COMPUTATIONAL ANALYSIS OF MOLECULAR SIGNATURES OF SELECTION AT PRODUCTION GENES FOR EGG AND MEAT IN LIVESTOCK AND WILD RELATIVES

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Abstract

In Kenya, indigenous chicken form the majority of poultry. Their egg and meat production is low compared to commercial chicken. However there is variation among the indigenous chicken caused by evolutionary forces such as Natural Selection. The aim of this study was to model evolution and subsequent detection positive selection on genes for egg and meat production. Genes for egg production were prolactin, vasoactive intestinal peptide (vip) and intestinal peptide receptor (vipr) while genes for meat production were growth hormone (gh), growth hormone receptor (ghr), insulin growth factor 1(igf1) and insulin growth factor 1 receptor (igf1r). This was achieved by data mining of the sequences of these genes from databases followed by performing reciprocal BLASTp and BLOSUM62 substitution matrix using chicken sequence for each gene as the query. Homologues with an expectation value greater than 1e-10 were selected for each gene. Thereafter, Multiple Sequence Alignment was done using MUSCLE which uses an iterative algorithm. The alignments were edited using Seaview. MEGA6 was used to test for heterogeneity in substitution rate and for determining evolutionary model using lowest Akaike Information Criterion. Finally, phylogenetic trees were constructed using Nearest Neighbour Interchange with subtree pruning and regrafting of FastME followed by analysis for signatures of selection using PAML4. This led to inferred phylogenetic trees that modeled evolution of the genes in the different species and identification of positive selection on one amino acid site on igf1r. This is an advanced genetic technology that may be used to improve egg and meat production through artificial selection.

Key words: poultry; growth hormone; natural selection; insulin growth factor1 receptor; codon-substitution models; prolactin; vipr1

1.0 Introduction

Chickens, quails and guinea fowls are poultry which are domesticated by humans for production of eggs, meat and feathers. In Kenya, as in other countries worldwide, chickens account for largest livestock species reared by man. In Kenya, there are approximately 30 million domesticated birds. Indigenous chicken form 70%, layers, broilers and the breeding stock form 25% while the rest of the domestic birds like quails, guinea fowls, ducks, geese, turkeys and pigeons make up 5% of the poultry population (Olwande *et al*., 2010). The eggs and meat from the poultry are a source of protein and a source of income when they are sold. Eggs and meat from Indigenous poultry are highly favored because of their taste. Indigenous chickens lay an average of 40-60 eggs per year while commercial chickens lay more than 300 eggs per year (FAO, 2010a). Their eggs are small weighing about 25-49g. Indigenous chicken are also slow in maturing and they have small bodies. Broilers are 2kg live weight at 5 weeks of age while indigenous cocks merely weigh more than 1kg in 20 weeks (FAO, 2010a). The government and some NGO poultry programs have attempted to improve indigenous poultry through crossbreeding with commercial genotypes which has not been successful.

Indigenous poultry are highly variable phenotypically in size, skin colour, live weights and egg production among other traits (Kingori *et al*, 2010). This variation is as a result of individuals of a population changing to adapt to their environment through the process of Natural Selection. There are three forms of Natural Selection: (1) Purifying Selection which eliminates deleterious mutations (2) Positive Selection which refers to mutations that are advantageous (3) Neutral Selection which does not confer any fitness advantage (Oleksyk *et al*., 2010).

One reliable way to detect positive selection is by comparing the rate of non-synonymous substitution (dN) to the rate of synonymous substitution (dS). Values of dN/dS<1 indicate purifying selection while values of dN/dS>1 indicate positive selection. When the dN/dS=1, It indicates neutral selection (Yu *et al*., 2011). There have been several methods that have been proposed to estimate the dN/dS rate (Nei, 2005). Codon-based maximum likelihood models by Nielsen and Yang are the most accurate for detecting positive selection among lineages and amino acid sites as they account for variability in selection pressure among sites (O'Brien and Suchard, 2009). These models have been used to detect positive selection in various genes like growth hormone gene (Yuri *et al*., 2008), Leptin gene (Yu *et al*., 2011) and Hyperglycemic hormone (Padhi *et al*., 2007). In this study, we constructed phylogenetic trees to understand the evolution of the production genes and analysis for signatures of selection. In Kenya and most developing countries, improvement of indigenous poultry is not advanced (Dana *et al*., 2010). Current technologies are not accurate as there is no evidence of the influence of genes on the phenotype. In this study, we used codon-based models to detect positive selection in genes for egg production and growth. Application of selection of genes in improving production is more accurate, economical and reliable (Cheng, 2010).

2.0 Methods

2.1 Retrieval of Sequences from Biological Databases

The full coding sequence or mRNA sequences of chicken Prolactin, Vasoactive Intestinal Peptide and Vasoactive Intestinal Peptide receptor genes implicated for egg production and Growth Hormone, Growth Hormone Receptor, Insulin-like Growth Factor I, Insulin-like Growth Factor I Receptor implicated for growth were retrieved using cross-database ENTREZ searches in GenBank. The chicken genome available at the annotated ENSEMBL Database was used as the reference genome.

2.2 Retrieval and Selection of Homologues to the Candidate Genes using Reciprocal BLAST

A reciprocal BLAST was done using the algorithm BLASTp which is available at NCBI to infer homology. The amino acid sequence of each candidate gene in the fasta format was used as the query in performing pairwise sequence alignments in non-redundant (nr) databases. The substitution matrix used was BLOSUM62. For egg production, homologues selected were birds with an E value greater than 1e-10. For meat production, the homologues selected were animals that are commonly eaten by man with an E value of greater than 1e-10. Amino acid sequences of the homologues were converted to their corresponding coding sequences and the stop codons manually removed to prevent interference with later analysis.

2.3 Multiple Sequence Alignment of Homologous Sequences

ClustalX2 (Larkin *et al*., 2007) which is a MSA software that uses a progressive algorithm that is heuristic in nature was used to align the different homologues for each candidate gene to confirm homology and as a preceding step in phylogenetic analysis. PRANK that also uses the progressive algorithm and MAFFT (Katoh and Standley, 2013) were used. This was followed by MSA using MUSCLE version1.3.8.31-1 (Edgar, 2004). Jalview version 2.8 (Waterhouse *et al*., 2009) and Seaview were used to view and edit the alignments.
A Comparison of the alignments from the three MSA softwares was done.

2,4 Selection of Substitution Model

MEGA6 was used to test for heterogeneity in substitution rate and select the evolution model using the Akaike Information Criterion.

2.5 Phylogeny Construction

Phylogenetic trees were inferred using Nearest Neighbour Interchange with subtree pruning and regrafting of FastME2 (Lefort *et al*., 2015). 1000 bootstraps were used to test for confidence of the inferred relationships. The inferred trees were saved in Newick format. Dendroscope version3 (Yang, 2007) was used for graphical visualization of the trees.

