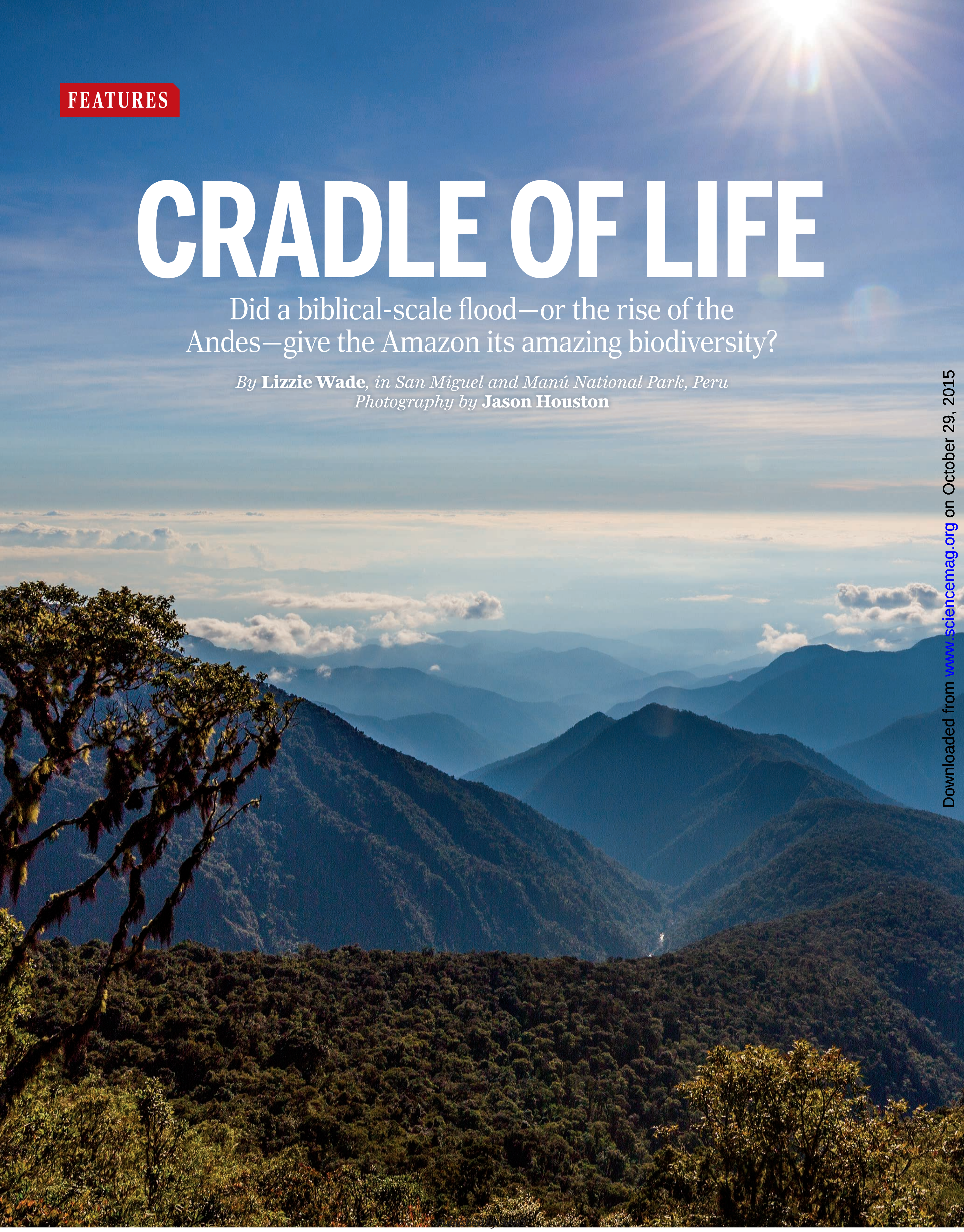
FEATURES

CRADLE OF LIFE

Did a biblical-scale flood—or the rise of the Andes—give the Amazon its amazing biodiversity?

By **Lizzie Wade**, *in San Miguel and Manú National Park, Peru*
Photography by **Jason Houston**



The foothills of the Peruvian Andes are home to some of the most diverse cloud forests in the world.

