Baldwin. Submitted. Characterization of the domestic goat γδ T cell receptor gene loci and gene usage. Immunogenetics, submitted Nov 2020.
14. Massa, Alisha T, Mousel, Michelle R, Herndon, Maria K, Herndon, David R, Murdoch, Brenda M, White, Stephen N. Genome-Wide Histone Modifications and CTCF Enrichment Predict Gene Expression in Sheep Macrophages. *Frontiers in genetics*. 2021;11. doi:10.3389/fgene.2020.612031.
15. Cinar MU, Mousel MR, Herndon MK, Taylor JB, White SN. Association of *TMEM8B* and *SPAG8* with Mature Weight in Sheep. Animals (Basel). 2020 Dec 15;10(12):2391. doi: 10.3390/ani10122391.
16. Herndon MK, White SN, Mousel MR. Assay to compare cell- and antibody-mediated immune responses in domestic sheep and goats. Vet Immunol Immunopathol. 2020 Dec;230:110125. doi: 10.1016/j.vetimm.2020.110125.
17. Ueti MW, Johnson WC, Kappmeyer LS, Herndon DR, Mousel MR, Reif KE, Taus NS, Ifeonu OO, Silva JC, Suarez CE, Brayton KA. Comparative analysis of gene expression between *Babesia bovis* blood stages and kinetes allowed by improved genome annotation. Int J Parasitol. 2020 Oct 15:S0020-7519(20)30275-7. doi: 10.1016/j.ijpara.2020.08.006.
18. Çınar MU, Akyüz B, Arslan K, White SN, Neibergs HL, Gümüşsoy KS. The EDN2 rs110287192 gene polymorphism is associated with paratuberculosis susceptibility in multibreed cattle population. PLoS One. 2020 Sep 3;15(9):e0238631. doi: 10.1371/journal.pone.0238631.
19. Guzman, M.R., Howard, Z.P., Oliveira, R.D., Massa, A.T., Omsland, A., Oliveira, R.D., White, S.N., Goodman, A.G. Natural Genetic Variation in Drosophila Reveals Genes Associated with Susceptibility or Tolerance to Coxiella burnetii Infection. Genetics. (in press)
20. Shringi, S. O'Toole, D., Cole, E., Baker, K., White, S.N., Donofrio, G., Li, H., Cunha, C.W. OvHV-2 glycoprotein B delivered by a recombinant BoHV-4 is immunogenic and induces partial protection against sheep-associated malignant catarrhal fever in a rabbit model. Vaccines. (in press)
21. Miles, A.M.†* and Huson, H.J.*, (2020) Graduate student literature review: Understanding the genetics underlying mastitis. Journal of Dairy Science 6 November. https://doi.org/10.3168/jds.2020-18297
22. Posbergh, C.J.† and Huson, H.J.*, (2020) All Sheeps and Sizes: A Genetic Investigation of Mature Body Size across Sheep Breeds Reveals a Polygenic Nature. Animal Genetics 21 October. https://doi.org/10.1111/age.13016
23. Stambuk, C.R.†, Staiger, E.A.‡, Nazari-Ghadikolaei, A.†, Heins, B.J., Huson, H.J.*, (2020) Exploring physiological and genetic variation of digital cushion thickness of Holstein and Jersey cows and bulls. Journal of Dairy Science 23 July. https://doi.org/10.3168/jds.2020-18290

24. Miles, A.M.† and Huson, H.J.*, (2020) Time- and population dependent genetic patterns underlie bovine milk somatic cell count. Journal of Dairy Science 103(9): 1-13, 1 July. https://doi.org/10.3168/jds.2020-18322; Selected as "Editor's Choice" article in JDS.
25. Posbergh, C.J.†*, Staiger, E.A. ‡, Huson, H.J. *, (2020) A stop-gain mutation within MLPH is responsible for the lilac dilution observed in Jacob sheep. Genes 4 June, 11,618 https://doi.org/10.3390/genes11060618
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27. Stambuk, C.R.†, Staiger, E.A.‡, Nazari-Ghadikolaei, A.†, Heins, B.J., Huson, H.J.*, (2020) Phenotypic characterization and genome-wide association studies of digital cushion thickness in Holstein cows. Journal of Dairy Science Apr;103(4):3289-3303. Epub 2020 Feb 7. https://doi.org/10.3168/jds.2019-17409.
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29. Leal Yepes, F.A.†, Nydam, D.V., Mann, S., Caixeta, L., McArt, J.A.A., Overton, T.R., Wakshlag, J.J., Huson, H.J.*, (2019) Longitudinal phenotypes improve genotype association for hyperketonemia in dairy cattle. Animals Dec 1, 2019, 9(12), 1059, https://doi.org/10.3390/ani9121059
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31. Posbergh, C.J.†, Thonney, M.L., Huson, H.J.*, (2019) Genomic approaches identify novel gene associations with out of season lambing in sheep. Journal of Heredity, Volume 110, Issue 5, Pages 577–586, https://doi.org/10.1093/jhered/esz014
32. Leal-Gutiérrez J.D., M.A. Elzo, C. Carr, and R.G. Mateescu. 2020. RNA-seq analysis identifies cytoskeletal structural genes and pathways for meat quality in beef. PLOS One. 15(11): e0240895. doi:10.1371/journal.pone.0240895
33. Leal-Gutiérrez J.D., F.M.Rezende, J.M Reecy, L.M. Krammer, F. Peñagaricano and R.G. Mateescu. 2020. Whole genome sequence data provides novel insights into the genetic architecture of meat quality traits in beef. Frontiers in Genetics. doi: 10.3389/fgene.2020.538640
34. Sarlo Davila K.M., A. Howell, A. Nunez, A. Orelien, V. Roe, E. Rodriguez, S. Dikmen, and R.G. Mateescu. 2020. Genome-wide association study identifies variants associated with hair length in Brangus cattle. Animal Genetics. 51:811-814. doi: 10.1111/age.12970
35. Mateescu R.G., K.M. Sarlo Davila, S. Dikmen, E. Rodriguez, and P.A. Oltenacu. 2020. The effect of Brahman genes on body temperature plasticity of heifers on pasture under heat stress. J. Anim. Sci. 1:98(5):skaa126. DOI: 10.1093/jas/skaa126
36. Leal J.D., M.A. Elzo, and R.G. Mateescu. 2020. Identification of eQTLs and sQTLs associated with meat quality in beef. BMC Genomics. 21:104. doi.org/10.1186/s12864-020-6520-5

Published abstracts and proceedings: 20

1. Davenport KM, Massa AT, Mousel MR, Herndon MK, White SN, Bhattarai S, McKay S, Smith T.P.L, Carnahan JK, Cockett N, Murdoch BM. Hitting the Mark: Characterizing Four Histone Modifications in Ovine Liver and Spleen. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
2. Murdoch BM, White SN, Mousel MR, Massa A, Worley KC, Archibald AL, Clark EL, Dalrymple B, Kijas JW, Clarke SM, Brauning R, Smith TPL, Hadfield T, Cockett N. Update of the Ovine FAANG Update. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.