2.6 Detection of Signatures of Selection

The PAML5 package (Yang, 2007) was then used for phylogenetic analysis using maximum likelihood.

3.0 Results

In the supplementary we find the orthologues for prolactin, vip, vipr, gh, ghr, igf1 and igf1r generated from a reciprocal BLAST approach. In Figure 1, the alignment from MUSCLE for vip gene is shown with the conserved domains. In Figure 2 and 3 respectively, the conserved domains are shown for prolactin and vipr1 genes.

Multiple Sequence Alignment of gh showing the different domains (Figure 4). Multiple Sequence Alignment of ghr with the different domains (Figure 5). Figure 6 and 7 shows the Multiple Sequence Alignment of igf1 and igf1r genes.

Phylogenetic tree in Figure 8 illustrates evolution of prolactin in the different species while Figure 9, 10, 11 respectively illustrate evolution of gh gene, igf1r gene and vipr1 gene. Figure 12 shows one positively selected amino acid site on igf1r gene.

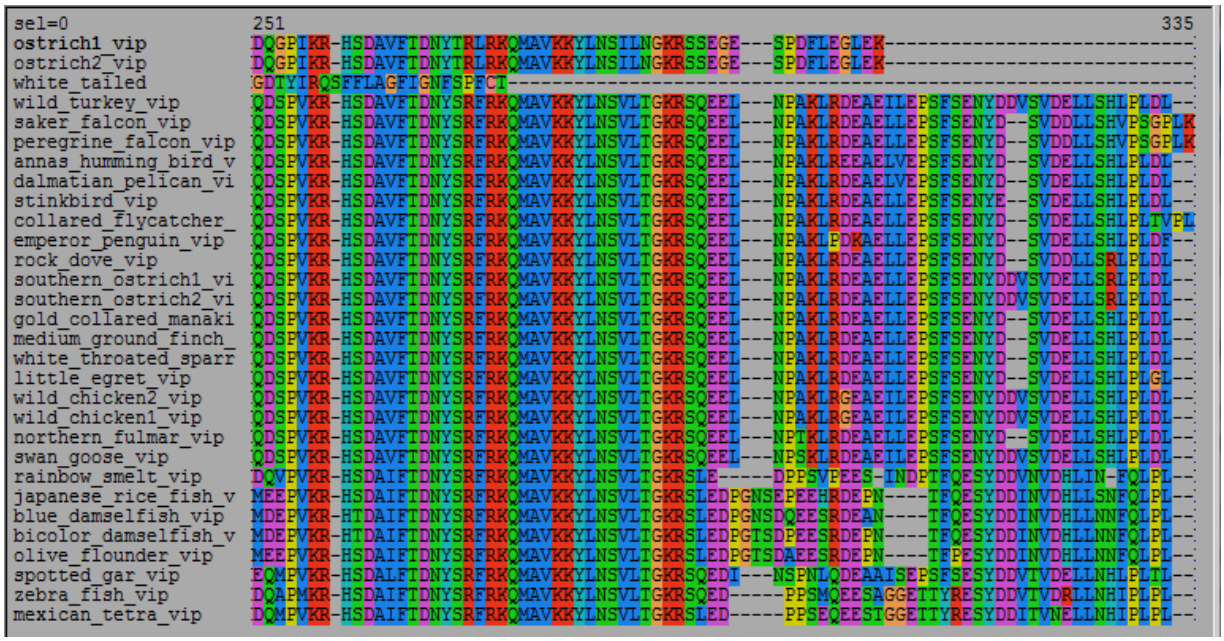

Figure 1: Multiple Sequence Alignment of vip showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

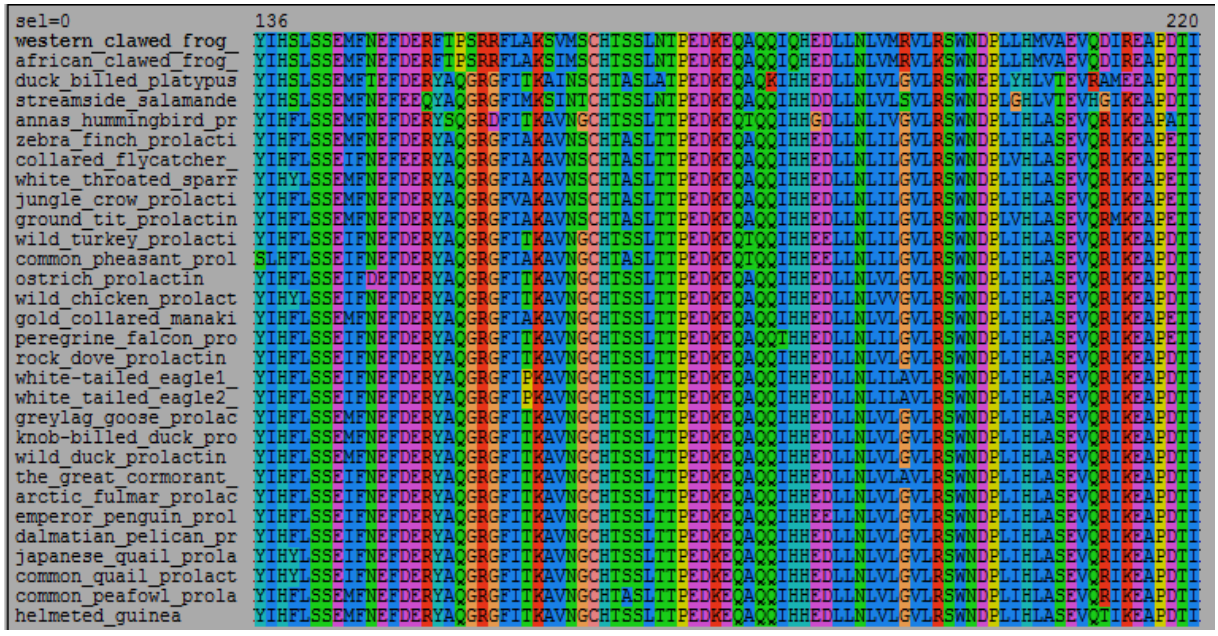

Figure 2: Multiple Sequence Alignment of prolactin showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