Trudging along the bank of a shallow creek in the Peruvian Amazon, Catherine Rigsby sinks knee-deep in mud. She calmly shimmies out of the muck, then grabs a branch lying on the bank for a walking stick. As stingless bees buzz around her head and macaws screech in the trees above, the sedimentologist gingerly resumes her search for a rainforest rarity: exposed rock. One of the few outcrops in this corner of the Amazon—a day's boat ride up the Manú River from the nearest town—is around here somewhere.

"I think we're in luck!" Sage Wagner, Rigsby's graduate student, calls from around a bend in the creek. Catching up, Rigsby, who splits her time at East Carolina University in Greenville and Yachay Tech University in San Miguel de Urcuquí, Ecuador, faces her quarry: a modest wall of sediment, about as high as she can reach. Deposited layer by layer some 9 million years ago, the outcrop holds clues to an enduring riddle: What gave rise to the Amazon rainforest's staggering biodiversity?

The western Amazon, which includes parts of Peru, Ecuador, Colombia, and northwestern Brazil, "is the most diverse region in the world in terms of plants," says Christopher Dick, an evolutionary biologist at the University of Michigan, Ann Arbor. "We have about 300 tree species in eastern North America. In the western Amazon, we have 300 tree species in a single hectare." And plant diversity is just part of the picture. All told, the Amazon Basin, a 6.7-million-square-kilometer area extending through Brazil all the way to the Atlantic, is home to 10% of the world's known species.

ONLINE

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Listen to a podcast with author Lizzie Wade at http://scim.ag/pod_6260.

Scientists agree that the rich biodiversity springs from the convulsive geological changes that the western Amazon has experienced—mountains rising, coasts shifting, rivers changing course. By fragmenting and transforming habitats, these landscape changes would have driven bursts of speciation. But the experts differ, often vehemently, about just what form those upheavals took and which of them supercharged Amazonian speciation.

The prevailing scenario invokes an incursion of the Caribbean Sea into the South American continent that ended less than 10 million years ago, creating a vast wetland studded with islands. Organisms would have had to adapt to a patchwork of biomes that varied between salty and fresh, aquatic and terrestrial—driving up diversity.

To advocates of this scenario, fossils of plankton, mollusks, and marine fish as well as present-day species like Amazon river dolphins provide powerful support. "I'm 100% positive" that at least twice in South America's long history, the Caribbean breached the continent's northern coast and poured into the western Amazon, 2000 kilometers away, says Carlos Jaramillo, a paleontologist at the Smithsonian Tropical Research Institute in Panama City.

But Rigsby's outcrop, which formed during the putative Caribbean invasion, challenges that picture because it suggests that the environment then was very much like today's. The 9-million-year-old sediment—coarse at creek level—becomes finer grained as Rigsby traces the layer upward. Half a meter or so up, the grains switch back to coarse, and the pattern repeats. Rigsby suspects she's looking at the traces of repeated river floods like those that occur today. The modern floods leave the same sedimentary signature of coarse grains giving way to fine grains as the waters subside.

Based on this outcrop and other geological evidence, Rigsby and others argue that the wellspring of the Amazon's extraordinary diversity was a much earlier event. Their favored candidate: the early uplift of the Andes on the basin's western edge, which began fitfully at least 65 million years ago. As species adapted to narrow ecological niches along the mountainsides, these researchers say, biodiversity soared and new species spilled from the Andes into the Amazonian rainforest.

With Dick and 18 other colleagues, Rigsby traveled to Peru in July in search of geological and biological data that could test their alternative theory about the Amazon's evolution. They examined not just rocks and fossils but also living plants, whose genomes hold clues to when and where Amazonian flora diversified. "We're going to get this huge amount of [genetic] data that, if we filter it correctly, can give us a lot of environmental history, geological history, climate history," says one of the team's leaders, Paul Baker, a geologist at Yachay Tech and at Duke University in Durham, North Carolina.

