How did forelimb function change as vertebrates acquired limbs and moved onto land?

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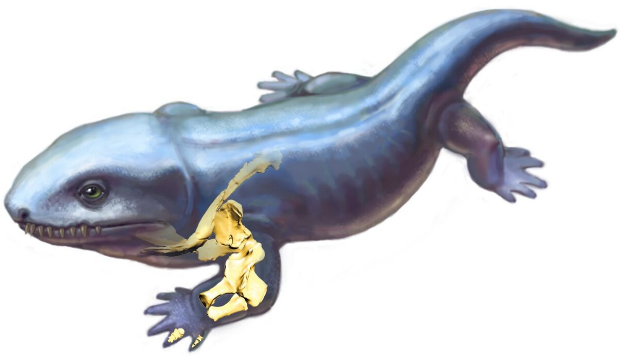

Life reconstruction of the early tetrapod Pederpes showing the forelimb bones beneath the skin. Credit: Image copyright 2021, Julia Molnar.

When tetrapods (four-limbed vertebrates) began to move from water to land roughly 390 million years ago it set in motion the rise of lizards, birds, mammals, and all land animals that exist today, including humans and some aquatic vertebrates such as whales and dolphins.

The earliest tetrapods originated from their fish ancestors in the Devonian period and are more than twice as old as the oldest dinosaur fossils. They resembled a cross between a giant salamander and a crocodile and were about 1-2 meters long, had gills, webbed feet and tail fins, and were still heavily tied to water. Their short arms and legs had up to eight digits on each hand and foot and they were probably ambush predators, lurking in shallow water waiting for prey to come near.

Scientists know how the fins of fish transformed into the limbs of tetrapods, but controversies remain about where and how the earliest tetrapods used their limbs. And, while many hypotheses have been proposed, very few studies have rigorously tested them using the fossil record.

In a paper published January 22 in *Science Advances* an international team of researchers examined three-dimensional digital models of the bones, joints, and muscles of the fins and limbs of two extinct early tetrapods and a closely related fossil fish to reveal how function of the forelimb changed as fins evolved into limbs. The research led by Julia Molnar, Assistant Professor at New York Institute of Technology College of Osteopathic Medicine and Stephanie Pierce, Thomas D. Cabot Associate Professor of Organismic and Evolutionary Biology at Harvard University, discovered three distinct functional stages in the transition from fins to limbs, and that these early tetrapods had a very distinct pattern of muscle leverage that didn't look like a fish fin or modern tetrapod limbs.

To reconstruct how limbs of the earliest known tetrapods functioned, Molnar, Pierce and co-authors John Hutchinson (Royal Veterinary College), Rui Diogo (Howard University), and Jennifer Clack (University of Cambridge) first needed to figure out what muscles were present in the fossil animals. A challenging task as muscles are not preserved in fossils, and the muscles of modern fish fins are completely different from those of tetrapod limbs. The team spent several years trying to answer the question, how exactly did the few simple muscles of a fin become dozens of muscles that perform all sorts of functions in a tetrapod limb?

"Determining what muscles were present in a 360-million-year-old fossil took many years of work just to get to the point where we could begin to build very complicated musculoskeletal models," said Pierce. "We needed to know how many muscles were present in the fossil animals and where they attached to on the bones so we could test how they functioned".

They built three-dimensional musculoskeletal models of the pectoral fin in *Eusthenopteron* (a fish closely related to tetrapods that lived during the Late Devonian period about 385 million years ago) and the forelimbs of two early tetrapods, *Acanthostega* (365 million years old living towards the end of the Late Devonian period) and Pederpes (348-347 million years old living during the early Carboniferous period). For comparison, they also built similar models of the pectoral fins of living fishes (coelacanth, lungfish) and forelimbs of living tetrapods (salamander, lizard).

To determine how the fins and limbs worked, the researchers used computational software originally developed to study human locomotion. This technique had been used recently to study locomotion in the ancestors of humans and also dinosaurs like *T. rex*, but never in something as old as an early tetrapod.

Manipulating the models in the software, the team were able to measure two functional traits: the joint's maximum range of motion and the muscles' ability to move the fin or limb joints. The two measurements would reveal trade-offs in the locomotor system and allow the researchers to test hypotheses of function in extinct animals.

The team found the forelimbs of all terrestrial tetrapods passed through three distinct functional stages: a "benthic fish" stage that resembled modern lungfish, an "early tetrapod" stage unlike any extinct animal, and a "crown tetrapod" stage with characteristics of both lizards and salamanders.

"The fin from *Eusthenopteron* had a pattern that was reminiscent of the lungfish, which is one of the closest living relatives of tetrapods," said Pierce. "But the early tetrapod limbs showed more similarities to each other than either fish or modern tetrapods."

"That was perhaps the most surprising," said Molnar. "I thought *Pederpes*, and possibly *Acanthostega*, would fall pretty well within the range of modern tetrapods. But they formed their own distinct cluster that didn't look like a modern tetrapod limb or a fish fin. They were not smack dab in the middle but had their own collection of characteristics that probably reflected their unique environment and behaviors."

The results showed that early tetrapod limbs were more adapted for propulsion rather than weight bearing. In the water, animals use their limbs for propulsion to move themselves forward or backward allowing the water to support their body weight. Moving on land, however, requires the animal act against gravity and push downward with their limbs to support their body mass.

This doesn't mean that early tetrapods were incapable of moving on land, but rather they didn't move like a modern-day living tetrapod. Their means of locomotion was probably unique to these animals that were still very much tied to the water, but were also venturing onto land, where there were many opportunities for vertebrate animals but little competition or fear from predators.

"These results are exciting as they independently support a study I published last year using completely different fossils and methods", said Pierce. "That study, which focused on the upper arm bone, indicated that early tetrapods had some capacity for land movement but that they may not have been very good at it."

The researchers are closer to reconstructing the evolution of terrestrial locomotion, but more work is needed. They plan to next model the hind limb to investigate how all four limbs worked together. It has been suggested that early tetrapods were using their forelimbs for propulsion, but modern tetrapods get most of their propulsive power from the hind limb.

"We plan to look for any evidence of a shift from forelimb driven locomotion toward hind limb driving locomotion, like modern tetrapods," said Molnar. Looking at the forelimb and hind limb together could reveal more about the transition from water to land and how tetrapods eventually came to dominate the terrestrial realm.

More information: "Evolution of forelimb musculoskeletal function across the fish-to-tetrapod transition" *Science Advances*, DOI: 10.1126/sciadv.abd7457