A mouse lemur shows its strength at a field lab in Madagascar before returning to the wild.

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MAKE WAY FOR THE MOUSE LEMUR

If a US biochemist has his way, the world's tiniest primate could become a top research animal for genetics.

BY LESLIE ROBERTS

Onja is struggling tonight — her hands keep slipping off a miniature grip bar used to measure her strength. "Come on, you can do better," coos Zeph Pendleton, who is gently supporting the mouse lemur as she tries to get a firm hold. Finally, the animal gets her fingers around the bar and gives it a tug. It records a force of 1 kilogram, impressive for a creature weighing only 41 grams. "Good," says Pendleton, a research assistant who is working here in the rainforest at Centre ValBio, a research station at Ranomafana National Park in Madagascar.

Bathed in dim red light, Pendleton, who has come here from Stanford University in California, puts Onja through her paces. He gets her to place her hands on an iPhone modified to measure her heart's electrical activity. He checks her length and weight — she has gained 2 grams in less than a week — then he snaps a mugshot, eventually logging the information into an ever-expanding database of one of the planet's smallest and most abundant primates.

Finally, Pendleton nudges Onja back into a cage and covers it with a black bag to protect the nocturnal creature's eyes while he carries her out into the bright hallway and back to the rainforest.

Onja, which translates as ocean wave, is one of about 500 animals studied so far in the mouse lemur project, a collaboration that aims to parse the genetics of this diminutive, prosimian primate. The brainchild of Stanford biochemist Mark Krasnow, the project is studying a large population of grey and brown mouse lemurs — *Microcebus murinus* and *Microcebus rufus*, respectively — in the wild to work out how their genes link to differences in biology, health and behaviour.

Krasnow thinks that the mouse lemur could become an important animal for genetics research, potentially rivalling the common laboratory mouse *Mus musculus*, at least for certain questions. Mouse lemurs are more closely related to humans, genetically speaking, yet still have many of the advantages of mice in terms of small size, rapid reproduction and relatively large litters. As such, they can shed light on some questions about human biology and disease that mice simply can't. "You hear a lot about successes in mice in elucidating human biology," Krasnow says. "What you don't hear are the aspects of human biology that are not mimicked in the mouse", from behaviours to disease and physical traits. Mouse lemurs could go a long way towards addressing that, he contends.

Although it's difficult to establish a new model organism, scientists and funding agencies are taking notice of the mouse lemur. Researchers have sequenced its genome (P. A. Larsen *et al. BMC Biol.* **15**, 110; 2017), and Krasnow and his collaborators at Stanford and the Chan Zuckerberg Biohub in San Francisco, California, will soon publish a single-cell atlas for the creature — a detailed description of the gene activity in hundreds of thousands of cells from all over the animal's body. It would be the second cell atlas of a mammal, after the mouse. And several labs around the world already have established colonies of mouse lemurs to explore topics as varied as Alzheimer's disease and evolution.

A decade into the project, Krasnow says it is already a "huge success". His team has identified dozens of distinctive mouse lemur traits, many of them medically relevant, such as cardiac arrhythmias, movement disorders and high cholesterol. The researchers are now starting the difficult work of connecting these traits to mutations found in the animals' genomes, an approach that could reveal the genetic basis of complex primate behaviours and human disease.

"I love the concept of using a new model," says Rochelle Buffenstein, a comparative biologist at Google's anti-ageing company Calico in South San Francisco. "Mice have given a huge contribution to biology, but they can only take it so far. Cures for obesity and Alzheimer's in mice have never translated to humans," says Buffenstein, who has pioneered efforts to use the naked mole rat (*Heterocephalus glaber*) as a model organism. And although mouse lemurs will probably never replace more established primate models such as the macaque, Buffenstein is all for expanding the repertoire. "The more models the better."

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A LEAP OF FAITH

The mouse lemur project had its origins during the hot, dry summer of 2009, when Krasnow's teenage daughter, Maya, and her friends were angling to work in his lab at Stanford. "We had asked Mark 1,000 times," says Camille Ezran, who is now a 24-year-old medical student at the University of Rochester in New York. "Finally, he relented." Ezran thought she might shadow a postdoctoral fellow for a couple of months. Instead, on day one, Krasnow charged Ezran, Maya and their friend Jason Willick with finding a new genetic model organism that was a closer mirror of human biology than a mouse.