Other teams are also rising to this grand challenge of biogeography. CLIM-AMAZON, a joint European and Brazilian project, is puzzling out the sedimentary history of the Amazon Basin to trace ancient water bodies. And a group led by Joel Cracraft, an ornithologist at the American Museum of Natural History in New York City, and Lúcia Lohmann, a botanist at the University of São Paulo in Brazil, is mapping the ranges of Amazonian birds, butterflies, primates, and two plant families to understand how

Graduate students Federico Moreno and Lauren Gonzalez sample sediment from a 9-million-year-old outcrop along the Manú River in Peru. Catherine Rigsby (pictured below) believes the sediments were left behind by a primeval river, pointing to an ancient environment very much like today's Amazon Basin.

the basin's history shaped its biogeography (*Science*, 13 July 2013, p. 234). All three teams will present findings next week at the Geological Society of America's annual meeting in Baltimore, Maryland.

The multidisciplinary synergy is "accelerating our view of Amazonia," Cracraft says. "We are on the cusp of understanding how this large-scale diversity happened."

FORTY YEARS AGO, scientists thought they had the Amazon all figured out. In a 1969 *Science* paper, ornithologist Jürgen Haffer mapped the distributions of Amazonian bird species and identified patches of high diversity (11 July 1969, p. 131). Those hotspots, he concluded, were the legacy of the ice ages. When temperatures across the world fell during the Pleistocene Epoch, starting about 2 million years ago, the Amazon dried out, he argued. Most of the basin became grassland, with pockets of rainforest hanging on in the most humid areas. Isolated in forest refuges, life forms diverged into new species. When warmer, wetter conditions returned, the forest expanded to cover the basin once again, bringing all those new species into contact. According to Haffer, this cycle repeated itself several times during the Pleistocene and the current Holocene Epoch—revving up speciation each time. The refuges would stand out today as diversity hotspots within the larger forest—exactly what he saw in his bird data.

"It's not a bad idea," Dick says. But Haffer's hypothesis crumbled when a 1990 *Nature* paper showed that the biodiversity hotspots were illusions—the inevitable result of scientists collecting in the same easily accessible areas of rainforest over and over again. Meanwhile, pollen cores and isotope studies revealed that climate cycles had never turned most of the Amazon into arid grassland. "In fact, in the South American tropics, [the global ice ages] were wet," says Sherilyn Fritz, a paleoclimatologist at the University of Nebraska, Lincoln, and co-leader on Baker's project. "In a simple sense, the Haffer hypothesis was wrong." Scientists were left scrambling for a new idea to explain the Amazon's biodiversity.

That's when Carina Hoorn came along. Then a graduate student, the young geologist and palynologist was tromping around the western Amazon, building a picture of what the area looked like long before the Pleistocene. Millions of years earlier, in the Miocene Epoch—from 23 million to 5 million years ago—"there [was already] a highly diverse rainforest out there," she says.

Hoorn knew that for more than a century, scientists had been finding fossil mollusk shells in the western Amazon that looked strikingly like ocean species. But she couldn't quite bring herself to believe that the Caribbean had reached so far inland. Then the fossilized remnants of foraminifera—single-celled organisms that live mostly in the sea—started showing up in her Miocene sediment samples from Peru, Colombia, and northwestern Brazil. "That's what did it for me," recalls Hoorn, now a researcher at the University of Amsterdam in the Netherlands and a leader of CLIM-AMAZON. Her inland samples also yielded pollen from mangroves, a tree that today grows in saltwater along tropical coasts.

"In the beginning I thought it was just a flooding event," she says: a one-time rush of seawater into an inland region dominated by freshwater rivers and lakes. But the deeper she delved, the more she was convinced that the presence of saltwater was "rather common."

She now pictures the western Amazon during the Miocene as an estuarine environment subject to saltwater incursions. Forested islands may have arisen, separating terrestrial populations and spurring speciation. Meanwhile, the seawater brought with it the marine ancestors of several river species found in Amazonia today, including dolphins and stingrays, says Nathan Lovejoy, an evolutionary biologist at the University of Toronto, Scarborough, in Canada. Still more signs of an ancient marine ecosystem came this summer when Pierre-Olivier Antoine, a paleontologist at the University of Montpellier in France, uncovered thousands of giant oysters, as well as fossil sawfish and stingrays. These scientists say that the estuary system—known as the Pebas wetland—endured for most of the Miocene.

Around the same time, however, the other potential driver of Amazonian speciation was stirring. The Andes were rising in fits and starts—and once that great wall was in place, South America would never be the same.