3. Becker GM, Davenport KM, Davis, AL, Duan AL, Eidman L, Schauer CS, Stewart WC, Murdoch BM. Identifying Novel Variants in Economically Important Genes through the Flock54 Low-Density Sheep Genome Panel. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
4. Thorne JW, Riley DG, Redden R, Murdoch BM, Waldron DF. Effect of the Booroola Mutation at BMPR1B on Range Lamb Production of Finewool Sheep. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
5. Henslee D, Murdoch BM, Yelich J, Taylor B, Ellison MJ. Comparative Genomics of Sheep Tas2r Genes to Cattle, Goat, Human, Dog and Mice. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11-15th, 2020.
6. Pablo J. Ross, Hamid Beiki, Angela Canovas, Sarah Corum, Clare Gill, Rui Hu, Honglin Jiang, Zhihua Jiang, Chandlar Kern, Colin Kern, Wansheng Liu, Pengcheng Lyu, Wenzhi Ma, Stephanie McKay, Juan Medrano, Jennifer J. Michal, Brenda Murdoch, James M. Reecy, Gonzalo Rincon, Monique Rijnkels, Tim P.L. Smith, Milton Thomas, Hongyang Wang, Xiaoqin X1, Xiaohui Zhang, Yunqi Zhang, Huaijun Zhou (2020) Functional Annotation of the Bovine Genome. Plant and Animal Genome Conference, FAANG Workshop, Jan 10, 2019. San Diego, CA. W474.
7. Hua, L.S., Ricon, G., Liu, W.-S. (2020) Sequence and Assembly of the Holstein Y Chromosome. Plant and Animal Genome Conference, Jan 11-15, 2019. San Diego, CA. PE0358.
8. Kern, C, and Liu, W.-S. (2020) Role of the Bovine PRAMEY Protein in Sperm Function. SSR, July 9-12, 2020. Virtual meeting.
9. Yang, Y., Oatley, J., and Liu, W.-S. (2020) Deletion of the *Pramel1* gene leads to germ cell reduction and subfertility in male mice. SSR, July 9-12, 2020. Virtual meeting.
10. Le Page, L., A. Gillespie, A. Yirsaw, C.L. Baldwin and J.C. Telfer. Genomic organization and expression of the swine WC1 multigenic array of hybrid coreceptor/PRR molecules. CRWAD virtual meeting Dec 5th, 2020 -July 1rst, 2021.
11. Yaser Ahmed, Hamid Ismail, Djafar Rehrah and Mulumebet Worku. 2020.Immunomodulatory effects of Gum Arabica in goat blood. Southern section of the American Society of animal sciences southern section meeting. submitted.
12. Yaser Ahmed, Hamid Ismail, Djafar Rehrah, and Mulumebet Worku. 2020.Effect of Gum Arabica (GA) drench on indicators of anemia in goats. Southern section of the American Society of animal sciences meeting submitted.
13. Sade Ford H Ismail M Worku.2020. Using NCBI PubMed to gather research articles about genes encoding ruminant galectins (LGALS 1,3,4, and 9). SNCURCS2020, 11/06,308.
14. Association of Nitrogen Permease Regulator-Like 3 and Enah/Vasp-Like genes with objective milk production traits in U.S. dairy sheep. 2020. M. K. Herndon, K. M. Hemmerling, T. W. Murphy, D. L. Thomas, S. N. White, and M. R. Mousel. Plant & Animal Genomes XXVIII, San Diego, CA, USA.
15. deCarvalho Balieiro J. C, J. D. Leal Gutierrez, C. Paschoal V.R., Carr, M.A. Elzo, and R.G. Mateescu. Comparative transcriptomic profile for meat tenderness in a multibreed Brahman-Angus Population. *66th International Congress of Meat Science and Technology.* (2020).
16. Sarlo Davila K., Howell A., Nunez A., Orelien A., Roe V. Rodriguez E., Dikmen S. and Mateescu R.G. *PRLR* and *PCCA* variants associated with hair length in Brangus heifers. *American Association of Animal Science Annual Meeting*. Virtual meeting (2020).
17. Mateescu R.G., Leal J.D., M.A. Elzo. Expression QTL mapping for meat quality in beef cattle. *American Association of Animal Science Annual Meeting*. Virtual meeting (2020).
18. Mateescu R.G., Sarlo Davila K., Dikmen S., Nunez A., Rodriguez E., and Oltenacu P.A. Phenotypic plasticity of heat tolerance in beef cattle. *American Association of Animal Science Annual Meeting*. Virtual meeting (2020).
19. Mateescu R.G., Leal J.D., M.A. Elzo. Integrated -omics approaches for meat quality improvement. *Plant and Animal Genome Meeting*, San Diego, CA. (2020).

20. Sarlo Davila K., Howell A., Nunez A., Orelien A., Roe V. Rezende F., Dikmen S. and Mateescu R.G. Genome-wide association study for hair length in Brangus heifers. *Plant and Animal Genome Meeting*, San Diego, CA. (2020).

Invited Seminars: (7)

1. Murdoch BM The functional annotation of the sheep and cattle genomes. Lecture in ANSC691 – Current Topics in Genomics, Instructor Luiz Brito Nov 10th, 2020.
2. Murdoch BM Genomic Testing for Sheep and Goats. University of Idaho Sheep Extension seminar series July 30, 2020.
3. Murdoch BM, White SN, Mousel MR, Massa A, Worley KC, Archibald AL, Clark EL, Dalrymple B, Kijas JW, Clarke SM, Brauning R, Smith TPL, Hadfield T, Cockett N. Update of the Ovine FAANG Update. The FAANG Workshop. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 10th, 2020.
4. Clark E, Salavati M, Caulton AJ, Clark R, Gazova I, Smith TPL, Worley KC, Cockett N, Archibald AL, Clarke SM, Murdoch B. The Ovine FAANG Project: A High-Resolution Atlas of Transcription Start Sites in the New Rambouillet Ovine Reference Genome. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 11th, 2020.
5. Murdoch BM, White SN, Mousel MR, Massa A, Worley KC, Archibald AL, Clark EL, Dalrymple B, Kijas JW, Clarke SM, Brauning R, Smith TPL, Hadfield T, Cockett N. Update of the Ovine FAANG Update. The International Sheep Genomics Consortium. The 28th International Plant and Animal Genome Conference San Diego Ca Jan 13th, 2020.
6. Davenport KM, Massa AT, Mousel MR, Herndon MK, White SN, Bhattarai S, McKay S, Smith T.P.L, Carnahan JK, Cockett N, Murdoch BM. Hitting the Mark: Characterizing Four Histone Modifications in Ovine Liver and Spleen. The 28th International Plant and Animal Genome Conference. San Diego Ca Jan 14th, 2020.
7. Worku, M. "Management and Selection for Goat Production held by the Faculty of Animal Science Universitas Gadjah Mada Indonesia on July 18, 2020.

