

## **IN SILICO DETECTION OF SIGNATURES FOR ADAPTIVE EVOLUTION AT SELECT INNATE IMMUNE AND HEAT STRESS GENES IN INDIGENOUS POULTRY**

**C. Sigei<sup>1</sup>, D. Kariuki<sup>2</sup>, E. Ndiema<sup>3</sup>, E. Wainaina<sup>4</sup>, S. Maina<sup>5</sup>, M. Makanda<sup>6</sup>, P. Oyier<sup>7</sup>, J. Lichoti<sup>8</sup> and S. Ommeh<sup>9</sup>**

<sup>1,2,4</sup> Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology Nairobi, Kenya

<sup>3</sup>Department of Earth Sciences, National Museums of Kenya

<sup>5,7</sup>Department of Information Technology, Jomo Kenyatta University of Agriculture and Technology

<sup>6,9</sup>Institute For Biotechnology Research (IBR), Jomo Kenyatta University of Agriculture and Technology, Nairobi-Kenya

<sup>8</sup>State Department of Veterinary Services, Ministry of Agriculture Livestock, and Fisheries

Email: [sommeh@jkuat.ac.ke](mailto:sommeh@jkuat.ac.ke)

### **Abstract**

Indigenous poultry such as chickens and ducks have unique embedded adaptive traits which have evolved hence allowed their survival under varied environmental conditions. They provide a cheap source of protein in developing countries especially those in Sub-Saharan Africa. However, indigenous poultry are threatened by emerging disease outbreaks that are virulent and often associated with climate change conditions such as drought. Therefore, the objective of this work was to identify genetic signatures of natural selection at Toll-like Receptor 3 (TLR3), Toll-like Receptor 7 (TLR7), Protein Kinase R (PKR), 2' 5'- Oligoadenylate Synthetase 1, Heat Shock Protein 90 (HSP90), Heat Shock Protein 70 (HSP70) and Small Heat Shock Proteins (sHSPs). A reciprocal BlastP algorithm was used to search a non-redundant protein database with an expectation value cut off of 1e-10 so as to obtain homologs. MUSCLE was used to perform Multiple Sequence Alignment, sequences edited in Jalview and MEGA v6 used for evolutionary substitution model selection and rate heterogeneity testing. Phylogeny reconstruction was built using FastME software that implements Nearest Neighbor Interchange and Subtree Pruning and Regrafting (SPR) algorithms. The reliability of the phylogeny tree generated was evaluated using 1000 bootstrap replicates. Codon substitution models in PAML were used to compute the rate of non-synonymous to synonymous substitutions in different amino acid sites and lineages through likelihood Ratio Tests (LRTs). Heat shock protein genes results revealed signatures of negative selection suggestive of high evolutionary conservation. The results from disease-resistant genes revealed signatures of positive selection at several amino acid sites that encode protein domains. This is a good primer towards genetic development of better adapted and highly productive poultry breeds. This will likely reduce disease and drought-related morbidity in poultry, hence alleviation of food insecurity and better economic growth.

**Key words:** Signatures, toll-like receptor, heat shock proteins, protein kinaseR, dN/dS, oligoadenylate Synthase 1

### **1.0 Introduction**

Indigenous chickens are believed to have been domesticated from their main wild progenitor, the Red Jungle Fowl (*Gallus gallus*), in multiple distinct centers in Southeast Asia, India and China (Miao et al., 2013; Storey et al., 2012). Intensive artificial selection led to the development of exotic breeds which however came with compromised immunity and inability to adapt to local environmental conditions. In an attempt to generate more income, both exotic and indigenous poultry farming has been embraced in domestic settings. This has occasionally led to extensive, random and indiscriminate crossbreeding of indigenous breeds with exotic breeds without proper characterization and proper conservation measures (FAO., 2000). The end result is genetic dilution and at the very extreme erosion of the valuable adaptive gene traits embedded within indigenous poultry species. Also, infectious avian disease-outbreaks coupled with the absence of an effective cure have occasionally caused catastrophic losses to small-scale farmers. For instance, Newcastle disease (NCD) has been shown to be a major constraint to rural poultry production in most developing countries (Jibril et al., 2014). Together with other viral diseases such as Infectious Bursal Disease (IBD) and Avian Influenza (AI), NCD can cause up to 100% mortality in flocks (Garcia et al., 2013; Gardner, 2014). In addition to causing nutritional and heat stress in animals, climatic extremes associated with climate change has also been shown to influence the spread, distribution, virulence and cross-species transmissions of infectious diseases (Howard & Fletcher, 2012; Vandegrift et al., 2010).