The students scoured the animal kingdom, compiling a spreadsheet of characteristics, including time to sexual maturity, litter size and conservation status, for each candidate. They considered the northern tree shrew (*Tupaia belangeri*), the pygmy marmoset (*Callithrix pygmaea*) and other prosimian primates, including bush babies (*Galagoides demidoff*) and the tarsier (*Tarsius tarsier*). But the mouse lemur stood out. It has a gestation time of 2 months, reaches sexual maturity in 6–8 months and is among the most fecund primates, with up to 4 offspring per litter. These factors make it possible to study several generations in just a few years. By contrast, the widely used macaque (*Macacca mulatta*) takes about 4 years to reach maturity, is pregnant for 5.5 months and has only one baby at a time.

Like all lemurs, mouse lemurs live only in Madagascar, and some species are critically endangered. But the common mouse lemurs are so abundant that they can be studied easily in the wild, Ezran says. For decades, field biologists have been implanting them with microchips and following them over their 5–10-year lifespan to study everything from vocal communication to foraging behaviour. Large lab colonies show that the animals can adapt well to living in captivity.

Krasnow was sold. Over school holidays, he and the students went on a 'tour de lemur', consulting with primate specialists and visiting mouse lemur colonies in North Carolina and Paris before heading to Madagascar in 2010. "We came back exhilarated. Researchers had welcomed us into their labs. Many would become our collaborators and mentors," says Krasnow.

Many of the researchers, Krasnow learnt, had never met each other. So in 2011, he and Megan Albertelli, a primate researcher and lab-animal veterinary physician at Stanford, organized the first-ever mouse lemur conference, held at the Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, Virginia. They drew in about 40 scientists, representatives from the 20 or so groups worldwide studying mouse lemurs, alongside human geneticists and model-organism specialists. People were enthusiastic, but there was a divide.

"It was immediately clear to us that field biologists viewed their research subjects differently from lab scientists," Krasnow says. Some of the powerful techniques of the lab scientists, such as genetic modification, were unthinkable to the field biologists. "They have a passion for their mouse lemur subjects, as do other primatologists, an almost human-like connection."

A scientific strategy emerged that aimed to use powerful genetics tools in a minimally invasive way. Researchers would work with animals in the wild, screening them for distinctive traits and collecting their DNA for further analysis. There would be no genetic modification and no bringing animals back to Stanford or other overseas research institutions. Aside from periodic captures, the animals would reside in the forest. And the team would train Malagasy researchers to be part of the project. "We wanted to find a respectful way to do genetics," Krasnow says.

Patricia Wright, a renowned lemur primatologist at Stony Brook University in New York who founded and runs the Centre ValBio field station, soon became a key collaborator. By the middle of 2012, Krasnow and the students had brought in several hundred thousand dollars worth of equipment and supplies to outfit a molecular biology lab there. It is now their home base.

CATCH AND RELEASE

On a late afternoon in May, Krasnow's team heads out to the rainforest to set traps. Expert tracker Victor Rasendry, who has worked with mouse lemurs in Ranomafana National Park for more than a decade, advises on setting the traps; the branches they're placed on need to be angled just so

JOZEPH L. PENDLETON, CAITLIN KARANEWSKY & MARK KRASNOW

These mouse lemur mugshots help scientists to track some physical traits, but also reflect the animals' unique personalities.

to catch a mouse lemur. The researchers bait about 30 traps with banana slices and then leave. Around 9 p.m., they return.

It's drizzling and pitch black, and the researchers have only headlamps to pierce the darkness. Rasendry darts expertly from tree to tree, checking traps with the help of Haja Razafindrakoto and her husband Mahery Razafindrakoto, both Malagasy biologists who have been working on the project almost since its outset. One after another, they come up empty. Then, near the bottom of the hill, Haja calls out excitedly, "I've got one." A few minutes later, another. One of these is Onja.

The team had hoped for more, but the weather is cooling down and the animals are moving into a period of reduced activity known as torpor. The team had planned to start its field work earlier, but had to delay the trip because of a typhoon and an unprecedented outbreak of bubonic plague in the country.

Inside the lab at Centre ValBio, Pendleton gets to work. In addition to Onja, they have captured a young male, Lahatra, whose name means something like destiny. The weaker of the two, Lahatra pulls a measly 300 grams on the grip-bar test. He is also much grumpier, twisting his head to bite Pendleton's thick gloves. He then lets out a low grumble. "He's agitated and letting us know," Pendleton says.

