**171 Genetic characterization of commercial broiler lines experimentally infected with Subgroup J Avian Leukosis Virus (ALV-J).** M Karaca*, J. K. Rosenberger, and S. S. Cloud, *University of Delaware, Newark,DE* .

This study was initiated to determine the genetic basis for weight gain or immune response in chickens infected with ALV-J. Embryos from 4 genetic lines of chickens were inoculated with the UD-4 isolate of ALV-J by the yolk sac route at 3 days of incubation. An equal number of embryos were sham inoculated. At hatch chicks were vaccinated with NDV and IBV and during grow-out exposed to infectious bursal disease virus, reovirus and chicken anemia virus to simulate commercial conditions. Body weights were measured every 2 weeks. At 5 weeks, chickens were assessed for their ability to clear *E.coli* following intravenous inoculation. This was done because ALV-J is believed to compromise resistance to bacterial infections. Biweekly body weights and *E.coli* blood clearance assays were analyzed using the GLM procedure of SAS. A total of 129 (A=42, B=40, C=47) chickens were genotyped using 33 microsatellite markers. The association of genotype with the phenotypic measurements of body weight and *E.coli* clearance were determined for sham vs ALV-J inoculated chickens. The heterogeneity of each locus was calculated within lines. The average observed heterogeneity of line A, B and C over 33 loci was 30.65 %, 33.31 % and 31.29 % with 3.24, 2.97 and 3.45 alleles per locus, respectively. The average allele sharing between lines A and B was 72.23 %, between lines A and C it was 66.07 % and 61.91 % between lines B and C. Overall, the lines shared 55.38 % of their alleles. The genetic distance between line A and B or C was found to be 25.82 or 27.41 % respectively. Both suggestive ($P<0.10$) and significant ($P<0.05$) associations of marker genotypes with quantitative trait loci (QTL) affecting biweekly body weights and clearance of *E.coli* were detected. Two marker loci were found to be significantly ($P<0.05$) associated with body weight at 2 and 4 weeks of age for line A. One marker genotype had a significant effect ($P<0.05$-$P<0.10$) on clearing pathogenic *E.coli*. Further characterization of the genetic lines is continuing by genotyping progeny tested sires with 100 microsatellite markers.

**Key Words:** Genotyping, Subgroup J Avian Leukosis Virus (ALV-J), Disease resistance

**172 Relationships between skeletal growth and body weight in Japanese quail selected for 4 week body weight.** J. M. Reddish*, A. El-Keredy, K. E. Nestor, and M. S. Lilburn, *Dept of Animal Science, The Ohio State University, Ohio Agricultural Research and Development Center*.

A weight selected strain of quail(HW) selected for increased 4 week body weight was compared with a randombred control to investigate the relationship of skeletal growth and selection for body weight. The heavy weight (HW) strain was derived from the randombred control strain (RBC). Body weight differences (HW>RBC) between strains were consistent throughout the experiment. The objective was to investigate the growth of the tibia and femur and determine if selection for resulted in differences between the HW and RBC strains. Measurements were taken of body weight, length and width of both the tibia and femur at weekly intervals from 1 to 35 days of age. Body weight difference (50%) was consistent throughout the experiment (HW>RBC) and growth patterns were similar. Differences in skeletal measures and body weight were only 15% for both RBC and HW ($p<.01$). Correlations for skeletal traits and body weight were positive, and significant($p<.01$) for RBC thru day 28 but HW showed disruption in this pattern. The results suggest that selection for body weight and skeletal growth are symmetrical in the RBC, but different in HW. The HW strain has proportional growth but does not have symmetry between skeletal traits and body weight as noticed in the RBC strain.

**Key Words:** selection, quail, growth

**173 The effect of selection for increased egg production in turkeys on incubation characteristics of embryos.** A. L. Antonelli*, K. E. Nestor, and M. S. Lilburn, *Department of Animal Sciences, Ohio State University/OARDC, Wooster, OH*.