Popular Press Publications:

1. *Impact of Brahman genetics on body temperature of heifers under heat stress.* R.G. Mateescu. Frontline Beef Producer. Fall 2020, Vol. 12, Issue. 2, pg. 61-62.
2. *Genomic heterosis estimation in crossbred cattle.* R.G. Mateescu. The Florida Cattleman & Livestock Journal. Nov 2020, Vol. 85, No. 2, pg. 86-87.
3. *The effect of Brahman genes on body temperature of heifers on pasture under heat stress.* Kaitlyn Sarlo Davila, Serdal Dikmen, and R.G. Mateescu. The Florida Cattleman & Livestock Journal. July 2020, Vol. 84, No. 10, pg. 84-85.
4. *Hot or not: understanding what makes a thermotolerant heifer.* Kaitlyn Sarlo Davila, Serdal Dikmen, Peter Hansen and R.G. Mateescu. The Florida Cattleman & Livestock Journal. April 2020, Vol. 84, No. 7, pg. 50-51.
5. *Genomic research on Brahman-influenced cattle presented during Plant and Animal Genome Conference.* R.G. Mateescu and Kaitlyn Sarlo Davila. The Florida Cattleman & Livestock Journal. March 2020, Vol. 84, No. 6, pg. 48-49.
6. *Using genomics to beef up meat quality in Brahman influenced cattle.* R.G. Mateescu. The Florida Cattleman & Livestock Journal. February 2020, Vol. 84, No. 5, pg. 40-44.

Presentations and Other Creative Activities:

1. Graduate student trainee Davenport DM, 3MT was "Characterizing Genetic Regulatory Elements in Sheep". The University of Idaho competition was February 7th and the statewide competition was February 19th, 2020.
2. Graduate student trainee Becker G, 3MT "Gaining a Better Understanding of Parasite Resistance in Sheep." The University of Idaho competition was February 7th and the statewide competition was

February 19th, 2020.
3. Graduate student trainee Jacob Thorne, Western Section Animal Science 3MT, July 19, 2020 awarded first place.
4. Graduate student trainee, Jacob Thorne, American sheep industry - sheep heritage scholarship 2020 awardee.

Graduate Student Education: In total five 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.

Active Grants and Contract Leveraged Through NRSP08 Research:
1. 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.
2. USDA ARS Host Genomics (2020) USDA PI: B Murdoch Awarded $156, 360.
3. 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.
4. 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.
5. RNA methylation as a mechanistic link between genotype and phenotype (2020) AFRI NIFA PI: S McKay, Co PI: B Murdoch. Awarded $200,000.
6. Flock54℠ a new genomic selection tool that enhances the U.S. sheep industry. (2020) IGEM PI: Murdoch B. Awarded $251,500.
7. 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.
8. 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.
9. Genomes to phenomes of cattle and sheep in Idaho. (2019) National Animal Genome Research Program – NRSP008 - Hatch Grant PI: Murdoch B.
10. Implementation of genetic selection technologies on Texas sheep ranches. (2019) Extension Enhancement Scholarship. PI: Redden R, Co-PI: Thorne J, Collaborators: Railey D, Murdoch B.
11. Ovine FAANG project. (2017-2020) AFRI NIFA, PI: Murdoch B, Co-PI's: Cockett N, Worley K, White S, Archibald A, Dalrymple B, Clarke S., Kijas J. and Brauning R. Awarded $500,000.
12. Understanding parasite resistance in organic livestock and using a systems approach for control. (2016 -2020) Organic Agriculture Research and Extension (OREI), NIFA PI: Joan Burke, Co–PI's: Bowdridge S., Coffey L., Lewis R., Terrill T. and Murdoch B. Awarded $1,991,149.00.
13. USDA, APHIS grant: Identifying genomic regions associated with chronic wasting disease in elk: A foundation for understanding an endemic disease; $60,147 September 2020 to September 2021

WORK PLANNED FOR NEXT YEAR:
1. Cattle FAANG project to finish transcriptional annotation of all tissues/cells studied and to integrate data generated from other assays, such as ATTS-seq, ChIP-seq, ATAC-seq, RAMPAGE and WGBS-seq, to identify and map all the functional and regulatory elements in the bovine genome.
2. Determine the function of the bovine PRAMEY protein during acrosome formation, sperm function and sperm-egg binding.
3. Continue to work on the ruminant sex (particularly) Y chromosome sequence, annotation, and function, and to finish the Holstein Y analysis.

4. Determine the functional role of PRAME during spermatogenesis by characterization of the mutant mice.
5. We intend to refine and correct current gaps in the reference assemblies and work towards a genome to phenome understanding of how WC1 expression from, and allelic variation in, conserved multigenic arrays in livestock species affects the immune response to important pathogens.
6. Continued work on identification of causal mutations underlying M. ovipneumoniae and entropion QTL.
7. Collaboration with Development of the Ovine Pangenome.

NRSP-8 Poultry Annual Report October 1, 2019 – September 30, 2020

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

The NRSP-8 Poultry Workshop held February 22-23, 2021 in conjunction with NC1170 Poultry Workshop at the virtual meeting and attendance overview: Attendance during the 2 x 0.5 day workshop averaged n=65 with peak attendance in excess of 90.

- Two keynote speakers presented poultry genetics, epigenetics, and genomics.
- Representatives of 16 agricultural experiment stations attended from across the US including the membership of NRSP-8 Poultry group: Iowa State, Michigan State, University of Arizona, University of Arkansas, Western University of Health Sciences, Mississippi State University, Univ of Delaware, Univ of Georgia, University of California Davis, University of Minnesota, Beckman Research Institute.
- Attendees also included members of the poultry layer and broiler breeding companies, and scientists from the United Kingdom, Germany, Canada, Sweden, Netherlands, Bangladesh, Australia and China.

Grants

University of Arkansas:

- Empowering US broiler production for transformation and sustainability; USDA-NIFA Sustainable Agriculture Systems; 9/2019 - 8/2024; $9,919,300; PD Bottje CoPIs Dridi, Rochell, Hargis, Erf, Kong, Kidd, Kuenzel, Owens, Kwon, Sun, Rhoads, Alrubaye, Tabler (MS), and others.

Douglas Rhoads, Univ of Arkansas:

Continuing

- Validation of a SNP panel for breeding against ascites in broilers. NIFA-AFRI; 3/2018-2/2021; $500,000; Rhoads (PI), Orolowski (CoI)
- Double-stranded RNA and bone attrition in BCO chickens: a translational model for human osteomyelitis. University of Arkansas Chancellor Innovation; 7/2019-6/2021; $90,000; S. Dridi (PI), Rhoads (coI).