The evolutionary mechanisms that accompanied poultry domestication and distribution to different parts of the world may have led to genetic variation at innate immune genes and stress genes. Natural selection plays a crucial role in adaptive evolution whereby different alleles and genotypes are favored while others are selected against; thus conferring fitness (Wade, 2008). As a result, the positively selected populations become more immuno-competent and better adapted to survive and reproduce despite environmental challenges. In developed countries, the availability of whole genome sequences and advanced technologies such as Genome-Wide Association Studies (GWAS), QTL mapping and High-density Single Nucleotide Polymorphism (SNP) genotyping chips have been used successfully to provide direct insight into DNA variation and subsequent breeding for desired traits in different livestock species including poultry ( Mukhopadhyay, 2012; Dekkers, 2012; Kranis et al., 2013; Wolc et al., 2013). However, for most developing countries, these developments are extremely costly in addition to being time-consuming and labor-intensive. The availability of genomic data and computational tools have made it possible to perform molecular evolution studies cheaply as a basis for identifying genomic regions that have been subject to selective pressures over evolutionary time. Such pressures can leave behind signatures on a gene which may alter a protein's fitness, stability, structure, expression, abundance and function. Various statistical methods that use intra-species population genetic data and inter-species comparative data have previously been used to detect such signatures. In this study, Signatures of selection were detected by comparing the rate of functional changes in amino acid substitutions in the coding region of a gene (non-synonymous mutations) with the rate of neutral changes (synonymous mutations) using the dN/dS ratio test ( $\omega$ ) implemented in PAML package (Yang, 2007). Values of  $\omega > 1$ ,  $\omega = 1$  and  $\omega < 1$  indicate positive selection, neutral evolution, and purifying selection respectively. These models have been extensively used to calculate positive  $\omega$  among lineages and also among amino acid. For instance, Lynn *et al.* (2005) used this models to identify signatures of positive adaptive selection on the CD4 gene that encodes glycoproteins in the bovine genome as well as on mammalian alpha defensins (Lynn *et al.*, 2004). More recently, (Zhu et al., 2010) and (Al-Daghri et al., 2012) have detected signatures of positive selection in avian MX genes and mammalian NPC1 genes respectively. Similarly, we hereby present results obtained from the analysis of TLR7, TLR3, OAS1 and PKR immune genes as well as HSP90, HSP70 and sHSP heat stress genes in indigenous poultry and other selected animal species for detection of signatures of adaptive evolution.

## **2.0 Materials and Methods**

### **2.1 Data Mining of Candidate Genes and Sequence Data from Biological Databases**

Candidate genes that have been shown to have a strong association with viral disease and drought response in poultry were selected through mining of bibliographic and biological databases, in particular PubMed, PubMed Central, HomoloGene, Genome, Nucleotide and Protein among others. To achieve this, ENTREZ cross-database query searches were performed at NCBI. TLR3, TLR7, PKR and OAS1 genes were identified to be commonly expressed in response to viral infections. These genes act through various signaling pathways to induce the transcriptional expression of genes that ultimately lead to production of pro-inflammatory cytokines, interleukins and interferons which lead to blockage and inhibition of viral replication. On the other hand, HSP70, HSP90 and sHSP family of genes were selected for drought stress response analysis in poultry.

### **2.2 Selection of Homologs through Reciprocal BLAST and Sequence Retrieval**

Across many other animal species, the selected candidate genes are normally expressed in response to viral infections and stress to cells through drought and heat. Therefore, a reciprocal BLAST was performed whereby BlastP program was used to search for homologous protein sequences from the Non-redundant protein sequences database (nr) of NCBI's GenBank using a chicken protein query. Homologs were selected as those having an expectation value cut off of 10 ( $e^{-10}$ ). All the complete amino acid sequences and the corresponding coding sequences for each homolog were then downloaded from GenBank. These sequences were saved in FASTA format and renamed in notepad for the downstream analysis. Stop codons were removed manually from coding sequences so as to avoid interference with the downstream analysis.

### **2.3 Alignment of Homologous Sequences through Multiple Sequence Alignment**

To assess alignments and confirm homology of the amino acid sequences of the selected homologs, a multiple sequence alignment was performed using the Multiple Sequence Comparison by Log- Expectation (MUSCLE) software that uses an iterative algorithm (Edgar, 2004a). SeaView version 4.5.3 (Gouy, Guindon, & Gascuel, 2010) was then used to view and edit the multiple sequence alignment outputs MUSCLE so as to ensure a good quality alignments. This step is significant since it provides a basis for calculation of sequence divergences for a true phylogeny that is representative of the evolutionary relationship among the taxa (Niranjan, 2011).



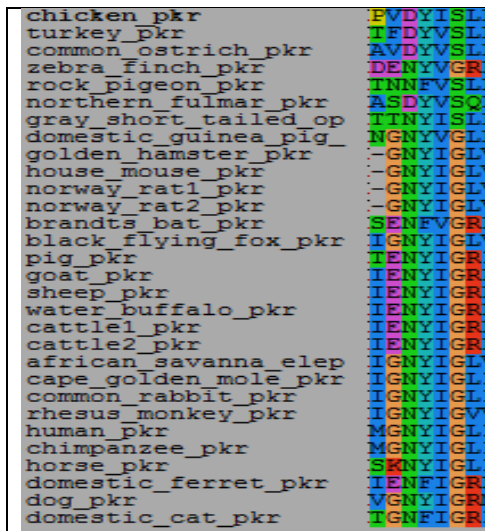


Fig. 1: A summary of Multiple Sequence Alignments showing sequence comparison similarities across all the selected homologs for each dataset, i.e, HSP70, HSP90, Shsp, TLR7, TLR3, OAS1 and PKR. The alignments were generated using MUSCLE (Edgar, 2004b).

