Annual Report for NC1170
Period Covered: January 1, 2017 to December 31, 2017
Prepared by: Behnam Abasht & Gale Strasburg

Institutional Stations (Institutional Abbreviation: Members)
Beckman Research Institute at the City of Hope (**COH**[1]: M. Miller[2])
Cornell University (**CU**: P. Johnson)
Iowa State University (**IA**[1]: S. Lamont[2,3], J. Dekkers)
Michigan State University (**MI**[1]: G. Strasburg[2,3])
Mississippi State University (**MS**[1]: C. McDaniel, B. Nanduri[2,3])
North Carolina State University (**NC**[1]: C. Ashwell, J. Petitte)
Pennsylvania State University (**PA**: A. Johnson, R. Ramachandran)
Texas AgriLife Research (**TX**[1]: G. Athrey[2,3])
University of Arizona (**AZ**[1]: F. McCarthy[2,3], S. Burgess)
University of Arkansas (**AR**[1]: W. Kuenzel[3], B. Kong, D. Rhoads[2,3])
University of California, Davis (**CA**[1]: M. Delany[2,3], H. Zhou[2,3])
University of Delaware (**DE**[1]: B. Abasht[2,3])
University of Florida (**FL**[1]: M. Edelmann[2,3])
University of Georgia (**GA**[1]: S. Aggrey[2])
University of Maryland (**MD**[1]: T. Porter[2,3], J. Song)
University of Minnesota (**MN**[1]: K. Reed[2,3], R. Sunde[3])
University of Tennessee (**TN**[1]: B. Voy)
University of Wisconsin (**WI**[1]: G. Rosa[2])
USDA-ARS-Avian Disease and Oncology Lab **(ADOL**[1]: H. Cheng[2,3], H. Zhang)
Virginia Tech (**VA**[1]: E. Wong[2,3], E. Smith)

1. Written annual report submitted.
2. Oral annual report given at the meeting.
3. Attended the annual business meeting.

Administration
Executive Director- Jeff Jacobsen, Michigan State University
Lakshmi Matukumalli- NIFA Representative
Christina Hamilton- System Administration
Susan Lamont - Admin Advisor

Poultry Workshop PAG, January 13-14:

a. Attendance: Saturday AM=73; Sunday AM= 70; peak attendance >110
b. Representatives of 17 stations attended at some point during the weekend
c. One survey showed 1 or more representatives from 15 other institutions including the poultry industry, U.S. government, Australia, Bangladesh, the United Kingdom, and China
d. Guest Speakers: Jim Reecy (IA State), Helen M. Sang (University of Edinburgh, Edinburgh, United Kingdom), Robert J Etches (Ligand Pharmaceuticals, Emeryville, CA), Erich Jarvis (Howard Hughes Medical Institute, New York, NY), Tad S. Sonstegard (Acceligen Inc. Animal Ag. Subsidary of Recombinetics, St. Paul, MN), Janet E. Fulton (Hy-Line International, Dallas Center, IA), Thomas Maloney (Revive & Restore, Sausalito, CA), Kevin Wells (University of Missouri), Muhammad Munir (The Pirbright Institute, Surrey, United Kingdom), Perot Saelao (UC Davis; Jorgenson Travel award winner),
e. Nine graduate students gave short presentations about their posters.
f. Updates: J Reecy on NRSP8 bioinformatics
g. 17 NC1170 members presented research updates

NC1170 Business Meeting
January 14, 2018

Attendees:
(DE) Abasht, Behnam, (TX) Athrey, Giri, (ADOL) Cheng, Hans, (CA) Delany, Marry, (FL) Edelmann, Mariola, (MI) Strasburg, Gale, (AR) Kuenzel, Wayne, (IA) Lamont, Susan, (AZ) McCarthy, Fiona, (USDA) Mirando, Mark, (MS) Nanduri, Bindu, (MD) Porter, Tom, (MN) Reed, Kent, (AR) Rhoads, Douglas, (DE) Schmidt, Carl, (WI) Sunde, Roger, (CSU Fresno) Tarrant, Katy, (VA) Wong, Eric, (CA) Zhou, Huaijun, (Rochester) Li, Xin

1. The meeting was called to order by Dr. Behnam Abasht
2. The agenda was approved by voice vote
3. Comments from Dr. Susan Lamont, USDA Administrative Advisor
    a. Status of NC1170 Project renewal: Dr. Lamont expressed thanks to Dr. Gale Strasburg for his leadership on the project rewrite. After review by the Administrative Advisor, the application has been sent for external review.
    b. Other comments: Dr. Lamont indicated that there are many things that the members of the group do very well including outstanding collaboration with other academics. However, we should be doing more to incorporate participation and representation from the poultry industry. Moreover, she stated that there are 33 members of NC1170, but the level of participation at the meeting could be improved.
4. Renewal of NC 1170 project: Comments from Dr. Gale Strasburg, Secretary
   Dr. Strasburg expressed his gratitude to everyone in the group for their quick responses to requests for information. In addition, he expressed special thanks to Drs. Carl Schmidt, Fiona McCarthy, Kent Reed, and Doug Rhoads for their assistance in the writing and editing process.

5. Renewal of NRSP8: comments from Dr. Mary Delaney
   Dr. Delaney indicated that this project rewrite is progressing along on a similar timeline to NC1170. She noted that unlike previous years, there were no poultry people leading the rewrite this year. Dr. Kent Reed added that this puts the poultry group at somewhat of a disadvantage as there was less emphasis on poultry in the final document.

6. NIFA/USDA Administrator comments: Dr. Mark Mirando, substituting for Dr. Lakshmi Matukumalli
    - federal agencies received a 2017 budget in May 2017; should have started in September 2016
    - The 2017 AFRI budget increased to $375 million, which represents a $25 million increase over FY2016. The 2018 budget request is still under Congressional review, so there is budget for this year; agencies are operating under continuing resolutions. The president proposed a reduction of the NIFA budget of about 5% for 2018; other agencies were cut >20%. The president's request for AFR was at $349,335,000 Appropriations discussions and budget approval are being impacted by political issues including a proposed wall at the US/Mexico border, Deferred Action for Childhood Arrivals (DACA). Both House and Senate appropriations committees have proposed a budget of $375,000,000 for AFRI.

    - Planning for the FY2019 budget is underway. President's 2019 budget proposal should come out in February 2018.

- AFRI grant awards: AFRI completed review and funding for the 2017 funding cycle. Although all decisions have been made and project directors notified, it appears not all funding decisions have been publicly announced.

- 2018 AFRE RFAs are being finalized with a planned release for February 2018, although it is more likely will be released in March. Planning for 2019 RFA is in progress; long term goal is to release future RFA's about 2 months earlier each year such that ultimately, RFA's will be released in September of each year and applications do in the December/January timeframe. Question from Dr. Tom Porter regarding the possibility of spreading out submission deadlines. Dr. Mirando responded that for animal-related projects, they've kept the same deadlines so proposals can be transferred between panels if needed. Moreover, panel meetings aret then clustered to meet at about the same time so that they can accommodate additional reviews of proposals if needed.

- Forthcoming RFAs

    - Foundational and Applied Science (FAS) funding to be ~$230M; includes all the animal research.
    - Education and Workforce Development program (includes pre/postdoctoral fellowships, Research and Extension Experiences for Undergraduates ($300-400K grants for student stipends, experiential learning opportunities).
    - SAS (Sustainable Agricultural Systems) $70-80M. Replaces challenge areas: 6-7 areas.

    - FACT program: workshops, tools, etc. Food and Agriculture Cyberinformatics and Tool initiatives. ($10M per year)

    - Question from Doug Rhoads on the fate of dual purpose/dual benefit program with NIH/NICHD. Mirando replied that the program is due to sunset after 2018 funding cycle. It's in its 9th year; typically, these programs run for about 10 years. NICHD is moving away from these interagency programs. There is significant stakeholder effort to renew again via lobbying through Congress. The program lost a significant supporter in Al Franken. There were 5 awards made last year (NIFA funding matched by NIH $70M)

    - Question from Carl Schmidt about possibly increasing award size because small grants inhibit collaboration among faculty at different institutions. Dr. Mirando agreed that this is a desirable goal but a limited budget means that increasing award size reduces number of awards.

    - Question from Fiona McCarthy: Could we look at NSF as an example for stand-alone awards that are linked but would not have the additional indirect costs. Dr. Mirando suggested looking at potential collaborators with Ireland, other European countries using this model.

    - Question from Tom Porter: is USDA exploring the possibility of collaborating with EPA for funding? Dr. Mirando agreed this could be desirable, but is unaware of any discussions.

7. Comments from NRSP8 Poultry coordinators (Drs. Mary Delaney and Hans Cheng)
    - NRSP8 has provided support for travel for students and PI's to travel to travel to meetings. Dr. Cheng pointed out that PI's should contact NRSP8 if a small pot of money is needed for funding of a piece of equipment or an initiative. NRSP8 has

tried to defray cost of some projects, e.g., targeted expenditures (Neogen SNP chip). Likewise, it has provided support for a lab to send a rep for an NIH workshop

- Committee is working on a 10-year blueprint in NRSP8. Looking at submitting 2-4 items that should be in an RFA. Providing more concrete goals and looking at ways to grow the pie. They are seeking to get more involvement e.g., get industry broiler and layer rep. Turkey rep.

- NRSP8 and NC1170 provided funds for the CRISPR/Cas9 minisymposium. Overall cost was ~$20,000 cost. In the past, they've brought in 1-2 people as outside speakers. This year was more expansive and expensive.

- Q from Dr. Mirando: have you submitted conference proposals? Some participants indicated that they had submitted; Doug had done one previously, but says he was told not to submit anymore. Dr. Mirando countered that a request for $5-10K is easy to fund; they like to match funds. He recommends communicating with several National Program Leaders to simultaneously to request funds. Don't be afraid to contact USDA (Send message TO or CC Mirando as AFRI science coordinator).

8. Location and Time for next year (2019)
   - Dr. Rhoads moved to have the meeting in San Diego in conjunction with The Plant and Animal Genome Meeting in January 2019. Motion seconded and approved by voice vote.

9. Creating transgenic Chickens: Wayne Kuenzel and Hans Cheng
   - Dr. Abasht thanked the group for organizing the mini-symposium. Carl Schmidt mentioned that NSF is encouraging NSF Science Centers; there is a proposal for other birds rather than chickens for gene editing. Further discussion focused on short term vs long term plans and needs, regulatory concerns regarding the technology. There could/should be some industry support for collaboration with Roslin Institute for additional research. There was some discussion that there should be a National Poultry Center similar to the National Swine Center at Missouri. Such a center would need industry, federal, university and international support, but could be highly beneficial for all stakeholders.

10. Other business
    - Gale Strasburg to be new Chair of NC1170
    - Kent Reed to be new NRSP8 Chair
    - Giri Athrey was elected NC1170 Secretary
    - Bindu Nanduri was elected NRSP8 Secretary

11. Meeting adjourned at 12:30 pm

Accomplishments by Objective

This document summarizes by objective the major accomplishments achieved by the NC1170 Multistate Research Project covering 2016. Achievements included the application of next generation technologies in sequencing to poultry genomes and numerous contributions to advances in bioinformatics, annotation, and transcriptomics as applied to a variety of fundamental disciplines in poultry biology. Our work is conducted under the auspices of many collaborative arrangements with stakeholders involved in the allied poultry industries for the purpose of improving poultry production. The scholarly publications resulting from this project

(see appendix) includes over 92 articles in peer-reviewed journals and books/chapters. Special note should be made of the large number of publications involving collaboration between members of this project as well as scholars from around the world. Participants were listed as PI or coPI on $18,675,963 in funding/grant support that was active during 2016. During the past four years this project has added new participants from Cornell University, Mississippi State University, Pennsylvania State University, University of Tennessee-Knoxville and Texas AgriLife Research, to a current membership of 32 participants.

Objective 1: Create and share data and technology to enhance the development and application of genomics and systems biology in poultry.

AZ

AgBase: supporting functional modeling in agricultural organisms. AgBase (http://www.agbase.msstate.edu) provides resources to facilitate modeling of functional genomics data and structural and functional annotation of agriculturally important animal, plant, microbe and parasite genomes. We provide 1,833,993 Gene Ontology (GO) annotations for 354,329 gene products from more than 40 agriculturally important species and their pathogens. During 2017 we worked on moving the AgBase database to new servers, which will enable continued growth and maintenance of this resource.

Standardized Gene Nomenclature. The Chicken Gene Nomenclature Consortium (CGNC; http://birdgenenames.org/cgnc) is the sole source globally for chicken gene nomenclature. During 2017 we mapped gene nomenclature to genes annotated for the new Galgal5 release to provide unambiguous nomenclature for a further 29,783 genes.

Chickspress – developing a tissue specific compendium of gene expression for chicken gene products. The Chickspress resource (http://geneatlas.arl.arizona.edu) provides a detailed "atlas" of chicken gene expression, collating experimental information from Red Jungle Fowl and chicken gene expression studies. The Chickspress genome browser information now utilizes the CoGe genome browser, providing access to more comparative analysis tools and leveraging cyberinfrastructure supported by the NSF funded CyVerse project. During 2017 we have remapped all expression data to the new Galgal5 annotations.

Host-Pathogen Interaction Database (HPIDB) – curation to support animal health data sets. During 2017 we continued manual biocuration of host-pathogen interaction data to support network modeling of animal health data sets as well as developing a set of predicted 75,323 "interologs" (developed in conjunction with Mississippi State). This includes curated and predicted interactions for Marek's Disease, Salmonella and Mycoplasma.

CA

Identification of Regulatory Elements in Livestock Species. The recent international FAANG (Functional Annotation of ANimal Genomes) initiative has stimulated efforts to functionally annotated important livestock species, which will ultimately be leveraged to improve production efficiency, animal welfare, and food safety. As one of the FAANG pilot projects coordinated by UC Davis, we present the current progress in generating and analyzing data from chicken, cattle, and pig. Samples were collected from adipose, cerebellum, cortex, hypothalamus, liver, lung, muscle, and spleen in two male biological replicates from each species, allowing the identification of both universal and tissue-specific functional elements. High depth of RNA-seq identified 9,393 long non-coding RNAs in chicken, 7,235 in cattle, and 14,428 in pig. From DNase-seq in chickens, 132,362 open chromatin regions were identified, many of which are tissue-specific. Genes present in these open chromatin regions show generally higher expression in our RNA-seq data. In chicken, we have identified a total of 31,174 H3K4me3 peaks, 79,144 H3K27me3 peaks, 34,091 H3K27ac peaks, 44,664 H3K4me1 peaks, and 21,710 CTCF peaks in liver, lung and spleen. For the same tissues in pig, 35,081 H3K4me3 peaks

were identified, 104,640 H3K27me3 peaks, 133,689 H3K27ac peaks, 38,247 H3K4me1 peaks, and 26,585 CTCF peaks. Preliminary chromatin state models built using this data show good correlation with gene expression and chromatin states representative of promoters, enhancers, and insulators. Work is ongoing to improve existing data and include the remaining brain, adipose, and muscle tissues, leading to the final genome-wide chromatin state predictions and comprehensive catalogs of regulatory elements for these three species.