New

- Biomin DON and FUM in feed; Biomin Corporation; 3/2020-8/2020; $35,000; coPI; Alrubaye (PI)
- 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. Alrubaye, A. (PI); Rhoads
- High Resolution Mapping of Genetic Determinants for Resistance to Infectious Bronchitis Virus in Broilers. Arkansas Biosciences Institute; 7/2020-6/2021; $74,521; Arkansas: Rhoads (PI), Alrubaye, Dridi, Pummill; Auburn: Toro coPI)

Wayne Kuenzel, Univ of Arkansas:

- Placement of 2D and 3D neuroanatomical visual data into a virtual environment to facilitate research and learning. Chancellor's Innovation & Collaboration Fund, 8/01/2020 – 7/31/2022, $84,661; PI: W. Kuenzel, Co-PIs: D.C. Fredrick, P.M. Gignac.
- A newly discovered function of a septal brain structure in poultry. Arkansas Biosciences Institute (ABI) Grant, 7/01/2020 – 6/30/2021, $25,000; PI: W. Kuenzel, Co-PI: A. Jurkevich

Byungwhi Kong, Univ of Arkansas:

- Gene editing and transgenic poultry production. Chancellor's Discovery, Creativity, Innovation, and Collaboration Fund. 7/01/2019-6/30/2021; $115,665; PI: B. Kong CoPI: W. Kuenzel.
- Determination of roles of mitochondrial small RNAs in metabolic disease phenotypes using isocitrate dehydrogenase 2 (IDH2) knock out mouse and genetically selected chicken models. Arkansas Bioscience Institute. 7/2017-6/2020; $148,500; PI: B. Kong.
- Plasma biomarker discoveries for early diagnosis of woody breast in broilers. Adisseo; 03/01/2020 – 2/28/2021; $50,400; PI: BW Kong Co-PIs: W. Kuenzel, C. Owens

Sami Dridi, Univ of Arkansas:

- Systems-based integrated program for enhancing the sustainability of antibiotic-restricted poultry production. USDA-NIFA Sustainable Agriculture Systems; 2020-2025; $10,000,000.00; PD: Kumar Venkitanarayanan, Co-PD: S. Dridi

Huaijun Zhou, University of California, Davis:

- NIFA: The project titled "Functional Annotation of the Bovine Genome" H. Zhou (PI), P. Ross, J. Mendaro et al. 1/15/2018-1/14/2022. $2,500,000
- USDA-NIFA, Leveraging High-Throughput Phenotypes and Biological Information into Genome-enabled Analysis and Prediction. H. Cheng (PI), H. Zhou (co-PI) 1/1/2021 – 12/31/2023 $446,473
- 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. 1/15/2018-1/14/2022 $2,500,000
- USDA NIFA High-Quality Reference Assembly And Annotation Of The Rainbow Trout Genome. Salem M. (PI) H. Zhou, 6/1/2019-5/31/2022 $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. $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 and Animal Health funds to Iowa State University

Marcia Miller; Beckman Research Institute, CA:

- MHC-Y-Directed Immune Responses during colonization of Chickens by Campylobacter; USDA NIFA; Grant No. 2016-10247; 06/01/2017-05/31/2020; $387,518.00.

Kent Reed, Univ of Minnesota:
- USDA National needs fellowship for enhancing animal production: Addressing national need in poultry production; USDA-NIFA-NNF; 2016-2021; $241,000; PI: Reed.
- Turkey breast muscle development and meat quality: The biological response to thermal challenge in production birds. USDA-NIFA, 2020-2024, co-Project Director w/ Strasburg (PD), Jones, and Velleman.

Hans. H. Cheng, USDA-ARS:
- 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.
- 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.

Giridhar Athrey, Texas AgriLife Research (Texas Ag. Experimental Station)
- USDA National Institute of Food & Agriculture. Genomic and Functional Basis of Wooden Breast in BroilersPI: Athrey, Funded $499,986(2019-2022)
- USDA National Institute of Food & Agriculture. Probiotic modulation of appetite to improve breeder pullet welfare. PI: Walzem, Funded $499,4338(2019-2023)
- USDA Multistate Hatch Equipment Grant. Funded $70,686.96–Used towards purchase of Agilent TapeStation 4200 and So-Low Ultra Freezer.
- Department of Defense. Assessment of Golden-cheeked Warbler population structure and genetic recovery. PI: Athrey, Funded 104,900 (2019-2022)

Rosemary L. Walzem, Texas A & M University (TAMU)
- Developing Primordial Germ Cell Techniques for Germline Transfer. USDA-TAMU Multi-State Project, Walzem (PI)

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 2019-20 research. Many of the efforts are focused on projects that directly impact poultry health and production.

- Validated two genes and demonstrated that we can breed for resistance to ascites without reducing production traits (manuscript in preparation).
- We have been using the existing assemblies for high resolution (10 kb regions) QTL mapping through whole genome resequencing.
- We have been improving the analysis pipeline for individual DNAs and identification through Minor Allele Frequency.
- We have used the pipeline to map regions for ascites in 3 commercial lines and 11 regions for bacterial resistance. We are working with primary breeding companies to validate these regions and to expand the analysis to commercial products. Validation of these regions for commercial breeding stocks will allow significant improvements in resistance to two diseases that cost the industry >$100 million annually.
- Utilizing MAS, resistant associated alleles were found that protected a developed line of broilers from displaying ascites when exposed to a hypobaric chamber challenge compared to controls.
- New molecular markers for heat stress, one extracted from feathers (HSP70) and a second known as 75 kDa glucose regulated protein (GRP75) appear to be effective markers monitoring heat stress in poultry.
- We have identified a sequence of activation of key neurons in the avian forebrain, cell types in the anterior pituitary and their receptors responsible for releasing the stress hormone, corticosterone, from the adrenal gland that could be useful to address stress problem occurring in chickens.
- Our recent work with BCO suggests that a single amino acid residue in a nucleotide recycling protein may be responsible for contributing to lameness in broilers.
- High plasma levels of CORT, changes in glucocorticoid receptors and several metabolite and fatty acid signatures are involved in woody breast myopathy.
- Continue to investigate the causes, epidemiology, transmission, and prevention of Bacterial Chondronecrosis with Osteomyelitis BCO lameness in broiler chickens. We have worked with several industry partners to try to identify feed additives that can help reduce the incidence of BCO lameness in broilers. BCO lameness is the main animal welfare issue facing the poultry industry in the US.
- Genetic variation was characterized in commercial, research and indigenous lines of chickens.
- Genes, pathways and genomic regions associated with important biological traits in chickens were identified.
- Defining MHC-Y function and a means for typing individual birds. We have demonstrated a correlation between MHC-Y genotype and immune response. This implies that genetic selection at MHC-Y might be valuable in tailoring disease resistance in chickens raised for meat and eggs.
- First showing the expression of LPL from the vascular endothelium in chickens. Our study also confirms the existence of slow myofiber-phenotype and provides mechanistic insights into increased lipid uptake and metabolism in Wooden Breast disease process. We also found intriguing parallels between myopathy of broilers and metabolic syndrome in humans, suggesting chickens as a potential model for studying complications caused by diabetes in humans
- We identified biomarkers of Wooden Breast severity and development. Additionally, gene expression analysis and ultrastructural evaluations provided evidence of vascular endothelial cell dysfunction in the early pathogenesis of Wooden Breast.
- We developed a new tool (VADT; https://github.com/mjtiv/VADT) for the detection of allele-specific expression (ASE) from RNA-seq data. What makes VADT especially unique is that the program implements two different statistical methods for ASE detection: (i) Detection of ASE variants across multiple samples: a meta-analysis to identify significant ASE variants across multiple samples; (ii) Detection of ASE samples among multiple samples: a multi-dimensional p-value adjustment to identify ASE samples among multiple samples.

