MOLECULAR EVIDENCE OF AVIAN EVOLUTION: PART 2

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doi: https://doi.org/10.52458/9789391842109.2022.ch02.eb.asu  Ch.Id:-ASU/EB/ELBDR/2022/02
EVOLUTION AND ORIGIN

Galliformes is the order of which the chicken is a member of, just like peafowl, quails, turkeys etc. this order consists of ground-feeding birds. Galliforms are the direct descendants of the Galliforms-like proto-birds that survived the cretaceous tertiary extinction event that obliterated all non-avian dinosaurs.[5]

As the ancestors of the galliformes were dependent only on the ground niche and not the trees, they survived the mass extinction by feeding off the ground, and gave rise to the modern birds.

In an experiment to show the evolutionary link between dinosaurs and birds, researchers created a chicken embryo with a dinosaur snout and palate, by switching on a recessive gene. Birds are therefore a useful model organism to study various aspects of evolution.[6]

Fig 2: Comparison of a regular chicken skull, a genetically altered chicken skull, and a crocodilian skull.


REVIEW OF LITERATURE

A. Speciation

Speciation is the evolutionary process by which a population of organisms becomes distinct enough that it cannot interbreed with any other population of similar biological characteristics. Speciation is a never-ending process and keeps forming new species out of earlier populations of similar characteristics. A single alpha species can give rise to multiple beta and gamma species. The alpha species now becomes a higher taxon that can be used as an umbrella term for the other species.[7]
The alpha species might become a genus, which then would slowly become an order and then a class and so on. It is important however that any taxon must have a unique set of genes that differ from rest of the fauna. For example, two species must have different genes which prevent them from interbreeding. In case of birds, we see that all birds have beaks and forelimbs modified to wings.[7]

They are also covered in feathers and are warm-blooded. The genes causing the above mentioned phenotypes are only active in birds, and no other class of vertebrata. Furthermore, Galliformes, an order of ground feeding birds has a set of genes that no other order of birds have, such as those that manifest into adaptations for surviving in the ground niche, without any dependence on trees. Finally, the fowl, which is a species of the galliformes order, must have distinct genes that no other galliformes have.

B. Unique Genes of a Taxon

To study the speciation of the fowl, we will look at various genes that fowls have and compare them to the corresponding genes from other bird species like ostrich or other vertebrate species like humans, platypuses, chimpanzee, zebra-fish, crocodiles etc. we do this to prove if the fowl has some unique genes if them or not. It is important for a population to have a set of unique genes to be categorized under a taxonomic group like a class, order, genus or even a species. Genes for production of chicken egg must be unique enough to distinguish themselves from the eggs of reptiles, amphibians, or egg laying mammals. The unique genes of a species may evolve by mainly three different mechanisms:-

**Gene formation:** This is an evolutionary phenomenon where new genes occur from duplication events, but are so different from the parent gene that they show no genetic similarity with it. Such genes seem to have been spontaneously generated in the genome.
If a phylogeny tree is constructed with this gene, we see minimum homology of this gene with any other counterpart from a different species. Birds may possess special genes for egg production that no other class possesses. Discovering such genes can be used as an evidence to prove distinct evolution or speciation.

**Gene divergence:** This is a mechanism through which a single gene slowly changes into two different variants.

Gene divergence leads to reproductive isolation, and as a result, speciation. The fowl must have genes that have a homologous counterpart in other bird species or vertebrates. If this is true, then we will observe high degree of similarity in the two genes, yet enough mutations in them to form two variants.

**Gene loss:** In this situation, some genes unique to the fowl may be a result of retention of those genes by the fowl throughout evolution. These genes would not be present in other birds due to the loss of such genes in them during evolution. If we identify such genes in the fowl, we can use the data as an evidence for the distinct evolution of the fowl.

We will use specific genes to confirm our theory such as ovocalyxin 36, an antibacterial gene found in the birds eggshell matrix.

HOX gene clusters A and D that code for the limbs of tetrapods and so are present also in the fowl. Such genes can be used to make phylogenic trees of the fowl with other vertebrates.[8]

**Genes of interest**

**Ovocalyxin 36**

Ovocalyxin 36 codes for an anti-bacterial protein which is found in the eggshell matrix of the chicken egg.

Ovocalyxin 36 or simply, OCX 36 is found in the areas of the fallopian tube, which are responsible for the formation for the eggshell of the chicken’s egg. OCX 36 seems to share 20% similarity with the BPI/LBP/PLUNC gene family of antibacterial proteins present across the tetrapod taxons. This BPI/LBP/PLUNC gene cluster is found in all the amniotes, yet the specific gene of OCX36 is unique to the genomes of the galliformes, best example being the fowl. This theory is supported by the fact that we couldn’t find this gene in any other tetrapod which further ignites our suspicion regarding the theory that OCX 36 gene was a result of aduplication event in early birds like the galliformes after the birds split from the rest of the amniotes.
Fig 4: Various eggshell matrix proteins like ovocalyxin 36 and ovocalyxin 32 etc.


This BPI/LBP/PLUNC super-family of proteins is responsible for the basic immunity of the body in the case of mammals; hence it is our hypothesis that OCX 36 also plays a role in the immunity of the egg and its embryo. Mammals don’t have this gene, the reason for which could be that mammals will not have genes for egg proteins, as true mammals or the eutherians don’t lay eggs. We can therefore, align the sequences of OCX 36 and the other BPI/LBP/PLUNC genes to see if the OCX 36 gene is a result of genetic divergence, or gene formation.

