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ON THE ORIGIN OF (AMAZONIAN) SPECIES

How did the Amazon achieve its stunning diversity? Some say great rivers are responsible, others point to vanished hills and seas. Now one team of zoologists is listening to what the rats have to say.

BY VIRGINIA MORELL

IT'S ONLY 8:45 A.M. IN THE CENTRAL AMAZON AND ALREADY THE forest is sweltering and thick with mosquitoes. Jim Patton, an evolutionary biologist from the University of California at Berkeley, is beaming nevertheless. He takes off his baseball cap, wipes the sweat from his forehead, and smiles at his colleague, Maria Nazareth da Silva. Da Silva, a biologist at the National Institute of Amazonian Research in Manaus, Brazil, has just trapped a spiny rat.

"Absolutely spectacular!" says Patton, looking at the rat in its cage.

"It's a good one," Da Silva says quietly but triumphantly. She raises the cage and watches the rodent poke its snout through the bars.

To the uninitiated, Patton and Da Silva might seem a bit overenthusiastic about a trapped rat. The animal, 14 inches from head to tail, *is* remarkably beautiful, its pelt of spine-tipped fur a stunning mélange of browns, cinnamons, and golds. But while Patton readily admires the rat's outward beauty, what he's really excited about is the chance to look at its DNA. The rat's genes, he thinks, may hold a clue to one of the great mysteries of evolutionary biology: What underlies the astonishing variety of species in the Amazon basin?

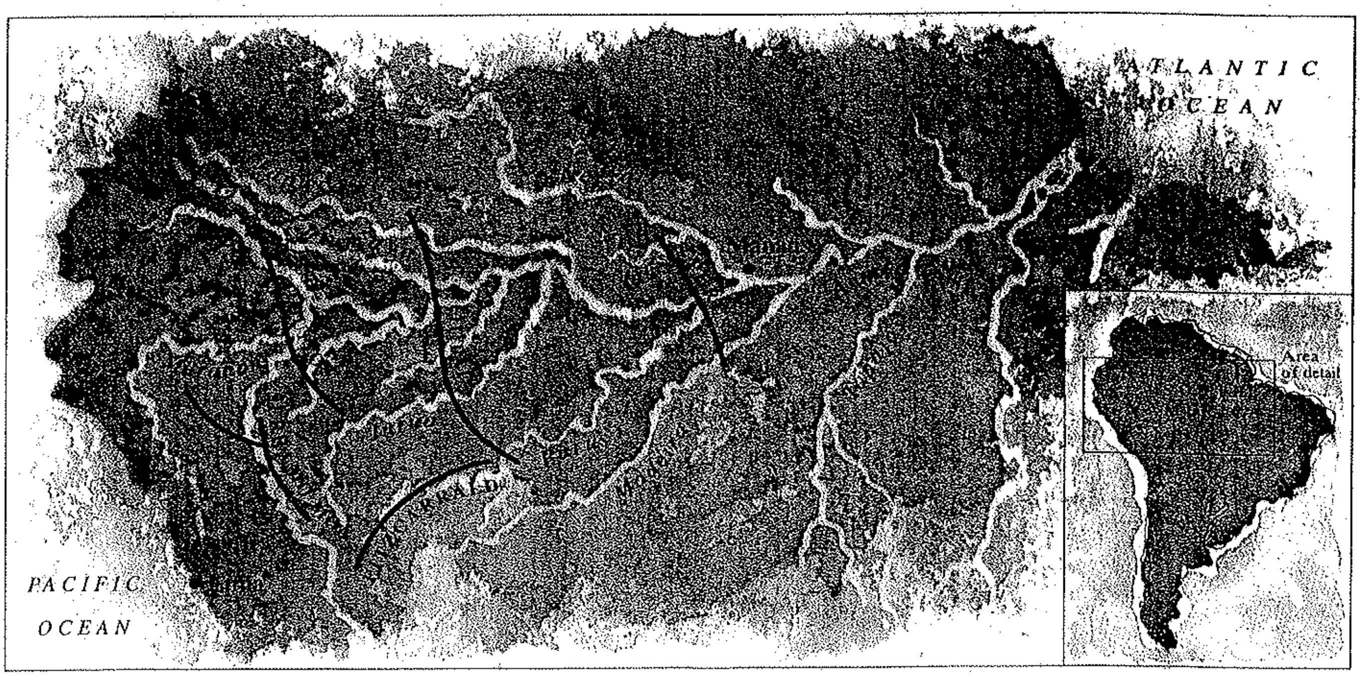
Naturalists have been staggered by the Amazon's diversity ever since the first European researchers set foot in the South American rain forest more than 200 years ago. Those naturalists, of course, hailed largely from temperate climes, where forests typically support no more than 50 to 60 species of tree. The Amazon, they quickly discovered, is far more lavish: twice as many species of tree can easily be found in an acre or two. And these plants support many times more species of bird, insect, and mammal than are found on an equivalent piece of temperate real estate. In one day at one site in the Amazon, entomologists gathered 440 species of butterfly—more species than can be found in the entire eastern United States. But despite the recognition that the Amazon is one of Earth's great centers of biodiversity, the underlying causes of its richness remain unknown.