OUTSIDE THE MINING TOWN of San Miguel, 4000 meters up on Peru's Altiplano, Carmala Garzione gasps for air. Armed with a set of GPS coordinates, Garzione, a geologist at the University of Rochester in New York, and her student, Federico Moreno, are leading the visiting researchers to an unmistakable relic of the Andes' dynamic past.

After a breathless 3-kilometer hike, they find their treasure: a petrified tree trunk lying incongruously on the grassy plain. The cellular structure of its wood shows it is a member of the legume family, and the age of nearby limestone outcrops suggest the tree is about 10 million years old. "Nothing like it lives at these altitudes," Garzione says—which means that the forest of which the tree is a relic grew at a much lower altitude. The finding jibes with her earlier studies of chemical traces left in ancient soils by rainwater, which suggest that between 18 million and 9 million years ago, the Altiplano was about half as high as it is today.

In Hoorn's scenario, the rise of the Andes to their full height between about 10 million and 5 million years ago spelled the end of the Caribbean incursions (*Science,* 12 November 2010, p. 927). Water vapor flowing across South America from the Atlantic couldn't surmount the mountains and fell as rain in the western Amazon. Fresh water poured down the eastern flank of the Andes, flushing out saltwater. The surge also eroded nutrient-rich sediments and carried them to the lowlands, enriching the soil of western Amazonia. The northern seaway connection to the Caribbean closed as the flow of water and sediments plowed eastward to the Atlantic. They broke through to the sea about 7 million years ago, and the modern Amazon River was born.

The notion of Miocene marine incursions ended by a burst of Andean uplift beginning 10 million years ago "is a beautiful story. It's just, I think, completely wrong," Baker says. Findings like the petrified tree leave little doubt that the East Andes shot up between 10 million and 5 million years ago, as Hoorn's model assumes. But the story's real beginning was much earlier, Baker says: the rise of the West Andes, a string of volcanoes that runs down South America's Pacific coast.

The West Andes' geological history is shrouded in mystery. Unlike the mountains of the East Andes, the volcanoes do not contain any ancient soils that Garzione uses to reconstruct environments long past. But the landscape itself gives a clue to the West Andes' age. As the mountain belt rose, Garzione says, its weight depressed the nearby continental crust, creating a broad "foreland basin" in what is now central Peru. The basin filled with sediment; later mountain-building episodes thrust it upward to form the Altiplano and the East Andes. From the age of the foreland basin and its sediments—now twisted inside the mountains and across the Altiplano—geologists estimate that the West Andes had risen to an elevation of some 3 kilometers by 53 million years ago, Garzione says.

Nothing like this fossilized tree grows on the Andean Altiplano today. When this member of the legume family was alive 10 million years ago, the mountains were barely half their current height.

To Baker, that means the Andean-driven processes—from speciation on the slopes to trapping moisture over the Amazon to pushing freshwater rivers east—could have started long before 10 million years ago, the date Hoorn and others propose. When the mountains rose, the burst of new habitats in both highlands and lowlands was as extreme as the geological transformation, Baker and others say. "If you had to point to one single event to explain the Neotropics' biodiversity, I think it was the uplift of the Andes," says Alexandre Antonelli, an evolutionary biologist at the University of Gothenburg in Sweden.

THE RELATION BETWEEN topography and species richness is vivid as Baker's group leaves the Altiplano and, following an old Incan trail, descends the Andes' eastern flank into the cloud forest. At about 3500 meters, the grassland gives way to trees draped in moss so dense it forms a tunnel around the path. At 2500 meters, the moss thins and woody vines snake through the trees. Lower still, the forest floor is lush with ferns. "There are no species that have ranges that extend the entire gradient," says Miles Silman of Wake Forest University in Winston-Salem, North Carolina, an ecologist who studies cloud-forest trees. Just moving up and down the mountain, he says, "you accrue a lot of diversity." The cloud forest is the roiling core where all these species mix, mingle, compete, and diversify, he says. "It's the hottest of all biodiversity hotspots."

Not only is there no need to invoke a Caribbean invasion to explain present-day diversity, it never actually happened, Baker and others contend. Marine incursions are "a pretty complicated story," Rigsby says. "A complicated story requires a lot of data to back it up. And the data are just not there." The sedimentary outcrop in the eastern Peru, recording river floods like those of today, is one strike against the story, she says. And there are others.

