"It has been disputed at what period of life the causes of variability, whatever they may be, generally act; whether during the early or late period of development of the embryo, or at the instant of conception."
— Charles Darwin

Out on a Limb

DEVELOPMENTAL BIOLOGIST MARTIN J. COHN STUDIES THE MOLECULAR BUILDING BLOCKS THAT SHAPE APPENDAGES FROM FEET TO FLIPPERS

By John Pastor

OPENING A FREEZER IN HIS LAB AT THE UF CANCER AND GENETICS RESEARCH COMPLEX, DEVELOPMENTAL BIOLOGIST MARTIN J. COHN REVEALS DOZENS OF GLASS VIALS CONTAINING TINY, EMBRYONIC ORGANISMS. HE REMOVES ONE OF THE BOTTLES. EVEN BLURRED BY CONDENSATION, THERE IS NO MISTAKING ITS CONTENTS. SHARKS ARE INSTANTLY RECOGNIZABLE, EVEN WHEN THEY ARE EMBRYOS.

Cohn removes additional containers filled with embryos from other animals — lampreys, mice, chickens, ducks, snakes, turtles, mollusks, more — a menagerie of rudimentary life, frozen in time.

"People in my lab look at so many different organisms because we go to the species that is best suited to answer our questions," Cohn says. "If we want to understand how limbs were lost in evolution, we're not going to learn much by studying a mouse with a mutation that causes it to have no limbs. Uncovering the path that was taken by snakes or legless lizards or eels or whales requires us to study those organisms."

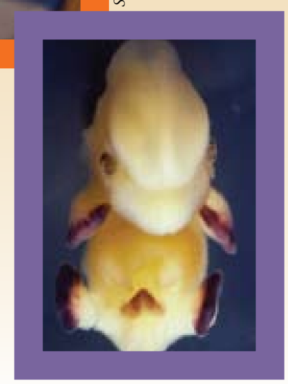
With the sensibilities of an anthropologist and the techniques of a molecular biologist — and the wide-eyed enthusiasm of a kid with a jar of tadpoles — Cohn says the embryos have taught him about evolution and the molecular building blocks that shape appendages from feet to flippers.

His findings have shed light on evolutionary processes and human problems such as birth defects, including the recent rise in the incidence of malformed genitalia in newborns. And they've

Ray Carson; Sarah Kiewel, inset.

Martin J. Cohn

Sarah Kiewel



By tagging the genes known to control limb development, Cohn's lab has shown that they also are present in genital development, as the purple areas on this mouse embryo illustrate.

Sarah Kiewel

earned him recognition as a Howard Hughes Medical Institute Early Career Scientist, the first in Florida and one of only 50 in the United States.

Each embryo in Cohn's collection provides its own take on life's multibillion-year-old story. But common genetic plotlines appear in all of them.

In chick embryos, for example, Cohn found the master switch for limb formation — a multifunctional protein called fibroblast growth factor, or FGF — that is now known to initiate limb development in all vertebrates, including humans.

From the embryos of the catshark and the lamprey, Cohn's research group traced the origin of the genetic instructions that build our own arms and legs and found that they were actually being perfected in the dorsal fins of fish nearly half a billion years ago — about 200 million years before limbs evolved — a completely new notion about life on Earth.

Shark embryos have been especially revealing. Cohn and his students discovered that the genes for development of fingers and toes were beginning to flicker on in the fins of sharks more than 200 million years before limbs made their debut.

Previous work suggested that the transition from fins to limbs involved the addition of a completely new phase of gene activity. Instead, Cohn's team demonstrated that what was thought to be an evolutionary innovation had actually existed in fish eons earlier than anyone suspected.

GENETIC TOOLBOX

"Evolution has been remarkably unimaginative when it comes to inventing new ways to solve problems," Cohn says, paraphrasing Lewis Wolpert, an emeritus professor in cell and developmental biology at University College London and one of Cohn's mentors.

"If you look at the broad classes of genes involved in developmental processes, there are relatively few, and they've been used time and again," Cohn says. "For instance, you see the same gene network involved in patterning a fly wing and a human limb. That is not because the limbs evolved from a common ancestral limb. Limbs evolved lots of times. But every

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— Martin J. Cohn

time limbs evolved, nature went back to the genetic tool kit, and used the same tools to fashion those appendages."

Now, Cohn is taking that thought a step further. Could the same gene network that initiated fins and then limbs have been employed to build genitalia?

About 365 million years ago, when animals with four limbs gradually began to spend more and more time in the terrestrial world, an external sex organ became necessary for reproduction.

In the water, the meeting of sperm and egg is possible outside the body and most fish and amphibians reproduce by external fertilization. But on dry land, a sex organ is essential for delivering sperm to an egg safely inside the mother. So, the conquest of land may have required not only the evolution of fingers and toes, but also external genitalia.

"We're pursuing the idea that the same gene network that builds fins and limbs was recycled yet again by evolution to build a new appendage in a new location, the genitalia," says Cohn, who is a professor in the Department of Molecular Genetics and Microbiology.

Already, Cohn's lab has noticed striking similarities between the processes that control limb development and those that regulate development of genitalia.