Patton (left) and Da Silva prepare their trapped mammals. Previous pages: The spiny rat *Proechimys steerei*.

Ancient ridges (purple) testify to geologic events that may have determined the course of Amazonian evolution.

Until a couple of decades ago many researchers attributed the region's biological wealth to its supposedly stable climate. With balmy weather for millennium after millennium, the thinking went, plants and animals had nothing to do but speciate. However, that notion was turned on its head in 1969, when Jürgen Haffer, a petroleum geologist and amateur ornithologist working in Colombia, suggested that the Amazon had experienced great climatic changes over the last few million years. With regularity, he said, the Amazon had dried and cooled, and the resident flora and fauna, rather than living in a perpetual Club Med, had to beat a retreat when the forests were nibbled away by spreading grasslands. In these remaining fragments of forests, tropical organisms could hide and wait out the ice ages. Members of old species, divided among these "refugia," split into new ones, creating the diversity we see today.

There are other ideas. The oldest, put forward in 1849 by the great evolutionist Alfred Russel Wallace, holds rivers responsible. Wallace imagined that the basin's many wide and meandering waterways split and separated populations, thereby creating conditions favorable for the rise of new species. In contrast, the newest hypothesis—so au courant it's still being formulated—attributes the bounty of species to geology. In this view the Amazon is not a



Photograph courtesy Virginia Morell

MAP BY BETTE DUKE

vast dull floodplain, as has long been thought, but rather a wounded and torn land, jarred and jolted by earthquakes and mountain building, whose effects may ultimately have boosted the land's biological diversity.

In the end, though, it is far easier to come up with an explanation for the Amazon's diversity than to actually test the hypothesis. That job falls to biologists like Patton and Da Silva—which is why they are trapping rats and other small mammals in this steamy forest along the Rio Jaú, a tributary of the mighty Rio Negro. By surveying the mammals here and later analyzing their genes, the biologists may be able to test both the oldest and newest Amazonian diversity models.

"I'd test the refugia model too, if I knew a way to do it," Patton says, kneeling to clean and rebait a trap. "But the boundaries of the fragmented forests have never been clearly defined, and without those it's impossible to determine whether animals in one refuge actually grew apart from those in another."

He stands up and hefts a white canvas bag stuffed with spare folded traps, granola-like bait, bananas, a canteen, and a machete onto his shoulder, then moves swiftly down the trail to the next trapping station. The sight is something like a Johnny Appleseed heading into the dark Amazonian woods. But where Appleseed might bend and plant, Patton sets a trap. Above him the forest soars in a green tangle of branches, leaves, and vines. It's oddly silent in this shadowy world. Aside from the buzz of insects, the occasional piercing bird call or unearthly moan of a howler monkey, one hears nothing but the metallic sounds of Patton and Da Silva working their traps. They've hidden them every 20 yards or so along the trail, nestling one against a log, tucking another under the lacy fronds of a palm, strapping others to the vines climbing up the trees. While most of the traps are empty, a few contain creatures like the spiny rat caught earlier.

