Cell

Biology Gone Wild

To study how genes, cells, or organisms operate in natural environments, researchers often need to leave the bench and venture into the field. Here are a few approaches that field biologists use in designing and conducting semi-wild experiments and the many challenges they face.

Traditionally, molecular and cell biologists have been more or less university-bound homebodies, while ecologists and evolutionary biologists venture into the forests, prairies, jungles, tundras, and oceans to ask questions about biology in the wilderness. Today, distinctions between the disciplines are blurring, but field researchers encounter many of the same challenges they always have.

"In the 1970s, there was a large gulf between these two research enterprises (molecular biology and eco-evolutionary biology), in part because it was thought they had little to offer to each other, and in part because each had its own vocabulary and special techniques," evolutionary biologists Peter and Rosemary Grant wrote in an email to *Cell*. The Grants are a husband-and-wife team who documented ongoing evolution in Galápagos finches, starting in the 1970s. They started taking blood samples from the finches early on, and as molecular techniques have advanced and preservatives have improved, they've been able to glean more information from DNA analysis by sending blood samples back to Princeton, where they are professors emeriti.

Ecologists and evolutionary biologists increasingly use molecular tools to tease apart relationships between organisms; meanwhile, the falling cost of gene sequencing enables researchers to incorporate new species with unique traits into controlled experiments as new model organisms.

However, in some aspects, the more biology fieldwork changes, the more it stays the same. "The main difficulty in working on a rocky uninhabited island was, and still is, the absence of electricity," the Grants wrote. "Suitable rechargeable batteries were not easy to operate in the field. This was a limitation on the use of electrophoresis for allozyme studies in the field. The main solution was to take a cylinder of liquid nitrogen in the field, and there were many challenges in doing that!"

Fieldwork requires both preparedness and a willingness to improvise. "Where we tend to go, they're places that are very isolated," says evolutionary biologist Jessica Ware of Rutgers University. "There's no electricity, sometimes no running water, and certainly no store nearby where you can run and get supplies." She recalls one trip when her team ran out of ethanol for preserving samples. Instead of flying back to the nearest city, they went to a local liquor store, bought bottles of vodka, and preserved their samples in vodka until they could transfer the samples to ethanol.

Ware, who studies insects such as dragonflies and termites, is part of the new guard of evolutionary biologists who rely heavily on genomics. Her field expeditions have taken her to Guyana, Ecuador, Namibia, and Sweden among other places, but she spends the majority of her time in Newark, NJ. Her lab work—cataloging and sequencing samples—isn't that different from what molecular biologists do. "We're using the same methods, and we're even asking similar questions," she says. "It's just that I use the word 'selection' more often in my discussions."

Still, Ware emphasizes that fieldwork is "a skillset in and of itself." Marshalling students who are away from home for the first time can be a challenge. Sorting out logistics, paperwork, and permits can be onerous. However, the very thing field biologists study—nature—presents many of the biggest obstacles to field research. Leaving the lab means working outdoors with unpredictable test subjects in highly variable (and sometimes extreme) conditions. Tales of equipment trucks getting stuck in mud, winds knocking over specimen-collecting devices, unsettling encounters with wild animals, and unexpected weather events abound.

Hopi Hoekstra handles a wild mouse at a field site in Florida. Image courtesy of Elizabeth Pennisi/AAAS.

Researchers from the Mitchell-Olds lab transplant plants in the mountains of central Idaho. Image courtesy of Evan Raskin.

However, in well-designed field studies, small doses of natural chaos can lead scientists to results they would never have predicted. "In the lab, we often think of variation as introducing noise, but in the field, the variation is the stuff that makes the experiment interesting," says Harvard evolutionary biologist Hopi Hoekstra. "It's a different way of looking at variation, whether it's a nuisance or whether it's something very exciting and powerful."

Hoekstra is a prominent example of a 21st century discipline-straddling evolutionary geneticist. Her portfolio includes behavioral experiments, population genetics projects, and developmental studies, all centered around understanding the evolutionary forces that shape rodents. One of her most ambitious projects is an outdoor genomics experiment that tests whether variation in wild deer mice's pelt color is a response to the environment. "We had this intuitive notion that the color of the mice and how that matched their local soil could be important to their survival, because their predators tend to be visually hunting predators," says Hoekstra. "But we had no experimental evidence that that was true, so we wanted to set up an experiment."

In designing her experiment, Hoekstra drew inspiration from microbiology. Experiments in bacterial evolution can be carried out in petri dishes stored on a lab bench, but key evolution drivers such as climate and predators are either highly controlled or totally absent in lab settings. Understanding those evolutionary forces requires leaving the bench and setting up experiments that are open to the elements.

Plant biologists have been pairing greenhouse research with outdoor experiments for decades. Common design schemes include randomized outdoor plots and transplantation studies, where plants from one spot are re-planted in another location. "Field experiments are high-risk, high-payoff approaches," says Tom Mitchell-Olds of Duke University. Over the course of his career, he and his team have transplanted more than 99,000 plants into new environments to identify genes that contribute to plant adaptation. "If things work, if the experiment survives, then we're measuring the things we most care about…. We can measure traits in the environment where the traits evolved."