Both animals have been caught before and have microchips that allow the researchers to identify them immediately. First-time visitors would get a more extensive examination, about 70 assays in all, during their 2-hour stay in the lab. After microchipping the animals, the team would take a tiny blood sample from the leg and collect a 2-millimetre ear punch — the scientists culture mouse lemur skin cells for a ready source of cells and DNA. Then the animals would go through a standard battery of tests.

The scientists check the lemurs' hearing and eyesight. They measure the base of the tail, where fat is stored, and the width of the skull, a good indicator of age. They analyse gait as the animals make their way across a clear tube dubbed the Prosimian Promenade, modified from a device used to measure the walking patterns of *Drosophila* fruit flies.

With such extensive studies of natural variation, Krasnow says, they find that about one in five animals has an interesting or rather extreme trait — it can be as minor as eye colour or as major as heart arrhythmias. So far, the team has identified more than 20 distinctive traits. The biomedically relevant ones include progressive eye disease, morbid obesity, early signs of diabetes and microcephaly, a smaller than normal head size.

The lemurs' personalities also come through loud and clear, often reflected in the names the researchers have given them. Many are docile and compliant, but Feisty will attack. Murderface, another aggressive one, lets out an uncharacteristic, high-pitched screech. Blinky and Stoic are just as they sound. Pendleton is especially fond of Alphy, a male they have caught every year except last. "He's a great customer. I think he really likes bananas," Pendleton says, although he worries he hasn't seen him as much recently.

Back at Stanford, the researchers are mapping the genes that underlie the animals' distinctive traits. So far, they've identified how some features cluster in families, and they are establishing pedigrees that will enable them to home in on responsible mutations. The researchers are focusing first on a movement disorder they call stutter-stepping, in which the animals lift one hand immediately after it contacts the ground. They are also looking at a sometimes-fatal condition seen in humans, known as sick sinus syndrome, in which the natural cardiac pacemaker, the sinus node of the heart, beats much slower or more variably than normal.

Hopi Hoekstra, an evolutionary biologist at Harvard University in Cambridge, Massachusetts, says that this idea of capitalizing on natural variation "has proven to be incredibly powerful". Hoekstra works with wild mice, which she argues are excellent models for human variation because, like humans, they are genetically diverse. And that variation is subjected to natural selection in harsh, wild environments, she says.

Krasnow's team is now starting phase two, a deep genomic screen to identify naturally occurring variants that render a gene inactive, known as a loss-of-function mutation. To find the mutations, Krasnow and his group intend to sequence the genomes of every animal they catch, not just those with distinctive traits, and compare them with the genome published in 2017. Krasnow hopes to end up with a living library of animals with loss-of-function mutations for almost all of the animal's estimated 20,000 protein-coding genes. It would be analogous to the International Mouse Phenotyping Consortium, a multi-centre collaboration working to determine the function of every gene in the mouse genome by

MINDEN PICTURES/ALAMY

Mouse lemurs are among the most abundant primates on Earth.

intentionally mutating them — knocking them out — one by one.

"While others generate mutations, we just spot them," Krasnow says. This approach is not only faster and cheaper, he argues, but it also avoids the technical and ethical issues of genetic engineering in non-human primates. Researchers interested in the function of a specific gene could look for the mutation in Krasnow's library and see whether there are any significant physiological traits in the animal. It's even possible that Krasnow's team could recapture the animal, or its kin, for follow-up study.

Buffenstein is all for it. "If Mark can exploit natural variability without doing genetic manipulation, that is fantastic."

HIGH HOPES

There's no telling whether the mouse lemur will ever enter the pantheon of established model organisms, Krasnow is the first to admit. But he has high hopes. He doesn't see the animal as a 'boutique' model to study a particular question, but what he calls a 'canonical', or all-purpose model, like the mouse, to explore many diverse aspects of primate biology, disease and ecology. Developing any model takes years. And although Krasnow's work is minimally invasive — just a speck of tissue from an ear punch and a 200-microlitre blood sample — any research on primates is sensitive.

