



The Secret Lives of Ants

By Sandeep Ravindran on Wed, 15 Mar 2017

The tiny brown clone walks alone, far away from its brethren. Its solitary path takes it round and round the edge of the small plastic dish as it completely ignores its siblings.

It's very unusual for an ant to be this anti-social. But it's not the ant's fault that it's a loner, rather that of Daniel Kronauer. The ant is unable to detect the others' social cues, the result of years of work by Kronauer and his team of researchers at Rockefeller University.

Their seemingly esoteric work could shed light on eusociality, one of the more unusual social structures in the animal world. Eusocial animals raise their young cooperatively while adhering to a strict division of labor—one class reproduces while others may tend to the young or forage for food. Deciphering eusociality could lead to a broader understanding of how complex social systems evolved. Already, Kronauer's lab has elucidated some of the first genetic underpinnings of ant sociality, and the tools they used to do that could transform how researchers study social insects.

Video: <http://www.pbs.org/wgbh/nova/next/wp-content/uploads/2017/03/ant-tracking.mp4>

Two ants, PP in pink and GG in green, have had a crucial olfactory receptor knocked out, leaving them unable to pick up on the others' social signals.

Scientists have long been fascinated by the complex societies and behaviors of eusocial insects such as ants and honeybees. Some ants collaborate to construct towering edifices, others undertake complex foraging expeditions or herd aphids and farm fungi for food. Still others can come together to form makeshift rafts when they are threatened by floodwaters.

In many ways, ant and bee colonies resemble superorganisms that cooperate and communicate to produce a whole that's greater than the sum of its parts, much like our cells give rise to us.

Just as studying a single neuron wouldn't give you a sense of a brain's complexity, a single ant doesn't tell you what a group of them can achieve. "You get these emergent system-level properties that you can't really predict from studying an individual unit," Kronauer says. "If you're interested in how individual units in complex biological systems interact—and how they evolve—ants are great model systems for these things," he says. "You can ask the exact same questions—and many people do—when they study humans."



A confocal laser scanning microscope produced this 3D projection of the clonal raider ant brain. Synapses are stained purple, blue is cell nuclei, and green is actin, which is enriched in neuron fibers.

To achieve their outsize feats, ants and bees need to work in concert, and to coordinate, they need a sophisticated means of communicating with each other. Ants use chemical signals called pheromones to communicate with each other, distinguish friend from foe, and decide what job to do for the colony. But despite ants' ubiquity, we still know very little about the molecular mechanisms and genetic underpinnings behind their complex behaviors and chemical signals. "There are so many really, really fundamental things about ants that we just don't know," says Waring "Buck" Tribble, a graduate student in Kronauer's laboratory.

That's primarily because ant researchers still lack the molecular biology and genetic tools that those who study many other organisms take for granted. Unlike in a model organism such as the fruit fly *Drosophila*, it's not yet possible to delete a gene or add in genes that express a fluorescent protein, for example. "It would be much easier if we could do in ants what we can do in *Drosophila*," says Patrizia Dettore, an ethology professor at University Paris 13 who studies chemical communication in ants.

"The bottom line is, currently there's no real genetic model system for social insects," Kronauer says. In fact, "there's no really good model systems for studying very complex social behaviors," he says. That's what he's spent the past several years hoping to change.

A Model Ant

Kronauer spent many years as a graduate student and postdoctoral researcher studying large swarms of ants, including driver ants in Africa and army ants in Costa Rica. Today, he spends his days far from the rainforests in his lab in Manhattan's upper East Side. Apart from a yearly trip or two to Costa Rica, his closest link to the field are the numerous ant photos that decorate his office.

As he was studying ants in the field, Kronauer realized that there was a limit to what he could learn without taking them back to the lab. "You really have to follow every colony. It feels like following a bunch of chimpanzees through the forest," Kronauer says. "Towards the end of my PhD, I figured if I

ever wanted to run a lab and do experiments and really get somewhere at the molecular basis of social behavior, I would need a model system that I could keep in my lab,” Kronauer says.



Daniel Kronauer looks over one of his ant tracking systems. Labeled ants are placed in the white dishes and tracked via webcams.

With more than 12,000 ant species to choose from, Kronauer certainly had options when it came to selecting a model organism. Traditionally, ant researchers have studied species that exhibited interesting behaviors in the wild. That’s great for understanding the range of ant diversity, but it’s not as useful when you want to study the mechanisms underlying eusociality. So Kronauer took the opposite tack—he chose an otherwise unremarkable species that might make a good lab model.

