Published: March 2017

Models and data collide in Madagascar

Cara Brook - PhD student in the Department of Ecology and Evolutionary Biology at Princeton University, as well as an ICI3D alum and junior instructor.

They say that those who drink the water from the Manangareza River always find their way back to Madagascar. And, indeed, I can't seem to pull myself away.

As a PhD student in Ecology and Evolutionary Biology at Princeton University, I have spent more than half of the past four years of my dissertation work on the so-called Eighth Continent of Madagascar, where I study the transmission dynamics of zoonotic viruses like SARs, Nipah, and Ebola, which jump the species barrier from bat reservoirs to human hosts. I spent the majority of my early doctoral research years chasing down fruit bats with mist-nets and collecting biological samples all across this remote island nation.

For one who was originally drawn to ecology out of a fervent love for the outdoors, I feel almost embarrassed to admit that I have spent most of 2016 coding equations into the R console (an opensource programming software) on my laptop, or sometimes, scrupulously measuring RNA into an Eppendorf tube at the lab bench. My only defence is to say that I have discovered a passion for science that burns with equal potency, and I have realized that my many months of bat netting under the inky black of a Malagasy sky can only tell me one part of the bigger truth.

My favourite author, John Steinbeck, said it best in his ecological treatise, *The Log from the Sea of Cortez:* "We could, if we wished, describe the sierra [a ray-finned bony fish] thus: "D. XVII-15-IX; A. II-15-IX," but also we could see the fish alive and swimming, feel it plunge against the lines, drag it thrashing over the rail, and even finally eat it… Spine-count description need not suffer because another approach is also used. Perhaps out of the two approaches, we thought, there might emerge a picture more complete and even more accurate than either alone could produce."

The pursuit of this more complete picture has taken me out of the field and into the office and the lab. The past few years have witnessed me develop into an epidemiological modeller in the same mould as so many SACEMA researchers. I code up systems of equations that describe populations of bats as they move between susceptible, infected, and recovered statuses, and I use statistical fitting methods to find the equations that best match my data. Most infectious disease modellers fit their equations to time series of public health data, but in my case, where data are not so easy to come by, I am forced to build my own time series—hence all the months and months of bat netting and, now, all the laboratory work that goes with it.

Classifying bats according to infectious states

My research investigates the extent to which bats might represent "special" reservoirs for emerging viruses. Bats show no demonstrable 'sickness' when infected by many of the same viruses so pathogenic in humans and other mammals, and researchers like me want to know why. In particular, we've noticed that transmission of these viruses—both between bats and from bats to humans—tends to occur at the end of the dry season in all countries studied. This observation has led us to two hypotheses for how viruses might persist in wild bat populations: (1) They could be immunizing in much the same way as childhood measles, infecting a new crop of baby bats each year with the annual dry season birth pulse, then getting cleared, and leaving immune bats in their wake. Or (2) they might act more like human herpesviruses, which are maintained in dormancy throughout much of the year but shed seasonally in individuals immunocompromised by nutritional or reproductive stress.

In order to differentiate between these hypotheses, much of my laboratory work attempts to identify a given bat's infection status with a particular pathogen at a given point in time—essentially, we class bats into the same "susceptible," "infected," or "recovered" states recapitulated in the models coded into my R console.

In the field, this means catching bats and collecting biological samples, which we examine for various pathogens. We collect blood, which we centrifuge and separate into serum and red blood cell. From red blood cell, we extract DNA, which we match to primers for bacterial pathogens like *Bartonella* spp. or protozoa like malaria. We amplify this DNA via PCR and read these results via electrophoresis on an agarose gel. If positive, we conclude that this bat might belong in the "infected" class for these pathogens.

Christian Ranaivoson (left) and Cara Brook (right) process bat samples in the field. Maromizaha, Madagascar. September 2014. Photo by Deborah Bower.

Such classification is harder for the elusive RNA viruses—my favourite zoonoses—because we do not lethally sample any bats to hunt for virus hiding in their tissues. Rather, we attempt to catch viral shedding events by extracting RNA from preserved samples of faeces, urine, and saliva collected upon capture. Sometimes we get lucky and we convert this RNA to cDNA and run it through our PCR pipeline. If not, we often turn to serological data to fill in the gaps.

In the lab, we conduct binding assays on bat serum to identify the presence of antibodies to different viruses, thus hinting at a history of past exposure and present immunity to the pathogen in question. In our models, we then place "seropositive" (antibody-positive) bats in the 'recovered' class.