COH

Sequence, gene content and organization of the MHC-Y gene region in the red jungle fowl reference genome. (Miller, Goto, Warden, Wu, McPherson and Delany). The MHC-Y region is unusual [1-4]. SMRT sequencing supported by NSRP-8 funds allowed us to determine and accurately assemble sequences for multiple RJF BAC clones. These sequences allowed us to map one hundred genes to the MHC-Y haplotype in the RJF reference genome. This includes 47 MHC-Y class I genes, 4 MHC-Y class IIβ genes, 36 c-type lectin-like genes, 6 LENG9 genes and 7 ZFP genes. MHC-Y is linked to additional interesting genes nearby including olfactory and scavenger receptor genes [5]. The YF genes are especially polymorphic. The YF molecules are structurally most similar to the monomorphic human molecule called MR1. Human MR1 presents microbial vitamin B metabolites to specialized T cells that make innate immune responses to microbial infections [6]. Our own studies suggest that MHC-Y class I also bind ligands of microbial origin. MHC-Y class I molecules are of interest for their potential contributions in immune responses to microbes. A manuscript describing genomic sequence of the RJF MHC-Y haplotype is well on its way to completion.

Development and evaluation of a method for MHC-Y genotyping (Miller and Goto). The sequence determinations described above allowed us to identify regions that might be suitable for defining MHC-Y genotypes using PCR fingerprints. Several candidate regions were tested. A suitable region containing a simple repeat of variable numbers near the MHC-Y class I genes was selected and has been evaluated for distinguishing MHC-Y genotypes. Several primer pairs encompassing this region were evaluated and found to distinguish standard haplotypes. PCR fingerprints made with these primer pairs reveal patterns that correctly identify the MHC-Y haplotypes segregating in fully pedigreed families.

Testing for MHC-Y contributions to immune responses and the genetics of colonization (Miller, Goto, and Zhang; Siegel and Honaker; Psifidi, Stevens and Fife; Fulton and Plastow). We are now using PCR fingerprinting to investigate the contribution of MHC-Y haplotype in immune trails. We are looking for other test populations that might be appropriate for investigating the contribution of MHC-Y in disease or immune responses.

FL

Phosphorylation is a key post-translational modification controlled by kinases, and it regulates essential cellular processes, therefore various anomalies in this modification have been implicated in animal diseases. However, kinases have never been systematically studied in chicken. Therefore, annotation of kinases in chicken is necessary for studying chicken health/disease. To map chicken kinases in tissue we used two different approaches. First, we used kinase domain mapping based on published data and Chickspress database (University of Arizona, http://geneatlas.arl.arizona.edu), which incorporates NCBI and Ensembl gene models with the protein expression data from specimens of multiple tissues from both genders. This bioinformatic approach allowed us to detect chicken kinases in various tissues by using peptide evidence, but it was limited to identification of 7 kinases in spleen and 2 in liver. Second, we utilized active-site directed probes combined with mass spectrometry to identify over 200 ATP- and ADP-binding sites in 140 chicken kinases in spleen and liver combined. This experiment led to identification of novel kinases, 71% of which were previously uncharacterized in chicken. By utilizing this chemical proteomics, which is invaluable in novel enzyme identification, we improved existing annotation of these enzymes, which drastically expands current annotations available via Uniprot and other resources. We compared several sequences of detected kinases

to known mouse, rat and human orthologues to understand conservation of these ATP-binding domains. Our study confirmed that chicken proteome contains multiple kinases, some of which have not been previously annotated. We also mapped novel nucleotide-binding lysine residues in these kinase and show that these kinases have a unique abundance in chicken tissue. This information will be made publically available in collaboration with AgBase (Arizona Univ. and Mississippi State University).

IA

Transcriptome data made public. Several data sets from RNAseq experiments on chickens were deposited in public databases upon submission for journal publication of the manuscripts describing those studies.

MS

MS continues to generate data and develop bioinformatics resources to facilitate the analysis of functional genomics data in agricultural species. Working with University of Arizona, MSU contributed to AgBase. AgBase (http://www.agbase.msstate.edu) facilitates structural and functional annotation of agriculturally important animal, plant, microbe and parasite genomes and provides tools and resources for biological interpretation of functional genomics data. AgBase provides 1,833,993 Gene Ontology (GO) annotations for 354,329 gene products from more than 40 agriculturally important species and their pathogens. MSU also contributed to Host-Pathogen Interaction Database (HPIDB): HPIDB provides predicted and curated host-pathogen protein-protein interaction data to support animal health/disease studies. During 2017 HPIDB continued manual biocuration of host-pathogen interaction data as well as developing a set of predicted 75,323 “interologs”. This includes curated and predicted interactions for Marek’s Disease, Salmonella and Mycoplasma. Working with University of Florida, MSU identified ~200 kinases in spleen and liver tissues in chicken based on their reactivity with the ATP and ADP desthiobiotin acyl phosphate probe. The assumed functions of these kinases were analyzed by comparing functional pathways and disease involvement of human, murine and rat orthologs of these kinases. Performing chemical proteomics with active site probes for deubiquitinases, we identified ~20 DUBs from chicken spleen and cecum.

TX

Athrey Lab at TX (new member since 2017) generated whole genome sequences from two wild Gallus species, and from three domestic breeds of Gallus to characterize structural variation in chicken genomes (14 whole genomes). Comparison against the Gallus reference genome V4 helped identify small and large structural variants. Further analysis of inversions and large deletions helped identify such variants that were unique or shared among compared varieties. Over 200 unique inversion variants have been identified and are being verified by standard laboratory techniques. In the process a new tool, named Girar, for classification of inversions was developed. Following additional testing of the tool, Girar will be made available for community use (estimated Summer 2018). In theory this tool is taxon- independent and could be valuable for other livestock and non-model organisms as well.
Also towards Objective 1, Athrey lab graduate student Rohit Rohra worked on an in-silico approach to validate currently predicted miRNAs in the chicken genome. The results of this work are under review for publication. This work also produced a short pipeline tool that will be shared with the community.

WI

predictive assessment of genetic correlations between traits in chickens using markers.
Genomic selection has been successfully implemented in plant and animal breeding programs to shorten generation intervals and accelerate genetic progress per unit of time. In practice, genomic selection can be used to improve several correlated traits simultaneously via multiple-trait prediction, which exploits correlations between traits. However, few studies have explored multiple-trait genomic selection. Our aim was to infer genetic correlations between three traits measured in broiler chickens by exploring kinship matrices based on a linear combination of

measures of pedigree and marker-based relatedness. A multivariate genomic best linear unbiased prediction model was designed to combine information from pedigree and genome-wide markers in order to assess genetic correlations between three complex traits in chickens, i.e. body weight at 35 days of age (BW), ultrasound area of breast meat (BM) and hen-house egg production (HHP). A dataset with 1351 birds that were genotyped with the 600 K Affymetrix platform was used. A predictive assessment was used to gauge genetic correlations. Our findings indicate that multiple-trait prediction may benefit from combining pedigree and marker information. Also, it appeared that expected correlated responses to selection computed from standard theory may differ from realized responses. The predictive assessment provided a metric for performance evaluation as well as a means for expressing uncertainty of outcomes of multiple-trait selection.

Objective 2: Facilitate the creation and sharing of poultry research populations and the collection and analysis of relevant new phenotypes including those produced by gene transfer.

ADOL

A major strength of ADOL is the large number of chicken lines that are characterized for a number of traits, especially those associated with viral diseases, and maintained under specific pathogen free (SPF) conditions. Besides providing unique genetic resources to ADOL, ~1,500 embryos or chicks are supplied yearly to academic institutions or companies in the United States. The lines and maintenance are briefly summarized below. ADOL maintains 35 chicken lines with special genetic characteristics for tumor or viral susceptibility that also differ remarkably for immunological and physiological traits. All but 3 (C, N and P) were developed at the ADOL over the last 67 years. These include 4 of the world's most highly inbred lines (6_3, 7_1, 7_2,and $15I_5$,), all of which are well defined for avian leukosis virus (ALV) receptor genes, endogenous virus loci (EV), and resistance to MD. Two of the lines are outbred, 2 of which are highly utilized worldwide for ALV analyses (0 and 15B1). Four congenic lines exist for analysis of EV genes; 3 (0.44-TVBS1- EV21, 0.44-TVBS3-EV21, and RFS) were developed from line 0 and 1 (100B) from line 7_2. Eight congenic lines exist for analysis of the influence of the MHC (*B* haplotype) on resistance to tumor diseases, immune responses or vaccinal immunity; 7 (15.6-*2*, 15.7-*2*, 15.15I-*5*, 15.C-*12*, 15.P-*13*, 15.P-*19*, and 15.N-*21*) were developed from line $15I_5$, and 1 (15.N-*21*) from line 0. Lines 6_3 and 7_2 differ markedly for MD resistance and immune function traits, as well as ALV and EV genes, but have the same B haplotype. Nineteen recombinant congenic strains (RCS) are under development to identify non-MHC genes that influence traits differing between lines 6_3 and 7_2. ADOL also developed one transgenic chicken line (0.ALV6) that is very beneficial for analysis of ALV.

ADOL lines are routinely tested by blood-typing using 40 antisera either to ensure purity or to maintain heterozygosity (EV21, 100B, and O.P-*13*) during annual line reproduction. The breeders are unique in that they are maintained in a quarantined state and, on the basis of frequent serologic tests for 11 pathogens, are considered free of infection from common poultry pathogens. With the planned moved to of ADOL staff to new facilities in Athens, GA, we hope efforts will be developed to successfully transfer all the lines prior to the planned move estimated to be 2022.

AZ

Chicken Phenotype annotation. During 2017 we added 1,774 avian anatomy terms to the avian ontology provided by Uberon. This project is finishing as we did not obtain funding for further work.

IA

Iowa State University chicken resource populations maintained. Iowa State University maintained thirteen unique chicken research lines [including highly inbred, MHC-congenic, closed populations; and advanced intercross lines (AIL)] to serve as resources for identifying

genes, genetic elements and genomic regions of economic importance; as well as defining unique aspects of chicken genomic architecture. All adult breeders were housed in individual cages and matings done by artificial insemination to ensure pedigree accuracy. All MHC-defined lines were blood-typed to verify MHC serologic haplotype. Two AILs (now at generation F27) were maintained to facilitate fine-mapping of QTL with the goal of identifying genomic regions and candidate genes controlling important phenotypes.

Utilization and sharing of research populations. The ISU genetic lines formed a discovery platform for research on the genomics of heat resistance in a USDA-AFRI-NIFA project (PD: C Schmidt, U Del) and a USAID project on genomics of resistance to Newcastle disease virus and heat (PD: H Zhou, UC-Davis) because of defined, distinct responses among lines. Genetic material (chicks, fertile eggs, blood, tissues, DNA or RNA) was shared with many cooperating investigators to expand studies on the chicken genome. Active collaborations utilizing ISU chicken genetic lines or biological materials include H Zhou, UC-Davis (NDV and heat-stress response); C. Schmidt, U Delaware (heat stress); R Coulombe, Utah State (aflatoxin sensitivity); B Abasht, U Delaware (allele-specific expression); E Wong, Virginia Tech (Eimeria response) and V Kapur, Penn State (NDV-embryo assays).

NC

Differences in jejunal gene expression of two chicken lines divergently selected for antibody response to sheep red blood cells. For over 30 generations 2 lines of White Leghorn chickens have been undergoing continuous divergent selection for high (HAS) or low (LAS) antibody titer to sheep red blood cells (SRBCs) at 5 d post injection. This has been a well utilized model for immunology and genetic trials, and many differences between the lines have been observed in terms of performance and response to diseases. However, little has been done to study native molecular differences between lines, so the purpose of this experiment was to examine differences in gene expression in the jejunum of non-SRBC injected birds. Eggs from both lines were obtained from Virginia Polytechnic Institute and State University (Blacksburg, VA) and co-incubated until hatch, after which all chickens were housed and raised together in mixed cages. At 46 d of age, 6 chickens from each line were euthanized and jejunum samples were collected for RNA isolation. RNA was isolated using the RNeasy kit by Qiagen and then sent to the NC State University Genomics Sciences Laboratory for library preparation and sequencing on the Illumina HiSeq 2500 sequencer. RNA sequence data were analyzed using NGS RNA analysis tools, TopHat and Cufflinks (statistical significance threshold of $q < 0.05$) available at galaxy.org. Significant differences in gene expression were observed between lines with over 4 times as many genes upregulated in HAS as compared with LAS. Not surprisingly, many of the upregulated HAS genes are involved with immune response, particularly interferon signaling and antigen processing. Genes upregulated in LAS largely involve fatty acid transport and cell membrane integrity. Understanding native gene expression provides insight into the ways in which energy resources are allocated within an organism. It has previously been reported that HAS is slower growing with delayed performance compared with LAS, and the increased immune system related gene expression could indicate priority resource allocations to these systems over growth and performance, further supporting those observations. More research is needed to better understand the effects of genetic selection on physiology to optimize genetic selection for performance and health.

Intestinal microbiome comparisons of two white leghorn lines divergently selected for high and low antibody response to sheep red blood cells. Interest is ever growing in research investigating the dynamic relationship between hosts and their resident microbes. While human biomedical research dominates, there is also need for research into the effects of the microbiome on the health of production animals. A well-studied model for avian immunogenetics exists, consisting of two lines of white leghorn chickens divergently selected for over 30 generations for high (HAS) or low (LAS) antibody response to sheep red blood cells at five days post injection, but so far only about 40% of the phenotypic variation in response has been

explained by genetics. Microbiome characterization of these lines could offer insight into the role of microbes in antibody response as well as the effects of genetic selection on microbiome diversity. For this experiment, eggs from each line were co-incubated to gether until hatch, and raised together in mixed cages for the duration of the experiment. At seven weeks of age, six birds from each line were euthanized and intestinal contents from approximately 4cm of duodenum, jejunum, and ileum were collected for microbial DNA isolation. DNA was isolated using theQIAmpDNAStoolkitbyQiagenandthensenttotheUNCMicrobiome. Core facility for 16S pyrosequencing and analysis. A small but significant difference in the microbial composition between lines was observed. HAS exhibited less over-all diversity, however a significantly higher percentage of the phyla Firmicutes, which include lactic acid bacteria (LAB), were observed. Some studies suggest that probiotic LAB feeding stimulates the gut immune systems. Potentially, the increased abundance of naturally occurring intestinal LAB of these birds may enhance their immune response, and possibly selection for high antibody response also selects for gut conditions conducive to LAB colonization. More research is needed examining the host-microbiome relationship to better understand how these microbes may affect host physiology.