"There are many different theories, and you want to listen to them all," Patton says, shouldering his bag again after collecting an arboreal rice rat. "But then what I like to do is find out what the rats have to say." And the Amazon's rats, it turns out, are surprisingly eloquent.

THE RODENTS BEGIN THEIR TEACHING IN Patton's field lab—a makeshift structure of wood and tarp, with mosquito netting nailed over the window openings. The floor is earthen; at night a single bare bulb provides light. In the center stands the preparation table, where Patton, Da Silva, and three Brazilian students are working on the animals they trapped that morning. Behind them, lab supplies line wooden shelves; on drying racks overhead hang three white boards, each bearing a load of stuffed and pinned mammals: mouse opossums, brown four-eyed opossums, bats, tree-dwelling and land-dwelling rice rats and spiny rats.

The Rio Jaú in the central Amazon is a "black water" river, and relatively species-poor.

Photograph courtesy Jim Patton

"We're collecting mammals from both sides of the river, and in two different habitats," explains Patton, as he carefully stuffs cotton into the skin of the rice rat he is preparing. The team has already made a similar collection at an upriver site and will do yet another sampling close to the Jaú's mouth—a mixture that allows them to "look for a pattern," as Patton puts it. "You want to see if there's something that ties these animals together, that gives them a shared evolutionary history across lineages."

Specifically, he and Da Silva are looking for a pattern that might link the rodents' ranges to rivers or to geology. The pickings along the Jaú are relatively slim because it is a black-water river: its waters are heavy with humic acids and tannins, giving it the color of strong tea. Black-water rivers are notorious for being species-poor, apparently because the forests that grow along their banks sprout from such nutrient-poor soils. They seem able to sustain only animals that can survive in a region that local Indians call the River of Hunger.

In other places Patton has trapped 50 animals or more every day. But here the team celebrates when they catch only a half dozen, despite having 300 traps in place. "It can get to you after a while," Patton says. Today the ten animals they have caught—including Da Silva's early-morning spiny rat—have lifted everyone's spirits. Now they'll take tissue samples and preserve the skins of the rodents, as well as any embryos the females may be carrying and the males' phalluses, which help distinguish closely related species. Any distinctive pelt patterns or colorations are noted, too, along with the size and swelling of the females' teats, which can reveal whether a rat has already had a litter and is in a new reproductive cycle.

"Our base level of knowledge about these critters is just so poor," explains Patton, threading a needle with white cotton thread, then quickly stitching up the rice rat's rust-colored belly. "We don't know what their breeding season is, their age at first reproduction, their life span—all that basic natural history. So anything we can find out is a plus."

This is the second trip Patton and Da Silva have made to test theories of the Amazon's diversity. They took their first journey five years ago along the Rio Juruá, spending an entire year trapping animals to see if Wallace's river hypothesis fit the facts of the Amazon. "It had always been appealing because it makes a lot of sense," says Patton. "You come to

For a year the zoologists hopscotched along the rive
But their stores of food began to dwindle, insect

the Amazon and what's the first thing you see? Big, wide rivers. Of course they're keeping the animals apart."

Wallace first ventured into the Amazon in 1848, when he was 25, expressly for the purpose of solving, as he put it, "the problem of the origin of species." (Ten years later he would publish ideas on this subject much like those of Darwin's.) Wallace couldn't have gone to a better place for clues: the lowland Amazon basin has an astonishingly high number of species, even for a tropical rain forest. Initially, that abundance of life nearly overwhelmed Wallace, as did the size and breadth of the basin's rivers. But he soon began to discern patterns in the jumble of life around him.

The rivers, Wallace decided, must have been the forceful creators of much of the diversity. Some, such as the Amazon, the Negro, and the Madeira, were as wide as big lakes and as turbulent in places as mountain streams. They cut broad, deep swaths through the forest, from the edge of the Andes to the Atlantic, splitting up the land. In some places they flooded the forest for six months at a time, leaving only the crowns of the trees exposed. And after the heavy rainy seasons, the rivers sometimes leaped over their banks to carve out new channels or a different meander, in the process paring off a segment of the forest and making it an island.