The very thing that makes social insects so fascinating—their sociality—also makes them tricky to use in the lab. A functional colony requires thousands of workers. They also need a queen to anchor the colony and replenish the workers. It’s a combination that makes colony maintenance both expensive and labor-intensive, and the colonies often need frequent reinforcements brought in from wild populations.



Clonal raider ants

Kronauer first read about the clonal raider ant, *Ooceraea biroi*, in 2006 when he was perusing some niche insect journals as a graduate student at the University of Copenhagen. “It’s essentially just a miniature pet lab army ant,” Kronauer says. What piqued his interest was a report that, instead of having a queen, every worker of this species could lay eggs. “You can start a whole population with just a few ants,” he says.

O. biroi has another trick up its sleeve—being clonal, the eggs that workers lay are genetically identical to the parent, eliminating a potential confounding variable. “You can see how factors other than genetics affect the behavior,” says Laurent Keller, professor of evolutionary ecology at the University of Lausanne, with whom Kronauer conducted his postdoctoral research. “I think it’s a fantastic system to study social behavior,” he says. “It will become one of the model species among ants for sure.”

But just finding the right species wouldn’t be enough. Kronauer’s also had to develop a set of standardized tools to encourage others to pick clonal raider ants when studying the mechanisms underpinning ant sociality. “If everybody has to reinvent the wheel every time, it’s not going to catch on,” says Yehuda Ben-Shahar, an associate professor of biology and medicine at Washington University in St. Louis, who works on behavioral genetics in honeybees and flies. “But if you come up with a series of protocols that will make adopting that species for whatever question you’re interested in, and it’s all streamlined and easy, then people will do it.”

“It’s hard work,” Ben-Shahar says. “He has to generate all the tools—and first principles to show what’s doable.”

Origin Stories

If anyone could stick with such a project, it’s Kronauer, whose interest in ants dates to his childhood. He would spend hours flipping rocks in his garden and collecting the ants and other critters that lay beneath. Back in his room, he assembled a menagerie of insects, lizards, scorpions, and tarantulas, which led to the occasional infestation—Kronauer recalls a particularly annoying outbreak of large Madagascan hissing cockroaches.



*A raiding column of *Eciton burchellii* army ants return to the bivouac in Brazil.*

Kronauer's childhood ant-collecting skills would come in handy when he first started collecting clonal raider ants from Okinawa and the U.S. Virgin Islands. "I spent eight to ten hours a day just flipping rocks or logs," he says. Often, the reward for a whole day's toil was just a single colony. But the advantage of *O. biroi* was that Kronauer only needed ten or so workers to start a new colony. "That's the cool thing about these guys. They're not so easy to find and collect, but once you have them in the lab, they're super easy to maintain," he says.

As Kronauer traveled the world collecting clonal raider ants, he found that most belonged to the same few variants. "You can go to Madagascar and collect an ant and St. Croix in the U.S. Virgin Islands and collect an ant, and you sequence the genome and they're virtually identical. It's the same clone," he says. It's a nifty biogeographic riddle, but for Kronauer, it was a bit of a headache. More variants would allow researchers to look at more natural variations in the ants' behaviors and interactions. But to find these variants, Kronauer would have to figure out where this ant species originated—no easy task. "Usually it's very difficult to say where invasive species come from, because you have no good way of knowing where to look," he says.

The key to tracking down these ants' origin turned out to be some samples that Kronauer received from an Indian colleague. The ants in that sample were distinct from the variants Kronauer had already collected. Putting on his detective hat, he reasoned that these soil-dwelling ants were likely to spread through ships, which take up local soil as ballast. Kronauer used Google Earth to plot the large harbors closest to the source of the North Indian ants he received. He pinpointed several large riparian harbors in Bangladesh that were worthy of a closer look.



Kronauer works with some Eciton army ants at La Selva Biological Station in Costa Rica.

Kronauer sent two of his graduate students, Sean McKenzie and Buck Tribble, all the way to Bangladesh. There, McKenzie and Tribble spent three weeks flipping over rocks and trash looking for ants, much to the bemusement of the locals. “There was a big conspiracy theory going around in some of the villages that we were eating the ants, that they were a weird American delicacy. Because why else do you go around finding ants?” McKenzie says. Ultimately, the expedition paid off, as McKenzie and Tribble found several new variants of the species that they could study in the lab.

Raising Ants

The store room in Kronauer’s lab is a warren of one-and-a-half gallon Rubbermaid containers partially filled with moist plaster of Paris. Thousands of clonal raider ants live in each container, from which researchers pluck a few for each of their experiments. The unmistakable smell of bleach permeates the room. “We’re a little bit paranoid of having parasites getting into the cultures, like mites or nematodes,” Kronauer says, which explains why they religiously wipe every surface with the disinfectant. That’s a lesson learned from experience—when Kronauer started his lab, a mite infestation killed off 80% of the ant stocks he had painstakingly built up as a postdoctoral fellow. As he slowly rebuilt from the 20% that remained, the incident just reinforced the need for a model ant system. “In flies, you would just order them again from the stock center,” Kronauer says. “With ants, what are you going to do?”