Gene-editing in Chicken Primordial Germ Cells. The lack of efficient, specific genome editing methods have been one of the major impediments to our ability to produce gene knockouts and targeted mutations in the avian genome. This has significantly limited agricultural scientists to address fundamental questions related to food animal health and disease resistance; however, the CRISPR/Cas system has been used successfully to edit the genomes of prokaryotes to humans, and with a specificity and efficiency unmatched by other genome editing platforms. As a proof of concept and because of their significance in host defenses against pathogens, a knockout strategy was developed to target iNOS (NOS2) and phox (NOX2), (Gene IDs395807 and 418581, respectively). Specific protospacer adjacent motifs (PAM) sites were selected using CHOPCHOP, a webtool for selecting the optimum target sites for CRISPR/Cas9 (Nucleic Acids Res. 42. W401-W407-2014). Rankings of possible target sequences were checked for off-target sequences in the genome using BLAST. Oligonucleotides of the selected PAM sequences were ligated in to CRISPR/Cas9 vectors and verified by sequencing. Before targeting PGCs, two avian macrophage cells lines were used to model functional knockout events that resulted through non- homologous end joining DNA repair (NHEJ). MQ-NCSU and HD11 macrophage cells lines were transfected with the specific NOS2and NOX2 CRISPER/Cas9 constructs generated. TheCas9 protein was fused to a GFP marker and allowed enrichment of Cas9 expressing cells using FACS. Subsequently, the GFP-expressing fraction of cells were cloned by limiting dilution. After 14 days, viable colonies of cells were expanded and cells banked. DNA isolated from individual clonal lines were used to amplify about a 450 bp region flanking the PAM site. Amplification products were gel purified and cloned into plasmid vectors for DNA sequencing. Gentoypes were classified as wild-type, monoalleic or biallelic. Sequence analysis of two targeted sequences for iNOS (NOS2) resulted in monoallelic and biallelic genotypes in MQ-NCSU cells. In all cases of a homozygous knock-out of NOS2, the sequence was either in-frame or generated a new start site for the correct transcription of a truncated protein. Functional induction of nitrous oxide production in such cases was no different than that observed for wild-type cells. New CRISPR/Cas9 targets will need to be generated and tested in the cells lines before use in PGCs. Sequence analysis of a targeted locus in NOX2 in MQ-NCSU and HD11 macrophages also resulted in monoallelic and biallelic genotypes in both cells lines. Stimulation of reactive oxygen species (ROS) production was tested in the biallelic knockout genotypes. In all cases, ROS production was inhibited. In the monoallelic cell lines, ROS production was roughly half of that produced by the wild-type cells and demonstrated a gene-dosage effect for NOX2 function.
Attempts to duplicate the results in PGCs were inconsistent. In most cases, PGCs appeared to be refractory to gene modification at the same loci tested in MQ-NCSU and HD11 macrophage lines using the same CRISPR/CAS9 vectors. This suggests that gene editing in avian cells will differ between cell lines. Hence, while model cell lines can be used as a starting point for gene editing in avian cells, efficient conditions for PGCs will require standardization.

TX

Towards Objective 2, Athrey lab has established and is maintaining a Red Junglefowl population of the Richardson strain. The current population size stands at 40, of which 12 are males. Through an undergraduate research project (Nicholas Faust), we have begun using molecular markers to generate a pedigree for this population. DNA samples are available from each of these birds for interested researchers for use in comparative analyses. In 2017, we provided samples to Hy-Line for comparative analyses on their MHC SNP panel.
Also, as these birds are generating more eggs than we can hatch out and place in the colony (due to space limitations), we are able to collect and ship fertile RJF eggs.

Objective 3: Elucidate genetic mechanisms that underlie economic traits and develop new methods to apply that knowledge to poultry breeding practices.

ADOL

Identifying driver mutations for Marek's disease. Marek's disease virus (MDV) is a highly oncogenic virus in susceptible chickens as lymphomas, characteristic of Marek's disease (MD), are induced as early as 2-4 weeks after infection. Unlike other herpesviruses, MDV integrates into the chicken genome and encodes an oncoprotein, known as Meq, a bZIP transcription factor that homodimerizes or heterodimerizes preferably with c-Jun. However, as all MDV-infected chicken do not develop gross tumors and most tumors are clonal, it is likely that somatic driver mutations are required for transformation. To identify potential driver mutations, ~200 line 6 x 7 F1 progeny were challenged with MDV (JM/102W strain to help promote large homogenous gonal tumors), which resulted in ~100 tumor samples. To identify somatic mutations, whole genome and transcriptome sequence analyses of 26 MD tumors and matched control tissues were conducted. In ~85% of the tumors, mutations or low gene expression were found in IKAROS family zinc finger 1 (IKZF1), a zinc-finger transcription factor associated with T-cell development and chromatin remodeling. Similar to human Acute Lymphocytic Leukemia (ALL), MD tumors contain dominant negative somatic mutations in zinc-finger binding domains suggesting its role as a tumor suppressor gene. Furthermore, pathways enriched for differential gene expression between tumors and matched controls mimic proposed Ikaros targets, suggesting that deregulated Ikaros may reprogram T cells for hallmarks of cancer. Collectively, these data suggest MDV Meq and host IKZF1 regulate the decision between viral replication and latency, which is perturbed by somatic mutations in IKZF1 leading to tumorigenesis.

Identifying the molecular basis for MD vaccine synergy Marek's disease (MD). vaccines utilize protective synergism, a phenomenon where the protective efficacy of two vaccines in combination is greater than either vaccine when administered alone. A key example is the bivalent MD vaccine of serotype 2 (SB-1) and serotype 3 HVT (FC-126). Despite this widespread usage, the biological mechanism of the synergistic effect has never been elucidated. We previously found that SB-1 replicated to the highest levels in spleen, bursa, and thymus, respectively, while HVT showed replication only in bursa at 1 dpi and could not be detected at any other time point or tissue type. Looking at later time points, we find that only SB-1 + HVT (bivalent MD vaccine) can control MDV replication compared to the other two MD vaccines administered alone. Currently, we are screening cytokines to determine if unique or enhanced expression of one or more might account for protective synergism. Hypothesizing that CD8 T cells may play a role in the protective ability of MD vaccines, we injected chicks with anti-CD8 antibody, which reduced CD8 T cell populations by 50-80%. Regardless of the MD vaccine administered, disease incidence was significantly higher in CD8-depleted birds, indicating that CD8 is responsible for at least part of the protection afforded by MD vaccines. The question is whether CD8 T cells are essential for anti-viral or anti-tumor response.

Comparative transcriptome analysis of genetically divergent lines of chickens in response to Marek's disease virus challenge at latent phase. To advance the understanding on genomic mechanisms underlying genetic resistance to MD, we continued to comparatively characterize

transcriptomic differences of the two unique experimental lines of chickens, the resistant line 63 and the highly susceptible line 72, in response to MDV challenge at the latent phase. A total of 64 and 106 differentially expressed genes were identified in resistant and susceptible lines across 10 and 21 dpi time points in contrast to their control counterparts, respectively. After a systematic close re-screening with considerations to gene expression patterns, gene functions, and overlap to reported QTL regions, we identified 27 genes that are promising candidates contributing to genetic resistance to MD. These genes encompass virus process (*F13A1* and *HSP90AB1*), immunity (*ABCB1LB*, *RGS5*, *C10ORF58*, *OSF-2*, *MMP7*, *CXCL12*, *GAL1*, *GAL2*, *GAL7*, *HVCN1*, *PDE4D*, *IL4I1*, *PARP9*, *EOMES*, *MPEG1*, *PDK4, CCLI10*, *K60* and *FST*), and tumor suppression (*ADAMTS2*, *LXN*, *ARRDC3*, *WNT7A*, *CLDN1* and *HPGD*). These findings advanced our current understanding on genetic resistance to MD.

AR

Identification of genes affecting ascites susceptibility. AR- performed RNAseq on right ventricles from three sets of male birds: ambient pressure, hypoxic challenge no disease, and hypoxic challenge with ascites. The RNAseq data have been partially analyzed to identify major gene expression changes (manuscript in preparation). AR used whole genome resequencing to identify 31 chromosomal regions as candidate QTLs for affecting ascites. Three of these regions have been further validated, representing the first validated QTLs for ascites. AR evaluated the developmental, gender and ontological aspects of mitochondrial biogenesis in broilers, primarily focused on skeletal muscle and the cardio-pulmonary system. Significant differences were found for gender, tissue, age, and ascites susceptibility. Muscle mitochondrial content was positively correlated with ascites phenotype. This may be an easily evaluated trait for selection against ascites.

3.2 Bacterial chondronecrosis with osteomyelitis and lameness in broilers

AR- performed two separate trials for reducing bacterial chondronecrosis with osteomyelitis (BCO) lameness. Trial one was with four different commercial probiotics. Trial two was with a commercial trace organics supplement. In both cases we identified specific treatments that significantly reduced BCO lameness. AR has been evaluating bacterial isolates from BCO lesions to understand the virulence determinants specific to colonization and pathogenesis in broilers. Virulence has been assessed in direct challenge to live broilers as well as phagocytosis assays using chicken macrophage in culture. Macrophage response may be a simple test to select for resistance to bacterial colonization.

Feed efficiency of broilers. AR- In collaboration with other investigators at AR, chicken small noncoding RNAs encoded by mitochondrial genomes (mitosRNAs) were identified by small RNA sequencing method and differential abundance of mitosRNAs was analyzed in breast muscles derived from chickens of rapid growth and large muscle mass (LARGE) compared with those from slow growth and small muscle mass (SMALL). A
total of 183,416 unique small RNA sequences were identified as potential chicken mitosRNAs. After utilizing a stringent filtering process, 117 mitosRNAs showing greater than 100 raw read counts were abundantly produced from all 36 mitochondrial genes. Of those, 44 mitosRNAs were differentially expressed in breast muscles of LARGE compared to SMALL: all mitosRNAs were upregulated in LARGE breast samples, except those produced from the 16S-rRNA gene. Our data demonstrate that in addition to 36 known mitochondrial genes, the mitochondrial genome also encodes abundant mitosRNAs, that may play an important regulatory role in the control of mitochondrial gene expression in muscle growth. Proteomics and bioinformatic analyses with breast muscle samples obtained from pedigree male (PedM) broilers exhibiting high feed efficiency (FE) or low FE phenotypes revealed that high FE muscles may retain functions of enriched ribosome assembly and protein translation. The results provided the fundamental cellular pathways in feed efficiency of broilers.

Genes and proteins involved in the stress response of broilers. AR- in collaboration with other investigators at AR and the University of Missouri has completed studies addressing the neuroendocrine regulation of stress in broilers. Following the use of a stressor, feed deprivation,

corticotropin-releasing hormone (CRH) neurons in a brain structure within the septum showed first activation. The septal structure is called the nucleus of the hippocampal commissure (NHpC). Hours later, CRH neurons in the paraventricular nucleus (PVN) of the hypothalamus, the principal nucleus involved in stress, showed significantly increased mRNA. Thereafter, arginine vasotocin (AVT) neurons in the PVN showed significantly increased mRNA. Hence, CRH neurons within the NHpC appear to be the first stress responders in the chick brain following feed deprivation. In a second study using an organotypic brain slice culture method coupled with immunocytochemistry, enabled testing how CRH neurons within the NHpC could be regulated. Results showed that CRH neurons along with a terminal field of vasotocinergic fibers occurred within the NHpC. Importantly, a receptor, the vasotocin 1a receptor (V1aR), was shown located in glial cells. When arginine vasotocin (AVT) was applied to brain slices containing the NHpC, significantly increased CRH gene expression as well as increased brain-derived neurotrophic factor (BDNF) gene expression occurred. Results suggest that CRH neuronal activity in the NHpC is modulated by AVT via V1aR involving BDNF and its receptor (TrkB) located in that structure.

AZ

Training workshops and community support via AgBase. We continue to provide training, outreach and support for poultry researchers via AgBase to ensure that they are able to better leverage their functional genomics data to understand key economic traits for poultry. During 2017, the AgBase resources were visited by 18,497 different researchers, with 28.7% of these visitors from the US (includes visitors from 48 states). AgBase was cited in 68 publications during 2017.