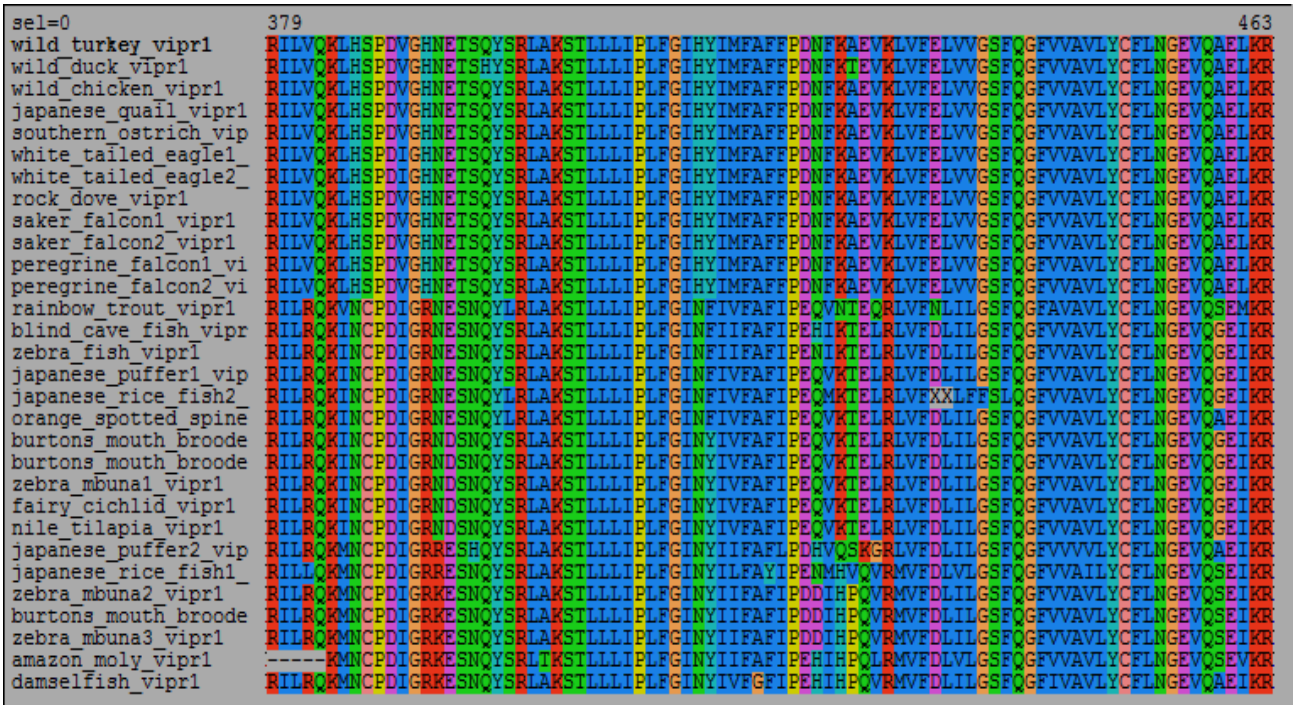

Figure 3: Multiple Sequence Alignment of vipr1 showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

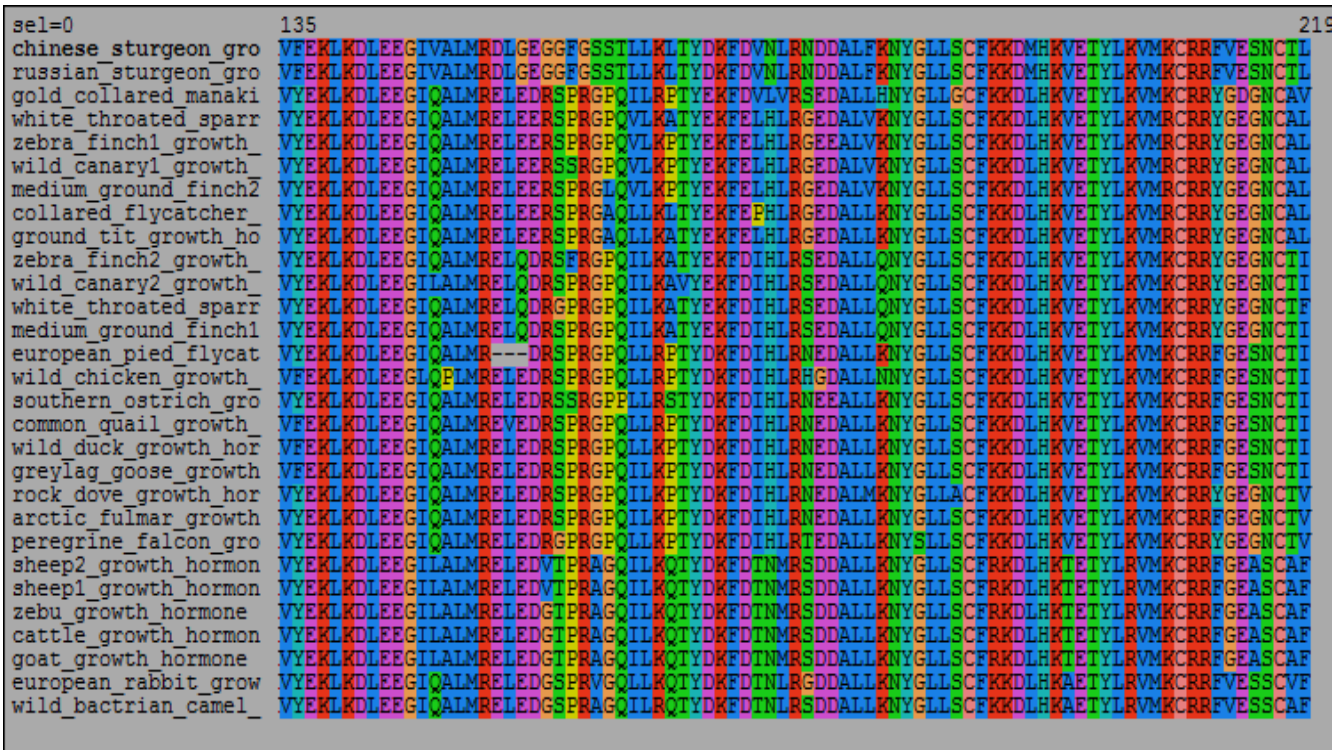

Figure 4: Multiple Sequence Alignment of gh showing conserved sites. MSA was done by MUSCLE v1.3.8.31-1 (Edgar, 2004)