"The embryo has to solve many of the same problems to build limbs and genitalia, like initiating outgrowth of an appendage, and telling cells whether they are positioned at the top or bottom or left or right. It makes biological sense that the same signals would be used to accomplish the same goals, albeit in different locations," he says.

This knowledge is being used to investigate why an increasing number of boys are being born with a birth defect called "hypospadias," which involves incomplete formation of the urethral tube.

"The incidence of genitourinary malformations in humans came as a huge surprise to me," Cohn says. "A staggeringly high frequency of one in 250 kids has a urethral tube defect, and that number has more than doubled in the past 30 years without an explanation."

Even more surprising to Cohn was how little was known about the genetic control of genital development.

He suspects that toxic chemicals in the environment, known as endocrine disruptors, are contributing to the problem, and that defective genes are not solely to blame. However, by identifying how these contaminants interfere with the gene networks that build the urethral tube, Cohn believes his research group will be a step closer to preventing the disruptions that cause the birth defect.

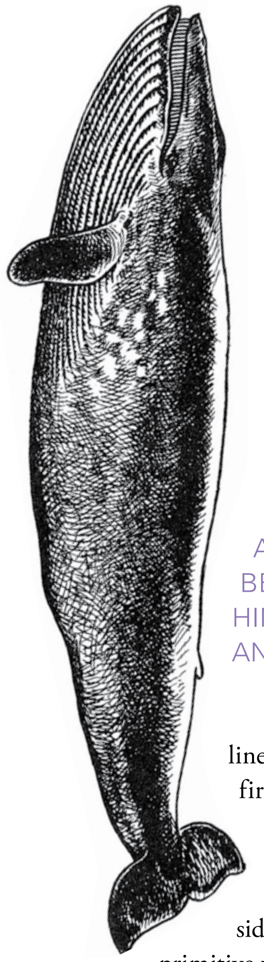
COMPARATIVE APPROACH

During the course of his research, Cohn has asked fundamental developmental questions: What initiates the formation of external genitalia? What is the trigger that makes limbs develop? What molecular processes are at work to position the limbs so precisely? You never see a vertebrate with six limbs, only pairs of upper and lower limbs. And people are not born with two left feet, no matter how it may seem on the dance floor. Toes grow in standard positions at the end of feet positioned at the ends of legs.

To find the answers, he looks to his assortment of embryos. If he wants to know how the first fins evolved, he identifies the event on the evolutionary tree of life, and then finds organisms that can "tell" him what happened.

"The ideal solution would be to go back in time and study an embryo of a primitive vertebrate that had no fins, then, study the first organism that developed fins," Cohn says. "We would determine what occurred differently in the body walls of these animals during their embryonic development. But since we don't have a time machine, we do the next best thing and take a comparative approach, using extant living organisms whose lineages can be traced to these critical positions in evolution."

That's why lampreys — jawless, eel-like creatures abundant in the Great Lakes — and sharks play important roles in Cohn's studies. The lamprey has hardly changed at all since its ancestors first appeared in the early Cambrian period 540 million years ago. The shark is only slightly more recent — its



THE GRADUAL SHRINKING OF THE WHALES' HIND LIMBS WAS THE RESULT OF SLOWLY ACCUMULATED GENETIC CHANGES. THEN, WITHIN A RELATIVELY SHORT FEW MILLION YEARS, THE LIMBS DISAPPEARED. WHAT HAPPENED, COHN'S TEAM DETERMINED, WAS THAT A GENE CALLED HAND2 BECAME INACTIVE IN THE HINDLIMB BUDS OF THOSE ANIMALS.

lineage goes back about 500 million years ago as the first jawed fish with paired fins.

"Lampreys tell us about the prefin condition because they retained that primitive, finless state," Cohn says. "We can then go to the other side of that event by looking at sharks, the most primitive vertebrate that has paired fins. These animals are no less evolved or specialized than we are, but the difference is their lineages are very ancient and even the modern versions retain some very primitive anatomy."

Similarly, by analyzing genes at work in embryonic porpoises and pythons, Cohn and his colleagues discovered how the ancestors of today's whales and snakes abandoned their legs over vast expanses of time.

Fossils show that the ancestors of today's whales and dolphins were tromping about on land more than 50 million years ago. They were four-footed animals about the size of large dogs. They became the sleek swimmers we recognize today during the next 15 million years, losing their hind limbs in a dramatic example of evolutionary change.

Cohn says the gradual shrinking of the whales' hind limbs was the result of slowly accumulated genetic changes. Then, within a relatively short few million years, the limbs disappeared. What happened, Cohn's team determined, was that a gene called Hand2 became inactive in the hindlimb buds of those animals. Hand2 — genes are often given names that make them memorable — is essential for turning on yet another gene absolutely critical for limb development in vertebrates, called Sonic hedgehog.

Without Sonic hedgehog — named after a video game character — no creature with a backbone has a leg to stand on, including whales.

But why would any animal evolve to grow limbs, only to further evolve to lose them?

"We've been able to answer questions about how limbs develop and evolve," Cohn says. "*Why* is harder."

But you can tell he is thinking about it.