So far, our data points strongly towards a story of persistent viral infection and periodic shedding, though it will likely be many more years before we truly understand the mechanisms at play in our system. In the meantime, we chip away at the problem, gathering evidence in pursuit of a more complete picture.

Reflections on models and data

There is something a little bit amazing about holding up an Eppendorf tube in the biosafety hood at Institut Pasteur of Madagascar and staring at its swirling contents full of viral RNA. There is something even more amazing about the fact that I caught these bats myself, and I recognize their sample numbers. Just last week, I extracted RNA from a faecal sample from ANDH1, an *Eidolon dupreanum* bat from Ankarana National Park in northern Madagascar. I remember him because his number is unusual—one of the few times we netted at a feeding tree instead of cave roost—and because we caught him on the night of my twenty-seventh birthday. When he comes up positive shedding paramyxovirus in our PCR, I process this information with quiet reverence. This is the awe-inspiring power of research: everything we learn adds some new knowledge to the world.

I had just-turned twenty-seven when we caught ANDH1—and already three years into my PhD—and I will be going on twenty-nine in a few short months. The shocking thing about American PhDs is that they are really very long. You're young when you start and not-so-young when you finish, and I am just about finished…

I remember so vividly the idealistic development worker who first came to Madagascar back in 2010, drank the water from the Manangareza, and has been inextricably bound to this country ever since. I have come a long ways since then—*we have* come a long ways, I should say, for my forever-collaborator, University of Antananarivo PhD student, and fellow International Clinics on Infectious Disease Dynamics and Data (ICI3D) alum, Christian Ranaivoson, is still by my side.

The inaugural class of E2M2 (both students and teachers) outside Centre ValBio Research Center, Ranomafana National Park, Madagascar. December 2016. Photo by Elizabeth Marshall (PIVOT).

E^2M^2: Ecological and Epidemiological Modelling in Madagascar

Bringing our work fittingly full circle, Christian and I journeyed to Centre ValBio Research Center in Ranomafana National Park in December 2016—where, long ago, at the beginning of my PhD, we once thought we might do field work. Instead of field supplies, though, we brought generous grant money from the healthcare NGO, PIVOT, and we taught the inaugural *E^2M^2: Ecological and Epidemiological Modeling in Madagascar*. This workshop was inspired by the ICI3D program's flagship course which we once attended, as students together, in South Africa. We are now students-turned-teachers in the never-ending life cycle of learning. For me, as for many in the ICI3D program, the meaningful union of the worlds of models and data has become one of my most fervent passions.

E^2M^2 was first born out of need. Knowing of my previous involvement with ICI3D, my scientific collaborators at Institut Pasteur of Madagascar approached me with a request to develop a similar course of study on the island. In many ways, our home. I am nearing the end of my PhD but only just embarking on a lifetime of scientific inquiry. And the future looks bright for my return to Madagascar—with five years of government grant money and a shiny postdoctoral fellowship glistening on the horizon. "Hiverina ve?" my Malagasy friends ask me, as I head to the airport. *Are you coming back?*

"Masava ho azy," I reply. "Hiverina foana."

Malagasy course is a hybrid of its two ICI3D predecessors, Dynamical Approaches to Infectious Disease, Dynamics, and Data (DAIDD) and the Meaningful Modelling of Epidemiological Data (MMED)—but in other ways, like the country in which it is based, E^2M^2 is a thing on its own. Madagascar is not called the Eighth Continent for nothing, and teaching to its students requires targeted approaches and language skills—both French and Malagasy—unique from those used in ICI3D. Like me, many of Madagascar's quantitative biologists hail from backgrounds in field ecology rather than public health and seek instruction in a scope of models beyond those traditionally used in epidemiology. I love to learn new things, and teaching is the best way to learn; teaching E^2M^2 gave me a unique opportunity to discover more new things about the world.

It is late December as I write this, and the days are long and sultry in Madagascar's capital city of Antananarivo. The exotic tang of *girofo*—cloves—and fresh vanilla bean hangs in the air, and Tana's market stalls abound with festive tinsel and brightly coloured paper. Once more, the holidays call me *Of course, I always come back.*

Cara Brook *- PhD student in the Department of Ecology and Evolutionary Biology at Princeton University, as well as an ICI3D alum and junior instructor. Research interests: epidemic dynamics of viruses that transmit from fruit bat reservoirs to human hosts.* caraeb@princeton.edu