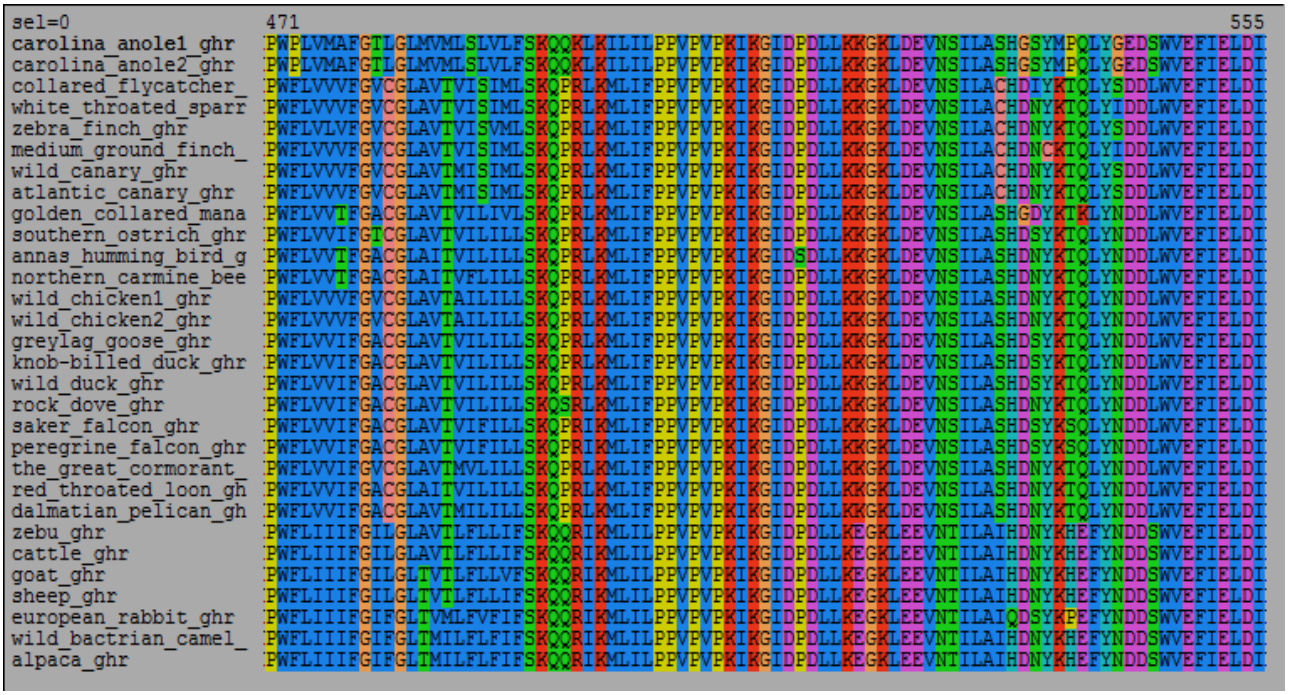

Figure 5: Multiple Sequence Alignment of ghr showing conserved sites. MSA was done by MUSCLE v1.3.8.31-1 (Edgar, 2004)

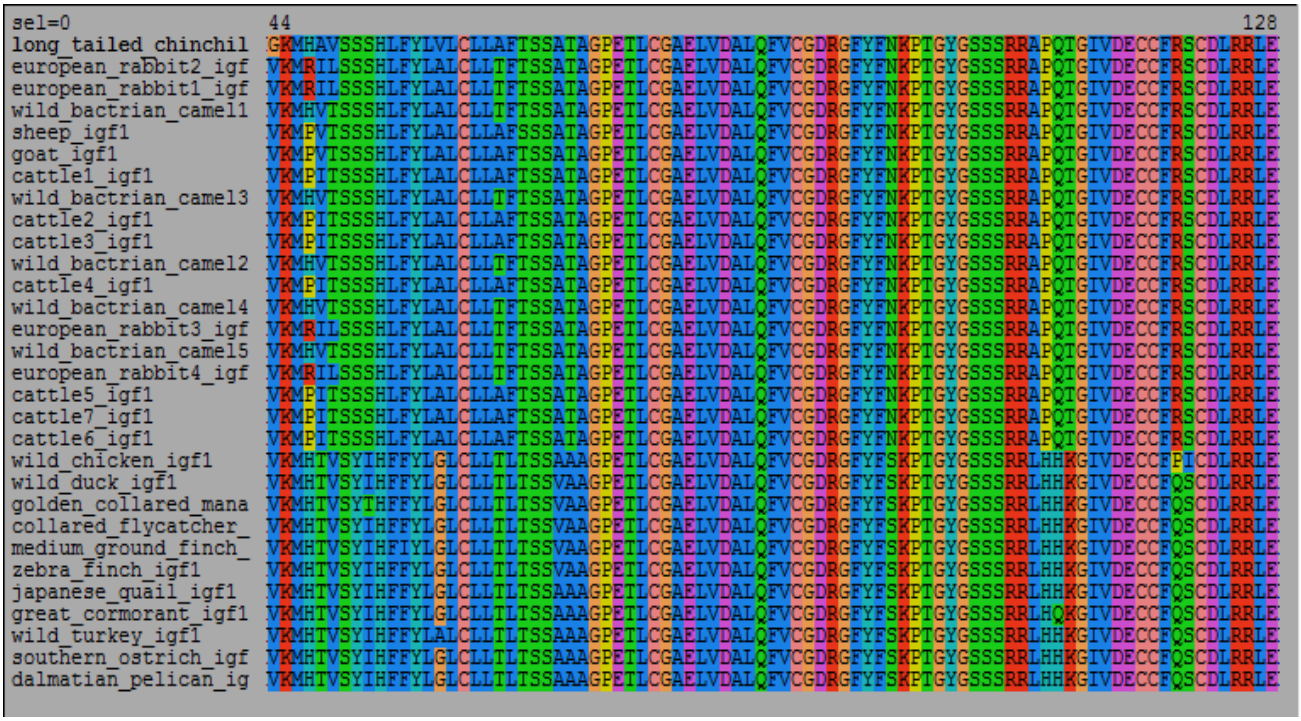

Figure 6: Multiple Sequence Alignment of igf1 showing conserved sites. MSA was done by MUSCLE v1.3.8.31-1 (Edgar, 2004)