### 3.2 Phylogeny Analysis

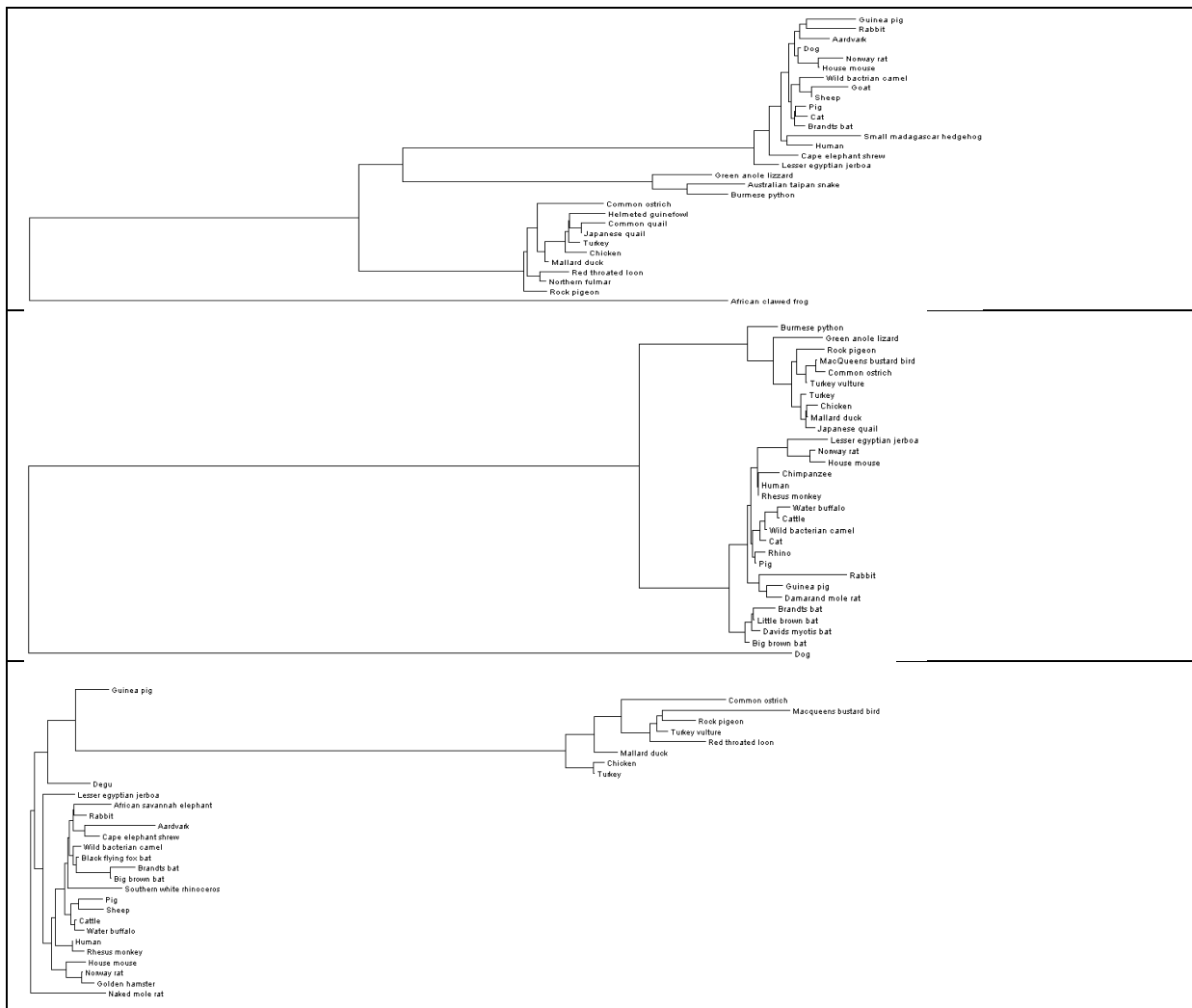


Figure 2: Phylogenetic trees of HSP 70, HSP90 and sHSP respectively. The trees were reconstructed using fastME software (Lefort et al., 2015)



Figure 3: Phylogenetic trees of TLR7, TLR3, OAS1 and PKR respectively. The trees were reconstructed using fastME software (Lefort et al., 2015)

Phylogenetic trees for each gene were generated based on the minimum evolution principle implemented in FastME software. In all our datasets, our species of interest, i.e, poultry clustered into one clade with almost similar topologies as shown in figure 2 and 3. Our bootstrap analysis showed large numbers of well-supported nodes > 50. Substitution pattern and rates were estimated under the Jones-Taylor-Thornton (JTT) model (+G). The estimated value of the shape parameter for the discrete Gamma Distribution was less than one for all datasets.

### 3.3 Detection of signatures of Adaptive Evolution

In all our datasets, our lineage results were highly significant based on the LRT comparisons of M0 and M1 with P-values less than 0.001 ( $P < 0.001$ ). Strong signatures of purifying selection were detected across all our heat stress genes with omega values less than 1 ( $\omega < 1$ ). Also, codon site analyses revealed no amino acid sites to be evolving under positive selection. In addition, P-values obtained were greater than 0.05 ( $P > 0.05$ ) hence we

failed to reject the null hypothesis of uniform distribution ( $0 < \omega < 1$ ). In contrast, signatures of adaptive evolution were detected across all the select innate immune genes. Signatures of adaptive evolution were inferred from codon site analyses based on BEB values  $\geq 0.95$ . These were found to occur at the N-terminus and C-terminus LRR domains of TLR7 and TLR3, the OAS1\_C and the NTase domains of OAS1 and the Protein Kinase and dsRBM II domains of PKR.