- The entire process is streamlined into one python script that can easily be run on an HPC system using a parameter file or through the HPC environment.
- Ikaros is the first MD driver gene identified and validated.
- Advancement in understanding the underlying genetic and epigenetic factors that modulate vaccine efficacy would empower knowledge-based design of more potent vaccines, which, thereby, would lead to significant improvement on disease control.
- Functional annotation of genome will be important for animal genome community for identifying genetic variants for economically important traits, therefore in improving animal production efficiency and food security.
- The project in collaborating with ISU colleagues in the 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.
- 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.
- We identified structural variants that are associated with wooden breast myopathy in broilers using a combination of novel genomics approaches. In addition, we also developed a new scalable assay to screen large populations for important candidate structural variants. The development of these assays can be valuable for rapid detection of wooden breast in breeder populations, and may help ameliorate this commercially important condition.
- We were the first to report the photoperiodic modulation of microbiota assembly in chicken in 2019. Since then we have built on this platform to investigate the mechanisms driving this feature. Secondly, given the fundamental discovery andits potential use for microbiota engineering in poultry, we are investigating the different downstream pathways that benefit from circadian signaling, including the modulation of immune response following vaccination.
- Genetic methods used to modify mammals do not work for a major branch on the tree of life: birds. The tremendous insight into mammalian biology, including human health, arising from genetic modification of animal models such as mice persuades that great benefit would develop if methods for genetic modification of birds were readily available. Birds are crucial contributors to ecosystems, the source of basic biological insights; have many utilities including food, vaccine production, and recreation. Global, methods to manipulate primordial germline cells (PGCs) in birds and transfer them to host embryonic gonads prior to incubation coupled with education and training opportunities for students and scientists will serve to lower the entrance bar for use of gene modification technologies in avians for biologic discovery.

2019-2020 Poultry Genome Committee publications (refereed journal articles)

1. Alrubaye, A., N. S. Ekesi, A. Hasan, D. A. Koltes, R. Wideman Jr and D. Rhoads (2020). Chondronecrosis with osteomyelitis in broilers: Further defining a bacterial challenge model using standard litter flooring and protection with probiotics. Poultry Science 99 (12): 6474-6480.
2. Alrubaye, A. A. K., N. S. Ekesi, A. Hasan, E. Elkins, S. Ojha, S. Zaki, S. Dridi, R. F. Wideman, M. A. Rebollo and D. D. Rhoads (2020). Chondronecrosis with Osteomyelitis in Broilers: Further Defining Lameness-Inducing Models with Wire or Litter Flooring, to Evaluate Protection with Organic Trace Minerals. Poultry Science 99(11): 5422-5429.
3. Baxter M.F.A., Greene E.S., Kidd M.T., Tellez-Isaias G., Orlowski S., Dridi S (2020). Water amino acid-chelated trace mineral supplementation decreases circulating and intestinal HSP70 and proinflammatory cytokine gene expression in heat-stressed broiler chickens. J Anim Sci. Mar 1;98(3). pii: skaa049. doi: 10.1093/jas/skaa049.

4. Cauble R., Greene e., Orlowski S., Walk C., Bedford M., Apple J., Kidd M., Dridi S (2020). Research Note: Dietary phytase reduces broiler woody breast severity via potential modulation of breast muscle fatty acid profiles. Poult Sci 99:4009-4015.
5. Dhamad A.E., Greene E., Sales M., Nguyen P., Beer L., Liyanage R., Dridi S (2020). 75-kDa glucose-regulated protein (GRP75) is a novel molecular signature for heat stress response in avian species. Am J Physiol Cell Physiol. 2020 Feb 1;318(2):C289-C303.
6. Ferver A., Dridi S (2020). Regulation of avian uncoupling protein (av-UCP) expression by cytokines and hormonal signals in quail myoblast cells. Comp Biochem Physiol A Mol Integr Physiol. DOI: 10.1016/j.cbpa.2020.110747
7. Flees J., Greene E., Ganguly B., Dridi S (2020). Phytogenic feed- and water-additives improve feed efficiency in broilers via modulation of (an)orexigenic hypothalamic neuropeptide expression. Neuropeptides. 2020 Jan 3:102005. doi: 10.1016/j.npep.2020.102005. [Epub ahead of print]
8. Greene E., Cauble R., Dhamad A., Kidd M., Kong B., Howard S., Castro H., Campagna S., Bedford M., Dridi S (2020). Muscle Metabolome Profiles in Woody Breast-(un)Affected Broilers: Effects of Quantum Blue Phytase-Enriched Diet. Front Vet Sci 2020 (https://doi.org/10.3389/fvets.2020.00458)
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10. Greene E.S., Zampiga M., Sirri F., Ohkubo T., Dridi S (2020). Orexin system is expressed in avian liver and regulates hepatic lipogenesis via ERK1/2 activation. Sci Rep 2020 Nov 5;10(1):19191. doi: 10.1038/s41598-020-76329-2.
11. Kadhim, H.K., M. Kidd Jr., S.W. Kang and W.J. Kuenzel. (2020). Differential delayed responses of arginine vasotocin and its receptors in septo-hypothalamic brain structures and anterior pituitary that sustain hypothalamic-pituitary-adrenal (HPA) axis functions during acute stress. Gen. Comp. Endocrinol. 286:113302. doi.org/10.1016/j.ygcen.2019.113302.
12. Kang, S.W., K.D. Christensen, D. Aldridge and W.J. Kuenzel. (2020). Effects of light intensity and dual light intensity choice on plasma corticosterone, central serotonergic and dopaminergic activities in birds, Gallus gallus. Gen. Comp. Endocrinol. 285: 113289. doi.org/10.1016/j.ygcen.2019.113289.
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Presentations

