Genome sequencing of “living fossil” fish sheds light on the evolution of land animals

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In a newly published article in the scientific journal *Nature* (496, 311–316), scientists report a significant step in the understanding of the evolutionary process that led certain ancient fish to develop limbs and emerge onto dry land, becoming the ancestors of all amphibians, reptiles, birds, and mammals. This transition is one of the most important in the development of life on earth.

The new research is based on the decoding of the genome of a so-called “living fossil” fish, the “coelacanth” (pronounced SEE-luh-canth). This group of fishes, known from fossils dating as far back as 400 million years ago, was long thought to have gone extinct 70 million years ago, in the Late Cretaceous Era. However, the discovery of a living specimen in the West Indian Ocean off of South Africa in 1938 demonstrated that a reclusive population still survived. More recently, a second species of living coelacanth, both belonging to the genus *Latimeria,* was discovered in Indonesia.

Coelacanths have been colloquially known as living fossils because of their close resemblance, at least outwardly, to fossil specimens dating back tens and even hundreds of millions of years. But perhaps the most interesting aspect of these fish is their apparent close evolutionary relationship to the first land vertebrates.

It has long been thought, based on paleontological and anatomical evidence, that all tetrapods (four-limbed, vertebrate animals—amphibians, reptiles, birds, and mammals) evolved, somewhere in the neighborhood of 400 million years ago, from fish closely resembling the now mostly extinct group of lobe-finned fish belonging to the class Sarcopterygia.

Lobe-finned fish have a well-developed bony skeleton inside their fins, in contrast to the common ray-finned (teleost) fish. Living representatives of this lineage include lungfish, which are actually able to breathe air with lungs that are homologous (have the same genetic origin) as those of tetrapods, and the coelacanth.

Basic anatomical similarities between the skeletal structures in the fins of lobe-finned fish and the limbs of tetrapods strongly suggested an evolutionary link. However, the details of the mechanism for the transformation of fins into limbs had yet to be understood. The newly reported research begins to fill in that gap.

The technique of DNA sequencing, which in recent years has permitted the decoding of the human genome and those of a growing number of other species, has now been used to document the coelacanth’s DNA sequence. Among the resulting discoveries is a genetic mechanism that controls the growth of both the lobe-shaped fins in the coelacanth and of limbs in tetrapods.

Scientists have long wondered whether lungfish or coelacanths were more closely related to tetrapods. In other words, which group is closer to the common ancestor of the earliest vertebrate animals to walk on land? Genetic sequencing holds the potential to answer such questions. Unfortunately, lungfish have an incredibly large genome, which cannot be effectively sequenced by existing methods. It should be noted that the size of a species’ genome has no necessary correlation with the complexity of the organism, due to duplications of DNA sequences and other “baggage” accumulated during the course of evolution, which is not a particularly neat process.

Despite this, the scientific team whose research was published in *Nature* was able to decode segments of the lungfish genome and compare it to their newly completed full coelacanth sequence as well as to those

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from a sample of tetrapods. This permitted the conclusion that lungfish are more closely related to tetrapods than are coelacanths. Nevertheless, since the two groups of lobe-finned fish are closely related to each other, knowledge of the coelacanth genome is revealing much regarding the evolution of the first land-dwelling vertebrates.

The first and highly necessary step for the investigators was to determine whether the living coelacanth had evolved only very slowly from its ancestors millions of years ago, as suggested by its ancient outward appearance. This was critically important because the relevance of further analysis would depend on whether the genetic patterns observed in the modern coelacanth could be expected to resemble those of the common ancestor of lobe-finned fish and tetrapods.

The results of this analysis indicate that coelacanths have evolved very slowly as opposed to a number of other species to which they were compared. Many factors influence the rate of evolution in any given lineage of organisms. Evolution is a dialectical interaction between a species and all the elements of its environment. A stable environment means that there is little selective pressure that would prompt adaptive changes. The relatively static environment in which the surviving coelacanths live, and a lack of predators, are likely to have been factors in their slow evolution.

With that understanding, the researchers focused on studying genes that control the expression of structural genes (i.e., genes that build body parts). Such control genes regulate such factors as the degree, rate, and timing of expression of the structural genes.

Among the categories of regulatory genes identified as being newly evolved in tetrapods (that did not exist in the ancestral lobe-finned fish) were ones associated with sensing of chemicals, through taste and smell, driven by the need to more fully perceive the complex terrestrial environment.

Other categories of new control genes include those related to radial pattern formation (body form), hind limb development, kidney development, and the immune system. Again, all of these would have been important in evolutionary adaptation to the new constraints and opportunities of terrestrial existence.

In all, the team identified over 44,000 control genes that evolved after the appearance of tetrapods.

The researchers paid particular attention to the genetic control of the development of hands and feet by comparing genes of teleost (ray-finned) fishes, coelacanths, and tetrapods. They found a specific gene sequence that is common to the latter two, but not found in the former. This sequence was identified as affecting limb development. Experimental insertion of the coelacanth genes into mouse embryos demonstrated that they could provide much of the regulation needed to produce tetrapod limbs, thus demonstrating that this gene complex existed in lobe-finned fishes and was repurposed in the evolution of land animals.

Also identified was a gene in coelacanths that appears later to have become important in the development of the mammalian placenta. Coelacanths give birth to live young which develop inside the mother's body, but don't receive direct nourishment from the mother during gestation, as is the case with placental mammals.

The research reported by this team demonstrates the great power and potential of genetic sequencing in helping to elucidate the patterns and mechanisms of biological evolution, complimenting evidence from the fossil record. In a broader sense, this work shows that biological evolution is a process that can be objectively studied and understood as part of the development of the material world.

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