CA

Improving food security in Africa by enhancing resistance to Newcastle disease virus and heat stress in chickens. Within a USAID funded Feed the Future Innovation Lab for Genomics to Improve Poultry project (H. Zhou, PI) through a partnership of the University of California at Davis (H. Zhou, D. Bunn, R. Gallardo), Iowa State University (S.J. Lamont, J. Dekkers), Sokoine University of Agriculture (SUA) -Tanzania, the University of Ghana (UOG), and the University of Delaware (C. Schmidt). The five-year research program will apply advanced genetics and genomics approaches to sustainably enhance innate resistance to Newcastle disease virus (NDV) and heat stress in chickens to improve production. This project directly addresses the President's FY2012 "Feed the Future" initiative. We are investigating two stressors (biotic: NDV and abiotic: heat stress). Birds of two genetically distinct and highly inbred lines (Fayoumi and Leghorn), and Hy-Line Brown were either exposed to NDV only (Iowa State) or NDV and heat stress (UCD). Measures of body temperature, blood gas parameters, NDV titers from tears, and antibody response in serum were taken on the live birds, and tissues were collected for transcriptome analysis. Three ecotypes each in Ghana and Tanzania will be exposed to NDV. DNA isolated from Hy-Line Brown were genotyped using chicken 600K SNP for GWAS. At UCD, the RNA-seq data of 144 individual cDNA libraries (focusing on infection: 3 tissues (lung, trachea, and harderian gland), 2 genetic lines of chickens, NDV challenge and control, 3 times points at 2, 6 and 10 dpi) and 96 individual cDNA libraries (focusing on heat stress: 3 tissues (liver, breast muscle, and hypothalamus) at 4 hours and 9 days post-heat treatment, 2 genetic lines of chickens, heat stress and control) were generated from the combined NDV challenge with heat stress study of highly inbred chicken lines. At ISU, the RNA-seq data of 192 individual cDNA libraries from an NDV challenge study of highly inbred chicken lines at ISU (3 tissues, 2 genetic lines of chickens, NDV challenge and control, 3 times) were generated.
All data were initially processed for quality control and then analyzed using bioinformatic programs. Genes that responded to NDV infection and differed between resistant and susceptible genetic lines were identified (for NDV infection, 500-800 genes at 2 dpi, 100-400 genes at 6 dpi, and 50-200 genes at 10 dpi; and for heat stress, 50-200 genes at both time points). Enriched gene groups and pathways were also identified and validated (NDV resistance: such as cytokine-cytokine receptor interaction, regulation of T cell activation, cell adhesion molecules, MHC class I protein complex antigen processing and presentation,

regulation of type I interferon T-helper 1 immune response etc.; heat tolerance: oxidative response, oxidative deethylation, and ossification etc.). To assess the response to heat stress, thirteen blood physiological parameters were measured using the iSTAT system.
Completed genome-wide association analysis for challenge experiments on Hy-Line Brown: All three replicate trials with over 1,100 challenged birds were completed at UCD and ISU. NDV titers were measured in tears collected at 2 and 6 days post infection. In addition, NDV serum antibody response was measured at 10 days post-infection. To assess the response to heat stress, thirteen blood physiological parameters were measured using iSTAT. ASReml estimated heritabilities were 0.34, 0.34, 0.04, 0.07, 0.19, and 0.05 for hatch weight, day 31 body weight, 0dpi and 10dpi antibody levels, and 2dpi and 6dpi ND viral load, respectively. In general, most of the economically important traits have low to medium heritabilities (0.1-0.4) except body weight with high heritability (0.5-0.7). Birds were genotyped using the 600K SNP panel to conduct a genome-wide association study (GWAS).
For African ecotype NDV challenges, replicate trials involving a total of 2,653 chicks (UOG) and 1789 chicks (SUA) were completed in the challenge facilities. For each replicate, blood was collected for DNA isolation. At four weeks of age, the chicks were challenged with NDV. Tear samples were collected at 2 and 6 dpi for NDV titers. Serum samples were also collected pre-challenge and at 10 dpi for NDV antibody titers. Body weight data was also collected at hatch, weekly until challenge, and at 6 and 10 dpi. To utilize replicates of previously challenged birds by La Sota NDV strain with available 600K SNP genotype data, the NDV resistance of indigenous African chickens in Tanzania and Ghana undergo further evaluation under field conditions. A RT-qPCR assay that specifically detects mesogenic and velogenic NDV strains was established and validated at SUA. The assay is being used to confirm natural exposure to velogenic NDV. Following natural NDV exposure, data on survival times, body weight, antibody response, and pathological lesion scores were collected. Data analyses are underway. Data processing and analysis pipelines have been developed to enable estimation of genetic parameters once all the data has been generated. More than 2900 birds (1490 from UOG and 1450 from SUA) have been genotyped with 600K SNP panel. Associations between SNPs and antibody titer in African ecotypes are underway.

Salmonella enterica serovars Enteritidis infection in young layer chicks. The main objective of the research project is to elucidate molecular and cellular mechanisms of Salmonella enterica subsp. enteric serovar Enteritidis (SE) persistent infection in chickens by studying the interaction of the following trio: host, pathogen, and microbiome using next generation sequencing (NGS). We had previously characterized the structural changes that occur in gut microbiota of chicks when challenged with Salmonella Enteritidis (SE) at one day of age. Our previous study showed that microbiota of SE infected chicks was significantly modulated compared to control with overall reduction in diversity and expansion of specific members of the community, Enterobacteriaceae family. Early inoculum of SE in newly hatched chicks was found to be the primary driving force behind alterations of the microbial community1. However, colonization by members of the microbial community expands gradually with time in avian hosts and the pathogen-commensal interaction during transitional development stage of microbial community has yet to be explored. Salmonella causes a significant economic loss to the poultry industry, as consumption of contaminated eggs and meats is the leading cause of human food- borne salmonellosis. The objective of the current study was to profile SE associated microbiome during the developmental stage of young chicks. Chicks were challenged with SE at two weeks of age and cecum microbiome was analyzed with 16S rRNA sequencing post-infection at 3, 7, 14 and 21 days. A total of 2,941,667 reads were generated with 59,762 different OTUs identified. Almost all alpha and beta diversity metrics measured between the SE-infected and control showed non-significant difference in microbial composition. On the other hand, Chao1 richness index indicated that as the course of SE infection progresses, total number of species present in the community also increases. This result suggests that as SE colonization continues to persist over time with infection, its functional activity could play a role in creating a beneficial environment for other incoming species. Overall, presence of a developing microbial community in 2 weeks old chicks was effective in inhibiting pathogen induced microbiota alteration. Further

in-depth analysis characterizing host metabolites over the course of infection is currently underway. Deciphering the linkage between microbiome and metabolome during infection will provide important insight into SE associated microbial profile and its functions that contribute to colonization persistence.

Development of colonization resistance in chicks. This research is in collaboration with Dr. Baumler (dual PI) funded by NIH/USDA Dual Purpose with Dual Benefit. The main objective to this project was to dissect the mechanism of SE colonization in newly hatched chicks by investigating aerobic respiration as possible mechanisms behind the bloom of facultative anaerobe in gut during SE infection. Early exposure to Salmonella Enteritidis (SE) in one-day old chicks could have consequential effect both on environmental condition of the gastrointestinal tract as well as on development of the microbial community. Furthermore, we also hope to further unravel the mechanism utilized by the SE pathogen to gain colonization advantages in gut of the newly hatched chicks. At one day old, newly hatched chicks were infected with SE and the effect of pathogen influence on microbiome community development was measured with 16S rRNA gene sequencing at 1,3, and 7 days post infection (dpi). Following SE infection, microbiota profiles of the infected chick host were altered with overall reduction in microbial diversity and luminal expansion of Enterobacteriaceae family most significant at 3 days post infection (dpi). Specifically, facultative anaerobic bacteria are able to outcompete against obligate anaerobe population. Alteration in microbiota profile was triggered partly by host inflammatory response to SE virulence factors secreted by its type III secretion system. Measurement of host inflammatory markers with qPCR indicated that the host inflammation response also peak at 3dpi in SE-infected group. To further assess the overall environmental condition in the gut that occurred with microbial dysbiosis, we use the exogenous hypoxia marker, pimonidazole (PMDZ) stain. We detected that SE utilized its virulence factors to modify the hypoxic condition of the gut to non-hypoxic condition (most noticeable at 3dpi) where there is increase oxygenation in gut lumen, which can be used by Salmonella as electron acceptor. This support the further growth and persistence of the pathogen by enabling its aerobic respiration in the otherwise anaerobic environment occupied mostly by strictly anaerobic members of the microbial community. SE utilized cytochrome bd1 oxidase, terminal oxidase in the respiratory electron transport chain to further expand its growth in the gut of the newly hatched chicks. We observed that mutant strain lacking the cytochrome bd1 (cydA mutant) oxidase failed to compete with wild type strain for colonization in co-infection model in all measured post-infection time points. Fitness advantage of competing wild type strains over cydA mutant strains is lost when virulence factors were deleted in both strains. Result indicated that the host inflammatory response triggered by Salmonella virulence factors contribute to microbial dysbiosis and increase oxygenation of the gut epithelial that drives the bloom of the SE growth in gut of the newly hatched chicks. To further assess the competition for available of oxygen in the gut between the facultative anaerobic members of the microbial community, we did in vivo bioluminescence imaging utilizing the transformed avian E.coli harboring the bacterial luciferase and fatty acid reductase genes that will emit visible light. A critical component of bacterial bioluminescence is requirement of oxygen presence. Emission of bioluminescence signal were measured both transcutaneously on whole animal as well as in post-mortem tissue and the magnitude of the signal is then quantified one days post infection (1dpi). In the co-infection study where chicks were infected with both SE wild type strain and Avian E.coli harboring the lux plasmid, we detected significantly lower emission signal from the group compared to the single infection group with E.coli. This result suggest that in the co-infection group, SE is actively competing with E.coli for utilization of the oxygen available in the gut for its aerobic growth which in turn reduces the light emission signal from E.coli. In another experiment, we tested the founder effect theory of niche occupancy of the early colonizer that may have profound effect on subsequent colonizer in the gut once again using the in-vivo & ex-vivo bioluminescence imaging system. The result suggested the early colonization by E.coli allow it to establish a colonization site in the gut that is closest in proximity to the oxygen rich niche. Subsequent colonization by SE later on however have to settle on colonizing site that were not already occupy by the first colonizer, thus resulting in SE having limited access to the

oxygen for aerobic growth and diminishing its ability to compete effectively against E.coli for oxygen utilization.

DE

Transcriptomics analysis of heterosis in Chickens. The superior performance of hybrids to parents, termed heterosis, has been utilized in animal and plant breeding programs for more than a century, but the understanding of molecular mechanism underlying heterosis remains inadequate and requires further study. RNA-seq provides a novel way to investigate the phenomenon of heterosis at the genome-wide level, because gene expression can be considered as an intermediate phenotype that connects the genetic information to observable phenotypes. We compared embryonic gene expression between the chicken hybrids and their inbred parental lines using RNA-seq in order to understand the gene expression basis of heterosis for embryonic weight in chickens. Two genetically distinct and highly inbred chicken lines, Fayoumi and Leghorn, were crossed reciprocally to obtain F1 fertile eggs. The polyadenylated RNA of brain and liver from Day 12 embryos was converted to cDNA and sequenced on an Illumina HiSeq sequencer. The resultant reads were mapped to the reference genome assembly Gallus gallus 5.0 and the differentially expressed genes were identified, pairwise, among the hybrids, parental lines and synthesized mid-parent expression values. Our results indicted expression of the majority genes in F1 crosses are not significantly different from synthesized mid-parental values, suggesting additivity is the predominant gene expression pattern between F1 and parental lines. The second and third prevalent gene expression patterns are dominance and over-dominance. Additionally, our results show that only 7-20% of the DE genes exhibit allele-specific expression in the F1, suggesting that differential trans – regulation is largely responsible for differential gene expression between parental lines and is the major regulatory mechanism leading to heterosis in the F1 cross.

Unraveling key morphological changes characterizing the onset and development of Wooden Breast Disease in commercial broiler chickens during the growth period. Wooden Breast Disease (WBD), a myopathy that frequently affects modern broiler chickens, is a disorder that has been associated with significant economic losses in the poultry industry. To examine tissue changes associated with the onset and early pathogenesis of this disorder, a time-series experiment was conducted using chickens from a high-breast-muscle-yield, purebred commercial broiler line. Birds were raised for up to seven weeks, with a subset of birds sampled weekly. Breast muscle tissues were extracted at necropsy and processed for analysis by light microscopy and transmission electron microscopy. Histologic presentation indicated localized phlebitis with lipogranulomas in Week 1, focal single-myofibril degeneration in Week 2 preceding an inflammatory response that started in Week 3. Lesions in Week 4 were characterized by multifocal to diffuse muscle fiber degeneration, necrosis, interstitial edema accompanied by increased lipid and inflammatory cell infiltration. Lesions in Weeks 5–7 revealed diffuse muscle degeneration, necrosis, fibrosis and fatty infiltration with lipogranulomas. Ultrastructural examination showed myofibrillar splitting and degeneration, irregular, displaced and degenerated Z-lines, mitochondrial degeneration and interstitial fibrosis with dense regular collagen fibers. This study, therefore, demonstrates that WBD exhibits an earlier onset in modern broilers than when detectable by clinical examination. Further, this study shows that the disease assumes a progressive course with acute vasculitis, lipid deposition and myodegeneration occurring in the earlier stages, followed by a chronic fibrotic phase.

Differential gene expression in the liver of commercial broiler chickens affected by Wooden Breast Disease. Research on Wooden Breast Disease (WBD) has been extensively concentrated on understanding the breast muscle damage associated with the disease. The current study focuses on exploring differential gene expression in the liver of commercial broiler chickens affected by Wooden Breast Disease. Liver tissue samples from 4 affected and 4 unaffected chickens previously used in high throughput mRNA sequencing using Illumina HiSeq 2500 were aligned to the most recent reference genome *Galgal5* with HISAT2. Differential gene expression analysis was performed using Cuffdiff. The 92 significant differentially expressed

(DE) genes are being used to determine pathways involved in changes associated with WBD. In affected birds, 47 of these DE genes were upregulated and 45 genes were downregulated. This study continues to indicate WBD is a physiologically complex abnormality of commercial broiler chickens that does not solely affect muscle.

Spatial and sex differences in gene expression in pectoralis major of broiler chickens. Wooden Breast Disease (WBD) is a novel myopathy affecting the breast muscle of modern broiler chickens. The etiology of the disease is currently unknown, but its recent emergence has been linked to increased feed efficiency and muscle yield in broiler chickens. The cranial region of the pectoralis major tends to be more severely affected than the caudal aspect. Additionally, male chickens have higher incidence rates of WBD and tend to display more severe symptoms than females. This study aims to characterize the biological differences in the p. major between sexes of birds and regions of the muscle to determine the cause of differential susceptibility to WBD. Samples were taken from cranial and caudal aspects of the p.major muscles of 3-week old, unaffected male and female birds for RNA sequencing. cDNA libraries were prepared, then sequenced using Illumina Hiseq2500. Sequence reads were aligned to the chicken reference genome with HISAT, then genes were analyzed for differential expression between sex and spatial groups using CuffDiff. There were 260 differentially expressed genes between male and female birds, and 12 between cranial and caudal samples. Genes involved in fat metabolism were up-regulated in samples from the cranial region and male birds. Other significant genes included those involved in muscle development, inflammatory processes, and oxidative stress. Results suggest increased fat deposition in male birds and the cranial aspect of the muscle. The up-regulated genes involved in oxidative stress in male birds support a hypothesis that the development of WBD is related to increased oxidative stress.

Comparison of bioinformatics software tools using RNA-seq data from three different populations of chickens with Wooden Breast Disease. Currently, there exist several bioinformatics tools for processing sequence reads generated from RNA-seq studies. Although the choice of a program in a RNA-seq study is dependent on the researcher's preference, information about comparison of the suites is limited. Moreover, studies on validation of results using non-sequencing-based techniques are lacking. This study aimed at comparing different bioinformatics programs using 22 RNA-seq samples comprising affected and unaffected groups from three distinct broiler populations with Wooden Breast Disease. Two aligners (HISAT, STAR) and two differential expression (DE) tools (Cuffdiff, DESeq) were used to generate four combinations of pipelines for the 3 datasets. Each combination generated a list of significant genes for each population. All the lists were evaluated for overlapping genes among the three populations to determine behavior and concordance. Although both aligners produced satisfactory read coverage; STAR had a higher percentage of mapping-rate (88%) than HISAT (76%). Combinations using Cuffdiff produced higher percentages of overlapping genes (25%) than those with DESeq (21%). Almost 100% of overlapping genes showed concordant expression patterns across all the three datasets for all combinations. Results also exhibited DE tools are not susceptible to aligners and produced similar outcomes. However, DE tools that used the same aligner produced different outcomes. Cuffdiff presented susceptibility to number of replicates and had better performance in larger groups. To corroborate RNA-seq study, an independent dataset was generated using a non-sequencing-based (Nanostring) technology; yielding a correlation of 0.85 between the two technologies. Overlap significant genes between Nanostring and RNA-seq also confirmed the behavior.

