

## **Genomic analysis of chicken ecotypes from Brazil, Sri Lanka, and Egypt: impact of different environmental conditions**

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### **Summary**

Extreme environmental conditions are a major challenge to livestock production worldwide and can cause detrimental effects to animal growth and reproduction. Furthermore, extremes of temperature or humidity are major contributors to oxidative cell damage and may also compromise the animal's immune system. The aim of this study was to examine genomic differences among chicken ecotypes from two different climatic regions (Brazil and Sri Lanka = Tropical; Egypt = Arid) and identify genomic regions that may be involved in the adaptation of chickens to these different environmental conditions. Comparisons were performed through Fst analyses. The analyses revealed a similarity between chickens from Sri Lanka and Brazil, including common regions of difference on chromosomes 2, 3 and 8 in the comparison with chickens from Egypt. The genes, TRMT1L and microRNA 6545 were detected on chromosomes 8 and 2, respectively, in the Brazil vs Egypt comparison. These results provide insights into genes that could be under selection pressure in the three populations relative to their adaptation to arid versus tropical environments.

*Keywords: chickens, environment, selection pressure, adaptation*

### **Introduction**

Extreme environmental conditions are a major challenge in livestock production. Changes in climate, particularly those that contribute to weather extremes like drought or extreme temperatures or humidity may result in reduced performance, reproduction and could comprise the animal's immune function (St-Pierre *et al.*, 2003). In chickens extreme environmental temperatures lead to generation of reactive oxygen species (ROS), causing oxidative stress and lipid peroxidation (Altan *et al.*, 2003). However, chickens may adapt to tolerate particular challenging environments. Domestication by human and subsequent breed formation has led to chickens being adapted in physiology, morphology, fertility, and behavior to increase production (Ericsson *et al.* 2014). Selection pressure is influential in enabling chickens to adapt to their

environments and demonstrate signatures of selection in chicken population genomes. Signatures of selection/selective sweeps reduce genetic variability of genomic regions. These regions may have genes with functional importance in particular processes and reflect allelic selection in under differing environmental conditions. To detect those regions of divergence, some studies have used Fixation Index ( $F_{st}$ ) analysis to detect selection signatures in both commercial and non-commercial breeds (Elferink *et al.*, 2012; Fleming *et al.*, 2017 ).

In this study we performed genome wide comparisons in indigenous chicken ecotypes from three countries that have different climates (Brazil and Sri Lanka = Tropical; Egypt = Arid) for regions where selection may have taken place and shaped the genome to enable the chicken adapt to different environments.

## **Material and Methods**

### **Sample collection**

Blood samples were collected from 156 Brazilian, 92 Sri Lankan, and 96 Egyptian chickens. The Brazilian chickens represented several ecotypes and were sampled from different farms, outside Porto Ferreira in the State of São Paulo. A total of 92 samples were collected from 3 Sri Lanka ecotypes: 27, 34, and 31 samples were collected from Gannoruwa town, Karuwalagaswewa, and Uda Peradeniya villages, respectively. A total of 95 samples were collected from Egypt ecotypes: 31 Baladi, 31 Fayoumi, and 33 Dandarawi from 3 villages in Qalyubia, 4 villages in Mid-Egypt, and 4 villages in Southern Egypt, respectively.

### **Genotyping and quality control**

Genotyping for all samples was conducted at GeneSeek (Lincoln, NE) using the Affymetrix Axiom®600k Array. SNP genotype data quality filtering was assessed with PLINK 1.9 software (Chang *et al.*, 2015) and only autosomal SNPs were screened based on parameters of > 90 % call rate (-geno 0.1) and minor allele frequency (MAF) > 0.02. In total, 523,186 SNPs were utilized for downstream analysis.

### **Population stratification analysis**

Multi-dimensional scaling (MDS) was performed to examine population structure for stratification in two dimensions using cluster algorithm in PLINK v1.9 (Chang *et al.* 2015)

### **F<sub>st</sub> analyses**

For the  $F_{st}$  analyses, three pairwise comparisons were performed for Brazil vs Egypt, Sri Lanka vs Egypt, and Brazil vs Sri Lanka ecotypes. For each comparison, mean  $F_{st}$  (m $F_{st}$ ) value was calculated in 100 kb sliding windows with a step size of 50 kb using an in-house script. Genomic regions with the highest peaks, 0.16% of the empirical distributions of the m $F_{st}$  values were considered for downstream analyses.

## Results

### Genetic diversity and population stratification

The MDS plot in Fig. 1 shows distinct separation among ecotypes from the three countries and separation of Brazil and Sri Lanka ecotypes from the Egyptian ecotypes.