#### **4.0 Discussion and Conclusions**

The evolution of species is believed to be majorly driven by forces of natural selection. Through evolution and speciation the current extensive species of birds are believed to have originated from a common reptilian ancestor known as *Archaeopteryx*. Domestication and adaptation to different environmental conditions and challenges posed by diseases and pathogens could have also contributed to divergence and evolution of new species. In this regard, the signatures of positive selection detected in our select innate immune genes could be driven by co-evolution with viral pathogens. In an attempt to evade recognition by host immune receptors, pathogens evolve constantly. Consequently, the host receptor must also mutate to keep pace with the mutating pathogen. Based on our results, this is highly likely since the domains that had high variability are regions that interact with pathogens prior to initiation of a signaling cascade to destroy the pathogens. This is consistent with previous studies that have been carried out in mammals and birds for TLR7 and TLR3 (Mikami et al 2012; Alcaide and Edwards 2011; Areal et al 2011; Grueber et al 2014) as well as in primates and bats for OAS1 (Ferguson et al., 2012; Hancks et al., 2015; Mozzi et al., 2015). Also, the rate of evolution of a gene is determined by the number of pathogens recognized by a receptor. Previous studies of PKR gene in selected vertebrates such as humans and primates have shown rapid evolution of PKR gene which be as a result of the multiple viral antagonists it recognizes (Brennan et al., 2014; Child et al., 2012; Elde et al., 2009; Rothenburg et al., 2009). Our results are also consistent with these finding since our results revealed high variability in the amino acid sequences of our PKR gene. The results from all our heat-stress genes revealed a high degree of conservation over evolutionary time which could be driven by functional constraints. In conclusion, our select innate immune genes, more so PKR, are a promising target for further experimental validation through *in vivo* and *in vitro* as this could give new clues into resistance, tolerance and susceptibility to viral infections in poultry. The results obtained can be used in their genetic improvement and conservation against virulent and emerging infections, hence sustainable food security for resource poor populations.

#### **Acknowledgements**

The authors are sincerely grateful to Jomo Kenyatta University of Agriculture and Technology, the State Department of Veterinary services, Ministry of Agriculture Livestock, and Fisheries and the National Museums of Kenya for providing all the necessary facilities to carry out this study.

## References

- Al-Daghri, N. M., Cagliani, R., Forni, D., Alokail, M. S., Pozzoli, U., Alkharfy, K. M., ... Sironi, M. (2012). Mammalian NPC1 genes may undergo positive selection and human polymorphisms associate with type 2 diabetes. *BMC Medicine*, *10*(1), 140. <http://doi.org/10.1186/1741-7015-10-140>
- Brennan, G., Kitzman, J. O., Rothenburg, S., Shendure, J., & Geballe, A. P. (2014). Adaptive Gene Amplification As an Intermediate Step in the Expansion of Virus Host Range. *PLoS Pathog*, *10*(3), e1004002. <http://doi.org/10.1371/journal.ppat.1004002>
- Child, S. J., Brennan, G., Braggin, J. E., & Geballe, A. P. (2012). Species Specificity of Protein Kinase R Antagonism by Cytomegalovirus TRS1 Genes. *Journal of Virology*, *86*(7), 3880–3889. <http://doi.org/10.1128/JVI.06158-11>
- C. S. Mukhopadhyay, D. K. (2012). SNP chip development and genome wide association studies in livestock (Theory & Practical Session).
- Dekkers, J. C. M. (2012). Application of Genomics Tools to Animal Breeding. *Current Genomics*, *13*(3), 207–212. <http://doi.org/10.2174/138920212800543057>
- Edgar, R. C. (2004a). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. <http://doi.org/10.1093/nar/gkh340>
- Edgar, R. C. (2004b). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. <http://doi.org/10.1093/nar/gkh340>
- Elde, N. C., Child, S. J., Geballe, A. P., & Malik, H. S. (2009). Protein kinase R reveals an evolutionary model for defeating viral mimicry. *Nature*, *457*(7228), 485–489. <http://doi.org/10.1038/nature07529>
- FAO. (2000). FAOSTAT. Statistical database of Food and Agriculture Organisation of the United Nations, Rome, Italy.
- Ferguson, W., Dvora, S., Fikes, R. W., Stone, A. C., & Boissinot, S. (2012). Long-Term Balancing Selection at the Antiviral Gene OAS1 in Central African Chimpanzees. *Molecular Biology and Evolution*, *29*(4), 1093–1103. <http://doi.org/10.1093/molbev/msr247>
- Garcia, S. C., Lopez, R. N., Morales, R., Olvera, M. A., Marquez, M. A., Merino, R., ... Afonso, C. L. (2013). Molecular Epidemiology of Newcastle Disease in Mexico and the Potential Spillover of Viruses from Poultry into Wild Bird Species. *Applied and Environmental Microbiology*, *79*(16), 4985–4992. <http://doi.org/10.1128/AEM.00993-13>
- Gardner, E. G. (2014). Livestock Risks and Opportunities: Newcastle Disease and Avian Influenza in Africa. *Planet@Risk*, *2*(4). Retrieved from <http://planet-risk.org/index.php/pr/article/view/99>
- Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView Version 4: A Multiplatform Graphical User Interface for Sequence Alignment and Phylogenetic Tree Building. *Molecular Biology and Evolution*, *27*(2), 221–224. <http://doi.org/10.1093/molbev/msp259>
- Hancks, D. C., Hartley, M. K., Hagan, C., Clark, N. L., & Elde, N. C. (2015). Overlapping Patterns of Rapid Evolution in the Nucleic Acid Sensors cGAS and OAS1 Suggest a Common Mechanism of Pathogen Antagonism and Escape. *PLoS Genetics*, *11*(5). <http://doi.org/10.1371/journal.pgen.1005203>
- Howard, C. R., & Fletcher, N. F. (2012). Emerging virus diseases: can we ever expect the unexpected? *Emerging Microbes & Infections*, *1*(12), e46. <http://doi.org/10.1038/emi.2012.47>
- Jibril, A. H., Umoh, J. U., Kabir, J., Saidu, L., Magaji, A. A., Bello, M. B., & Raji, A. A. (2014). Newcastle Disease in Local Chickens of Live Bird Markets and Households in Zamfara State, Nigeria. *International Scholarly Research Notices*, *2014*, e513961. <http://doi.org/10.1155/2014/513961>
- Kranis, A., Gheyas, A. A., Boschiero, C., Turner, F., Yu, L., Smith, S., ... Burt, D. W. (2013). Development of a high density 600K SNP genotyping array for chicken. *BMC Genomics*, *14*(1), 59. <http://doi.org/10.1186/1471-2164-14-59>
- Lefort, V., Desper, R., & Gascuel, O. (2015). FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. *Molecular Biology and Evolution*, *msv150*. <http://doi.org/10.1093/molbev/msv150>
- Miao, Y.-W., Peng, M.-S., Wu, G.-S., Ouyang, Y.-N., Yang, Z.-Y., Yu, N., ... Zhang, Y.-P. (2013). Chicken domestication: an updated perspective based on mitochondrial genomes. *Heredity*, *110*(3), 277–282. <http://doi.org/10.1038/hdy.2012.83>
- Mozzi, A., Pontremoli, C., Forni, D., Clerici, M., Pozzoli, U., Bresolin, N., ... Sironi, M. (2015). OASes and STING: Adaptive Evolution in Concert. *Genome Biology and Evolution*, *7*(4), 1016–1032. <http://doi.org/10.1093/gbe/evv046>
- Niranjan, R. B. (2011). Basics for the Construction of Phylogenetic Trees. *WebmedCentral BIOLOGY*, *2*(12).