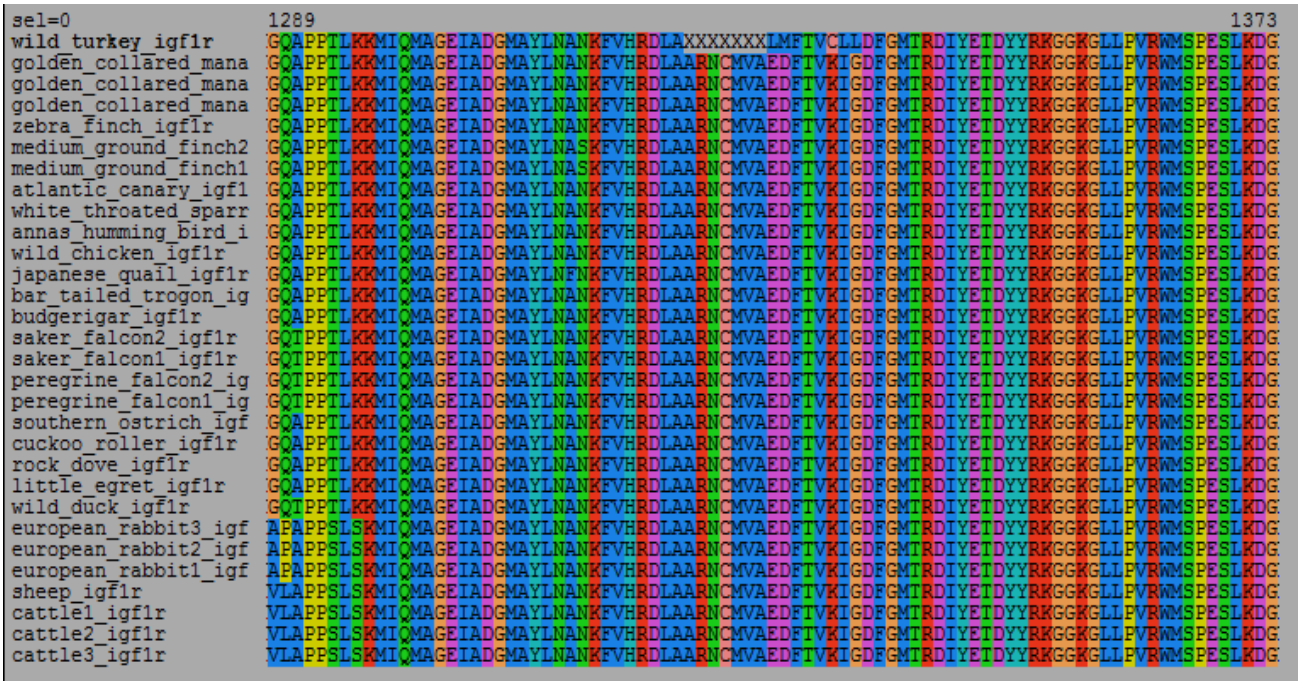

Figure 7: Multiple Sequence Alignment of igf1r showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

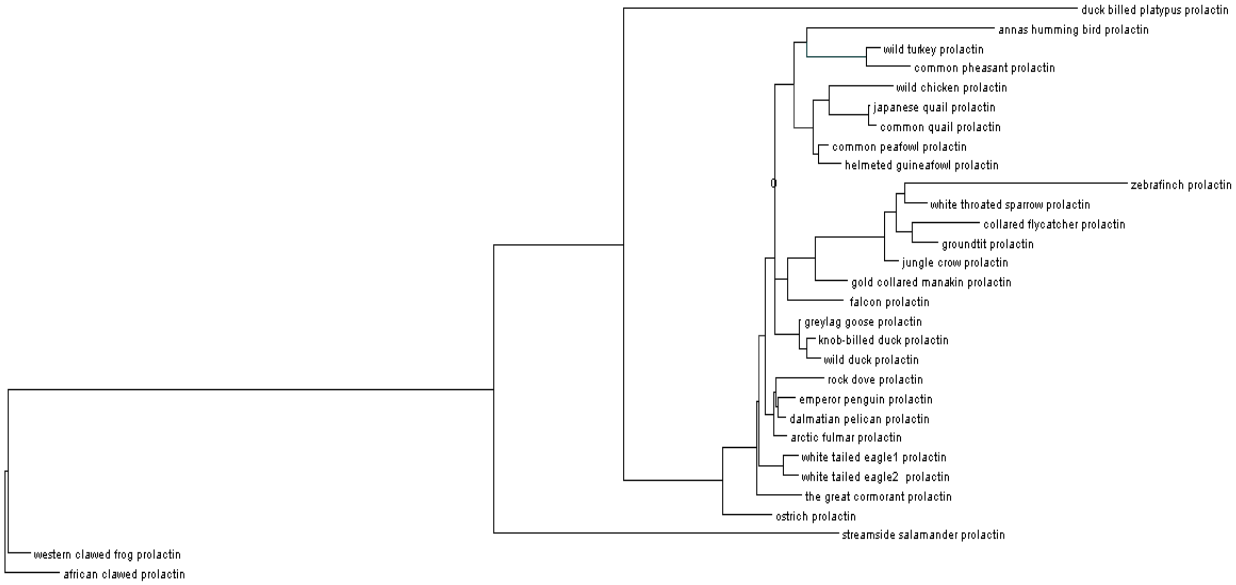

Figure 8: Phylogeny illustrating evolution of prolactin. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation.

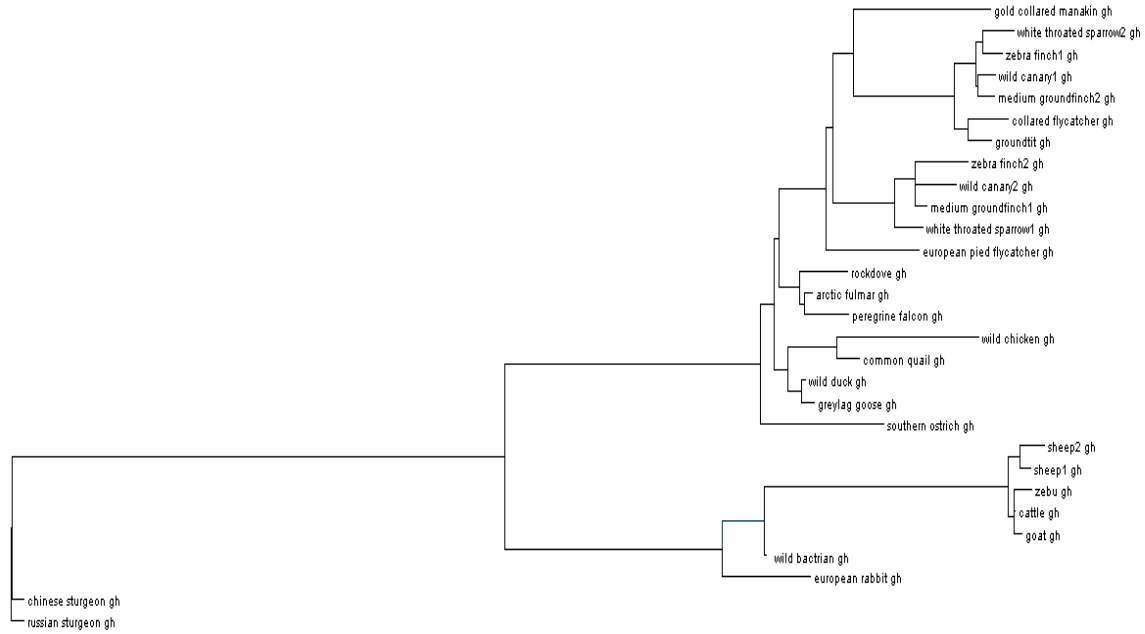

Figure 9: Phylogeny of growth hormone. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation

