



PRESS RELEASE

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HOW THE GENETIC BLUEPRINTS FOR LIMBS CAME FROM FISH

A study led by Denis Duboule shows that these appendages have emerged during evolution by modernisation of a preexisting DNA structure



Expression of fish Hox genes in a mouse embryo

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The transition from water to land is one of the most fascinating enigmas of evolution. In particular, the evolution of limbs from ancestral fish fins remains a mystery. Both fish and land animals possess clusters of *Hoxa* and *Hoxd* genes, which are necessary for both fin and limb formation during embryonic development. Denis Duboule's team, at the UNIGE and the EPFL, Switzerland, compared the structure and behavior of these gene clusters in embryos from mice and zebrafish. The researchers discovered similar 3-dimensional DNA organization of the fish and mouse clusters, which indicates that the main mechanism used to pattern tetrapod limbs was already present in fish. However, when inserted into transgenic mouse embryos, the fish *Hox* genes were only active in the mouse arm but not in the digits, showing that the fish DNA lacks essential genetic elements for digit formation. The study, published in the January 21, 2014 edition of *PLoS Biology*, thus concludes that, although the digital part of the limbs evolved as a novelty in land animals, this happened by elaborating on an ancestral, pre-existing DNA infrastructure.

Our first four-legged land ancestor came out of the sea some 350 million years ago. Watching a lungfish, our closest living fish relative, crawl on its four pointed fins gives us an idea of what the first evolutionary steps on land probably looked like. However, the transitional path between fin structural elements in fish and limbs in tetrapods remains elusive.

An ancestral regulatory strategy ...

In animals, the *Hox* genes, often referred to as 'architect genes', are responsible for organizing the body structures during embryonic development. Both fish and mammals possess clusters of *Hoxa* and *Hoxd* genes, which are necessary for fin and limb formation. The team of Denis Duboule, professor at the University of Geneva (UNIGE) and the Ecole polytechnique fédérale de Lausanne (EPFL), Switzerland, had recently shown that, during mammalian development, *Hoxd* genes depend on a 'bimodal' 3-dimensional DNA structure to direct the development of the characteristic subdivision of the limbs into arm and paw, a division which is absent from fish fins.

'To determine where the genetics behind this subdivision into 'hand' and 'arm' came from during evolution, we decided to closely compare the genetic processes at work in both fin and limb development', says Joost Woltering, researcher at the Department of Genetics and Evolution of the UNIGE Faculty of Science and lead author of the study. Surprisingly, the researchers found a similar bimodal 3-dimensional chromatin architecture in the *Hoxd* gene region in zebrafish embryos.

These findings indicate that the regulatory mechanism used to pattern tetrapod limbs probably predates the divergence between fish and tetrapods. “In fact this finding was a great surprise as we expected that this ‘bimodal’ DNA conformation was exactly what would make all the difference in the genetics for making limbs or making fins” adds Joost Woltering.

...that just needs to be modernized

‘...our digits evolved during the fin to limb transition by modernizing an already existing regulatory mechanism.’

Does this imply that digits are homologous to distal fin structures in fish? To answer this question, the geneticists inserted into mice embryos the genomic regions that regulate *Hox* gene expression in fish fins. ‘As another surprise, regulatory regions from fish triggered *Hox* gene expression predominantly in the arm and not in the digits. Altogether, this suggests that our digits evolved during the fin to limb transition by modernizing an already existing regulatory mechanism’, explains Denis Duboule.

‘A good metaphor for what has probably happened would be the process of ‘retrofitting’, as is done in engineering to equip outdated machine frames with new technology. Only, in this case, it was a primitive DNA architecture which evolved new ‘technology’ to make the fingers and toes’, says Joost Woltering.

Fin radials are not homologous to tetrapod digits

The researchers conclude that, although fish possess the *Hox* regulatory toolkit to produce digits, this potential is not utilized as it is in tetrapods. Therefore, they propose that fin radials, the bony elements of fins, are not homologous to tetrapod digits, although they rely in part on a shared regulatory strategy.

New lines of investigation are to find out exactly what has changed between the DNA elements in fish and tetrapods. ‘By now we know a lot of genetic switches in mice that drive *Hox* expression in the digits. It is key to find out exactly how these processes work nowadays to understand what made digits appear and favor the colonization of the terrestrial environment’, concludes Denis Duboule.

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