Ovocalyxin 32

OVOCALYXIN 32 or OCX 32 is another such eggshell protein found in the eggshell matrix that shares about 50% similarity with the proteins: latexin or LXN and retinoic acid receptor responder protein 1 or RARRES1. It can be hypothesized that the gene OCX 36 is an ortholog of LXN, as this gene is present in the bony fish, which could mean that OCX is duplicated from LXN and so is a paralog of LXN gene, while being an ortholog of RARRES1 gene as it also is found in the mammals, but not in the birds. A theory can be formed here which states that LXN gave rise to a gene which further evolved into two different genes like RARRES1 in mammals and in LXN in birds. Also, OCX is present in birds other than the galliformes like zebra-finch and the gene for OCX 32 lies next to LXN in chromosomes 9 or 11, depending on the species. When it comes to fish like the zebra-fish, we observe that the RARRES1 gene is present at an orthologous position to OCX-32 gene. Even though these three genes are homologous to each other in each in different ways, the function of these genes are quite diversified: OCX 32 is an antimicrobial eggshell protein, while the LXN gene is the sole endogenous carboxypeptidase A inhibitor in mammals, playing a crucial role of the regulation of hematopoietic stem cell activity, whereas RARRES1 gene is assumed to play the role of a tumor suppressor in humans. These facts point to the direction of the hypothesis that
OCX 36 and RARRES1 genes may be a result of gene divergence.

**Hox gene family**

Initially it was thought that the Hox genes plays a critical role during vertebrate limb growth, but recently it proved was proved by a group of scientists by a removing the Hox (A-11 & D-11) that codes for wrist in mice & it was observed that progeny of that mice did not had wrist (refer figure 17). In fact, deletions of both the Hox B and Hox C clusters did not evoke an irregular phenotype in the limbs. The combined deletion of both Hox A and Hox D, however, results in an early arrest of limb growth. Further it was found that hox genes are not only responsible for formation of structure of an organism, but it also has control over the positioning, size of morphology of organisms.(10)

Hox A and Hox D genes belonging to identical paralogous class (located at the same respective cluster positions) exhibit close, but not identical, domains of expression during limb development. Vertebrate limb growth begins with the development of buds in the embryonic body wall, then these buds eventually expand. Initially, the buds consist of undifferentiated mesenchyme cells found in ectoderm, but cells in the portion of the bud closest to the body wall tend to divide to form the humerus/femur as the bud elongates. When the bud grows out, the backbone is then eventually laid down in series with the digits being shaped last of all. Classical embryological studies in chick limb buds have shown that in the developing limb bud there are 3 sets of cell-cell connections, one related to patterning along each of the three axes: proximo-distal, dorso-ventral, and antero-posterior axes. Laying down the limb’s proximo-distal axis is connected to the outgrowth of the bud that is regulated by the apical ectodermal ridge, the thickened epithelium that surrounds the extremity of the limb; dorso-ventral patterning relies on epithelial-mesenchymal connections between the ectoderm along the sides of the bud and the mesenchyme which is underlying it; and antero-posterior patterning includes signaling at the posterior margin of the limb bud by the polarizing area, a region of mesenchyme cells. Anterior cells are specified to form posterior structures when a polarizing area is grafted to the anterior margin of a chick wing bud, and an additional sequence of digits develop in mirror-image symmetry with the regular collection. Two Polarizing region signaling also leads to maintenance of the apical ectodermal ridge over the posterior part of the limb bud; in turn signaling by the tyapical ridge maintains polarizing region signaling. The apical ectodermal ridge and polarizing region are also indicated.(11)

Hox Gene clusters A and D are the primary contributors to the development of limbs in vertebrates. Staining using unique antibodies revealed that Hox C-6 is expressed in many different vertebrates in the anterior-proximal area of early forelimb buds, including Xenopus, zebrafish, mouse, and chicken. Hox D genes (Hox D 9-13), then
known as Hox 4 genes, are expressed in overlapping domains centered on the posterior-distal margin in both fore and hind limb buds in mouse embryos. There is both temporal and spatial co-linearity of expression of these genes in the early limb bud, with 3’ genes being expressed before 5’ genes and 3’ genes being expressed in more proximally than 5’ genes.(12)

**Fig 5:** a) Stylopod stage

Inside the expression pattern of the adjacent 3* gene, the expression pattern of each additional 5’ gene in the cluster is covered. In early limb buds in chick embryos, identical patterns of Hox D gene expression are also shown and progressive expression of genes in the cluster in a 3 ‘to 5’ direction was seen to occur very rapidly within a few hours. 5’ Hox A genes were found to be expressed in similar overlapping domains in chick wing buds along the proximo-distal axis but without a clear posterior bias. A systematic and thorough study of the expression of all Hox genes, including genes from all four clusters, in developing chick limbs. In anterior/proximal regions of either wing
or leg bud, or both, Hox C genes are usually expressed. One cautionary finding was that Hox C 6 transcripts were detected in both fore and hind limbs whereas, as previously reported, Hox B 6 protein was only detected in forelimbs. Hox B 9 is expressed in the anterior of the leg bud and Hox B 8 expression in the posterior of the early wing bud has subsequently been described. With respect to Hox A and Hox D genes, there are at least 3 different phases of expression. Hox D 9 and Hox D 10 genes are expressed in the lateral plate mesoderm during the first period of expression as it starts to thicken to form the bud. Prior to this, several different Hox genes are expressed in dynamic patterns in lateral plate mesoderm in presumptive limb regions, including other Hox 9 paralogs such as Hox B 9. It has been suggested that the expression patterns of these genes at these pre-limb stages may serve to position the presumptive limbs along the main head to tail axis of the embryo.(12)