To Wallace's mind, those dynamic waters couldn't be affecting only the landscape: they had to shape the forest animals as well. He recorded which monkeys lived on which shore of the rivers, then argued that the rivers, so broad and uncrossable, were acting much like fences, keeping the species apart. If a single species of monkey was split by a river and the two groups kept separated long enough, he reasoned, they might eventually become two new species. And if that action happened again and again over the millennia, it could explain the Amazon's bounty of species.

Of course neither Wallace nor Darwin knew exactly how isolation and heredity could create new species, because they didn't know genetics. Only in the 1940s, as the nature of genes was becoming clear, could Harvard biologist Ernst Mayr offer an explanation. To make new species, he said, you need

Mammals trapped in the Amazon are brought back to California, where researchers can analyze their genes.

PHOTOGRAPH BY DAVID LIITTSCHWAGER AND SUSAN MIDDLETON

·er cutting trails, collecting animals—a naturalist's dream.

:ts ravaged the team, tempers flared, and then the boat sank.

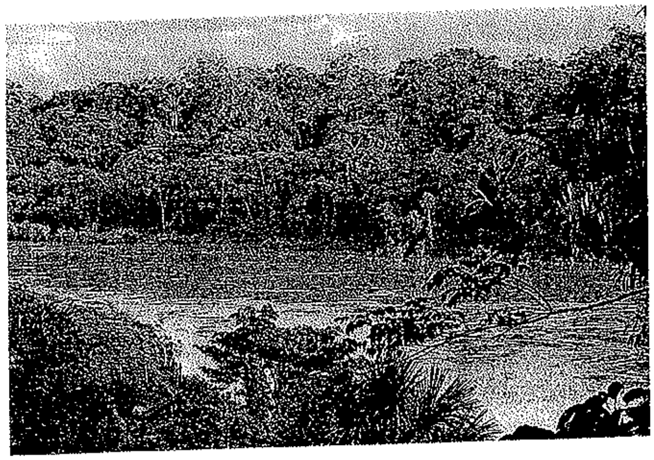
some change in the environment that splits a population. The sudden rise of a mountain, a prolonged climatic change, the shift of a river—all will suffice. If the two groups are kept apart for a sufficiently long time, unable to breed with each other, natural selection and random mutations will force their genes to diverge. If the old barrier to the two populations then drops and the organisms mingle again, they can no longer interbreed because their genes are no longer compatible. They have become two species.

Framed by Mayr's insights, Wallace's river hypothesis made eminent sense—perhaps too much sense, since biologists didn't actually bother to test it until the 1980s. The first to do so was Illinois State University ornithologist Angelo Capparella. Some Amazonian bird species—particularly the sedentary sorts that lived in the understory of the forest—seemed to be corralled by the rivers: their ranges coincided with the rivers' boundaries. Even those species that managed to leap the barriers and live on both shores of rivers showed marked genetic differences in their populations, suggesting that the two groups had not exchanged genes for some time.

But Capparella's work was not enough to vindicate Wallace. "Proving a barrier exists for one species does not make that model universally applicable to all species," Patton says. "You have to test every blasted organism, because each one has a different time of origin, a different rate of genetic change, a different set of ecological requirements. So some organisms are going to be impacted by rivers and others are not." Patton and Da Silva joined a team of biologists who set out in July 1991 to sample the reptiles, amphibians, and small mammals on both sides of the Juruá, a major tributary of the Amazon. "We wanted to collect in both kinds of Amazonian forest, the *terra firme*, where the soil is dry year-round, and the *várzea*, where it's flooded six months of the year," says Patton. "The Juruá was perfect for this because it meanders through both." By sampling at four sites—at the mouth, the headwaters, and two other sites between—the scientists thought they would see how the river's varying width and turbulence affected the animals living in both types of forest.