Developing an RNA-Seq analysis pipeline for the detection of ASE SNPs. This pipeline follows GATK's Best Practices for calling of variants and also corrects for reference allele bias. For detection of ASE SNPs custom python scrips were developed that test for ASE using the binomial test. This pipeline was used to analyze transcriptomic data from three tissues (breast muscle, abdominal fat and liver). At this time period, all data have been analyzed, variants called and ASE SNPs identified; however, further development of the ASE software needs to be done.

A new tool to prioritize candidate genes and characterize sample behavior in differential expression analysis of transcriptomic data. High-throughput sequencing technologies and differential expression analysis tools have facilitated the profiling of differential gene expression, resulting in sometimes extensive lists of candidate genes. However, prioritization of these candidate genes remains a challenge, especially in non-model systems where data mining techniques cannot easily be used to inform the analysis. As a result, many researchers use fold-change (FC) and p-value to prioritize genes, which does not account for the ability of individual samples to skew the results. ARB (Analysis of RNA-seq Data Behavior) addresses this problem by classifying genes based on the degree of separation of expression values between experimental groups. This is accomplished with a supervised subsetting algorithm that incrementally excludes samples up to a cutoff set by the user to achieve non-overlapping separation between groups. If a subset can be assembled that meets the user's constraints, then the following is calculated: (i) inter-group separation index (IGSI); 0-2 scale where a value of 2 corresponds to a subset where no samples have been removed (ii) FC using only that subset of samples, and (iii) the identities of the samples that have been excluded from the subset. With this information, a researcher can easily select the genes with the highest IGSI and FC for further scrutiny. ARB also includes functions to identify outliers, rank samples based on relative expression across genes, and identify misclassified samples based on frequency of exclusion. The program is designed to run using output from Cuffdiff and is available with sample data at https://github.com/AbashtLaboratory/ARB.

GA

mRNA analysis of amino acid, protein utilization and nutrient transporter in meat-type chickens under heat stress. Global temperatures have increased in the past few decades, and climate change will lead to frequent heat waves and longer hot seasons. Heat stress causes critical molecular dysfunction and cellular changes that affect productivity and potentially compromises bird's welfare. Molecular mechanisms that underlie nutrient partitioning and metabolism of poultry under heat stress would allow for strategies to mitigate the effects of heat stress. We investigated the immediate and long term transcriptomics changes in chickens under heat stress. Forty-eight Cobb500 male birds were divided into two groups and raised under either constant 25^{o}C or 35^{o}C from 14-26 days of age in individual cages and fed ad libitum on a diet containing 21% CP and 3100kcal ME/kg. Five birds per treatment at 1 and 12 days after heat treatment were euthanized and the Pectoralis (P.) major was sampled for gene expression analysis. At d 33, ileal contents were collected and used for digestibility analysis. Broilers under HS had reduced growth and feed intake compared to controls. Although the apparent ileal digestibility (AID) was consistently higher for all amino acids in the HS group, it was not significant except for hydroxylysine. The amino acid consumption and retention were significantly lower in the HS group when compared to the control group. The total consumption and retention of protein and fat were significantly lower in the HS group compared to the control group. Meanwhile, the retention of crude protein per BWG was significantly higher in the HS group compared to the control group. Meanwhile, the retention of amino acids per BWG was higher in the HS group when compared to the control group except for hydroxylysine and ornithine. The dynamics of amino acid transporters in the P. major and ileum was influenced by HS. In P. major and ileum tissues at d 1, transporters SNAT1, SNAT2, SNAT7, TAT1, and b0,+AT, were down- regulated in the HS group. Meanwhile, LAT4 and B0AT were down-regulated only in the P. major in the treatment group. The amino acid transporters B0AT and SNAT7 at d 12 post HS were down-regulated in the P. major and ileum, but SNAT2 was down-regulated only in the ileum and TAT1 was down-regulated only in the P. major compared with the control group. In P. major and ileum tissues at day 1, transporters FATP1 and SGLT1 were down-regulated in the HS group. Meanwhile, FABP1 and PepT1 were down-regulated only in the ileum of the HS group. The converse was shown in P. major. The nutrient transporter FABP1 at day 12 post-HS was down-regulated in the P. major and ileum, but GLUT1 and PepT2 were down-regulated only in the ileum, and PepT1 was down-regulated only in the P.

major compared with the control group. These changes in nutrient transporters suggest that high ambient temperature might change the ileum and P. major amino acids, lipids, glucose, and oligopeptide transporters.

Genomic differentiation as a tool for SNP prioritization for genome-wide association of phenotype prediction in animals. Genome-wide association studies (GWAS) have been successful in detecting associations between single nucleotide polymorphisms (SNPs) and phenotypic variation and in identifying several causative mutations. However, SNPs with significant association identified using GWAS tend to explain only small fraction of the phenotypic variations. GWAS are affected by lack of power due to small sample size, large numbers of highly correlated markers, and the moderate to small effects of most quantitative trait loci (QTLs). This situation is further complicated by the continuous increase in marker density, especially with the availability of next-generation sequencing (NGS) data. The latter generates an unprecedented number of marker variants, with a complex linkage disequilibrium (LD) structure limiting the advantage and adequacy of existing methods that internally try to prioritize (filter) SNPs (e.g. BayesB, and BayesR). Consequently, it is becoming necessary to either filter SNPs before conducting the association analysis or to enlist additional sources of information. Methods that include biological prior information (e.g. BayesRC) are limited by the amount and quality of available prior information. Knowledge of genetic diversity based on evolutionary forces is beneficial for tracking loci influenced by selection. The fixation index (FST), as a measure of allele frequency variation among sub-populations, provides a tool to reveal genomic regions under selection pressure. In order to evaluate its usefulness as an additional source of information, a simulation was carried out. A trait with heritability of 0.4 was simulated and three subpopulations were created based on the empirical phenotypic distribution (< 5% quantile; > 95% quantile; and between 5% and 95% quantiles). Marker data was simulated to mimic a bovine chip of 600 K, 1 million, and 3 million SNP marker panels. Genetic complexity of the trait was modelled by the number of QTLs, their distribution, and the magnitude of their effects. Using different empirical cut off values for FST, most QTLs were correctly detected using as few as 2.5% of SNP markers in the panels. Furthermore, the genomic similarity, calculated based on the selected SNPs, was very high (>0.80) for individuals with similar genetic and phenotypic values despite having limited to no pedigree relationship. These results indicate that filtering SNPs using FST could be beneficial for use in GWAS by focusing on genome regions under selection pressure. High functional genomic similarity based on selected markers indicates similarity in SNP signatures, regardless of relatedness, and translates into high phenotypic correlation that could be used in decision making.

IA

Selection signatures in Sri Lankan, Brazilian and Egyptian ecotypes assessed using a 600K SNP chip. Understanding the genetic strategies that indigenous, non-commercial breeds have evolved to survive in their environment could help to elucidate molecular mechanisms underlying biological traits of environmental adaptation. We examined the genomes of poultry from diverse breeds and climates of Egypt, Sri Lanka and Brazil for selection signatures that have allowed them to adapt to their indigenous environments. Birds were genotyped with the 600K SNP chip. Selection signatures were studied using a combination of population genomic methods that employed F_{ST}, and runs of homozygosity procedures. The analyses revealed unique differences in the genomic regions under selection pressure in hot and humid versus hot and arid climates, as well as runs of homozygosity that were shared amongst the populations indigenous to both climate types. This information may guide us toward identification of genomic regions of important in controlling resilience to high ambient temperatures.

Cecal tonsil transcriptome of laying hens altered by exposure to high ambient temperature. Egg-laying chickens are under high physiological demands as they come into peak egg production, and they can be further challenged by suboptimal environmental conditions such as heat stress. To better understand the genetic mechanisms that control response to heat stress in laying hens, we studied the genomics of the hens' response to heat stress by using two

complementary methodologies. To assess gene expression changes, we studied the transcriptomes of the cecal tonsil of hens subjected to heat stress (or control) conditions. Tissues were harvested at 3 times (1 day, 2 weeks and 4 weeks) after initiation of heat treatments. For the 3 contrasts across time within the control group, there was only 1 DE gene detected: *CLEC2B*, a cell adhesion protein involved in cell signaling. Another 18 DE genes were detected in the remaining 6 contrasts involving the heat group. As expected for layers that recently started egg production, we detected changes in genes related to cholesterol biosynthesis and metabolism. A surprising result is the large number of genes involved with epigenetic regulators that were found to be differentially expressed. Although epigenetic regulators are currently not well studied in chickens, epigenetic regulatory mechanisms such as histone modification and alternative splicing have been shown in mammals to have dramatic impact in gene expression changes. In all, the 19 DE genes represent pathways and mechanisms that layer CT tissue utilizes under heat stress that were previously unknown. This result will add to the breadth of knowledge for potential gains in layer production through future investigation of specific pathways associated with heat stress in different tissues.

Chromosomal regions associated with feed efficiency of laying hens under heat stress identified by GWAS. To identify genomic regions associated with the response of hens to heat stress, we conducted a genome-wide association study (GWAS) of the feed efficiency of hens under heat stress using the 600K SNP panel. Feed efficiency is defined in terms of the feed intake per mass of eggs produced. Genome wide analysis revealed the same regions on chromosomes 1 and 9 seem to be associated with both feed efficiency and feed intake at 2 weeks after initiation of cyclic heat exposure. Shared regions between these two traits were not unexpected because the traits are not independent. Not all regions were shared however, such as the novel chromosome 4 association with feed efficiency. Feed efficiency and feed intake 4 weeks after initiation of cyclic heat exposure also shared regions of genomic control, however these regions were different than those of week 2. Regions on chromosome 1, 6 and 22 were identified. Genomic investigation of response to heat stress in commercial laying hens has provided valuable information that will help inform production and breeding decisions and help feed a growing population.

Genetic evaluation models developed and tested. Models were developed and compared for the genetic evaluation of egg shell quality in layers. Results showed that, for traits with repeated records at different ages, repeatability within and across ages as well as genetic correlations should be considered while choosing the number of records collected per individual and the model for genetic evaluation.

Related studies. Several studies complementary to those reported in the NC1170 project are reported as part of the Iowa contribution to the NE1334 project or elsewhere. These include (1) investigating the interaction of two stressors [heat stress and exposure to an inflammation-inducing PAMP (LPS)] on the transcriptome of birds of two distinct lines (broiler, Fayoumi) using RNA-seq of individual samples of thymus, bursa and spleen, (2) using two highly inbred lines (Fayoumi and Leghorn) and RNA-seq, identifying genes and pathways associated with NDV challenge, (3) using a commercial egg-laying line (Hy-Line Brown), conducting GWAS of response to NDV challenge, with NDV titer and anti-NDV antibodies as phenotypes, (4) conducting NDV challenge studies and associated genetic and genomic analyses on local African chickens in Tanzania and Ghana, and (5) genomic, molecular and cellular characterization of the host-pathogen interactions between chickens and avian pathogenic *E. coli*.

MD

Gene Expression Differences Associated with Egg Production Rates in Turkey Hens. Variation in egg production exists in commercial turkey hens, with low producing hens costing more per egg produced. Ovulation is governed by the hypothalamic- pituitary-gonadal (HPG) axis, and total egg production is correlated with ovulation frequency. Each ovulation is stimulated by a

preovulatory surge of progesterone and luteinizing hormone (LH), caused by release of gonadotropin releasing hormone (GnRH). Ovulation can be inhibited by release of gonadotropin inhibiting hormone (GnIH). Differences in the HPG axis between low and high egg producing turkey hens were explored by determining mRNA levels for key genes of the HPG axis, both outside and during the preovulatory surge. From the top and bottom 15% in egg production of 200 commercial line hens, six high egg producing (HEP) hens and six low egg producing (LEP) hens were sampled, collecting the hypothalamus, pituitary gland, and granulosa layers of the largest follicle (F1G) and the fifth largest follicle (F5G), half outside of the preovulatory surge and half during the preovulatory surge (n=3 per group). Levels of mRNA for key genes in the HPG axis involved in ovulation and ovarian steroidogenesis were examined using RT-qPCR. LEP hens exhibited increased mRNA levels for genes associated with ovulation inhibition, such as hypothalamic GnIH (NPVF) and pituitary GnIH receptor (NPVFR), as well as decreased mRNA levels for genes associated with ovulation stimulation, such as pituitary follicle stimulating hormone (FSHB) and LH (LHB). Interestingly, LEP hens demonstrated increased mRNA levels for both the FSH receptor (FSHR) and the LH receptor (LHCGR) in the F5G, as well as the progesterone receptor (PGR) in the hypothalamus. Genes associated with progesterone production, steroidogenic acute regulatory protein (STAR), cholesterol side-chain cleavage enzyme (CYP11A1), and 3β-hydroxysteroid dehydrogenase (HSD3B1), tended to be up-regulated in the F1G of HEP hens, while in LEP hens these genes appear to be up-regulated in the F5G instead. Different degrees of stimulation and inhibition within the HPG axis at the mRNA level were noted in LEP and HEP hens.