'ARDI' AND MARTY

The "why" questions of natural selection — Darwin's mechanism by which favorable traits such as limbs, or lack thereof, are passed along to succeeding generations — began to grip Cohn while he was an undergraduate studying anthropology at the University of Texas. Later, when he met biological anthropologist C. Owen Lovejoy, his graduate adviser at Kent State University, Cohn figured out how to get a grip on the answers.

"Of course Marty is haunted by 'why,'" says Lovejoy. "Anybody who goes into anthropology is always going to be haunted by why. Here we are cognitive animals, talking about our evolution. It's a merry-go-round."

Lovejoy is renowned for reconstructing the near-complete skeleton of "Lucy," a tiny female who was part human, part ape, and who lived at the edge of an African rainforest about three million years ago. But in October 2009, Lucy's days as the earliest-known human ancestor were at an end.

Lovejoy and colleagues had found an ancient ancestor that preceded "Lucy" by an astounding million and a half years. The skeleton of a 4.4-million-year-old human-ape ancestor, a female dubbed "Ardi," was about to rewrite the story of human origins, and Lovejoy was reviewing proofs of 11 papers being prepared for the journal *Science*.

Even though sufficiently occupied with "Ardi," Lovejoy wanted to talk about "Marty."

"His most defining characteristics are a curiosity and drive that are unending," Lovejoy says. "There is no bottom to it. He gets so excited by every discovery — and I'm sure that is infectious for his students — but it is like he has the discovery virus. When you have it, you can't put an experiment down until you finally have it all figured out."

Cohn met Lovejoy at a pivotal time in the field of developmental biology, not long after fibroblast growth factor was discovered. Notions were growing about how FGF in its many forms seemed essential for normal development of vertebrate animals, and Cohn and Lovejoy were paying attention.

"The whole discovery process of FGF and the early advanced work in limb bud development was going on," Lovejoy says. "Those reports were being made almost weekly, and we would meet and read the latest article and get more and more excited."

With Lovejoy's encouragement, Cohn spent a summer learning laboratory techniques at University College London from Cheryll Tickle, a world authority on the mechanisms of embryonic development. After three months, Cohn was offered Ph.D. funding, and Lovejoy advised him to accept.

Red shows activity of a key developmental control gene known as Sonic hedgehog in the mouse embryo.

Sarah Kiewel

Cohn and post-doctoral researcher GuangJun Zhang study a spotted catshark embryo.

"By studying these weird animals and taking advantage of the diversity that evolution has produced, we are getting unexpected insights into disease."
— Martin J. Cohn

Kent State University

C. Owen Lovejoy

Cohn wrapped up his master's research with Lovejoy and would go on to receive his doctoral degree in developmental biology from University College London.

"Anthropology tends to lag behind the other sciences because it concentrates on descriptive things, like fossils, and we were both so excited by development," Lovejoy says. "So I thought he should take the offer. He did, and the next thing you know, everyone is reading about how he discovered that FGF is responsible for formation of the limb bud."

FOLLOWING THE BLUEPRINT

At this point, Cohn knew that if he was interested in anatomical changes over time, whether it involved hundreds of millions of years of evolution or 30 years for an increase in a human birth defect, his path was in developmental biology.

"Owen (Lovejoy) really got me thinking in a different way, less about why and more about how, which is a very mechanistic approach," Cohn says. "I realized that if I wanted to understand how animal form changes during evolution, such as how limbs evolved from fins or how snakes lost their legs, I had to understand development, because that's when the genetic blueprint for the body is being executed."

Since arriving in Gainesville in 2003, Cohn and his UF colleagues have discovered the evolutionary origin of the genetic program for fin development, shown how this program was modified to form fingers and toes, and identified the molecular basis for the loss of legs during whale evolution. The group has also published widely on the genetic control of external genital development.

Ultimately, it's hard to predict where Cohn's explorations into evolutionary processes will lead, Lovejoy says. Similar work has given the world the techniques of DNA analysis now used in medical diagnostics and criminal investigations, although that was never its original intention.

Cohn frequently cites the example of the developmental biologists who devoted 12 years of work studying a gene called "patched" in developing fruit flies. The same gene would later be implicated in the most common type of human skin cancer.

"As soon as the link between patched and basal cell carcinoma was found, cancer biologists could utilize over a decade's worth of work on the function and regulation of that gene in the fly wing," Cohn says. "If none of that work had been done in the fly, I don't know if it would have even been discovered that this gene was involved in cancer.

"That's the beauty of basic science. We're in the business of finding out how things work and we don't always know what sort of application will result," he continues. "My lab is trying to understand what causes evolutionary changes, and what causes birth defects, and the interesting part is how each of those areas can inform the other. By studying these weird animals and taking advantage of the diversity that evolution has produced, we are getting unexpected insights into disease."

Reaching into his laboratory freezer, he removes another test tube and clears the mist from the glass to reveal a lump of unfamiliar contents — nothing like the shark embryo, which looks like a miniature version of its adult self.

It's a duck embryo, and Cohn says its genetic thread in life's long, long story has some answers about the ever-increasing problems of birth defects in human genitalia — once again, evolution and development, one informing the other.

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