- Rothenburg, S., Seo, E. J., Gibbs, J. S., Dever, T. E., & Dittmar, K. (2009). Rapid evolution of protein kinase PKR alters sensitivity to viral inhibitors. *Nature Structural & Molecular Biology*, *16*(1), 63–70. <http://doi.org/10.1038/nsmb.1529>
- Storey, A. A., Athens, J. S., Bryant, D., Carson, M., Emery, K., deFrance, S., ... Matisoo-Smith, E. (2012). Investigating the Global Dispersal of Chickens in Prehistory Using Ancient Mitochondrial DNA Signatures. *PLoS ONE*, *7*(7), e39171. <http://doi.org/10.1371/journal.pone.0039171>
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. <http://doi.org/10.1093/molbev/mst197>
- Vandegrift, K. J., Sokolow, S. H., Daszak, P., & Kilpatrick, A. M. (2010). Ecology of avian influenza viruses in a changing world. *Annals of the New York Academy of Sciences*, *1195*, 113–128. <http://doi.org/10.1111/j.1749-6632.2010.05451.x>
- Wade, M. (2008). Evolutionary Genetics. In E. N. Zalta (Ed.), *The Stanford Encyclopedia of Philosophy* (Fall 2008). Retrieved from <http://plato.stanford.edu/archives/fall2008/entries/evolutionary-genetics/>
- Wolc, A., Arango, J., Jankowski, T., Settar, P., Fulton, J. E., O'Sullivan, N. P., ... Dekkers, J. C. M. (2013). Genome-wide association study for Marek's disease mortality in layer chickens. *Avian Diseases*, *57*(2 Suppl), 395–400. <http://doi.org/10.1637/10409-100312-Reg.1>
- Zhu, Y. F., Li, H. F., Han, W., Shu, J. T., Song, W. T., Zhang, X. Y., & Chen, K. W. (2010). Detecting Adaptive Evolution of Galliform and Anseriform Avians Mx Genes. *Journal of Animal and Veterinary Advances*, *9*(13), 1811–1815. <http://doi.org/10.3923/javaa.2010.1811.1815>