1. Alrubaye, A. (invited speaker), Rhoads, D. How feed additives influence lameness, Poultry Science Association, July 2020
2. Alrubaye, A. (invited speaker), Rhoads, D. Benefits of ZPM on Lameness in Broilers, International poultry webinar Sep 2020.
3. Alrubaye, A. (invited speaker). Recent Findings on BCO Lameness, Zinpro International Conference, Chicago, August 2019.
4. Alrubaye, A.A. (invited speaker). Reduction of the presentation of lameness in broilers, Mexico, August 2019.
5. Alrubaye, A., Rhoads, D. Staphylococcus agnetis as a Model Microorganism to Induce Bacterial Chondronecrosis with Osteomyelitis in Broilers. The American Society for Microbiology, June 2020. Rhoads D, A. Alrubaye, A Shwani, NS Ekesi, A Hasan. Bacterial chondronecrosis with osteomyelitis in broilers: pathogen genomics, and management strategies. Conference of Research Workers in Animal Diseases, virtual, November 2020.
6. Rhoads DD, K Lee, S Orlowski. Validation of a SNP panel for selection for ascites resistance in broilers. Conference of Research Workers in Animal Diseases, virtual, November 2020.
7. Rhoads D, A Alrubaye A System for Evaluating Strategies to Reduce Bacterial Lameness in Broiler Chickens. Annual Delmarva Poultry Federation Meeting, virtual, Sepember 2020.
8. Ö. Arda, D D Rhoads, M. Ö Özyigit. Investigation of Vacular Endothelial Growth Factor (VEGF) Activity in Ascites Syndrome of Meat Type Chicken. 10th National and 1st International Veterinary Pathology Congress (VETPAT-2020), October 2020. (Collaboration with University I Turkey)
9. Hasan A, DD Rhoads, A Alrubaye. Role of Gut Integrity and Cellular Tight Junctions in Lameness of Broiler Chickens. Plant and Animal Genome XXVII Conference, San Diego, CA, January 2020
10. Almansaf D, S Dey, A Parveen, D Rhoads. Molecular Analysis of the CPQ Gene, a Quantitative Locus for Pulmonary Hypertension in Broilers. Plant and Animal Genome XXVII Conference, San Diego, CA, January 2020
11. Ekesi NS, AA Alrubaye, A Hasan, A Parveen, RF Wideman, DD Rhoads. Genomic, and Virulence Comparisons of Different Bacterial Isolates from BCO Lesions in Broilers. Plant and Animal Genome XXVII Conference, San Diego, CA, January 2020
12. Al-Mitib L, K Al-Zahrani, K Lee, NB Anthony, DD Rhoads. Further evaluation of differences in mitochondrial biogenesis in broiler muscles that correlate with ascites susceptibility. Plant and Animal Genome XXVII Conference, San Diego, CA, January 2020
13. Rhoads D, E Sheikhsamani, A Parveen, K Lee, K Tarrant, S Dey, N Anthony. Genetics of Ascites Revealed by Whole Genome Resequencing in Two Distinct Broiler Lines. Plant and Animal Genome XXVII Conference, San Diego, CA, January 2020

14. Hasan A, N Ekesi, A Alrubaye, D Rhoads. Investigation of Gut Integrity and Cellular Tight Junctions in Stressed Broiler Chickens. Plant and Animal Genome XXVII Conference, San Diego, CA, January 2020
15. Shwani A, S Zaki, S Lenaduwe, S Ojha, D Rhoads. Directed Genome Evolution Identifies Deoxyribose Phosphate Aldolase as a Macrophage Survival Factor in Staphylococcus agnetis. Plant and Animal Genome XXVII Conference, San Diego, CA, January 2020

Swine NRSP-8 Genome Committee Report
January 1, 2020 – December 31, 2020

2020 Chair (for 2021 Workshop): Joan Lunney, Animal Parasitic Diseases Laboratory, BARC, ARS, USDA, Beltsville, MD, USA, joan.lunney@usda.gov

2020 Chair-elect: Wen Huang, Michigan State University, East Lansing, MI, USA, huangw53@msu.edu

The 2021 NRSP-8 Swine Workshop was held online through Zoom on January 21, 2021. The workshop included presentations from 17 scientists at different career stages (PIs, postdocs, and graduate students) from five universities and two USDA ARS laboratories. Approximately 35 participants attended the workshop. The research presentations represent a wide range of topics performed at participating stations, including gut and vaginal microbiomes, genetics of disease resilience, functional genomics of the porcine genome, gene editing, genomic selection, and development of tools. In addition, PIs provided updates projects previously supported by the NRSP-8 swine genome coordinator funds.

Following the research presentations, Max Rothschild from Iowa State University provided an update on the Visions III conference, which will take place in November of 2021. Chris Tuggle, Swine NRSP-8 Co-coordinator, led discussion on NRSP-8 progress and future plans, as well as ways to stimulate collaboration supported by the NRSP-8 fund. Jack Dekkers provided an update on AG2PI (seed grants and workshops) and how this new initiative may offer new opportunities for NRSP-8 investigators.

Finally, Nick Serão, Iowa State University, was elected as the chair-elect for 2021. 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 UNL, candidate genes and SNPs located in the QTL region for fertility related traits identified by GWAS, genome and RNA sequencing could explain some of the observed phenotypic variation. Following validation, some of these polymorphisms could be used in selection to improve sow fertility. This information was shared with industry to assist them in genomic selection applications.
2. The project conducted at MSU 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. NCSU scientists have identified genomic regions and candidate genes associated with growth and carcass parameters across populations. They have identified genes related to heat stress mechanisms and heat tolerance. They have obtained estimates of GxE for heat tolerance for fertility in diverse breeds. They have identified microbial features related to growth, carcass composition and feed efficiency and implemented methods to investigate causality and pinpoint the mediated effect of microbiome on growth carcass composition and feed efficiency.

4. At ISU, additional sequence information and annotation of this sequence in the pig will help inform agricultural and biomedical researchers for future research. Many lines of research were initiated and/or ongoing including the use of new approaches to predict breed from pigs without pedigree information; mapping QTLs, and importantly candidate genes, for meat quality, feed efficiency, response to PRRSV and PEDV infections. Antibody response to vaccination to PRRSV in crossbred commercial gilts is highly heritable, controlled by the MHC, and highly genetically correlated with subsequent farrowing performance, providing a novel and efficient tool for genetic selection. The vaginal microbiome of gilts is heritable, with several bacteria controlled by a locus on SSC12, and is predictive of reproductive performance. Water intake duration and number of visits are potential indicator traits to select for disease resilience because of their high heritability and had moderate genetic correlations with treatment rate and mortality. Vulva size traits in pigs are highly heritable and predictive of reproductive performance. Natural genetic variants in CD163, CD169, and RGS16 could be used to select for resistance to PRRSV and/or PRRSV-PCV2b co-infection and appear to interact with the resistance QTL in the *GBP5* gene. The first chromatin state map of alveolar macrophages, demonstrating the role of histone modifications, especially H3K27ac, in response to bacterial and viral mimics has been published and now can be used to better analyze SNPs associated with viral diseases involving macrophage function. Bulk RNAseq analyses of sorted PBMC and single cell RNA sequencing have identified cell types as well as revealed potential, previously unidentified cell populations. Use of random forest and regression approaches and a limited number of SNPs are useful to predict breed from pigs without pedigree information.