And those genes show that these eight spiny rats, though they may look virtually identical, are in fact eight separate species.

THE JURUÁ, WHICH emerges out of Peru's Andean foothills and then sweeps 600 miles to its confluence with the Amazon, is a white-water river. White water in the Amazon, however, means not a maelstrom of foaming, turbulent rapids but instead a river carrying so much silt that its waters are the color of café au lait and are absolutely opaque. "When you dive into a river like that," says Patton, "you can't see two inches in front of your face."



The Rio Juruá was the site of Patton's 1991 mammal survey (and his fifth boat sinking).

To reach their first collection site, the researchers motored 500 miles up the Amazon from the city of Manaus in a double-decker riverboat they had outfitted as both a lab and living quarters. At the confluence with the Juruá, they turned south and traveled another 300 miles along its course. The trip took them a full month. "To tell you the truth, it was boring," says Patton. The landscape hardly varied: just a white-water river edged by green forest for 800 miles. To lessen the monotony, Patton spent his mornings making a record of the river dolphins he saw. And as soon as the boat stopped each night, he and his wife, Carol, jumped off to set snare traps and mist nets in order to catch small mammals along the forest's edge, just to see what was there. Although these animals were not part of the official speciation study, they were nevertheless important—in many parts of the basin, the mammals have never been systematically studied. "Mammals are the most poorly known group of vertebrates in the Neotropical forest," Patton says, "so anything we got was valuable scientifically."

The team finally reached its first site at the end of August. To avoid statistical sampling errors, they tried to trap the fauna at two locales on both banks of the river. Thus, at each major stop they would trap in two *terra firme* sites and two *várzea* sites. The procedure was to cut five parallel trails in the forest, 150 yards apart from each other. Along each trail, the biologists then placed two ground traps at 15 different sites, for a total of 150 traps. Because many rodents also favor the treetops, Patton put traps on wooden platforms that could be raised and lowered with a pulley. In addition, the team tried to trap mammals on river edges, stream edges, flooded grasslands, forests that were growing back after fire damage—all in the effort to maximize the total diversity of the catch. Altogether they put out as many as 450 traps each night. After running the traps for a week in the *terra firme* forest, they crossed the river to the corresponding *várzea* site and set up the whole scheme again.

For a year they lived like this, hopscotching along the river, cutting trails, setting traps, collecting animals. From a distance, such a trip might sound romantic, a naturalist's dream come true. But the stores of food dwindled, insects ravaged the team (six botfly larvae burrowed into one zoologist's hand, turning it nearly gangrenous), tempers flared, and then the boat sank. They were three days out of Cruzeiro do Sul, near the Juruá's headwaters, when they hit a submerged log. Within minutes the boat went down, taking most of their equipment with it. It wasn't a complete disaster, because, providently, they had just dispatched their specimens and field notes to the States. Partly because of that stroke of good luck, and partly because Patton was a veteran of boat sinkings (in 30 years of collecting, he'd been aboard four others that sank), the group persevered. "We got another boat and new equipment and continued," recalls Patton, "although the work took us a little longer."

By trip's end they had amassed the largest, most diverse collection of mammals ever gathered from any single area in the Amazon. They took tissue samples from each creature and then prepared its skin for the university's collection. The team often worked in the boat's lab until the wee hours of the morning and caught a few hours of sleep before rising at dawn to collect the next batch of mammals and rebait the traps. All told, Patton netted more than 3,000 small mammals from 52 species, including tamarins, rodents, bats, squirrels, and marsupials. At least seven of the species are new to science. Some of them belong to *Proechimys*, a genus of spiny rat on which Patton is an expert.

"I thought there were only four species and that I knew them all—but there were eight along this river, so that was a big surprise. And then there was the spiny mouse—when we first trapped it, I didn't know what the hell it was." Patton finally decided it must be a new species in a genus previously known only from the Andean foothills in Ecuador, 900 miles away.

Among the rodents testifying to the Juruá's effects on speciation are the region's humble rice rats.