In strains of turkeys selected for egg production (E; Nestor et al., 1980, 1995), genetic increases in egg production are proportional to decreases in the weight of the albumen and yolk, as well as the total egg weight when comparisons are made to a randombred control line (RBC1). In the present study, E line hens were mated to E line toms or RBC1 toms, and RBC1 hens were mated to RBC1 toms. Egg weight at set was 67.2 $\pm$ .39g for E/E eggs, 65.6 $\pm$ .32g for E/RBC1 eggs, and 87.16 $\pm$ .68g for RBC1/RBC1. At 21 days of incubation, E/E yolk-free embryo weight was 5% less than in E/RBC1 embryos, yet at 25 days there was no differences. At hatch, E/E yolk-free embryo weight was 5% greater than that of E/RBC1 embryos. Overall, the E/E line embryos had a 64% increase in yolk-free weight between 21 and 25 days with overall weight gain of 95%. E/RBC1 had only a 56% increase in yolk-free weight between 21 and 25 days with overall weight gain of 70%. RBC1/RBC1 embryosl had an 80% increase in yolk-free weight between days 21 and 25 with an overall weight increase of 118%. At 21 days of incubation, RBC1/RBC1 yolk-free embryo weight was 11% heavier than the E/E line and these differences increased to 25% and 26% at 25 d and hatch (28 d), respectively. The data suggest that genetic differences in embryonic development are maximally expressed from 21 to 25 days of incubation, concomitant with the period of maximal yolk lipid transfer to the development embryo.

**Key Words:** Turkey, Incubation, Embryo

**174 Germ-line transmission of a *lacZ* gene in chickens using an avian Spleen Necrosis Virus-based vector.** S. Borwornpinyo*, D.W. McCoy, P.E. Mozdziak, and J.N. Petitte, [1]*North Carolina State University*.

Replication-defective retroviral vectors based on the avian spleen necrosis virus (SNV) have been successfully used to produce transgenic chickens. One particular vector, SNTZ, expresses nuclear-directed beta-galactosidase. The SNTZ vector has been used as a cell lineage marker for the analysis of early embryonic development of the chick. The purpose of the present study was to produce transgenic chickens using SNTZ. High titers ($2.0 \times 10^7$ virion/ml) of SNTZ were injected into the subgerminal cavity of 66 stage X white Leghorn embryos followed by *ex ovo* embryo culture. Of the 66 infected embryos, 16 hatched (24%). To date, of the 16 hatched chicks, 12 have reached sexual maturity. Genomic DNA from blood and semen samples from these $G_0$ birds were tested for the presence of *lacZ* sequences using the polymerase chain reaction in combination with primers specific for *lacZ*. Out of 7 hens, 2 birds had detectable *lacZ* sequences in their blood. Of the 5 cockerels, 3 were carrying the bacterial *lacZ* gene in the semen. To date, test mating of males and females yielded germ-line transmission from one male at a frequency of 1 out of 20 offspring. No correlation was observed between the presence of *lacZ* sequences in circulating red blood cells and semen. The results of this study suggest that the SNTZ vector can be used to develop a line of transgenic chickens that expresses nuclear-directed bacterial beta-galactosidase. Support was provided through funds under projects NC05293 (PEM), NC06590 (PEM), and NC0168 (JNP).

**Key Words:** retroviral vectors, germ-line transmission, transgenic

**175 Molecular characterization of the genomic chicken prolactin receptor (cPRLR) gene from a native Chinese chicken (Wai Chow strain).** Angela Hui* and Frederick Leung, *University of Hong Kong*.

The aim of this study is to clone and characterize the genomic cPRLR gene from a native Chinese chicken (Wai Chow strain). Prolactin receptor (PRLR) participates in a number of physiological functions in birds including reproduction, maternal behavior and osmoregulation. It is widely distributed in many tissues including the brain, the testis and, particularly abundant, in hypothalamus and the anterior pituitary gland. The cDNA of PRLR has been previously shown to consist of an extracellular ligand-binding domain, a single transmembrane and an intracellular domain involved in signal transduction. However, the genomic molecular structure of the cPRLR gene is still lacking. We used the Polymerase Chain Reaction (PCR) trapping method. By designing different pairs of primers flanking the possible intron splice sites, fragments of introns can subsequently be amplified for subcloning and further sequencing analysis. Three introns have been successfully trapped, and the estimated sizes are 1.4kb, 1.5kb and 1.7kb. These DNA fragments were subcloned and further confirmation by DNA sequencing. Consequently, these sequences will be analysed in the databank for any

significance discovered, which helps further understanding of the gene expression pattern and to reveal important pieces of evidences on the gene diversity. Tissue culture will also be carried out to further characterize the promoter region using deletion expression approach.