One of the biggest threats to field experiments with plants is herbivory. Evolutionary ecologist and plant geneticist Johanna Schmitt of University of California, Davis recalls one experiment where her team was mapping quantitative trait loci (QTLs) that contribute to flowering time. The project was going well until her experimental plots were invaded by leaf-eating rabbits. "Luckily, they didn't eat all of them, so in addition to doing a QTL [analysis] for flowering, we also mapped the QTLs for rabbit herbivory," she says. Many plant biologists have similar stories, but the exact nature of the experiment-devouring beast varies by location. Field researchers build fences around their experimental plots to keep wandering cows and elk out, and the fences often extend underground to stymie burrowing animals such as gophers.

The difficulty of maintaining the field site's boundaries increases when the test subjects are the burrowing rodents themselves. For her coat color evolution experiment, Hoekstra needed to build two enclosures of areas large enough to support more than a hundred mice on two sharply contrasting patches of ground. "We had to go out and find a field site," Hoekstra says. "And then we had to convince people to let us build these enclosures. Then, we had to actually build the enclosures, which involved heavy machinery and 15,000 tons of steel and backhoes and all sorts of things…. We also had to worry about things like rattlesnakes that were in the area and at least in one case in the enclosure. This was in Nebraska, where there are also tornadoes, so we dealt with tornadoes."

The post doc leading the project—Rowan Barrett, now an associate professor at McGill University—ended up befriending many locals from the nearby town over the course of the study. Not surprisingly, this type of labor-intensive and equipment-heavy field experiment with animals is rare, but the project is still going strong, despite harsh winters, fires, and other small natural disasters. "You have to be nimble," Hoekstra says. "The things you didn't expect can be real opportunities."

Another strategy for harnessing natural variation is bringing the wild organisms into a lab setting, as opposed to taking the experiment to the animals. Neurobiologist Nachun Ulanovsky of the Weizmann Institute in Israel has done both. His work focuses on the neurobiology of navigation in bats. In the past, his team has done outdoor GPS tracking studies, where Egyptian fruit bats fly around freely in their natural habitat, but more recently, they've been conducting experiments where bats with neural recording devices mounted on their heads fly around a large ''flight room'' in the lab. ''This allows us to have the benefits of both worlds, to have a controlled experiment where the bat is doing the same thing over and over but also naturalistic in the sense that it is a very large scale,'' Ulanovsky says. ''You don't have to let animals loose outdoors and have them run around completely beyond control.''

These naturalistic flight room experiments have allowed Ulanovsky and his colleagues to gain insight into how bats' neurons encode three-dimensional flight paths. These bats are not established model organisms; Ulanovsky and his colleagues have to go into the field and capture their test subjects with butterfly nets, and housing wild animals in a lab can be difficult. However, Ulanovsky thinks the benefits of working with unusual organisms outweigh the difficulties.

''If you are able to formulate a question that is very interesting and you find a species that is very well-suited to address this . . . if you study closer to its natural behavior, then it's kind of a winner-take-all because you have a distinct advantage [in] that you can do something that's impossible perhaps to do in the classic model,'' says Ulanovsky.

One strategy for solving the lack-of-protocols problem is to pattern the new organism's lab set-up on protocols for an established model organism. When evolutionary geneticist Dario Valenzano of the Max Planck Institute for the Biology of Ageing started working with wild-caught African turquoise killifish, he didn't know what to feed them, what temperatures were best for them, or how many could comfortably live in a tank. The African turquoise killifish is a freshwater fish with a lifespan that only lasts the length of the three- to four-month rainy season in sub-Saharan Africa, making this small fish a useful model for studying aging. However, even a short-lived experimental organism needs to be able to survive well in a lab. To address this conundrum, Valenzano looked to two model fish species: the stickleback and the zebrafish.

''Basically, my thought has always been [that] if I can make it easy for a zebrafish research to switch over, to adopt a new model organism, that would be fantastic,'' says Valenzano. ''If you have some very similar species that's already a fantastic model and your organism provides a completely different perspective into some aspects of biology, then you can all of a sudden benefit from all the tools that have been developed in that other species. So you don't have to reinvent all of the tools from scratch.'' Finding the best protocol for the killifish still required a lot of trial and error, but using existing lab protocols as the baseline has made it easier for other researchers to adopt the killifish as a model organism and replicate Valenzano's results.

On the other hand, sometimes, when bringing a new organism into the lab, scientists simply have to invent new equipment. For his experiments, Ulanovsky needed a lightweight wireless neural recorder that could fit on a bat's head without disrupting their flight pattern. That technology didn't exist, so Ulanovsky recruited team members with the skill to miniaturize existing technology.

For both Ulanovsky and Valenzano, the question or area of interest drove the choice of non-traditional model organism. ''When I started the lab in Cologne, I really wanted to focus on the immune system. I wanted to use the killifish to study in particular B cells and T cells and whether they matter for the aging process,'' says Valenzano.

Using wild-caught or more unconventional species to address topics that traditionally fall under laboratory biologists' purview—such as neural encoding, immune systems, and microbiomes—is far from being the only interface between field biology and the molecular realm. Medical research centers increasingly hire evolutionary biologists to help unravel the behavior of cancer cells and microbial ecologists to understand interactions between bacteria in the human body. Field observations and experiments can also shed new light on well-characterized genes and pathways.

Though field experimentalists and molecular mechanism decoders often end up in separate academic departments, Schmitt has found that setting up collaborations is usually straightforward. ''When I reach out to people who are interested in mechanisms, they've been interested in what's going on out in the field,'' says Schmitt. ''People are interested in finding out what the genes they study are actually doing out in the wild.''

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