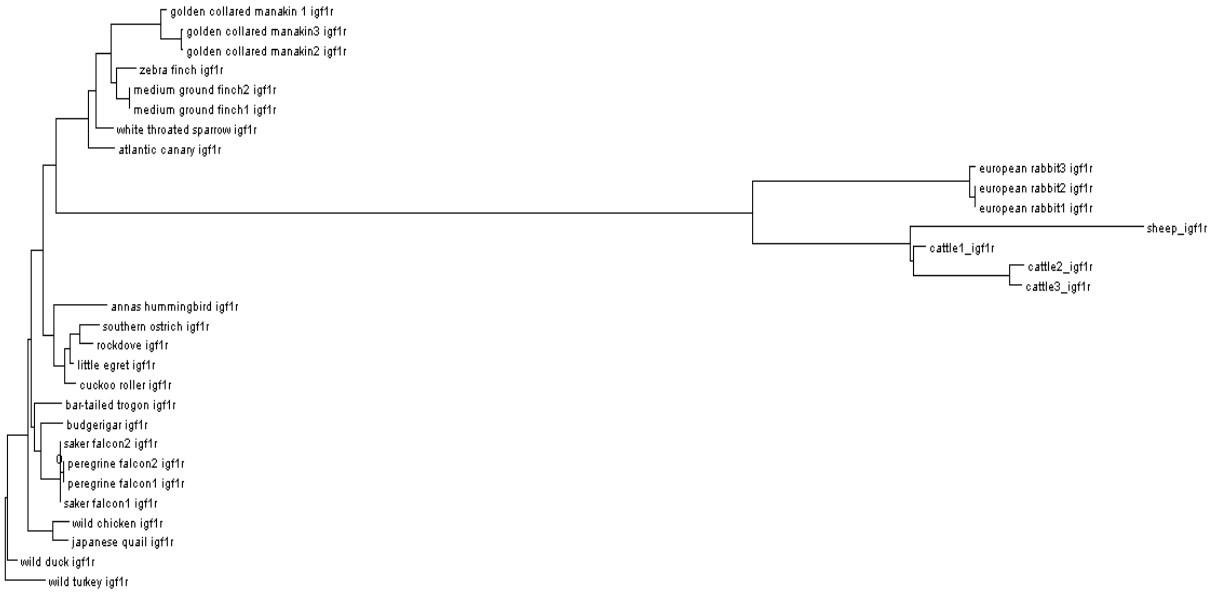

Figure 10: Phylogeny of igf1r. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation.

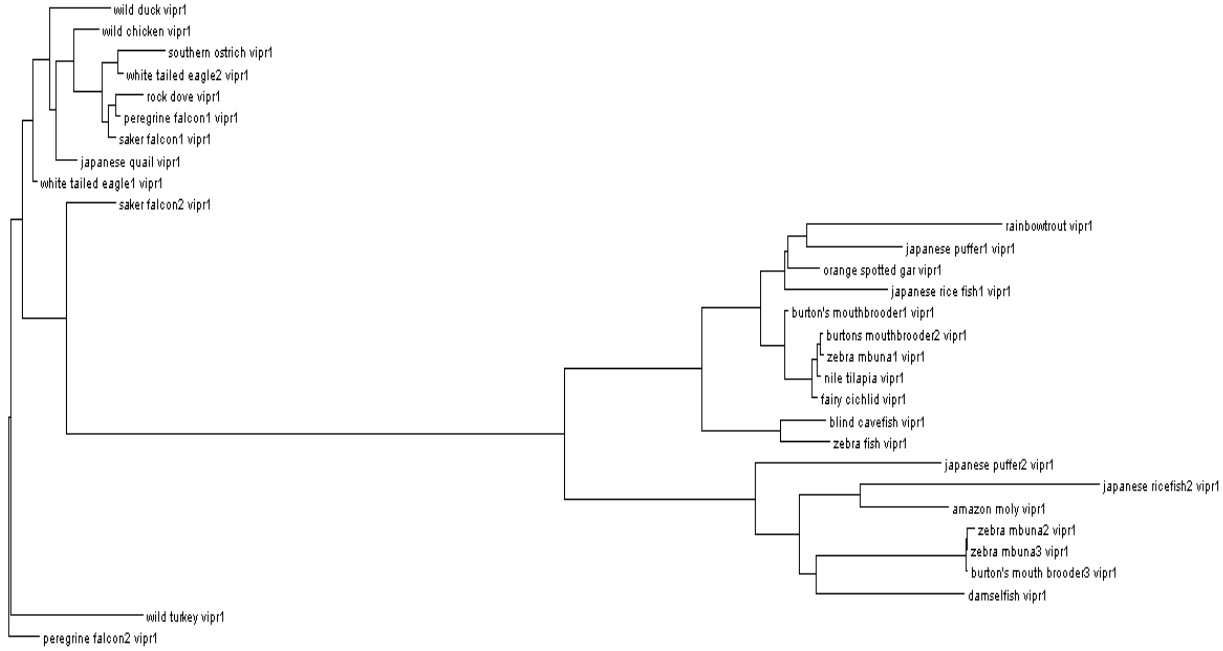

Figure 11: Phylogeny of vipr1. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation.

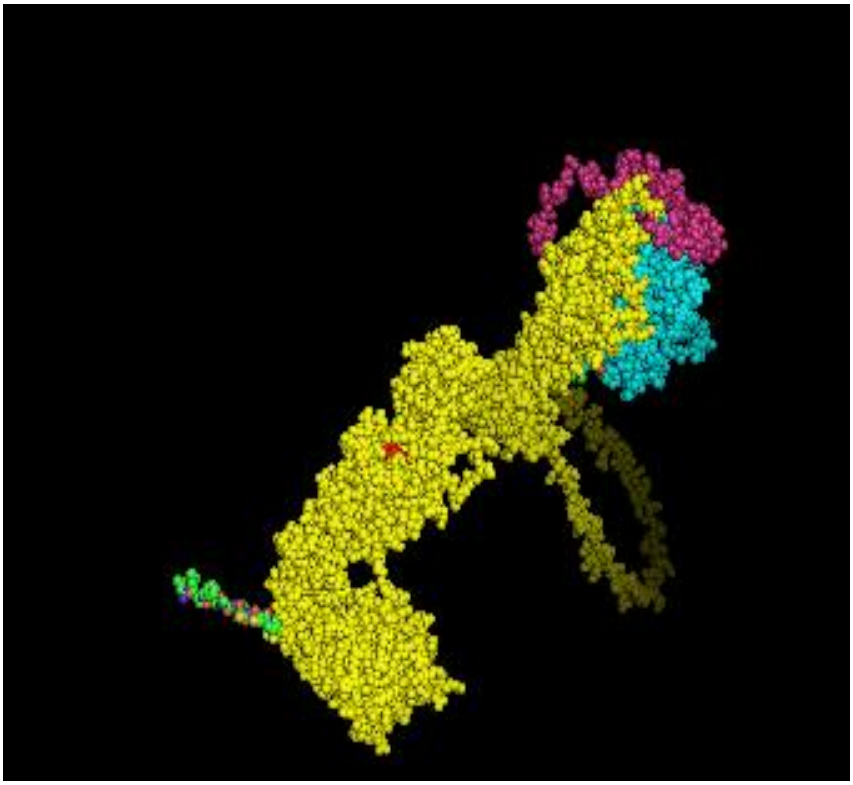

Figure 12: The 3D structure of igf1r showing the different domains. The site shown in red is under positive selection. The site is located in the L2 domain (shown in yellow) which is important for binding insulin. This structure was displayed by pymol v1.7.6 (DeLano, 2002).