The  $F_{st}$  analyses for the comparisons between Brazil or Sri Lanka vs Egypt generally indicated the strongest peaks on chromosomes 2, 3, and 8 ( $mF_{st} > 0.28$ ). A total of two regions, were detected only in the Brazil vs Egypt comparison, on chromosomes 8 (10.45-10.55 Mb) and 2 (71.85 - 71.95 Mb) that contained the TRMT1L and MicroRNA 6545 genes, respectively.

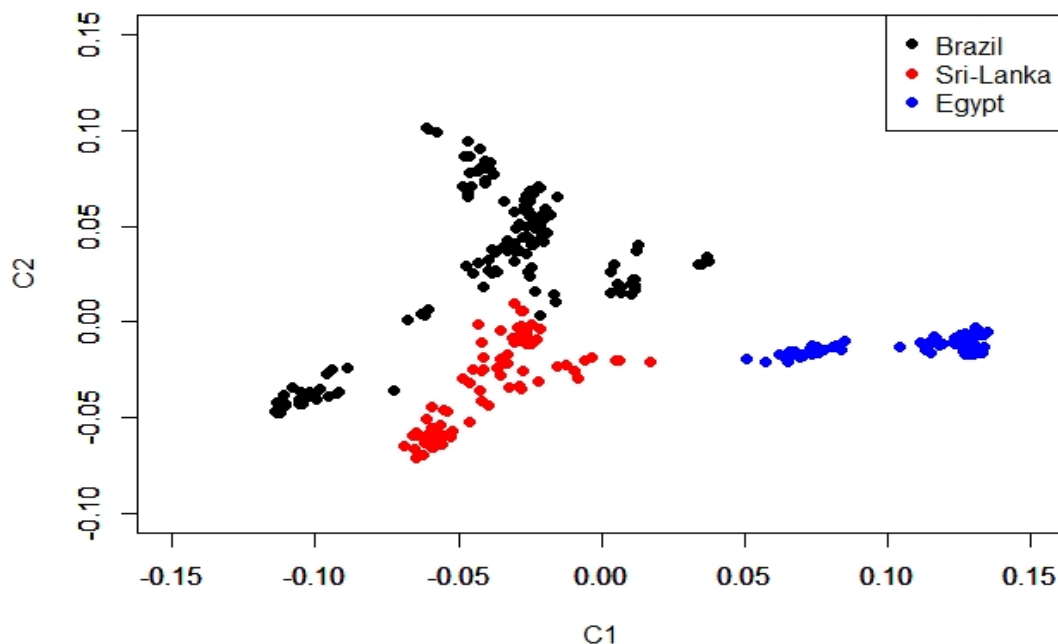


Figure 1. Multi-directional scaling plot showing Brazil, Sri Lanka, and Egypt distinct chicken ecotypes for  $F_{st}$  analyses.

TRMT1 catalyzed tRNA modification is required for redox homeostasis to ensure proper cellular proliferation and oxidative stress survival. Cells that are deficient in the TRMT1 will exhibit a decrease in proliferation rates, alteration in protein synthesis and perturbation in redox homeostasis including hypersensitivity to oxidizing agents (Dewe *et al.*, 2017). The second gene, MicroRNA 6545, is reported to be involved in reproductive processes and embryogenesis, including TGF- $\beta$  and Wnt that specifies the neutral fate of the blastodermal cells (Shao *et al.*, 2012). For the Sri Lanka vs Egypt comparison, a region on chromosome 3 (64.65 -64.75 Mb) was detected and contained the HS3ST5 gene. This gene is involved in immunity and defense molecular functions (Szauter *et al.*, 2011). There were also common regions between the two analyses of chickens from Brazil or Sri Lanka vs Egypt. A total of three common regions were identified on chromosome 2 (25.25 - 25.35 Mb; 25.35 - 25.45 Mb; and 26.15 - 26.25 Mb) with 38, 40, and 45 SNPs, respectively. Chromosomes 3 and 8 had each one common region of

111.25 - 111.35 Mb and 650 to 750 Kb with 4 and 44 SNPs, respectively. The Brazil vs Sri-Lanka comparison had generally lower mFst values in a similar environment.

## Conclusions

Results support that there are several signatures of selection on chromosomes 2 and 8 for the Brazil vs Egypt comparison, and on chromosome 3 for the Sri Lanka vs Egypt comparison and common differences between Arid (Egypt) and Tropical (Sri Lanka and Brazil). The differences between tropical and arid chicken populations could indicate the differences in chicken adaptations due to different climatic conditions in the respective countries

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