Characterization of Copy Number Variation's Potential Role in Marek's Disease. Marek's Disease (MD) is a highly contagious pathogenic and oncogenic disease primarily affecting chickens. Chicken Lines 6_3 and 7_2, as well as their recombinant congenic strains (RCS) with varied susceptibility to MD, are ideal models to study the complex mechanisms of genetic resistance to MD. In this study, we investigated copy number variation (CNV) in these inbred chicken lines using the Affymetrix Axiom HD 600 K SNP genotyping array. We detected 393 CNV segments across all ten chicken lines, of which 12 CNVs were specifically identified in Line 7_2. We then assessed genetic structure based on CNV and observed markedly different patterns. Finally, we validated two deletion events in Line 72 and correlated them with genes expression using qPCR and RNA-seq, respectively. Our combined results indicated that these two CNV deletions were likely to contribute to MD susceptibility. Besides, we also reported DNAmethylation level and gene expression related to vaccination are different between the two inbred lines and recombinant congeneric strains (RCS). The genetic and epigenetic mechanisms underlying both genetic resistances to MD and vaccine protective efficacy Besides, we also reported DNA are complex. Therefore, continuous and systematic efforts on such study are warranted.

linc-GALMD3 and Gga-MIR-219b in Marek's disease. Long intergenic non-coding RNAs (lincRNAs) are transcribed from non-coding DNA sequences. Studies have revealed that aberrant expressions of lincRNAs are associated with various types of cancers and neurological disorders. Marek's disease (MD) is a highly contagious T-cell lymphoid neoplasia of chicken induced by Marek's disease virus (MDV). In this study, we first identified and validated linc-GALMD3 highly expressed in MDV-infected CD4+ T cells by RNA-Seq and qRT-PCR. By RNA-Seq analysis in MDCC-MSB1 cells after loss of function of linc-GALMD3 by shRNA, we found that linc-GALMD3 could positively cis-regulate its downstream gga-miR-223 gene expression. In contrast, it could trans-regulate the 748 differentially expressed genes (FDR < 0.01) that were mainly enriched into mitochondrial structure and cell cycle processes using GO analysis. Of these, the most significantly expressed gene EPYC might cause iris lesion in MD. The other eight genes, NDUFA4, NDUFB6, NDUFV1, NDUFS8, SDHB, UQCRC1, UQCRC2, and COX7A2, actively participated in oxidative phosphorylation in mitochondrial dysfunction and cell death. Most importantly, we found that the MDV replication was repressed when linc-GALMD3 was knocked down in CEF

cells. Interestingly, we also found gga-miR-219b was significantly downregulated in MDV-induced lymphoma. One of its potential target genes, B-cell chronic lymphocytic /lymphoma 11B (BCL11B), was predicted. We further investigated the function of gga- miR-219b, and the gain/loss of function assay showed gga-miR-219b inhibited cell migration and reduced cell proliferation by promoting apoptosis not by cell cycle arrest. Gga-miR-219b also suppressed expression of two cell invasion-related genes MMP2 and MMP9. The results indicated suppressive effect of gga-miR-219b on MD tumorigenesis. The gene BCL11B was verified as a direct target gene of gga-miR-219b. RNA interference was performed to block BCL11B. As expected, the effects triggered by BCL11B downregulation were in accordance with that triggered by gga-miR-219b overexpression, suggesting that BCL11B was a stimulative regulator of MD transformation. Moreover, both gga-miR-219b and BCL11B influenced the expression of Meq gene, the most important oncogene in MDV. Additionally, gene expression level of anti-apoptotic genes BCL2 and BCL2L1 was downregulated and pro-apoptotic gene TNFSF10 was upregulated in MSB1 cells with gga-miR-219b overexpression or BCL11B knockdown, which suggested gga-miR-219b promoted cell apoptosis via regulating gene expression in the apoptosis pathways. Our results suggested that linc- GALMD3 and gga-miR-219b might be critical regulators in chicken MD and could be used as a candidate-promising mark for MD prevention, diagnosis, and treatment.

Dynamic Expression and Regulatory Mechanism of TGF-β Signaling in chicken stem cells. We investigated the dynamic expression and regulatory mechanism of TGF-β signaling involved in embryonic stem cells (ESCs) differentiation into male germ cells. Candidate genes involved in TGF-β signaling pathway were screened from RNA-seq, which were further validated by quantitative real time PCR(qRT-PCR). Bone morphogenetic protein 4 (BMP4) was used to induce differentiation of ESCs in vitro Inhibition of TGF-β signaling pathway was reflected by western blot of SMAD 2 and SMAD 5 expression. Differentiating efficiency of germ cells was evaluated by immunofluorescence and fluorescence activated cell sorting (FACS). Germ cell marker genes were assessed by qRT-PCR in the differentiation process, with activation or inhibition of TGF-β signaling pathway. Our study reveals the mechanism regulating SSCs and lays the basis for further understanding of the regulatory network. Now, we are using CRISPR/Cas9-Mediated deletion to study stem cell differentiations.

Threshold points for gene expressions under multiple biological conditions. Temporal gene expression data is of importance in the classifications of gene functions and have been extensively used in biomedical studies, such as cancer diagnostics. However, since temporal gene expressions vary over time, after the initial time periods, many genes exhibit some kind of stability, which means that gene expressions keep constant or fluctuate slightly after those time points. Thereby, this threshold point is a key in the study of behaviors of gene expressions, which can be used to decide the measuring time period and to distinguish the gene expressions. We used three methods to detect the threshold points for the gene expressions. In particular, the first-order and second-order change rates are used to construct the test statistics for detecting the threshold points. The simulation study shows that the proposed methods have a good performance for the detection of threshold points. A real dataset with 21 genes in P. aeruginosa expressed in 24 biological conditions is used to illustrate the proposed methodology.

MI

Influence of thermal challenge on turkey muscle development and meat quality. This project, conducted in collaboration with Ohio State University and the University of Minnesota, is studying the effect of thermal challenge on: 1) cultured turkey muscle satellite cells; and 2) posthatch turkey poults by characterizing changes in transcriptional profiles, muscle microstructure, and meat quality characteristics. In addition, one of the aims of the project is the use of thermal manipulation of embryos to enhance thermotolerance in the birds and thereby enhance turkey meat quality in the market age birds. Exposure of newly hatched turkey poults to hot or cold thermal stress often results in detrimental effects on breast muscle growth and development. Typical changes include increased lipid deposition and damage to muscle

ultrastructure, leading to inferior meat quality with consequent economic losses to producers and processors. Likewise, exposure of market-weight turkeys to acute heat stress immediately prior to harvest, frequently results in a high incidence of inferior meat quality characterized by pale color, reduced marinade uptake and water-holding capacity, and poor protein functionality. Thermal manipulation of embryonic development has met with some success as a strategy to improve thermotolerance of broilers. We hypothesized that exposure of turkey eggs to a mild heat challenge at a critical developmental stage would alter muscle growth and development, thereby setting the stage for improved thermotolerance of the growing bird. Eggs from RBC2 (slow-growing) and F (fast-growing) turkey lines were exposed to a control temperature of 38C throughout 28 days of incubation or 12h of 39.5C between days 21-25. Following hatch, birds were brooded at temperatures of 31C, 35C (control), or 39C for 3d, followed by brooding at 35C until 14d of age when the birds were sacrificed. Post mortem analysis included body weight, breast muscle weight, muscle fiber diameter and perimysial spacing. Results from this preliminary study suggest that mild thermal manipulation of turkey eggs results in changes to muscleultrastructure that may be associated with altered thermotolerance in the growing bird. Based on this study, we have selected an embryonic thermal manipulation time of 3h at the elevated temperature from day 21-25 for the next phase of the study.

Differential gene expression in normal and pale, soft, exudative meat from slow- and fast-growing turkey lines.

Intensive genetic selection for poultry growth has resulted in substantial advances in growth rate and breast muscle mass. However, these gains have been offset to varying degrees by muscle myopathies which pose economic challenges to the poultry meat processing industry. One of these ongoing concerns is the prevalence of pale, soft, exudative (PSE) meat, which is observed with increased frequency in response to the onset of a sudden increase in environmental temperature. PSE meat is generally thought to result from an unusually high rate of postmortem metabolism causing a rapid drop of pH while the temperature is still warm, resulting in denaturation of meat proteins. However, the specific mechanism underlying the accelerated postmortem metabolism is still poorly understood. Recent RNA-Seq and western blot analyses from our laboratory have shown that expression of the pyruvate dehydrogenase kinase isozyme 4 (*PDK4*) gene and the PDK4 protein are significantly downregulated in PSE turkey muscle. PDK4 serves as a modulator of glycolytic metabolism by regulating pyruvate dehydrogenase (PDH) activity. Phosphorylation of PDH by PDK4 results in inactivation of PDH with a shift to anaerobic metabolism and lactate production. We hypothesized that reduced PDK4 levels would result in decreased phosphorylation of PDH, thereby providing a mechanistic basis for the development of PSE meat. Randombred Control Line 2 (RBC2) turkeys, representing the turkey of the 1960s maintained without selection pressure and commercial turkeys, were raised to market age, and slaughtered and processed according to industry standards. Pectoralis major muscle samples were collected at 5 min postmortem, snap-frozen in liquid nitrogen, and stored at -80°C until further use. Breast muscle samples were classified as normal or PSE based on marinade uptake and cook yield at 24h postmortem. PDH and phospho-PDH abundance were quantified (n=6 normal and 6 PSE) by western blotting. Phospho-PDH (Ser293, Ser300, or Ser232) abundance was normalized relative to that of PDH. Total PDH was significantly reduced (FC = -0.31; $p<0.05$) in commercial but not RBC2 turkey muscle; however, there were no significant differences in phosphorylation of PDH despite the differences in PDK4 expression. These results suggest that despite variation in PDK4 abundance, other isozymes of PDK in muscle may affect postmortem muscle metabolism.

MN

Influence of thermal challenge on turkey muscle development and meat quality. This project in collaboration with Michigan State University and Ohio State University seeks to quantify climate change impacts on poultry breast muscle growth and development, morphological structure, intramuscular fat deposition, and protein functionality to develop appropriate strategies to mitigate the undesirable changes in meat quality. To this effect we completed analysis of satellite cell RNAseq data for two experiments. Results of these have been published. Analysis

of data from a third RNAseq experiment examining skeletal muscle gene expression in your turkey poults (30 libraries) has been completed and a manuscript is in preparation (Barnes et al).

Genomics to increase aflatoxin resistance in turkeys. To investigate the response to aflatoxin exposure we are using RNA-Seq approaches to characterize the transcriptome level changes in the liver, intestine and spleen of birds exposed to AFB_1. We have completed analysis of a liver RNAseq database from an AFB_1 challenge of 16wk wild and domestic turkeys conducted at our collaborating institution (Utah State University, RA Coulombe). A manuscript detailing this study is in revision. Using the same tissues examined for liver mRNA we have collected a miRNA-seq dataset being analyzed in Roger Coulombe's lab. A manuscript detailing this study is in preparation. In addition to studying the liver, we are currently summarizing an RNAseq study of the intestine (primary site of AFB_1 absorption) specifically focusing on the cecal tonsil. A manuscript is in preparation (Reed et al.) A third data set from this same experiment involves the spleen transcriptome (site of secondary immune response) this dataset is in analysis phase.

Antibiotic-free alternatives to improve health and performance in commercial turkeys. The goal of this project is to advance our understanding of the interactions between the turkey gastrointestinal microbiome and host during maturation and microbiome modulation. We seek to change the paradigm by which alternatives to antibiotics are developed, using systematic and science-grounded approaches. First, we are examining host-microbiome relationships that occur during turkey development and gastrointestinal microbiome modulation. Caged performance experiments were conducted using turkey poults (n=400). Treatments included a negative control, a GroGel carrier control, continuous subtherapeutic bacitracin methylenedisalicylate administration in feed, a commercial probiotic (FM-B11) , and an experimental 10-strain probiotic derived from turkey gastrointestinal bacteria administered daily. Tissues from birds were collected at days 3, 6, and 13 of age (spleen, ileum, cecum, trachea) and used for assessments of intestine health, host gene expression via RNA-Seq (96 libraries), host signaling pathways via kinomic immune peptide arrays, and bacterial and fungal communities via amplicon sequencing. We are conducting data analysis is underway for the RNAseq portion of this study.

Related studies. Determining Turkey Selenium Nutrition and Requirements Using Molecular Biology, University of Wisconsin, Multi-state Hatch Project 2016-2108. Roger Sunde (PD). This project utilizes RNA-Seq to characterize the effect of selenium on the turkey liver transcriptome. We are working with Dr. Sunde and his graduate student Rachel Taylor to provide bioinformatics support for data analysis.

NC

The effects of feeding a diet high in methyl donors on Japanese quail. Much speculation surrounds the parental influence or epigenetic effects on progeny performance. Several genomic modifications have been shown to be inherited from parent to offspring including methylation of DNA in the form of 5- methylcytosine. Changes in DNA methylation leads to changes in gene expression and ultimately phenotypes, which can affect the health and growth of the bird. The addition of methyl side chains to DNA inhibits the access of transcription machinery to DNA for the transcription process. Dietary nutrition has been shown to impact the DNA methylation pattern and a diet that is high in methyl donors may increase DNA methylation that can be seen in the progeny. For the first generation of this multi- generational trial, 300 Japanese quail were placed in a brooder at day of hatch.150 chicks received a control diet, while the remaining 150 received a control diet with the addition of methyl donors added on top. The methyl donors included 7030 mg/kg choline chloride, 5 mg/kg betaine, 1.5 mg/kg Vitamin B12, 7.5 mg/kg folic acid, 12 mg/kg pyridoxine, and 99 mg/kg zinc sulfate. Weekly BW were recorded at 28 d and the body composition was analyzed from 60 quail, 30 from the high methyl donor diet and 30 from the control. All comparisons were analyzed using JMP ANOVA. The

remaining birds continued the 2 diets for reproductive performance analysis. At 21 d, quail receiving the diet high in methyl donors exhibited a significant increase in BW ($P < 0.001$). Impact on body composition was observed by significant increases in liver as a result of the high methyl donor diet ($P = 0.003$). Analysis of global DNA methylation in liver from the 2 diets showed a significant increase in %5-methylcytosine in birds fed the high methyl donor diet from 1.1 to 1.6% ($P = 0.03$). Continuation of this trial will evaluate dietary methyl donor effects on reproductive and growth performance, gastrointestinal microbiome, gene expression, and individual gene methylation patterns. This data indicates a change in DNA methylation, which can alter gene expression without altering the genome itself. Similar studies in mice have shown that feeding a diet high in methyl donors can lead to phenotypic changes along with improved health and life span of individuals. Manipulation of breeder diets to impart positive epigenetic effects on progeny may be a useful alternative to growth promotor use in market birds and be of significant economic impact to the poultry industry.