**Key Words:** Chicken, Prolactin receptor, Intron

**176 Molecular characterization of the chicken prolactin (PRL) gene:genomic gene structure, its polymorphism and promoter analysis.** Florence Au* and Frederick Leung, *University of Hong Kong*.

Prolactin (PRL) is a polypeptide hormone of the anterior pituitary gland and has been shown to have a diverse spectrum of biological activities and functions in all vertebrates. Characterization of the chicken PRL gene and its polymorphism is the first step in establishing the genotypes and traits association. Polymerase chain reaction (PCR), cloning and sequencing were used to obtain the four intron sequences of the chicken PRL genomic gene. PCR products indicated that the sizes of the four introns are of 1.5 kb, 0.5 kb, 1.3 kb and 2.0 kb respectively. Sequence analysis of the four PRL introns of the chicken PRL gene revealed that they share high homology with that of the turkey PRL gene and the other strains of chicken PRL gene. In addition, PCR-restriction fragment length polymorphism (PCR-RFLP) was used to identify polymorphic sites within the four introns. An Ava II enzyme cut site in the first intron was found to be polymorphic in Chinese native (Guangdong Xing Hua) chickens. PCR-RFLP was also applied to other chicken strains such as broiler, layer and other native Chinese strain (Shek kai). However, No polymorphism was identified. Present results enable extended study in the PRL gene diversity. In addition, an approximated 2 kb 5' flanking of the cPRL was also obtained by PCR and confirmed by DNA sequencing. Deletion analysis and luciferase reporter gene assay will be used to characterize and define the promoter region in PRL gene expression.

**Key Words:** Chicken, Prolactin, Polymorphism

**177 Detection of a single nucleotide polymorphism in exon 10 of the chicken growth hormone receptor gene.** Joanna Lau* and Frederick Leung, *University of Hong Kong*.

Interaction of Growth Hormone (GH) with its receptor (GHR) is required for normal growth in both mammalian and avian species. Defect in GHR gene functions caused by mutation is one of the major causes of genetic disorder, eg. Laron Syndrome and Dwarfism in human and chicken respectively. Recent studies suggest that polymorphisms on both the GH and GHR genes in chicken might serve as genetic markers for phenotypes of commercial values. In this study, a new restriction fragment length polymorphism (RFLP) at exon 10 of the GHR gene has been identified in native Chinese strain chicken, using polymerase chain reaction (PCR) and Alu I restriction enzyme digestion. The same study has been extended to broiler and layers strains and no RFLP was detected. The PCR-RFLP results were further verified by direct sequencing analysis and confined to a single nucleotide polymorphism (SNP) silent mutation at position 924th nucleotide (GA) counting from the translational start site on the published cDNA sequence, resulting in no change in the amino acid. Future studies will be set up to examine whether such mutation has any association with phenotypic traits.

**Key Words:** Chicken growth hormone receptor, Restriction fragment length polymorphism (RFLP), Single nucleotide polymorphism (SNP)

**178 Candidate genes and reproductive traits in a commercial broiler breeder population, an association study.** I C Dunn*[1], Y-W Miao[1], A Morris[2], M N Romanov[1], D Waddington[1], P W Wilson[1], and P J Sharp[1], [1]*Roslin Institute, Roslin, Midlothian EH25 9PS, Scotland,* [2]*The Cobb Breeding Company, East Hanningfield, Essex, CM3 8BY, England*.