Although Patton recognized that the spiny mouse was something new, he did not see any telltale differences in the other mammals until he returned to Berkeley, where

Not all of Patton's small mammals are rodents: there are more than a dozen species of the mouse opossum in the Amazon.

he could study the specimens closely, examining diagnostic features of their skulls and teeth. He then confirmed their uniqueness as species by analyzing their DNA. Analysis at the genetic level also let him determine whether the Juruá acted like a species barrier, as Wallace had hypothesized. While most DNA in a cell is housed in its nucleus, an extra bit sits in its mitochondria, which are energy-generating factories distributed throughout the cell. Mitochondrial DNA can be particularly useful for studying evolution because animals inherit it only from their mothers. Without the changes that occur when the genes of both parents mingle in nuclear DNA, mitochondrial DNA is altered only by occasional mutations. By estimating the rate of these mutations over millions of years, biologists can calculate how long two populations or species have been separated.

For certain primates, such as the saddleback tamarins Patton had collected, the mitochondrial DNA did indeed confirm Wallace's predicted river effect. At the river's widest points, the tamarins on either side registered genetically as two distinct subspecies. That difference confirmed what Patton could see with his own eyes, since one subspecies sports a white coat, while the other is dark brown. "Along the central portion of the river, you can stand on one bank and see troops of all white tamarins," Patton says, "then look across at the other bank and see the same animals, only they're brown."

At the river's narrow headwaters, though, the pattern changes. "There you see tamarins that have clearly hybridized," Patton says—a mixing that was also evident in their genes. The local people explained to Patton how decades earlier the river had lurched to the right, cutting a new channel. White tamarins had lived on the right side of the river and brown ones on the left, so the river's jump corralled a population of the white tamarins on a sort of temporary island. On its right was the new channel, and on the other side was the old one, which was degenerating into an elongated, dying lake. As the lake dried and filled with sediments, the island joined the old left bank of the river, and now the white tamarins found themselves mingling with their brown counterparts. The two groups proceeded to mate, and since they had diverged only into subspecies during their time apart rather than full-fledged species, they were able to produce hybrids. "It's exactly what the riverine hypothesis predicts," says Patton.

Another marsupial, the woolly opossum, stretches over two feet from the tip of its nose to the tip of its tail.

But Patton discovered that many of the rodents defied Wallace's predictions. Rather than diverging across the river, as the tamarins do, these smaller mammals were genetically split instead into upriver and downriver lineages. "Eleven out of the 17 rodent species we got show that kind of divergence," says Patton, "and that includes *terra firme*, *várzea*, terrestrial, and arboreal specialists. When I saw that pattern, I thought, 'Wow, what is going on here?'" From all outward appearances, the upriver and downriver lineages looked identical, but when he studied their DNA back at Berkeley, Patton discovered that they are genetically distinct. "They differ by as much as 13 percent in their mitochondrial DNA, which is enormous," he says. "It suggests they've been separated for a long time, around several million years."

In fact, all 11 species of rodent that Patton studied are separated at almost the same geographic spot along both sides of the river. "I scratched my head over it a long time," Patton says. "Because when you see a pattern like this in a series of unrelated lineages, you know that they must have experienced some common history. So, what was this historical event they shared?"

THE ANSWER MAY NOT BE IN THE FOREST and its rivers but underneath them. As Da Silva discovered for herself last year, geologists have been devising a radically new view of the Amazon. "It was always taken for granted that there were no geologic differences in the basin," explains Jukka Salo, a paleoecologist at the University of Turku in Finland. "It was thought of as just a green carpet overlying a floodplain." Of course researchers knew that the geology wasn't totally uniform under the Amazon, but it didn't seem to be at all biologically significant.