Comparison of the microbial diversity of the tibia in ducks with tibial dyschondroplasia. The complexity of the microbiome has revealed that an organism's health can be correlated with the diverse microbial populations present. For example, the microbiome has been shown to influence many diseases in various organisms. The relationship between the microbiota and abnormal bone development has not been well studied, especially when specifically examining ducks with tibial dyschondroplasia (TD). TD is the primary cause of lameness in commercial poultry and is associated with fast growth affecting bone and cartilage development and often results in bone deformations and infections. This clearly has a negative impact on industry costs and the welfare of the bird. Differences in the microbiota of ducks with TD could provide information for microbiota selection and probiotic administration in developing a healthier duck as well as providing insight for environmental interactions that could influence different microbial communities. For this experiment, ducks were incubated and raised together on a slanted floor system, with a 45-degree slope, to induce lameness (i.e. TD). At 39 days of age, ducks were harvested and tibia samples were taken from individual ducks who exhibited lameness and from those who were not lame. Sixteen tibia samples, 8 from ducks who were lame and 8 from ducks that appeared normal, were sent to Zymo Research (Irvine, CA) for microbial DNA isolation, to 16S sequencing, and analysis. Lame ducks demonstrated abnormal cartilage at the end of the tibiotarsal growth plate that was neither vascularized or mineralized, unlike that of the non- lame ducks. The taxa analysis displayed multiple differences in the microbial community between lame ducks and non-lame ducks. At the phyla level, ducks who exhibited lameness were found to have a three percent upward shift in Firmicute and a three percent downward shift in Proteobacter when compared to ducks that appeared normal. While it is generally accepted that Firmicutes are the predominate phyla in the gastrointestinal tract (GIT), our data supports previous research suggesting that Proteobacter predominate in the tibial joint. Additionally, when evaluating genus-level organisms, there was a tendency for microbes typically present in water and soil to be relatively increased in lame ducks when compared to normal ducks. More research is needed to determine whether the differences in tibia bacteria in ducks exhibiting TD is the cause of the condition or a result of stress associated with TD. Key Words: microbiota, lameness, tibial dyschondroplasia, duck

TN

Dietary manipulation of adipose development in broiler chicks. Commercial broiler chickens accumulate excess adipose tissue due to inadvertent consequences of genetic selection for rapid growth. Feed converted into fat is effectively wasted, increasing costs to producers. The metabolic influence of excess adipose tissue may also impair lean tissue growth and contribute to fertility and skeletal issues in broiler-breeders. To be most efficient, strategies to control fatness in broilers should focus on limiting the initial deposition of excess adipose tissue, which begins shortly after hatch. Based on previous transcriptomic, metabolomic and functional studies, our lab works under the premise that specific fatty acids and other lipid mediators play important roles in the regulation of adipogenesis and adipocyte lipid metabolism in broilers. The hypothesis guiding our current research is that the lipid component of the diet, either in chicks or

in hens, can be manipulated to limit fatness in broilers. In 2017, we continued to investigate maternal programing by dietary fatty acids, and the potential to manipulate chick growth and body composition through the diet of the hen. Previously, we demonstrated that enriching the hen diet in fish oil, relative to corn oil, reduced adiposity in broiler chicks up to at least 14 days after hatch. In 2017, we performed RNAseq of abdominal adipose tissue from these chicks at 7 and 14 days of age, to identify cellular pathways that contribute to reduced adipose deposition. We found that maternal fish oil significantly affected sets of genes involved in cellular growth and differentiation, as well as lipid metabolism. Several of the genes that responded to maternal fish oil have been shown to play roles in obesity in other species. This dataset provides a foundation from which to develop and test follow-on hypotheses to address the mechanism through which maternal dietary fat source can alter body composition in chicks.

TX

Towards Objective 3, we have focused on the commercial important woody breast disease. We completed live bird trials and also analyzed the pectoralis major transcriptome from these birds. These data were then used in comparative analyses against a variety of transcriptome datasets from commercial and heritage broiler strains. One of the important takeaways from our analyses is that there is an age dependent expression pattern that is associated with woody breast. The second, more intriguing result is that there normal phenotypes from the same genetic background are not good negative controls for woody breast gene expression analyses. This work generated a manuscript (PhD Student Shawna Peer) which is currently under revision. In the next year, we will complete analyses of time series and other tissue data that were collected during the same trial. A second area of interest has been the acquisition of gut microbiota and the stimulation of the immune system under different photoperiods in layers and broilers. We completed one study which showed that photoperiods can be used to modulate gut microbiota acquisition. This work by post doc Charlotte Hieke is currently under review. In the next year we will complete additional studies and analyses connecting immune maturation to microbiota structure.

VA

Localization of absorptive and stem cells in the small intestine of chickens. The uptake of nutrients is mediated by transporters located at the brush border membrane of enterocytes that line the intestinal villi. These enterocytes along with other differentiated cells in the villi arise from a population of stem cells. We have used in situ hybridization to identify enterocytes that express the peptide transporter PepT1 and stem cells that express Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) and Olfactomedin 4 (Olfm4). Stem cells expressing Olfm4 and Lgr5 mRNA were localized to the intestinal crypts. At e19, when the intestinal crypts were still rudimentary, cells staining for Olfm4 mRNA were already present. Enterocytes lining the intestinal villi expressed PepT1 mRNA. At day of hatch (doh), there was a population of cells located between the intestinal crypt cells and mature enterocytes that expressed neither PepT1 nor Olfm4 mRNA. These cells are likely transit amplifying (TA) cells, which serve as intermediate progenitor cells.

Expression of host defense peptides in the intestine of turkeys. The host defense peptides (HDP) play an important role in innate immunity. In aves, the HDP consist of avian beta defensins (AvBD), cathelicidins, and liver expressed antimicrobial peptide 2 (LEAP2). The mRNA expression profiles of six AvBD, two cathelicidins and LEAP2 have been determined in the intestine of turkeys from day of hatch (doh) until day 28 post-hatch. AvBD1, AvBD8 and AvBD13 mRNA increased from doh to day 28 in the duodenum, while AvBD10 decreased from doh to day 7 in the duodenum, jejunum, and ileum. LEAP2 mRNA increased from doh to day 7 post-hatch in the duodenum and jejunum.

Expression of host defense peptides in the intestine of chickens challenged with *Eimeria.* The mRNA expression profiles for the avian beta defensins (AvBD) and liver expressed antimicrobial peptide 2 (LEAP2) in chickens challenged with different species of *Eimeria* (*E. acervulina*, *E.*

maxima, and *E. tenella*) have been investigated. There was downregulation of LEAP2 mRNA in the duodenum, jejunum, and ileum of *E. maxima* challenged chickens; however, changes in AvBD mRNA expression were not consistent between two different *E. maxima* challenge studies. Using in situ hybridization, LEAP2 mRNA was found to be expressed in intestinal enterocytes, which suggests that LEAP2 but not AvBD plays an important role in modulating an *Eimeria* infection.
The chicken LEAP2 gene was screened for sequence variation that was associated with expression levels of LEAP2 mRNA or symptoms of coccidiosis following an *E. maxima* challenge. A number of polymorphisms were identified that were located in the introns (7), exons (2) and the putative promoter region (7) of the LEAP2 gene. These polymorphisms, however, did not appear to be associated with gene expression level or symptoms of coccidiosis such as weight gain depression and lesion score.

Expression of host defense peptides and nutrient transporters in the intestine of chickens challenged with *Campylobacter.* The mRNA expression profiles of nutrient transporters and host defense peptides (HDP) was examined in the intestine of broilers challenged at day of hatch with three doses of *Campylobacter jejuni* (10^6, 10^7, and 10^8 colony forming units, cfu). Gene expression was measured in the duodenum, jejunum, ileum and cecum at days 7 and 14 post challenge. There were dose-, tissue-, and age-specific changes in gene expression. There was upregulation of selected amino acid and monosaccharide transporters along with the zinc transporter in the 10^6 cfu group. For the HDP, which included the avian beta defensins (AvBD) and liver expressed antimicrobial peptide 2 (LEAP2), there was a delayed response with only upregulation of a few AvBD at day 7 post challenge, but upregulation of all AvBD at day 14 post challenge in the 10^6 cfu group. These results suggest that at a low dose of *C. jejuni* (10^6 cfu), there is an increase in nutrient transporter and AvBD mRNA to try to counter the infection, but that at higher doses there is suppression of the cellular response.

Genetic analysis of inflammatory response genes in the turkey (*Meleagris gallopavo*). Though progress has been made in the genome analyses of the turkey, *Meleagris gallopavo*, our understanding of the genotype: phenotype relationships continue to lag those of other agriculturally important livestock and poultry species including the chicken. Here, we report initial investigations in our lab of the genetics of inflammation in the turkey using comparative information from the chicken. Using chicken genes for NLRX1 and turkey genes for IL8, we designed and tested primers using heritage turkeys exposed to LPS. We screened IL8 and NLRX1 nucleotide variants that may be informative for the turkey's response to lipopolysaccharide that causes inflammation A total of 344 sequences for both genes were screened. Six SNPs were identified and validated. The SNPs do not appear to be correlated with response to LPS. The association of the SNPs with inflammation is now underway. It is hoped that further examination will uncover how genetic variations are associated with differences in inflammatory response to LPS and furthermore, aiding in the understanding of phenotype: genotype correlation.

Brief Impact Statements

ADOL

- Ikaros is the first Marek's disease driver gene. Based on human and mouse studies, somatic mutations in the Zn-finger binding domains will lead to uncontrolled proliferation of T cells. Meq is likely to prevent apoptosis of these rapidly growing cells by inhibiting bcl-xL.
- HVT and SB-1 MD vaccines appear to replicate differently with respect to time after vaccination and organ, which may explain their interaction to enhance vaccinal protection.
- MDV induces specific immune responses that alter the colonization of the gut

- A total of 170 candidate genes were identified for the first time, which may contribute to genetic resistance to MD.

AR

- A new brain structure involved in stress has been identified. It is called the nucleus of the hippocampal commissure and may function as part of the classical hypothalamo-pituitary- adrenal (HPA) axis in avian species.
- Providing new molecules and additional key mechanisms into the cellular pathways for muscle growth, muscle mass, and feed efficiency in breast muscle of broilers will contribute to improving production efficiency and the prevention of metabolic diseases.
- Identification of the genetics of ascites will allow breeders to select against ascites and reduce production losses
- Development of management strategies to reduce lameness is critical for reducing a significant animal welfare issue in broilers.

AZ

- Our work provides fundamental annotation information (both structural and functional) that underpins functional modeling of genomic data sets. This enables poultry researchers to more accurately identify genes involved in the systems they are studying and translate long lists generated by functional genomics into a biological model that they can use to improve poultry production.

CA

- ChIP-seq and ATAC-seq assays developed and other omic data generated for regulatory elements annotation will be important for animal genome community.
- Identification of genes that are associated with resistance to heat stress and Newcastle disease virus and can be used to genetic enhancement of disease resistance of chicken in adaption to hot climate.
- Knowledge of genes associated with enhanced immune response may inform further information on vaccine efficacy in poultry production.
- Understanding impact of gut associated pathogen on microbiota composition at different development stages will provide great insights in improve gut health and subsequently increase production efficiency and animal well-being.

COH

- The genetic variability in the MHC-Y region may differentially influence immune responses with some MHC-Y haplotypes being more valuable in the genetic control of resistance to infection or colonization than others. Continuation of this work will help to define the role of MHC-Y in immunity and the interactions of chickens with the microbes that colonize them.

DE

- Our results from RNA-seq analysis of tow inbred chicken lines and their F1 reciprocal crosses indicate that *additivity* is the predominant gene expression pattern in embryonic liver and brain at Day 12. The second and third prevalent gene expression patterns are dominance and over-dominance. Additionally, our results show that only 7-20% of the DE genes exhibit allele-specific expression in the F1, suggesting that differential trans–regulation is largely responsible for differential gene expression between parental lines and is the major regulatory mechanism leading to heterosis in the F1 cross. [*manuscript is in preparation*]
- Our study shows that development of Wooden Breast myopathy in chickens assumes stepwise structural perturbations involving various tissues within the breast muscles during the growth period, key among them being the vasculature, components of the

extracellular matrix and the myofibers. Further, lipid infiltration (indicative of disturbances in fat metabolism) appears to play a key role in the pathogenesis of the myopathy. [*manuscript in preparation*]

- Differential gene expression data show changes in the liver of WBD affected chickens suggesting the myopathy is more physiologically complex than previously thought. [*work in progress*]
- Results suggest increased fat deposition in male birds and the cranial aspect of the muscle. The up-regulated genes involved in oxidative stress in male birds support a hypothesis that the development of WBD is related to increased oxidative stress. [*work in progress*]
- Results exhibited that differential expression analysis tools, Cuffdiff and Deseq, are not susceptible to aligners and produced similar outcomes. Cuffdiff presented susceptibility to number of replicates and had better performance in larger groups. [*manuscript is in preparation*]
- We have identified over 3 million variants (SNPs and indels) between the three tissues tested for ASE, of which 325,000 had testable variants. Out of those testable variants ~12% of them showed ASE in at least one sample tested. The overlap of ASE SNPs among the 3 tissues was found to be only ~11%, suggesting that ASE is a tissue dependent mechanism in chickens. [*work in progress*]
- We developed a new tool to prioritize candidate genes and characterize sample behavior in differential expression analysis of transcriptomic dataARB (Analysis of RNA-seq Data Behavior) is a simple, robust and convenient tool that allows for evaluation of data behavior as well as prioritization of DE genes derived from RNA-seq data. ARB can be run on all operating systems in high-performance computing environment as well as locally on personal computer, and is publicly available at https://github.com/AbashtLaboratory/ARB. The program paper was just submitted (Dec 2017) to BMC Bioinformatics for review.

FL

- Chicken is an important agricultural species as one of the most important sources of animal foods worldwide. To further the research on chicken health, the enzyme annotation is necessary as this class of proteins often represents a key part of many regulatory and signaling pathways. Phosphorylation is an important post-translational modification controlled by kinases and it regulates essential cellular processes. Moreover, this modification as well as kinases regulating it, have been implicated in animal diseases. Utilizing functional genomics data to understand disease in poultry requires the availability of functional information for genes. Our work adds to this foundational knowledge that helps effective use of investments made in sequencing the chicken genome that is critical for applying “omics” approaches.

GA

- Heat stress affect growth possible via changes in nutrient and amino acid transporters
- Broiler chickens maintain the same level of amino acid digestibility under heat stress
- Heat stressed birds incorporate more amino acids into growth than birds raised in a thermos-neutral environment
- Cysteine is the amino acid incorporated most under heat stress.
- Using different empirical cut off values for FST, most QTLs were correctly detected using as few as 2.5% of SNP markers in the panels.
- Genomic similarity, calculated based on the selected SNPs, was very high (>0.80) for individuals with similar genetic and phenotypic values despite having limited to no pedigree relationship
- Filtering SNPs using FST could be beneficial for use in GWAS by focusing on genome regions under selection pressure.

- High functional genomic similarity based on selected markers indicates similarity in SNP signatures, regardless of relatedness, and translates into high phenotypic correlation that could be used in decision making.

IA

- The feasibility of applying molecular genetics and genomics to analysis of variation in structure, function and gene expression within the chicken genome was demonstrated.
- Genes, pathways and genomic regions associated with important biological traits in chickens were identified.
- Genetic variation in commercial research lines, research lines and indigenous lines of chickens from around the globe was characterized.
- Heat exposure was found to enhance gene expression changes over time in laying hens.
- Important factors for optimal use of genetic evaluation models were identified.