Scientists who support the marine incursion theory "use proxies of things they think are only found in marine systems," Dick says. "But all of the proxies are becoming, on inspection, weaker and weaker." Many of Hoorn's foraminifera species, for example, are found in water with a wide range of salinities, including fresh water. As for marine-derived species like the dolphins and stingrays in today's Amazon River, it's possible that their ancestors lived near the mouths of rivers and ventured upstream gradually, adapting to fresh water along the way, Baker says.

> ***"We are on the cusp of understanding how this large-scale diversity happened."***
> **Joel Cracraft**, American Museum of Natural History

Geochemical clues also hint that western Amazonia in the Miocene may have been a freshwater system. Werner Piller, a paleontologist at the University of Graz in Austria, studied fossilized ostracods—small crustaceans with a clamlike shell—from the western part of the Brazilian Amazon. By analyzing the ratio of oxygen isotopes in the shells, Piller could tell whether they formed in saltwater or fresh water. "We get a clear freshwater signal," he says.

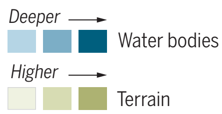
In her own work, Hoorn says she has examined "meters and meters of sediment that look purely freshwater," with vestiges of saltwater species like plankton and mangrove pollen limited to just a centimeter or two. "It's a mixed message in the sediments"—one she admits can be confusing. But it's also what she would expect to see in an estuary created by marine incursions: a shifting pattern of freshwater and saltwater dominance.

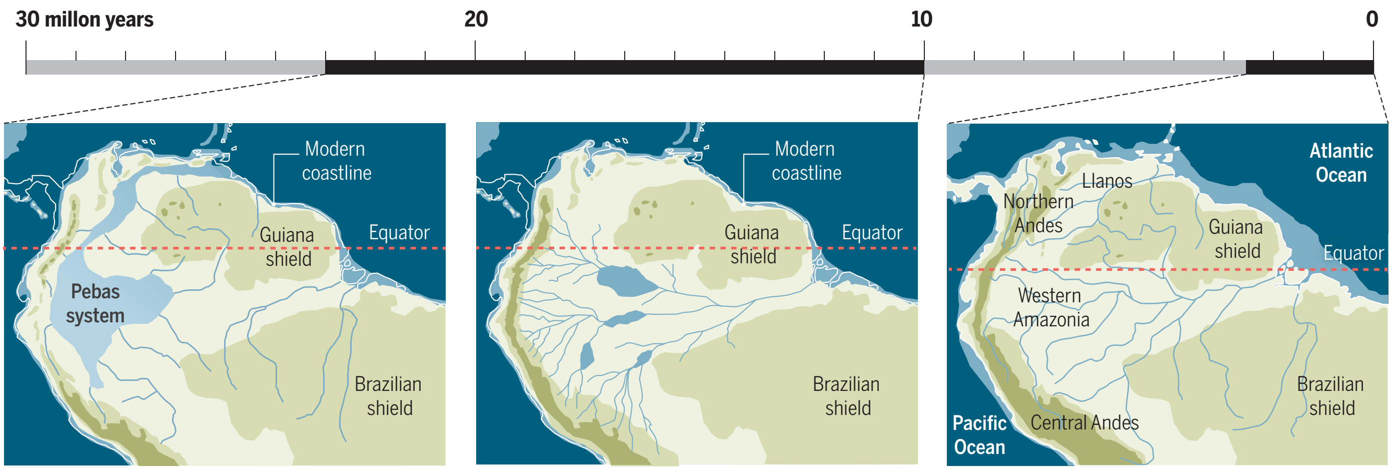
Lovejoy agrees, noting that the closest marine relatives of many Amazonian fish species, including freshwater stingrays, live in the Caribbean along the northern coast of South America—just where the marine incursions are thought to have originated. What's more, their family trees suggest, he says, that "those [marine] lineages have only moved into the Amazon once or maybe twice," a hint that they invaded the Amazon during a marine incursion rather than slowly colonizing fresh water from river mouths.

"There are really a lot of different types of evidence," Hoorn says. "You cannot disregard that."