To take advantage of programmes to identify candidate genes for variation in traits of economic importance, methods to test these genes in selected pedigree populations need to be developed. To this end we have carried out a study of association between candidate genes and reproductive traits in a pedigree line of broiler breeders. Gonadotropin releasing hormone (GnRH), its receptor (GnRHR), growth hormone receptor (GHR) and neuropeptide Y (NPY) were selected for their role in controlling aspects of reproduction. Genetic markers for NPY, GnRHR and GHR alleles were detected using bulk PCR-restriction fragment length polymorphism or BESS-T Scan (Epicentre Technologies). Genotyping of 772 hens from one generation was by PCR-restriction fragment length polymorphism. Total number of eggs, age at first egg (AFE) and number of double yolked eggs (DY) for each hen were recorded. Additive and dominance effects were fitted for the autosomal GnRHR and NPY genes; additive effects were fitted for the sex linked GHR gene. To control for some of the background genetic variation, candidate genes were assessed within heterozygous sire families. A dominance effect of NPY (14 sire families) on AFE and an additive effect of GnRHR (36 sire families) on DY, were found ($P<0.02$). If the latter effect were true, selection could increase overall flock performance by 0.13 usable eggs per hen. A simplified model, omitting sires, was also fitted. This analysis gave four significant associations ($P<0.05$), a surprisingly large number. In conclusion it is possible to detect association between economic traits and candidate genes in a population undergoing selection, and test if a candidate gene explains some of the trait variation. However, statistical associations between trait and genes require to be treated with caution and models should account for as many genetic and environmental variables as possible.

**Key Words:** Reproduction, Candidate genes, Association

**179 Mapping QTL Loci Affecting Growth And Disease Resistance to Avian Coccidiosis.** J Zhu*[1], H Lillehoj[1], C Van Tassell[2], M Emara[3], P Allen[1], H Cheng[4], D Pollock[5], M Sadjadi[5], and T Sonstegard[2], [1,2]*U.S.Department of Agriculture, BARC, Beltsville, MD,* [3]*University of Delaware, Newark, DE,* [4]*U.S.Department of Agriculture, ADOL, East Lansing, MI,* [5]*Perdue Farms, Inc., Salisbury, MD*.

Selection of commercial poultry stocks with improved disease resistance using classical genetic breeding techniques has been unsuccessful due to technical difficulties. Although selection based on progeny tests can be used, this is labor-intensive, time consuming, and costly. In order to develop a DNA marker-assisted selection strategy to improve disease resistance against avian coccidiosis in commercial breeder chickens, chicken genes controlling resistance to coccidiosis are being identified. Three hundred and twenty four F2 offspring for mapping quantitative trait loci (QTL) affecting disease resistance were produced from 12 full-sib families of a commercial broiler breeder. The F2 offspring were inoculated with 104 sporulated oocysts of Eimeria maxima at 4 weeks of age. Body weight gain and fecal oocyst shedding were determined as a measure of infection. One hundred and twenty chicken microsatellite markers with an average genome distribution of 20 cM were used for genotyping the F1 and F2 generations. Genotypic data were analyzed with CRIMAP version 2.4 to construct a marker linkage map. A minimum LOD score of 3.00 was used as the statistically significant threshold for declaring linkage. The QTL analysis was conducted using SOLAR on genotypes of the F1 and F2 and the phenotypes of the F2 chickens. A locus on chromosome 1 was identified that was significantly associated with reduced oocyst shedding and 3 potential loci affecting growth were identified on chromosomes 1 and 6. (Supported by Fund for Rural America, Grant No 9704985 and partially by ARS CRIS).

**Key Words:** Coccidiosis, QTL mapping, Disease resistance

**180 The use of molecular markers to associate feather color alleles with tissue pigmentation in broiler chickens.** R Okimoto*, *University of Arkansas*.

Consumers dislike black melanin pigment in the abdominal skin and fascia. It has been demonstrated that certain feather color alleles are associated with this pigmentation. The dominant white allele ($I$) and sex-linked barring allele ($B$) are known to reduce the incidence of abdominal pigmentation when coupled with the extended black allele ($E$) of the $E$ locus. Since most commercial broilers are white feathered the cryptic feather color alleles that are segregating within the population cannot be determined without test mating. Molecular PCR based markers would facilitate selection against unwanted alleles. We have developed PAMSA (PCR amplification of multiple specific alleles) tests that can distinguish the various $E$ locus alleles. These tests detect specific nucleotide substitutions in the melanocortin 1-receptor gene that result in amino acid substitutions in the receptor sequence associated with specific alleles of the $E$ locus. In order to test the efficacy of using these tests in selection against tissue pigmentation a cross between two broiler dam lines was made to create an $F_1$ population that was nearly