**Appendices***Table 1: A list of homologs, accession numbers and E-values for HSP70*

<b>Animal Species</b>	<b>Protein Accession Nos.</b>	<b>E-values</b>
Gallus gallus (chicken)	AAP37959.1	0.0
Anas platyrhynchos (mallard)	XP_005022715.1	0.0
Coturnix japonica (Japanese quail)	BAF37039.1	0.0
Meleagris gallopavo (turkey)	XP_003206814.1	0.0
Fulmarus glacialis (northern fulmar)	XP_009574733.1	0.0
Columba livia (rock pigeon)	XP_005506432.1	0.0
Coturnix coturnix (common quail)	ACC85671.1	0.0
Numida meleagris (helmeted guineafowl)	BAC24791.1	0.0
Gavia stellata (red-throated loon)	KFV42297.1	0.0
Struthio camelus australis (common ostrich)	XP_009673875.1	0.0
Python bivittatus (Burmese python)	XP_007435687.1	0.0
Oxyuranus scutellatus scutellatus (Australian taipan snake)	AAV33973.1	0.0
Anolis carolinensis (green anole lizard)	XP_003214318.1	0.0
Jaculus jaculus (lesser Egyptian jerboa)	XP_004649317.1	0.0
Ovis aries (sheep)	XP_004010768.1	0.0
Elephantulus edwardii (Cape elephant shrew)	XP_006891865.1	0.0
Mus musculus (house mouse)	NP_032327.2	0.0
Orycteropus afer afer	XP_007940478.1	0.0
Canis lupus familiaris (dog)	XP_537479.1	0.0
Echinops telfairi (small Madagascar hedgehog)	XP_004698706.1	0.0
Capra hircus (goat)	AFP43992.1	0.0
Felis catus (domestic cat)	XP_006932972.1	0.0
Sus scrofa (pig)	XP_003356782.1	0.0
Oryctolagus cuniculus (rabbit)	XP_002719605.1	0.0
Camelus ferus (Wild Bactrian camel)	XP_006177814.1	0.0
Rattus norvegicus (Norway rat)	NP_068635.1	0.0
Homo sapiens (human)	NP_068814.2	0.0
Myotis brandtii (Brandt's bat)	XP_005874088.1	0.0
Cavia porcellus (domestic guinea pig)	XP_005004828.1	0.0
Xenopus laevis (African clawed frog)	NP_001079632.1	0.0

*Appendix Table 2: A list of homologs, accession numbers and E-values for HSP90*

<b>Animal Species</b>	<b>Protein Accession Nos.</b>	<b>E-values</b>
Gallus gallus (chicken)	NP_001103255.1	0.0
Anas platyrhynchos (mallard)	XP_005020783.1	0.0
Coturnix japonica (Japanese quail)	BAI23206.1	0.0
Struthio camelus australis	XP_009673798.1	0.0
Cathartes aura (turkey vulture)	KFP47363.1	0.0
Columba livia (rock pigeon)	XP_005506148.1	0.0
Python bivittatus (Burmese python)	XP_007437399.1	0.0
Anolis carolinensis (green anole lizard)	XP_003224109.1	0.0
<b>Chlamydotis undulata macqueenii (MacQueen's bustard)</b>	KFP45671.1	0.0
Meleagris gallopavo (turkey)	XP_003206775.1	0.0
Homo sapiens (human)	NP_005339.3	0.0
Sus scrofa (pig)	NP_999138.1	0.0
Macaca mulatta (Rhesus monkey)	NP_001182596.1	0.0
Myotis lucifugus (little brown bat)	XP_006100595.1	0.0
Eptesicus fuscus (big brown bat)	XP_008152985.1	0.0
Camelus ferus (Wild Bactrian camel)	XP_006175722.1	0.0
Ceratotherium simum simum (southern white rhinoceros)	XP_004434322.1	0.0
Fukomys damarensis (Damaraland mole rat)	KFO22274.1	0.0
Myotis davidii (david's myotis)	XP_006766684.1	0.0
Felis catus (domestic cat)	XP_003988041.1	0.0

Bos taurus (cattle)	NP_001012688.1	0.0
Pan troglodytes (chimpanzee)	BAK63243.1	0.0
Bubalus bubalis (water buffalo)	XP_006079133.1	0.0
Cavia porcellus (domestic guinea pig)	XP_003463143.1	0.0
Myotis brandtii (Brandt's bat)	XP_005877923.1	0.0
Rattus norvegicus (Norway rat)	NP_786937.1	0.0
Mus musculus (house mouse)	XP_006515548.1	0.0
Oryctolagus cuniculus (rabbit)	XP_002721768.1	0.0
Jaculus jaculus (lesser Egyptian jerboa)	XP_004665641.1	0.0
Canis lupus familiaris (dog)	XP_005627459.1	0.0