Amazonian upheavals

Experts agree that between 23 million and 10 million years ago, during the Miocene Epoch, convulsive geological changes rocked South America and supercharged the Amazon rainforest's biodiversity. But just what those changes looked like is a matter of heated debate. Here are two competing theories about the Amazon's past—and what the Amazon looks like today.





Marine incursion theory

Traces of plankton and marine fossils suggest that during the Miocene, the Caribbean Sea invaded South America. The western Amazon became a brackish estuary known as the Pebas system. The shifting patchwork of terrestrial, saltwater, and freshwater environments drove speciation.

Ancient Andes theory

Sediments left behind by primeval rivers and chemical analyses of fossil shells cast doubt on marine incursions. Instead, high peaks in the western Andes trapped precipitation, fed freshwater rivers, and created an abundance of mountainside habitats that pumped new species into the rainforest.

Current state of Amazon Basin

Cloud forests connect Andean peaks to lowland rainforest. Fresh water pours off the 4-kilometer-high mountains, feeding the Amazon River and its tributaries. The 6.7-million-square-kilometer basin is home to 10% of the world's known species, making it one of the most species-rich regions on the planet.

GRAPHIC: A. CUADRA/SCIENCE

CRACK! CRRRRRACK! Christopher Dick pulls a rope to snap shut a lopping shear at the end of a long pole. The blades bite into a branch of a small, spine-covered tree in the old growth rainforest around Cocha Cashu, an isolated research station in Manú National Park. The branch snaps and plunges into a palm frond below. Dick shakes it to the ground and targets the freshest looking leaf, writing the date, sample number, and the code for its species on it with a Sharpie. He snips out this smaller section and bags it for DNA analysis back at the University of Michigan.

Dick hopes to find clues to the origins of today's species by decoding the environmental history embedded in their genes. The spines covering the tree he has sampled identify it as *Poulsenia armata*. *Poulsenia* grows on both sides of the Andes, in the Amazon as well as in western Ecuador and even as far north as Panama. But the species isn't a restless pioneer: It tends to stay put in the rainforest, dispersing its seeds nearby with the help of animals. There's no way it could climb over the Andes, or even move around them, once the mountains were at their full height, Dick says. In all likelihood, *Poulsenia* was already growing from Panama to Peru before the Andes rose.

The rising mountains separated populations of *Poulsenia*, setting them on different evolutionary paths. Since then, each *Poulsenia* population has accumulated random mutations in its DNA. By tallying up those changes, Dick can estimate when populations on opposite sides of the mountain range formed one continuous group—and, therefore, estimate the age of the peaks. "Anytime you have a geographic barrier, [*Poulsenia*] reflects how old that barrier is in the DNA," he explains.

When Dick did this for *Poulsenia* samples from opposite sides of Ecuador, he found that the populations had a common ancestor about 9 million years ago—about the time Garzione says the Andes were going through their most recent growth spurt. Now, Dick wants to compare the Ecuadorian populations with *Poulsenia* samples from Peru. He hopes his results will yield clues to the timing and extent of the Andes' earlier growth spurts—and help to test ideas about the role they could have played in speciation. "Geogenomics," as Baker calls it, can be a particularly useful approach in a place like the Amazon, where outcrops are scarce.

In a remote rainforest in Manú National Park, Christopher Dick clips leaves from the tree *Poulsenia armata*. He hopes to find signs of ancient geological changes lodged in the tree's genome.

Baker has a plan for expanding geological sampling as well. He's gathering funding for an international project that would drill five or more sediment cores from the mouth of the Amazon River to the base of the Andes. That, Hoorn says, would give scientists an unbroken look at the landscape over the course of 65 million years or more, rather than forcing them to "piece it all together" from disparate and hard-to-date samples. The drilling project "is essential," Cracraft agrees. "It will do more to help reconstruct the environmental and hydrological history of Amazonia than anything we've done previously."

But for now, Baker and his colleagues are making do with the precious snippets of Amazonian history they've gathered on this trip: armored leaves, a rare outcrop, and a petrified tree. They will resume their quest for the origins of its staggering diversity back in their labs. For now, as they chug up the Manú River and out of the jungle, they're content to watch the Amazon's landscape—at once dynamic and seemingly eternal—glide by. ■