4.0 Discussion and Conclusion

In this study, we successfully modeled evolution of the different production genes and performed analysis for positive selection. The computational method used is fast in modeling evolution compared to previous methods that took a long time.

Growth hormone is a polypeptide hormone which is present in all vertebrates (Kawauchi *et al*., 2002). It has a crucial function in growth and promoting differentiation at different target sites. In birds, growth hormone has other secondary functions such as reproduction, egg production and aging (Zhao *et a*l., 2004).
According to the phylogenetic tree of gh (Figure 9), we find that birds are clustered together and ruminants are clustered together. It is believed that the rate of evolution for any particular protein is constant although the rates differ significantly from one protein to another. However, the evolution of gh shows a pattern of variable evolutionary rate which is unusual (Forsyth and Wallis, 2002). The evolution of gh is generally slow because of the important roles it plays and perhaps the constraints imposed by multiple functions.

The sequences are highly conserved in the birds as can seen in Figure 4 while there are some substitutions in the ruminants. This is as a result of bursts of rapid change that occurred in some mammals. These bursts of rapid change occurred in two occasions: i) during primate evolution and ii) during evolution of artiodactyls. Most of the changes that have occurred during gh evolution occurred during these bursts.

An accepted explanation for the rapid evolution is adaptive natural selection although there lacks a well-defined associated functional change (Forsyth and Wallis, 2002).

This study is significant in understanding the process of evolution of the production genes. This is an important milestone in that *in vitro* and *in vivo* studies could be carried out to confirm the effect of positive selection of igf1r gene on growth. Subsequently, improvement of egg and meat production may be done using the positively selected genes as makers in molecular breeding.

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Supplementary Material

Table 1: Prolactin homologues

Specie	E value	Accession Number
Common quail	7e-150	BAD10927.1
Wild turkey	5e-147	AAB60604.1
White throated sparrow	3e-148	XP 005481833.1
Gold collared manakin	3e-148	KFW 77597.1
Peregrine falcon	7e-145	XP 005235773.1
Arctic fulmar	5e-163	KFV 94966.1
Greylag goose	5e-145	XP 007653890.0
Ostrich	4e-143	BAF81528.1
Wild chicken	5e-162	AAG01026.1
Common peafowl	1e-160	BAG68293.1
Japanese quail	8e-151	BAJ61717.1
Helmeted guinea fowl	2e-151	BAG68294.1
Dalmatian pelican	2e-143	KFQ 6004.1
Wild duck	3e-144	BAD14942.1
Emperor penguin	2e-134	KFM 11481.1
Knob billed duck	1e-143	CAJ55836.1
White tailed eagle 1	2e-139	KFQ 01370.1
Common pheasant	2e-143	BAG68292.1
The great cormorant	2e-143	KFW 89232.1
White tailed eagle 2	1e-139	XP 007867890.0
Rock dove	2e-134	ADK73557.1
Jungle crow	2e-139	BAJ61712.1
Ground tit	4e-139	XP 005525306.1
Collared flycatcher	1e-138	XP 005041658.1
Anna's humming bird	2e-114	XP 908765780.0
Zebra finch	5e-121	XP 004186110.1
Streamside salamander	7e-123	AP93863.1
Duck billed platypus	5e-114	XP 007657220.1
African clawed frog	6e-114	NP 001086486.1
Western clawed frog	5e-114	NP 001093699.1

Table 2: Vip homologues

Vip Gene		
Species	Accession Number	E value
Wild chicken 1	0.0	NP 990697.2
Swan goose	0.0	XP 008776654.0
Collared flycatcher	0.0	XP 005643344.4
Wild turkey	0.0	XP003204177.1
Southern ostrich 1	0.0	KFV78141.1
Northern fulmar	0.0	AA99877.0

Gold collared manakin	0.0	BAB98877
Rock dove	0.0	BAB99879
Saker falcon	0.0	AA98765
Peregrine falcon	0.0	KFW56765.4
Little egret	0.0	KFV87765.5
Emperor penguin	0.0	XP 004543220.0
Dalmatian pelican	0.0	XP 005465365.1
Wild chicken 2	0.0	NP 7866878.0
Southern ostrich2	0.0	NP 8979898.9
Stinkbird	0.0	NP 64763764.8
Medium groundfinch	0.0	KFW7878787
Anna's hummingbird	0.0	KFQ2334434
White throated sparrow2	0.0	XP 008987487.0
Spotted gar	0.0	XP 006574638.6
Ostrich1	0.0	BAB768567
Ostrich2	0.0	AA6758.0
White tailed eagle	0.0	NP 8976565.0
Zebra fish	0.0	BAB87765
Mexican tetra	0.0	BAB56432
Japanese ricefish	0.0	AA69870.0
Bicolor damselfish	0.0	XP 005644322.3
Olive flounder	0.0	XP 008887766.0
Rainbow smelt	0.0	XP 003476654.1
Blue damsel fish	0.0	XP 007876548.0

Table 3: vipr1 homologues

Vipr1 Gene		
Specie	Accession Number	E value
Wild chicken	BAA95164.1	0.0
Japanese quail	AED87510.1	0.0
Wild duck	EOA98591.1	0.0
Rock dove	EMC82014.1	0.0
Southern ostrich	BAA76574.1	0.0
Saker falcon1	XP 005442369.1	0.0
Peregrine falcon1	XP 005229590.1	0.0
White tailed eagle1	XP 007856847.0	0.0
Wild turkey	Q91085.2	0.0
Saker falcon2	XP 005442370.1	0.0
Peregrine falcon2	XP 005229591.1	0.0
White tailed eagle2	AAB67768.0	0.0
Blind cavefish	XP 007249106.1	0.0
Zebra fish	AAI162971	0.0

Nile tilapia	XP 003439239.2	0.0
Fairy cichlid	XP 006802769.1	0.0
Burton's mouthbroooder1	XP 005912343.1	0.0
Zebra mbuna1	XP 005463577.0	0.0
Zebra mbuna2	XP 006756847.8	0.0
Damsel fish	XP 007876487.0	0.0
Orange spotted spinefoot	ACC78770.1	0.0
Japanese rice fish1	AA787879.0	0.0
Burton's mouthbroooder2	XP 005933737.1	0.0
Zebra mbuna3	XP 007878780.0	0.0
Japanese rice fish 2	XP 004081326.1	0.0
Japanese puffer1	CAC82587.1	0.0
Japanese puffer2	XP 003977758.1	0.0
Rainbow trout	AAU29499.1	0.0
Burton's mouth brooder3	XP 007457657.6	0.0
Amazon moly	XP 007548620.1	2e-159