Appendix Table 3: A list of homologs, accession numbers and E-values for sHSP

Animal Species	Protein Accession Nos.	E-values
Gallus gallus (chicken)	NP_990507.1	7e-121
Meleagris gallopavo (turkey)	XP_003212808.1	2e-120
Anas platyrhynchos (mallard)	XP_005030928.1	1e-117
Cathartes aura (turkey vulture)	KFP47238.1	7e-114
Columba livia (rock pigeon)	XP_005500801.1	3e-112
Gavia stellata (red-throated loon)	KFV52607.1	1e-110
Struthio camelus australis (common ostrich)	KFV78909.1	6e-110
Chlamydotis undulata macqueenii (MacQueen's bustard bird)	KFP45078.1	2e-105
Jaculus jaculus (lesser Egyptian jerboa)	XP_004666540.1	1e-92
Cavia porcellus (domestic guinea pig)	NP_001166547.1	1e-92
Octodon degus (degu)	XP_004646410.1	1e-91
Oryctolagus cuniculus (rabbit)	NP_001075876.1	5e-91
Pteropus alecto (black flying fox)	XP_006912883.1	9e-91
Homo sapiens (human)	NP_001876.1	1e-90
Macaca mulatta (Rhesus monkey)	NP_001247830.1	2e-90
Camelus ferus (Wild Bactrian camel)	XP_006190435.1	2e-90
Eptesicus fuscus (big brown bat)	XP_008148383.1	3e-90
Mus musculus (house mouse)	NP_034094.1	4e-90
Bos taurus (cattle)	NP_776715.1	4e-90
Ceratotherium simum simum (southern white rhinoceros)	XP_004427396.1	4e-90
Orycteropus afer afer (aardvark)	XP_007934744.1	5e-90
Sus scrofa (pig)	XP_005667376.1	5e-90
Rattus norvegicus (Norway rat)	NP_037067.1	1e-89
Heterocephalus glaber (naked mole-rat)	NP_001266786.1	1e-89
Bubalus bubalis (water buffalo)	XP_006065322.1	1e-89
Ovis aries (sheep)	NP_001012475.1	1e-89
Elephantulus edwardii (Cape elephant shrew)	XP_006890834.1	2e-89
Mesocricetus auratus (golden hamster)	XP_005069569.1	2e-89
Loxodonta africana (African savanna elephant)	XP_003415672.1	2e-89
Myotis brandtii (Brandt's bat)	XP_005856898.1	2e-89

Appendix Table 4: A list of homologs, accession numbers and E-values for TLR7

Animal Species	Protein Accession Nos.	E-values
Gallus gallus (chicken)	ACR26208.1	0.0
Gallus lafayetii (Ceylon junglefowl)	ACR26206.1	0.0
Perdix perdix (Grey partridge)	AGO86775.1	0.0
Coturnix japonica (Japanese quail)	BAL02987.1	0.0
Meleagris gallopavo (turkey)	XP_003203134.1	0.0
Anser cygnoides (swan goose)	AFK29095.1	0.0

Anas platyrhynchos (mallard)	XP_005029236.1	0.0
Columba livia (rock pigeon)	AIK67344.1	0.0
Ceratotherium simum simum (southern white rhinoceros)	XP_004435171.1	0.0
Sus scrofa (pig)	ABG47422.1	0.0
Equus burchellii cuninghamei (Plains zebra)	AGK25872.1	0.0
Canis lupus familiaris (dog)	ABC69204.1	0.0
Rousettus leschenaultii (Leschenault's rousette fruitbat)	BAH02556.1	0.0
Loxodonta africana (African savanna elephant)	ABC95782.1	0.0
Felis catus (Domestic Cat)	NP_001073602.1	0.0
Capra hircus (goat)	XP_005701170.1	0.0
Bos taurus (cattle)	ABN71673.1	0.0
Cavia porcellus (domestic guinea pig)	XP_003462941.2	0.0
Bos indicus (Bos taurus indicus-zebu breed harianna)	ACY25086.1	0.0
Macaca mulatta (Rhesus monkey)	NP_001123898.1	0.0
Pan troglodytes (chimpanzee)	NP_001123605.1	0.0
Eptesicus fuscus (big brown bat)	XP_008154799.1	0.0
Myotis brandtii (Brandt's bat)	XP_005881008.1	0.0
Myotis lucifugus (little brown bat)	XP_006088669.1	0.0
Myotis davidii	XP_006763859.1	0.0
Mus musculus (house mouse)	NP_001277687.1	0.0
Homo sapiens (human)	AAF78035.1	0.0
Ovis aries (sheep)	NP_001128531.1	0.0
Pteropus alecto (black flying fox)	NP_001277093.1	0.0
Rattus norvegicus (Norway rat)	NP_001091051.1	0.0

Appendix Table 5: A list of homologs, accession numbers and E-values for TLR3

Animal Species	Protein Accession Nos.	E-values
Gallus gallus (chicken)	ABL74502.1	0.0
Meleagris gallopavo (turkey)	XP_003205822.1	0.0
Anas platyrhynchos (mallard duck)	XP_005009038.1	0.0
Cairina moschata (Muscovy duck)	AFK29094.1	0.0
Columba livia (rock pigeon)	XP_005500267.1	0.0
Struthio camelus australis (common ostrich)	XP_009674995.1	0.0
Anser anser (domestic goose)	AGJ98456.1	0.0
Loxodonta africana (African savanna elephant)	ABC95781.1	0.0
Felis catus (domestic cat)	XP_006930623.1	0.0
Myotis brandtii (Brandt's bat)	XP_005863156.1	0.0
Myotis lucifugus (little brown bat)	XP_006092716.1	0.0
Eptesicus fuscus (big brown bat)	XP_008150129.1	0.0
Myotis davidii	XP_006772770.1	0.0
Macaca mulatta (Rhesus monkey)	ABY64988.1	0.0
Canis lupus familiaris (dog)	XP_005630024.1	0.0
Ceratotherium simum simum (southern white rhinoceros)	XP_004428823.1	0.0
Rousettus leschenaultii (Leschenault's rousette)	BAH02555.1	0.0
Oryctolagus cuniculus algirus	AGU70373.1	0.0
Rattus norvegicus (Norway rat)	XP_008769488.1	0.0
Bubalus bubalis (water buffalo)	ADY18594.1	0.0
Capra hircus (goat)	AHJ90636.1	0.0
Sus scrofa (pig)	ADQ00195.1	0.0
Pan troglodytes verus (West African chimpanzee)	ADH84437.1	0.0
Bos indicus (Bos taurus indicus)	ACU16426.1	0.0
Bos taurus (cattle)	ABN71661.1	0.0
Homo sapiens (human)	ABC86908.1	0.0
Cavia porcellus (domestic guinea pig)	NP_001166500.1	0.0
Ovis aries (sheep)	NP_001129400.1	0.0