MD

- The results support the hypothesis that variations in egg production rates among turkey hens are due in part to differential expression of genes in the HPG axis involved in ovulation and ovarian steroidogenesis. These and our future results will be potentially useful to poultry breeders interested in increasing egg production in turkey hens.
- By high density SNP array, we found Copy Number Variations in two prestigious inbred lines. Song and his collaborators will validate the CNV in deep sequencing data. The validated CNV will be genetic marks in the future MD resistance study.
- The chick embryo has a long and distinguished history as a major model system in developmental biology. We explored genomics and epigenetics studies in chicken stem cells and found a lot regulatory elements and pathways. We do believe that in combination with classical techniques, the chicken is now one of the most versatile experimental systems available for variety of research and human health.
- Today, from genomics and epigenetics studies, the analysis of temporal gene expression is still a challenge. We proposed to detect threshold point in temporal gene expressions under multiple biological conditions, which will be of importance in gene regulatory network analysis.

MI

- Thermal challenge poses a multi-dimensional threat to poultry production systems, with direct impacts on meat production and quality. The information derived from these studies will be used to develop more effective breeding, nutritional, and management strategies that can be used by poultry breeders and growers to advance the production of consistent, high quality muscle food products.

MN

- 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. Likewise, AFB_1 causes annual industry losses estimated in excess of $500 M. Increasing innate resistance to AFB_1 could result in numerous health benefits. Transformational improvements in AFB_1 resistance require a multidisciplinary approach to identify protective alleles with potential to reduce disease. Genetic markers to improve AFB_1-resistance have a potentially high commercial value and positive economic impact to industry, owing to improvements in health and well-being, productivity, and a safer product for consumers. The gastrointestinal health of an

animal is key to its successful growth and development. Elimination of subtherapeutic antibiotics for growth promotion and health in poultry will leave a critical void. This project will improve our mechanistic understanding of host-microbiome interactions in the avian host, and identify feasible approaches towards modulating the turkey intestinal microbiome resulting in enhanced health and performance.

MS

- This work provides functional annotation that enables poultry researchers to accurately identify the role of genes identified using functional genomics experiments and expedites knowledge discovery from long lists of genes/proteins for improving poultry health and production. During 2017, the AgBase resources were visited by 18,497 different researchers, with 28.7% of these visitors from the US (includes visitors from 48 states). AgBase was cited in 68 publications during 2017. HPIDB is one of the12 databases that are partners in the International Molecular Exchange Consortium (IMEX) and the only database that provides detailed, manual curation for livestock pathogens.

NC

- Gene editing in avian cells appears to be cell-type specific. While model cell lines can be used as a starting point for gene editing in avian cells, efficient conditions for PGCs will require standardization.

TN

- This study suggests that the chick genome can be programmed in a way that reduces fat deposition, and raises questions about the role of the hen diet in epigenetic control of chick metabolism.

VA

- We have identified and are studying the ontogeny of stem cells in the yolk sac and small intestine. Because stem cells are precursors of absorptive cells necessary for nutrient uptake, this knowledge will enhance our understanding of growth and development of the embryonic and post-hatch chick.
- We continue to investigate the expression profiles of the host defense peptides in chickens and turkeys during normal and diseased challenged states. We have shown that the host defense peptide LEAP2 is downregulated following *Eimeria* challenge and may play an important role in mediating an *Eimeria* infection. However, there does not appear to be an association between LEAP2 polymorphisms and symptoms of coccidiosis.

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Books and Chapters in Books

1. Aggrey, S. E, F. González-Cerón and R. Rekaya, 2017. Gene associated with functional traits in poultry: implications for sustainable genetic improvement. In: Achieving sustainable production of poultry meat, Volume 2. Breeding and Nutrition, Editor: Todd Applegate, Burleigh Dodd Science Publishing Ltd, Cambridge, UK Pg 3-24. DOI: 10.19103/AS.2016.0011.02
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PhD Dissertations and Master Theses

1. Beckford, R. 2017. Unraveling the molecular mechanism of fat deposition through dietary manipulation and feed restriction. PhD Dissertation. University of Tennessee.
2. Dey, S. 2017. Genome Wide Association and Next Generation Sequencing Approaches to Map Determinants of Ascites in Broiler Chickens. Ph.D. Dissertation. University of Arkansas, Fayetteville.
3. Garcia, J. 2017. Temporal and tissue specific changes in expression of nutrient transporters and host defense peptides in young broilers during Salmonella and Campylobacter infections. PhD Dissertation. Virginia Tech.
4. Nagarajan, G. 2017. Central role of vasotocin in the neuiroendocrine egulation of stress responses and food intake in chickens, *Gallus gallus*. Ph.D. Dissertation. University of Arkansas, Fayetteville.
5. Ojha S. 2017. Identifying Genetic Factors Influencing Sperm Mobility Phenotype in Chicken using Genome Wide Association Studies, Primordial Germ Cell Transplantation, and RNAseq. Ph.D. Dissertation. University of Arkansas, Fayetteville.
6. Zhuo, Z. 2017. RNA Sequencing to Study Differential Gene Expression and Allele Specific Expression in Chickens. PhD Dissertation. University of Delaware.

Funding and Leveraging

Grant	Funding
Adapting chicken production to climate change through breeding. USDA-NIFA-AFRI; PI: Schmidt; CoPIs: Lamont, Rothschild, Persia, Ashwell	$4,700,000
Antibiotic-free alternatives to improve health and performance in commercial turkeys. USDA-NIFA-AFRI. 2016-2018; (co-I w/ Johnson (PI), Baumler, Knights, and Noll	$464,000
Antistress compounds as effective tools for addressing chronic stress. ABI; 07/2017-05/2018; PI: Kuenzel; CoPIs: Kumar, Christensen, Kang.	$50,000
ARS CRIS Project, Employing Genomics, Epigenetics, and Immunogenetics to Control Diseases Induced by Avian Tumor Viruses. PI Cheng	NA
ARS CRIS Project, Genetic and Biological Determinants of Avian Tumor Virus Pathogenicity, Transmission, and Evolution. PI Cheng	NA
Verification of Allele Specific Expression in Chicken Embryonic Brain and Liver by using Minor Allele Finder. Delaware INBRE Core Center Access Award August 2016- January 2017, Supported by the NIH NIGMS IDeA Program (P20 GM103446); PI: Behnam Abasht	$3,960
Development of colonization resistance in chicks NIH-NIFA Dual purpose with Dual benefits. 2015-67015-22930. A. Baumler, H. Zhou	NA
Development of Methods for Knockout Chickens: CRISPR-Cas Genome Editing To Understand Foodborne Pathogen-Host Interactions in Poultry, USDA- National Institute of Food and Agriculture, 2015-2017; PI: Koci; Co-PIs: Petitte, Hassan	$100,000
Effect of AFB1 on immune tissues of turkeys from diverse genetic backgrounds. USDA-UMN Multi-State Project, 2016-2018. Strasburg	$94,221
Egyptian Ministry of Education 2016; PI: Aggrey, S. E.	$7,000
Follicle Selection and Development in Chickens. USDA NIFA Foundational Program 2017- 2020; PI: Johnson.	$499,745
Follicle Selection and Differentiation in the Avian Ovary. NSF, 2014-2017(IOS-1354713); PI: A.L. Johnson.	NA
Genome wide identification and annotation of functional regulatory regions in livestock species USDA NIFA 2015-67015-22940 H. Zhou, P. Ross, I. Korf	NA
Genomics for improving animal production USDA NIFA National Need Training Grant 2014-38420-21796 H. Zhou, J. Murray, P. Ross.	NA
High throughput characterization of gene transcript variants by full-length single-molecule sequencing to improve farm animal genome annotation. USDA NIFA 2016. P. Ross, H. Zhou. J. Medrano	NA
Improving food security in Africa by enhancing resistance to disease and heat in chickens; Feed the future innovation lab for genomics to improve poultry, USAID AIDOAA-A-13-00080; PI: H. Zhou; CoPIs: D. Bunn, R. Gallardo, S. J. Lamont, J. Dekkers etc	$6,000,000
Improving the Efficiency of Livestock Production Using Genetic Selection. USDA-National Needs training grant; PI: Lamont (original PI: Spurlock); coPI: Rothschild, Garrick	$262,500
Industry funding. Aviagen Limited, EW Group, Hy-Line, International, Iowa Egg Industry Center Lamont, Dekkers, et al.	NA
Inferring Causal Phenotype Networks Using Genomic Information. USDA-AFRI; 3/2011-2/2016; PI: Rosa	$467,290

Influence of thermal challenge on turkey muscle development and meat quality. USDA-NIFA-AFRI. 2014- 2018. PD: G.M. Strasburg; co-PDs: Kent Reed, Sandra Velleman, and William Atchison.	$975,000
Marker Assisted Selection for Ascites Resistance in Broilers. NIFA-AFRI; 11/2014-10/2017; PI: Rhoads; coPI: Anthony, Kong, Schmidt	$467,000
MHC-Y-directed immune responses during colonization of chickens by Campylobacter USDA NIFA 2016. Miller	NA
NIH-NIAAA P60 AA007611. Genomics and epigenomics of alcohol preference; PI: Muir; coPI: Feng Zhou	$750,000
NLRP3 Inflammasome activation and lameness in chickens. USDA Animal Health; 7/16-6/19; PI: Dridi; CoPI: Rhoads	$45,000
PACBIO P6-C4 Sequencing of Chicken Chromosome 16 BAC Clones USDA NRSP-8 funding. Miller	
Polymorphic MR1-like Molecules in Immunity to Microbial Pathogens, Caltech-City of Hope Biomedical Initiative with Pamela Bjorkman. Miller	NA
Regression of Rous Sarcoma Virus-Induced Tumors in Arkansas Regressor Chickens –Mechanisms and Implications for Tumor Treatment. ABI; 07/2016-05/2019; PI: Anthony; coPI: Kong, Dridi, Greene.	$150,000
Replacement Autoclave for Ferritor Hall. ABI; 7/2016-6/2017; PI: Rhoads	$47,170
Role of mitochondrial hormone receptors in cell bioenergetics; relevance to metabolic syndrome. ABI; 07/2016-05/2019; PI:Bottje; coPI: Kong, Dridi, Rochelle, Hakkak, Baum	$150,000
Strategies to enhance de novo biosynthesis of methionine in organic chicken. USDA-NIFA 3/14-2-18; PI: Aggrey, S.E.	$500,000
Targeting Mitochondrial Health in the Prevention of Cancer-Cachexia Induced Muscle Atrophy. ABI; 07/2016-05/2017; PI: Greene; coPI: Kong, Washington.	$30,000
The biological mechanisms that underlie dietary methionine in the mitigation of the effects of heat stress in the broiler chicken. Evonik Nutrition and Care GmbH, Germany 2016 (10/16-12/18). PI: Aggrey, S.E; CoPIs: R. Rekaya and W. K. Kim	$365,000
UMD-UMB Research and Innovation Grant 2015-2017	NA
US Poultry, Cattle and Swine Genome Coordinators Funds for farm animal ENCODE Aviagen Limited National Pork Board CA no PI reported	NA
US-UK Collaborative Research: Host Resistance to Avian Pathogenic E. coli. USDA-NIFA-AFRI/BBSRC; PI: Lamont; coPIs: Wolc, Kaiser, Stevens, Vervelde	$499,999
USDA AFRI Competitive grant ARZT-3013680: Enabling network analysis of host-pathogen interactions. 2015-2017. PI McCarthy; co-PI: Nanduri	$487,987
USDA National needs fellowship for enhancing animal production: Addressing national need in poultry production. USDA-NIFA-NNF. 2016-2021. Strasburg	$241,000
USDA-NIFA Grant No. 2016-67015-25027) 03/01/2016 - 02/29/2020 Title: Genome-wide Identification and Functional Validation of Genes Causing Susceptibility to Wooden Breast Disease in Commercial Broiler Chickens My role; PI: Behnam Abasht; CoPIs: Jack C.M. Dekkers, Sandy G. Velleman and Carl J. Schmidt	$500,000

USDA, AFRI, award no. 2013-67015-21330, Genome biology of Marek's disease: Viral integration and genome alterations in genetically resistant and susceptible stocks. PI: Cheng; coPIs: Mary Delany (UC Davis) and Bill Muir (Purdue U.).	$499,997
Evaluation of Hansen probiotics for protection against BCO lameness on litter with exposure to *Staphylococcus agnetis*. Chr Hansen; 2-12/2017; PI: Rhoads, CoPI: Alrubaye, Koltes.	$33,614
Evaluation of Zinpro micronutrients for protection against BCO lameness and improving bone health for broilers raised on wire flooring. Zinpro; 9/1/2017-12/31/2017; PI:Rhoads, CoPI: Alrubaye, Dridi.	$39,461
Global Expression Pathway Analysis Training: Target Obesity. Chancellor's Discovery, Creativity, Innovation, and Collaboration Fund. 9/2017-7/2019; PI:Bottje, coPI:Rhoads, Kong	$76,500
Determination of roles of mitochondrial small RNAs in metabolic disease phenotypes using isocitrate dehydrogenase 2 (IDH2) knock-out mice and genetically selected chicken models. ABI; 07/2017-05/2018; PI- Kong, CoPI- Kim, Bottje, Anthony, Owens, Dridi	$48,500
US Poultry, Cattle and Swine Genome Coordinators Funds for farm animal ENCODE Aviagen Limited, National Pork Board, Zoetis. PI H. Zhou	NA
Research was and is supported by a USDA NIFA Foundational Program grant in the area of Understanding Antimicrobial Resistance. Award Number: 2017-67017- 26570, by a small NSRP-8 award for SMRT sequencing, and by City of Hope. PI Marcia M. Miller	NA
USDA-NIFA-Agriculture and Food Research Initiative #2013-00809 Glucocorticoid induction of endogenous growth hormone (GH) in chicken embryos. 1/1/2014-12/31/2017; PI T. Porter	$499,816
Maryland Agricultural Experiment Station-Competitive Grant Program Identification of mechanisms and gene networks associated with differences in egg production in turkey hens. 1/1/2016-6/30/2017 PI T. Porter	$30,000
USDA-NIFA-Agriculture and Food Research Initiative #2016-08370 Mechanisms affecting posthatch growth following embryonic induction of growth hormone in broiler chickens 3/1/2017-2/28/2020, PI T. Porter	$484,000
USDA-NIFA-Agriculture and Food Research Initiative #2016-09789 Mitigation of heat stress in broiler chickens through early-life thermal conditioning 5/1/2017-4/30/2020; PI T. Porter	$500,000
Identifying active deubiquitinases and kinases in chicken. USDA AFRI grant # 2015-67016-22939, 2014-2018, PI: Nanduri; co-PI: Edelmann	$149,958
The nutrient transporter research has resulted in a USDA-NIFA grant. PI Eric Wong	NA
Total	**$20,218,718**