5. USDA BARC scientists are collaborating on mapping the SLA complex genes, a detailed map of which will facilitate efforts to affirm the importance of SLA alleles in antigen presentation and immune and disease responses, as well as for vaccine design and swine biomedical models. Fetal outcome in response to pregnant gilt PRRSV infection is determined by both fetal and placental responses but is initiated only after fetal infection, revealing complex mechanisms underpinning fetal PRRS susceptibility. 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. Importantly, they continued to use samples collected and stored through the PRRS host genetics consortium (PHGC) and use data accumulated in the PHGC database to facilitate phenotype/genotype association analyses.

2020 Swine Genome Committee Publications (published refereed journal articles only)

1. Bai, X., Putz, A.M., Wang, Z., Fortin, F., Harding, J., Dyck, M.K., Dekkers, J., Field, C.J. and Plastow, G.S. (2020) Exploring Phenotypes for Disease Resilience in Pigs Using Complete Blood Count Data From a Natural Disease Challenge Model. Frontiers in genetics, 11, p.216.
2. Bergamaschi M, Christian Maltecca, Constantino Schillebeeckx, Nathan P McNulty, Clint Schwab, Caleb Shull, Justin Fix, Francesco Tiezzi. (2020) Heritability and genome-wide association of swine gut microbiome features with growth and fatness parameters. Scientific Reports.
3. Bergamaschi M, C Maltecca, J Fix, C Schwab, F Tiezzi. (2020) Genome-wide association study for carcass quality traits and growth in purebred and crossbred pigs. Journal of Animal Science 98 (1).
4. Bergamaschi, M, Francesco Tiezzi, Jeremy Howard, Yi Jian Huang, Kent A. Gray, Constantino Schillebeeckx, Nathan P. McNulty & Christian Maltecca. Gut microbiome composition differences among breeds impact feed efficiency in swine. 2020. Microbiome
5. Byrne K., C.K. Tuggle, C. L. Loving. (2020) Differential induction of innate memory in porcine monocytes by beta-glucan or bacillus Calmette-Guerin. Innate Immunity, 2020 Aug 30;1753425920951607. doi: 10.1177/1753425920951607.
6. Chen, C., Zhu, W., Steibel, J., Siegford, J., Han, J., & Norton, T. (2020) Recognition of feeding behaviour of pigs and determination of feeding time of each pig by a video-based deep learning method. Computers and Electronics in Agriculture, 176, 105642.
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10. Cheng, J., Putz, A.M., Harding, J.C., Dyck, M.K., Fortin, F., Plastow, G.S., Canada, P. and Dekkers, J.C., (2020) Genetic analysis of disease resilience in wean-to-finish pigs from a natural disease challenge model. Journal of Animal Science, 98(8), p.skaa244.
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12. Corredor FA, LP Sanglard, RJ Leach, JW Ross, AF Keating, NVL Serão (2020) Genetic and genomic characterization of vulva size traits in Yorkshire and Landrace gilts. BMC Genetics, 21:28.
13. Dawson HD, Sang Y, Lunney JK. (2020) Porcine cytokines, chemokines and growth factors: 2019 update. Research in Veterinary Science. 131: 266-300. https://doi.org/10.1016/j.rvsc.2020.04.022
14. Esfandyari, H., Thekkoot, D., Kemp, R., Plastow, G. and Dekkers, J., (2020) Genetic parameters and purebred–crossbred genetic correlations for growth, meat quality, and carcass traits in pigs. Journal of Animal Science, 98(12), p.skaa379.
15. Hammer SE, Ho C-S, Ando A, Rogel-Gaillard C, Charles M, Tector M, Tector AJ, Lunney JK. (2020) Importance of the porcine MHC (SLA) complex in swine health and biomedical research. Annual Review of Animal Biosciences. 8: 171-198. doi: 10.1146/annurev-animal-020518-115014.
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17. He Y, F. Tiezzi, J. Howard, C. Maltecca. (2020) Predicting bodyweight in growing pigs from feeding behavior data using machine learning algorithms. Computers and Electronics in Agriculture.
18. Herrera-Uribe J., H. Liu, K.A. Byrne, Z.F. Bond, C.L. Loving, C.K. Tuggle. (2020) Changes in H3K27ac at Gene Regulatory Regions in Porcine Alveolar Macrophages Following LPS or PolyIC Exposure. Frontiers in Genetics (Epigenomics and Epigenetics). https://doi.org/10.3389/fgene.2020.00817.
19. Hewitt, D.J., Dekkers, J.C., Antonick, T., Gheisari, A., Rakhshandeh, A.R. and Rakhshandeh, A., (2020) Effects of divergent selection for residual feed intake on nitrogen metabolism and lysine utilization in growing pigs. Journal of Animal Science, 98(5), p.skaa152.
20. Khanal P, C Maltecca, C Schwab, J Fix, M Bergamaschi, F Tiezzi. (2020) Modeling host-microbiome interactions for the prediction of meat quality and carcass composition traits in swine. Genetics Selection Evolution 52 (1), 1-13
21. Khanal P, Christian Maltecca, Clint Schwab, Justin Fix, Francesco Tiezzi. (2020) Microbiability of meat quality and carcass composition traits in swine. Microbiability of meat quality and carcass composition traits in swine. Journal of Animal Breeding and Genetics.
22. Liu J, Tan S, Huang S, Huang W (2020) ASlive: a database for alternative splicing atlas in livestock animals. BMC Genomics 21(1):97
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24. Maltecca C, M Bergamaschi, F Tiezzi. (2020) The interaction between microbiome and pig efficiency: A review Journal of Animal Breeding and Genetics
25. Maltecca C, F Tiezzi, JB Cole, C Baes. (2020) Symposium review: Exploiting homozygosity in the era of genomics—Selection, inbreeding, and mating programs. Journal of Dairy Science.