Mus musculus (house mouse)	AAH99937.1	0.0
Pteropus alecto (black flying fox)	NP_001277098.1	0.0

Appendix Table 6: A list of homologs, accession numbers and E-values for PKR

Animal Species	Protein Accession Nos.	E-values
Gallus gallus (chicken)	NP_989818.1	0.0
Meleagris gallopavo (turkey)	XP_003204013.1	0.0
Fulmarus glacialis (northern fulmar)	XP_009579791.1	0.0
Struthio camelus australis (Common ostrich)	XP_009684950.1	0.0
Columba livia (rock pigeon)	XP_005503897.1	0.0
Taeniopygia guttata (zebra finch)	XP_002191239.2	0.0
Loxodonta africana (African savanna elephant)	XP_003416160.1	9e-93
Monodelphis domestica (gray short-tailed opossum)	XP_007476152.1	2e-91
Myotis brandtii (Brandt's bat)	XP_005864773.1	3e-87
Mustela putorius furo (domestic ferret)	XP_004812890.1	9e-87
Mesocricetus auratus (golden hamster)	NP_001268874.1	3e-86
Felis catus (domestic cat)	XP_003984379.1	5e-86
Homo sapiens (human)	AAF13156.1	1e-85
Sus scrofa (pig)	NP_999484.1	3e-85
Canis lupus familiaris (dog)	NP_001041600.1	9e-85
Pan troglodytes (chimpanzee)	NP_001138509.1	3e-83
Rattus norvegicus (Norway rat) 1	NP_062208.1	3e-83
Rattus norvegicus (Norway rat) 2	XP_008762647.1	6e-83
Chrysochloris asiatica (Cape golden mole)	XP_006839514.1	1e-82
Bos taurus (cattle)1	XP_005212627.1	2e-82
Capra hircus (goat)	XP_005686545.1	2e-82
Bubalus bubalis (water buffalo)	XP_006056900.1	2e-81
Oryctolagus cuniculus (rabbit)	NP_001075682.1	5e-80
Bos taurus (cattle)	BAC66440.1	5e-80
Equus caballus (horse)	NP_001137272.1	3e-79
Ovis aries (sheep)	XP_004007349.1	1e-72
Cavia porcellus (domestic guinea pig)	XP_003472952.2	4e-72
Rhesus monkey	NP_001077417.1	2e-70
Mus musculus (House mouse)	NP_035293.1	1e-61
Pteropus alecto (black flying fox)	XP_006910452.1	1e-79

Appendix Table 7: A list of homologs, accession numbers and E-values for OAS1

Animal Species	Protein Accession Nos.	E-values
Gallus gallus (chicken) 1	BAB19016.1	0.0
Gallus gallus (chicken) 2	BAB19015.1	0.0
Struthio camelus australis (Common ostrich)	XP_009671383.1	0.0
Columba livia (rock pigeon)	XP_005508920.1	0.0
Taeniopygia guttata (zebra finch)	XP_004176346.1	0.0
Ceratotherium simum simum (southern white rhinoceros) 1	XP_004429952.1	1e-128
Felis catus (domestic cat)	XP_003994784.1	7e-127
Canis lupus familiaris (dog)	NP_001041558.1	9e-126
Equus caballus (horse)	XP_001488427.3	1e-125
Mustela putorius furo (domestic ferret)	XP_004753444.1	2e-125
Loxodonta africana (African savanna elephant)	XP_003419366.1	5e-119
Oryctolagus cuniculus (rabbit)	XP_002722162.1	9e-119
Elephantulus edwardii (Cape elephant shrew)	XP_006890566.1	4e-117
Cavia porcellus (domestic guinea pig)	XP_003477780.1	7e-117

---

Octodon degus (degu)	XP_004636401.1	1e-116
Echinops telfairi (small Madagascar hedgehog)	XP_004709754.1	5e-115
Chrysochloris asiatica (Cape golden mole)	XP_006865560.1	8e-112
Pteropus alecto (black flying fox)	ELK15543.1	7e-110
Mus musculus (house mouse)	NP_035984.2	1e-108
Mesocricetus auratus (golden hamster)	XP_005079016.1	7e-108
Rattus norvegicus (Norway rat)	NP_001009682.1	3e-105
Macaca mulatta (Rhesus monkey)	XP_001091486.1	7e-105
Monodelphis domestica (gray short-tailed opossum)	XP_007490032.1	8e-103
Pan troglodytes (chimpanzee)	NP_001267398.1	3e-101
Myotis davidii	XP_006769000.1	1e-98
Ceratotherium simum simum (southern white rhinoceros) 2	XP_004429953.1	2e-97
Eptesicus fuscus (big brown bat)	XP_008141141.1	6e-93
Bos taurus (cattle)	NP_835209.1	1e-92
Sus scrofa (pig)	AAT34965.1	8e-92
Homo sapiens (human)	ABE27977.1	2e-90

---