Now, Kronauer and his team can quickly rebuild stock colonies in the lab by creating the right conditions for them to prosper. But determining those conditions wasn’t obvious at the start. Setting up a new lab organism requires first figuring out how to efficiently feed it. Clonal raider ants raid the nests of other ant species for ant brood—their pupae and larvae—to feed their own larvae. But regularly collecting other ant species from the wild just so they could serve as food is not terribly efficient or scalable. So Kronauer decided to get creative, and after some trial and error, he discovered that the ants enjoy Mexican haute cuisine. “We feed them on what is called *escamoles*. It’s like a Mexican delicacy,”

Kronauer says. The dish consists mainly of ant pupae and larvae collected from fields of agave, which is used to make tequila. Now, replenishing the food stocks just requires sending a lab member to Mexico to buy them in bulk.

Chemical Communications

After years of work getting the ants set up in the lab, Kronauer moved on to deciphering their means of communication.

The most important form of communication chemicals are the pheromones present on their outer skin, what's known as the cuticle. Ants use these hydrocarbons to assess all kinds of things, such as whether an ant is from their colony or another colony. "A lot of social dynamics are regulated via hydrocarbons," Kronauer says. "We've known a lot about the pheromones that they use, but how ants have evolved to perceive the pheromones was really unclear," he says.

Ants pick up the pheromones using odorant-detecting hairs on their antennae called basiconic sensilla. In clonal raider ants, they are all present on one side of the antenna, so to investigate the genes expressed in these sensilla, McKenzie had to cut the antennae in half. "The antennae are the width of a human hair, so I was literally splitting hairs," he says. "That just took long weeks without caffeine to get the steadiest hands possible."



Ants that lack olfactory sensors lose track of the colony and wander listlessly about the dish.

Those weeks without coffee were worth it, as McKenzie found that a specific group of genes responsible for detecting odors was highly expressed in the basiconic sensilla. It's the same group of genes that other researchers discovered was highly enriched in ant genomes compared with most other insect genomes. In addition, McKenzie found that the area in the ant brain that processes smells is enlarged, and that's the same place where neurons from the sensilla terminate. "Essentially, it's this really cool

evolutionary scenario where the ants have evolved a massive group of genes to decode the chemical language that they use,” Kronauer says.

Ant Hacks

The next piece of the puzzle was the genetic component. That’s become somewhat easier lately with the development of a genome editing technique called CRISPR, which allows scientists to insert, delete, or modify genes in many different species. “It also works in ants very nicely,” Kronauer says. “But ants have all this complex, interesting life history, that also brings with it a lot of complications,” he says. “Colonies are massive, generation times are in years, and you can’t breed them in the lab,” Kronauer adds. “They’re nightmares as genetic systems.”

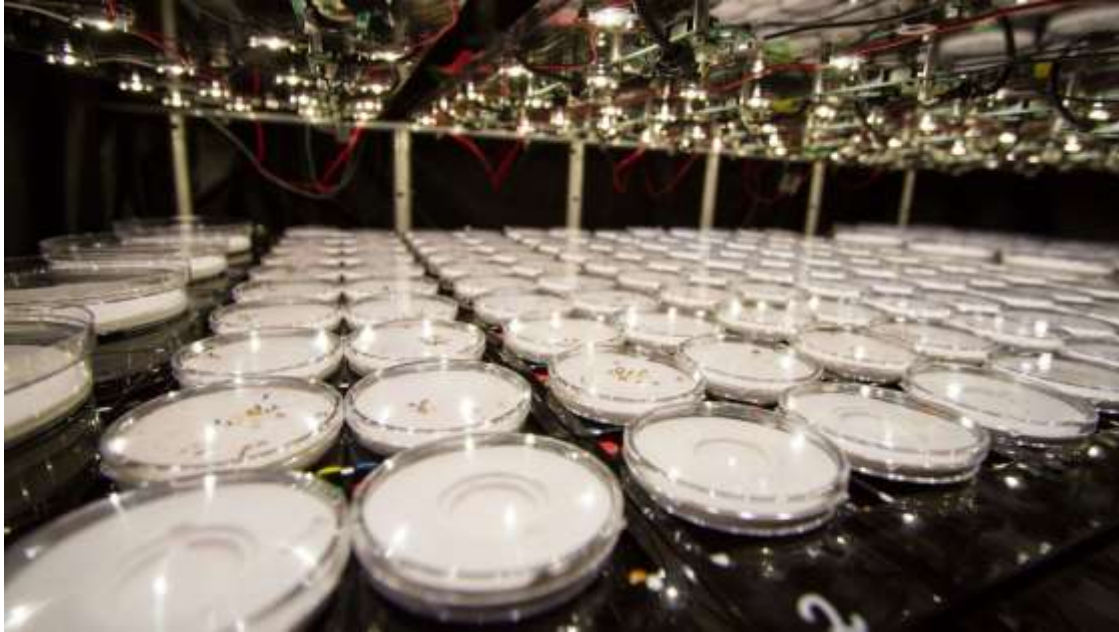
Clonal raider ants overcome most of these drawbacks by growing fast and cloning themselves. “We knew we had a pretty good species to do this, and if it would work in ants, this would be probably the best shot,” says Kronauer. But to get it to work, the scientists would have to inject the necessary molecules into ant eggs, where they induce mutations. The eggs then grow into mutated adults, which give rise to mutated offspring. “It’s not actually that easy to inject an ant egg, and nobody’s ever done this,” Kronauer says. To modify an ant, researchers stick the eggs—about the size of a grain of rice—onto a glass slide and use a joystick to painstakingly maneuver a fine needle into each egg. They then inject a nifty bit of molecular biology and move on to the next one, assembly line style. The researchers incubate the eggs on each slide until they hatch into larvae.