26. Quan J, Wu Z, Ye Y, Peng L, Wu J, Ruan D, Qiu Y, Ding R, Wang X, Zheng E, Cai G, Huang W, Yang J (2020) Metagenomic characterization of intestinal regions in pigs with contrasting feed efficiency. Front Microbiol 11:32
27. Rauw, W.M., Rydhmer, L., Kyriazakis, I., Øverland, M., Gilbert, H., Dekkers, J.C., Hermesch, S., Bouquet, A., Gómez Izquierdo, E., Louveau, I. and Gomez-Raya, L., (2020) Prospects for sustainability of pig production in relation to climate change and novel feed resources. Journal of the Science of Food and Agriculture.
28. Sanglard LP, RL Fernando, KA Gray, DCL Linhares, JCM Dekkers, MC Niederwerder, NVL Serão (2020) Genetic analysis of antibody response to Porcine Reproductive and Respiratory Syndrome vaccination as an indicator trait for reproductive performance in commercial sows. Frontiers in Genetics, 11:1011.
29. Sanglard LP, PigGen Canada, BE Mote, P Willson, JCS Harding, GS Plastow, JCM Dekkers, NVL Serão (2020) Genomic analysis of IgG antibody response to common pathogens in commercial sows in health- challenged herds. Frontiers in Genetics, 11:593804.
30. Sanglard LP, S Schmitz-Esser, KA Gray, DCL Linhares, CJ Yeoman, JCM Dekkers, MC Niederwerder, NVL Serão (2020) Investigating the relationship between vaginal microbiome and host-genetics and their impact on immune response and farrowing traits in commercial gilts. Journal of Animal Breeding and Genetics, 137(1):84-102.
31. Sanglard LP, S Schmitz-Esser, KA Gray, DCL Linhares, CJ Yeoman, JCM Dekkers, MC Niederwerder, NVL Serão (2020) Vaginal microbiota diverges in sows with low and high reproductive performance after porcine reproductive and respiratory syndrome vaccination. Scientific Reports, 10:3046.
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34. Van Goor A, Walker K, Pasternak A, Malgarin C, MacPhee DJ, Harding JCS, Lunney JK. (2020) Fetal Controlled Immune Response to PRRS virus reveals Placenta Initiates Fetal Demise while Dysregulation in Fetal Thymus is indicative of Viral Load. BMC Genomics. 21:763 https://doi.org/10.1186/s12864-020-07154-0
35. Warr, A., N. Affara, B. Aken, H. Beiki and 36 other authors including C.K. Tuggle. (2020) An improved pig reference genome sequence to enable pig genetics and genomics research. GigaScience Jun 1;9(6):giaa051. doi: 10.1093/gigascience/giaa051. Published: 16 June 2020.
36. Wijesena HR, Kachman SD, Lents CA, Riethoven JJ, Trenhaile-Grannemann MD, Safranski TJ, Spangler ML, Ciobanu DC. (2020) Fine mapping genetic variants associated with age at puberty and sow fertility using SowPro90 genotyping array. J Anim Sci. Oct 1;98(10):skaa293. doi: 10.1093/jas/skaa293.
37. Zhuang Z, Ding R, Peng L, Wu J, Ye Y, Zhou S, Wang X, Quan J, Zheng E, Cai G, Huang W, Yang J, Wu Z (2020) Genome-wide association analyses identify known and novel loci for teat number in Duroc pigs using single-locus and multi-locus models. BMC Genomics 21(1):344

Bioinformatics NRSP-8 Coordination Program 2020 Activities

Supported by Regional Research Funds, Hatch Act

James Reecy, James Koltes, and Fiona McCarthy,
Joint Coordinators

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.

FACILITIES AND PERSONNEL: James Reecy (ISU) James Koltes (ISU), and Fiona McCarthy (UA) serve as Co-Coordinators. Iowa State University and University of Arizona provide facilities and support.

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.

PROGRESS TOWARD 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.

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

PROGRESS TOWARD 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 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 2020, a total of 33,301 new QTL/association data were curated into the database, bringing the total number of curated data to 211,792 QTL/associations. Currently, there are 31,455 curated porcine QTL, 160,659 curated bovine QTL, 12,783 curated chicken QTL, 2,472 curated horse QTL, 3,562 curated sheep QTL, and 861 curated rainbow trout QTL in the database. https://www.animalgenome.org/QTLdb/). In 2020, we also laid the groundwork to have the goat linkage maps, genome maps, SNP mapping data, trait management space, and other required information ready for goat QTL/associations to be curated into the database. Continued efforts to also curate data into the Animal CorrDB resulted in an addition of 2,398 correlation data and 1,055 heritability data in 5 animal species. Currently there are a total of 21,036 correlations data on 754 traits, and 4,320 heritability data on 1,127 traits in 5 livestock animal species. The NAGRP data repository continues to play an active role hosting genomics study data for the community.

The collaborative site at CyVerse continues to play an integral role as a backup/recovery site, sharing some the web traffic load (e.g. http://i.animalgenome.org/jbrowse), and a platform for developmental experiments. New data sources and species continue to be updated. The virtual machine site to host the Online Mendelian Inheritance in Animals (OMIA) database (Dr. Frank Nicholas at the University of Sydney; http://omia.animalgenome.org/) and the Hybrid Striped Bass website (Benjamin Reading of North Carolina State University; http://stripedbass.animalgenome.org/annotator/index) continue to be used by researchers.

Ontology development

This past year we continued to focus on the integration of the Animal Trait Ontology into the Vertebrate Trait Ontology (http://bioportal.bioontology.org/ontologies/VT). Seven (7) dataset updates were released to the public throughout 2020. 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 (http://bioportal.bioontology.org/ontologies/CMO). Traits specific to livestock products continue to be incorporated into a Livestock Product Trait Ontology (LPT), which is available on NCBO's BioPortal (http://bioportal.bioontology.org/ontologies/LPT). Six (6) updates of Livestock Breed Ontology (LBO; https://www.animalgenome.org/bioinfo/projects/lbo/) 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://github.com/AnimalGenome/vertebrate-trait-ontology) 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

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. The efforts continued to accommodate multiple genomes for QTL/association mapping/curation. 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 or re-develop the CorrDB continued. 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 works 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 2020, 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://www.animalgenome.org/repository). 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://osf.io/) for better long-term data security. In 2020, the data sharing platform was still actively used for researchers to share data individually or in a group.

The data downloads from the repository generated over 2.27 TB of data traffic in 2020. Throughout the year, over 87 cases were handled through our helpdesk at AnimalGenome.ORG which include 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 have been hosting a couple dozen mailing lists/websites for various research groups in the NAGRP community (https://www.animalgenome.org/community/). This includes groups like AnGenMap, FAANG international consortium, and CRI-MAP users, new meetings, and user bulletin boards to facilitate these meetings, among other user forums (https://www.animalgenome.org/community).

The Functional Annotation of ANimal Genomes (FAANG) website (https://www.faang.org/) 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 500+ 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. Increases in the number of web hits and data downloads continued in 2020. AnimalGenome.org received over 2.9 million web hits from 388k individual sites (visitors), resulting in about 3.6 TB of traffic or data downloads.

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://www.animalgenome.org/community/angenmap/) 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 2020, consistent with the pace/pattern of the past 16 years (https://www.animalgenome.org/bioinfo/updates/).

PLANS FOR THE FUTURE

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:

1. Hamid Beiki, James E. Koltes, Zhi-Liang Hu and James M. Reecy (2020). Analysis of Divergent Transcription Factors across Different Cattle Tissues Reveals Their Interesting Biological Functions. Plant & Animal Genomes XXVII Conference, January 11-15, 2020. Town & Country Convention Center, San Diego, CA.
2. Zhi-Liang Hu, Carissa Park, James M. Reecy (2020). An Update on Database Growth and Improvements of Animal QTLdb and CorrDB. Plant & Animal Genomes XXVII Conference, January 11-15, 2020. Town & Country Convention Center, San Diego, CA.