But beginning in the 1980s, Salo and his team began doing what he terms "good old-fashioned geology"—in other words, nothing more remarkable than putting together a single geologic map of the entire basin. No one had bothered to do it before. When they did, they saw that far from being flat, the Amazon has been shaped by recent dynamic upheavals. To the west of the Amazon, the South American continent has been riding over a tectonic plate and pushing it into the depths of Earth. As a result, the edge of South America has crumpled into the Andes, and the effects of the mountain building have been felt hundreds of miles to the east. Parts of the basin were pushed up and other parts sank, and ridges formed between them. For a time, a vast shallow sea cut the entire basin in half, and although the sea has vanished and the ridges are now barely detectable, they have left their marks. In their heyday they controlled erosion rates and the flow of tributaries in each geologic zone and created unique soil types. Thus, the forests that have sprung from these soils—while they may look like a uniform green carpet—are actually more like a mosaic. "There are not just ten different kinds of forests in the Amazon," says Salo, "but hundreds."

Da Silva was struck by Salo's maps of ancient ridges and wondered how Amazon mammal ranges might compare, so she and Patton lined them up. They were astounded by the correlation between geology and biology. The point at which one of the ridges, called the Jutai, cut perpendicularly across the Rio Juruá was also the point where the mammals diverged genetically. "Patton's results are absolutely stupendous," says Salo. "He has the first molecular data that definitely indicate there was a border or barrier there." And that, in turn, means that the Amazon's little understood geologic past has played a far bigger role in shaping its biodiversity than suspected.

The toro (*Isothrix bistriata*)—a tree-dwelling bushy-tailed rat—can be found across the western and central Amazon basin.

SO FAR, THOUGH, ONLY THE RATS ALONG the Juruá say that Salo is right. What about the rodents elsewhere in the Amazon? It was to get their opinion that Patton and Da Silva launched this collecting expedition last summer, on the Jaú this time. "Salo's maps show that there's another hidden ridge here," Patton explains in their lab one evening. "It's called the Purus Arch, and we're collecting at sites we think fall on either side of it to see if it's had any effect."

Already Patton and Da Silva have begun to discern certain biogeographic patterns among the Jaú rodents. The arboreal rice rats, for example, are extremely varied in coloring and size. "I wouldn't be surprised if we had four different species here," Patton says. "On the Juruá, we recognized five species in the field, but after we did the DNA analysis, we had seven." He's equally certain that some of their spiny rats, from the genus *Proechimys*, represent new species. "The most common species of *Proechimys* we're finding here has a phallus that I've not seen before—and believe me, I've handled a lot of *Proechimys* phalluses in my time," he says with a laugh. On the other hand, the terrestrial rice rats appear identical to those they trapped on the Juruá; yet others caught at the mouth of the Jaú look like rice rats from the northeastern Amazon. "That break between the two types happens somewhere between the mouth of the Juruá and the mouth of the Jaú," says Patton. "It doesn't map with the rivers; it maps with something else. And what else is there? Well, there's the Purus Arch. Maybe it maps with that."

The final answer will come, of course, after Patton and Da Silva analyze the genes of these animals, and for that they need as many small mammals as they can find. Early the next morning, they are back in the forest, walking swiftly along their narrow, machete-hacked trails, stooping every 20 or so yards to check the traps hidden on either side of the path. Above them the high trees meet in a canopy of entwined branches and leaves. Fat brown lianas and dark green vines twine among the trees, climbing, like everything else here, to the sun. Bromeliads, orchids, and mosses cover the branches of the trees, while smaller, broad-leaved plants and lacy forest-floor palms grow in their shade. And everywhere is the incessant whirring, buzzing, and clicking of insects: cicadas, mosquitoes, flies of a thousand different kinds. Sometimes troops of red howler monkeys or black squirrel monkeys crash through the treetops in the distance, while the occasional blue morpho butterfly with wings as broad as two hands floats by. Male screaming pihas—nondescript brown birds—let female pihas far and wide know they are desperate to mate by letting out sharp, piercing cries all day long.

This spiny rat recently found along the Rio Jaú is yet another Amazonian mammal species that is new to scientists.

Even in the Jaú's species-poor forest, life is piled on life. Will the rodents here be as eloquent about this diversity as were those on the Juruá? "Who knows?" Patton wonders, tapping the spoiled bait out of an empty trap. "All I can say is that they will have a story to tell." And when Patton is ready to share it, many scientists who have puzzled over the Amazon's biological riches will be listening. ◘