He thinks he'll know in a year or two whether other biologists will embrace it. "Once there's a community exchanging results, techniques and reagents, then the more you know about that animal, the more likely it is that someone else will want to come in and use it," Krasnow says. That is why he is particularly excited about the US$1-million collaboration between Stanford and the Chan Zuckerberg Biohub to build a single-cell atlas.

The atlas came about through the unfortunate demise of Stumpy, one of a few aged mouse lemurs retired from a study elsewhere. Stumpy was about 10 years old, the human equivalent of around 100, when he came down with pneumonia. Aware of Stumpy's failing health, Krasnow and Stanford bioengineer Steve Quake, who is co-president of the Biohub, organized a team of 52 experts to mobilize on the animal's death. They worked quickly to separate cells from each organ and perform single-cell RNA sequencing. The resulting atlas shows the set of expressed genes in 250,000 cells from 30 major organs. Quake says the researchers will make the data available this year before they publish a paper on the project.

The atlas "will really turbocharge the mouse lemur effort", he says. Judging from the mouse cell atlas published last year, Quake predicts researchers will use it "for all sorts of things"; for instance, to look at the relationships between tissues, to explore male–female differences or to find new drug targets.

"That would be an incredible resource," says Anne Yoder, an evolutionary biologist who specializes in mouse lemurs at Duke University in Durham, North Carolina. Because so many diseases are tissue-specific, an atlas can offer insights into the genetics of disease risk and other aspects of health and biological function, she says. And when the Human Cell Atlas is done, researchers will be able to probe the evolutionary roots of diseases.

Krasnow says the ultimate measure of success will be whether the model can answer important questions that scientists hadn't thought to pursue when the project began, such as identifying genes that influence the ability to survive deforestation or climate change.

Still, others caution that Krasnow's quest could well fall short of his bold vision. Jeffrey Rogers of Baylor College of Medicine in Houston, Texas, who with colleagues sequenced the mouse lemur genome, calls the plan "awesome". But he doesn't see researchers switching from macaques or baboons, the most widely used primate models, or marmosets, which are coming up fast, because so much is already known about them. "Sometimes I think Mark is a little too dismissive of other primate models," says Rogers. "The idea that any model can be the model is missing the point," he says.

And Daniel MacArthur of the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, is sceptical about some of Krasnow's ambitions. MacArthur, who studies naturally occurring loss-of-function mutations in humans, is "highly doubtful that studying naturally occurring mouse lemur knockouts will give us transformative insights into human biology". That's because important, disease-causing loss-of-function mutations tend to be very rare, he says. "If you want to study naturally occurring knockouts, the best model is human," he adds, because sequence data are already available for more than 2 million people. MacArthur says that although he is bullish on the prospect of adding a new primate model to researchers' toolkits, he worries that even with a very careful approach, studying mouse lemurs in the wild could disrupt those populations.

And Krasnow is bound to meet resistance from peers. Buffenstein worked tirelessly to develop the naked mole rat, a long-lived rodent that dwells in underground colonies, as a model for ageing and pain research. She predicts that "Mark will have every obstacle thrown at him. Study sections will say, 'You don't have the state-of-the-art tools for the kind of research you are doing. Why not work with mice?'"

> "MARK WILL HAVE EVERY OBSTACLE THROWN AT HIM."

But Krasnow is already looking for ways to expand his efforts in Madagascar. His goal is to "help transform" biology education in the country by getting students to explore what he calls the "living laboratory" right outside. Krasnow envisions a citizen-science project in which Malagasy high-school students will screen thousands or hundreds of thousands of mouse lemurs in their own backyards and then sequence their genomes, greatly expanding the databases. As a first step, he is teaching mouse lemur genetics to biology graduate students at Madagascar's University of Antananarivo.

The team is also contacting conservation groups to explain its project and underscore the importance of maintaining mouse lemur habitat in the face of Madagascar's unrelenting environmental destruction. "Anyone who works with mouse lemurs is a de facto conservationist," Yoder says.

For now, mouse lemurs are thriving here in Ranomafana National Park. At midnight in late May, the rainforest is shrouded in fog when the team emerges from the lab and clambers back up the slippery hill to release Onja and Lahatra. The researchers make a point to place them on the same branches where they were caught. The animals hesitate before leaving their cages, as if to get their bearings, then they scamper up the trees. For a while, the scientists can follow the creatures' large, shiny eyes before the lemurs finally disappear into the rainforest. ■

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