But an ant larva can’t survive on its own—it has to be fed by other worker ants. “You have to then return it to the colony somehow without the workers freaking out or without it dying,” Tribble says. He and research specialist Leonora Olivos-Cisneros found that if they had at least ten larvae, they could trick worker ants into rearing the larvae into adulthood. “It took us two years of really hard work to just stumble into these weird things,” Tribble says.

After injecting thousands of ant eggs, the results have been worth it. “The genetic system that Kronauer and his team have developed is the most advanced in ant biology,” says Keller, the Swiss biologist.

Society’s Secrets

In another section of Kronauer’s lab, a passel of cheap webcams stare unblinkingly for weeks at a time upon 120 Petri dishes, the temporary homes of the ant colonies under behavioral observation. Kronauer developed this tracking system with postdocs Jonathan Saragosti and Yoko Ulrich, to assess how ants behave and interact with one another. Saragosti developed software that can identify each individual ant based on two colored dots painstakingly painted on its back. “It’s all very low tech,” Ulrich says. Previous tracking efforts used expensive high-resolution webcams to read barcodes on each ant’s back and could only analyze a handful of colonies at a time. The low cost of painted dots and low-resolution webcams allows for tracking at a much larger scale. “You can really replicate things very precisely, and you can get very high resolution behavioral data,” Kronauer says.



The lab's tracking system is relatively low-tech, with webcams trained on white dishes containing the labeled ants being studied.

What the cameras record acts as a ground truth, of sorts, for the genetic experiments. Kronauer's first experiments began by taking a crude but effective approach, targeting a co-receptor that is necessary for all of an ant's odorant receptors to function. "We knocked out the co-receptor and basically ablated the function of all of these olfactory receptor genes," he says.

Without functioning odorant receptors, "basically ant sociality completely breaks down," Kronauer says. "This blocks the chemical communication of the ants and creates ants that don't respond to the chemical language of the colony anymore," he says. The mutant ants appeared unable to follow chemical pheromone trails, and many spent their time wandering the Petri dish alone rather than nesting with other ants. These loners also lacked most of the usual enlargements in the brain region associated with processing smells. Together, these results suggest that the expansion of the olfactory receptors in the ant genome may have played a crucial role in allowing ants to evolve their complex social communication and behaviors.

That breakthrough sets the stage for a slew of future experiments. Blocking the functioning of all the olfactory receptors was just a brute-force proof-of-principle, and Kronauer can now start to interrogate them in smaller groups, answering basic questions about how they might interact and function to decode different smells. By figuring out what combinations of receptors might respond to different pheromones, for example, Kronauer could begin to decode ants' pheromone language.



Labeled ants navigate the white dishes of the tracking system.

If he succeeds at those, he'll probably have an even better chance of convincing other scientists to adopt clonal raider ants as a model species. Already, his efforts are bearing fruit. "I'm starting to get requests from people who are interested and want to study specific questions and think that this would be a good model system," Kronauer says. "It's still pretty early in the process, but my hope is that over the next few years other labs are going to start working on this species," he says.

It certainly wouldn't be very hard to get started. "If you write me an email," Kronauer says, "I could send you a little plastic tube with 200 ants overnight FedEx and you could start the experiments the next day."