Table 4: gh homologues

Gh Gene		
Specie	Accession Number	E value
Wild chicken	AHM95535.1	9e-35
Arctic fulmar	EOA56765.0	8e-67
Wild duck	EOA99704.1	5e-34
Greylag goose	AAN37412.1	9e-34
Common quail	ACJ73931.1	5e-34
Peregrine falcon	XP 005238874.1	2e-30
Rock dove	EMC85315.1	6e-32
White throated sparrow1	XP 005487217.1	2e-28
Medium groundfinch1	XP 005425878.1	1e-29
South ostrich	EOA76764.0	4e-34
Wild canary1	ABB56767.0	5e-33
Medium groundfinch2	XP 005431290.1	8e-27
European pied flycatcher	ABB70042.1	5e-33
Zebra finch1	XP 002196167.1	1e-27
Gold collared manakin	XP 003686878.1	5e-33
Wild canary2	XP 002435767.0	1e-25
Ground tit	XP 005524208.1	2e-29
Collared flycatcher	XP 005059628.1	1e-25
White throated sparrow2	XP 006576477.8	4e-27
Zebrafinch2	XP 002187284.1	3e-28
European rabbit	XP 007636368.7	2e-28
Wild Bactrian camel	XP 006177464.1	4e-23

Zebu	XP 001122366.7	2e-23
Chinese sturgeon	XP 006356356.0	2e-23
Russian sturgeon	ABK74674.6	9e-24
Sheep1	ABK59498.1	2e-23
Sheep2	ABO21737.1	5e-23
Cattle	ABK67647.0	5e-23
Goat	ADX66303.1	9e-24

Table 5: ghr homologues

Ghr Gene		
Specie	Accession Number	E value
Wild chicken1	AGG38006.1	0.0
Wild chicken2	NP001001293.1	0.0
Dalmatian pelican	XP 0876532.0	0.0
Greylag goose	ACY38605.1	0.0
Saker falcon	XP 005433804.1	0.0
Peregrine falcon	XP 005242027.1	0.0
Wild duck	ACT 20710.1	0.0
Knob billed duck	ACT 20711.1	0.0
Rock dove	EMC76968.1	0.0
Southern ostrich	EMC9876.0	0.0
Anna's humming bird	ACY2165.0	0.0
Golden collared manakin	ACY3476.0	0.0
Red throated loon	ACY5876.1	0.0
White throated sparrow	XP 005493766.1	0.0
Zebra finch	XP 002193695.2	0.0
Medium groundfinch	XP 005422066.1	0.0
Wild canary	XP 3454267876.0	0.0
The great cormorant	NP6473676787.0	0.0
Collared flycatcher	AA018173.1	0.0
Northern carmine bee-eater	XP 6376387878.0	0.0
Atlantic canary	XP 0087765409.0	0.0
European rabbit	1401239A	0.0
Wild Bactrian camel	AA987430.0	0.0
Alpaca	AA897322.0	0.0
Carolina anole1	XP 008101043.1	0.0
Carolina anole2	XP 0008101044.1	0.0
Goat	XP 0077863233.0	0.0
Zebu	ABM92307.2	0.0
Cattle	AAU94310.1	0.0
sheep	NP001009323.1	0.0

Table 6: igf1 homologues

Igf1 Gene		
Specie	Accession Number	E value
Wild chicken	AGG38005.1	2e-94
Japanese quail	AAF67202.1	2e-94
Great cormorant	XP 008766769.2	1e-64
Wild turkey	XP 003202426.1	7e-95
Wild duck	ABS76279.1	2e-94
Zebra finch	XP 006754322.0	2e-62
Collared flycatcher	XP 005040114.1	7e-94
Golden collared manakin	XP 006921111.4	1e-63
Medium ground finch	XP 005421104.1	1e-93
Dalmatian pelican	AAF98765.0	2e-94
Southern ostrich	AAF34222.0	3e-75
Wild Bactrian camel1	XP 006186100.1	1e-64
European rabbit1	XP 008254938.1	2e-62
Cattle1	AAF56222.1	2e-62
Long tailed chinchilla	XP 005374627.1	7e-65
Goat	BAB77524.1	3e-75
Sheep	ACG49835.1	1e-72
Wild Bactrian camel2	XP 006186101.1	5e-52
Wild Bactrian camel3	XP 006186102.1	7e-66
Wild Bactrian camel4	XP 006186103.1	1e-64
Wild Bactrian camel5	XP 006186104.1	6e-79
Cattle2	AAF22156.2	1e-63
European rabbit2	XP 008254939.1	7e-50
Cattle3	AAF42111.0	7e-50
Cattle4	AAF11114.2	3e-75
European rabbit3	XP 008254940.1	2e-62
European rabbit4	XP 008254941.1	5e-77
Cattle5	AAF75333.2	3e-75
Cattle6	AAF73432.0	3e-75
Cattle7	AAF23407.1	2e-62

Table 7:igf1r homologues

Igf1r Gene		
Specie	Accession Number	E value
Wild chicken	AGG38009.1	0.0
Japanese quail	BAF73401.1	0.0
Saker falcon1	XP 005436689.1	0.0
Peregrine falcon1	XP 005242493.1	0.0
Saker falcon2	XP 005436690.1	0.0

Peregrine falcon2	XP 005242494.1	0.0
Medium ground finch1	XP 005424278.1	0.0
Zebra finch	XP 002199843.1	0.0
Medium ground finch2	XP 005424279.1	0.0
Golden collared manakin1	XP 004687532.2	0.0
Golden collared manakin2	XP 000997654.8	0.0
Golden collared manakin3	EMC 77848.3	0.0
Wild duck	EOB07472.1	0.0
Little egret	EOB23699.0	0.0
Rock dove	EMC 77329.1	0.0
Southern ostrich	EMC 76589.5	0.0
Wild turkey	XP 0032009598.1	0.0
Atlantic canary	XP 0034509876.0	0.0
White throated sparrow	XP 0012567876.5	0.0
Anna's humming bird	XP 0035779654.0	0.0
Budgerigar	XP 0045885434.3	0.0
Cuckoo roller	XP 0056328797.1	0.0
Bar tailed trogon	XP 0011187072.0	0.0
Cattle1	XP 0078656766.5	0.0
European rabbit1	XP 0066666988.2	0.0
European rabbit2	XP 0011765445.3	0.0
European rabbit3	XP 0044498885.2	0.0
Sheep	XP 0040085983.1	0.0
Cattle2	XP 0067333333.3	0.0
Cattle3	XP 0078899906.2	0.0