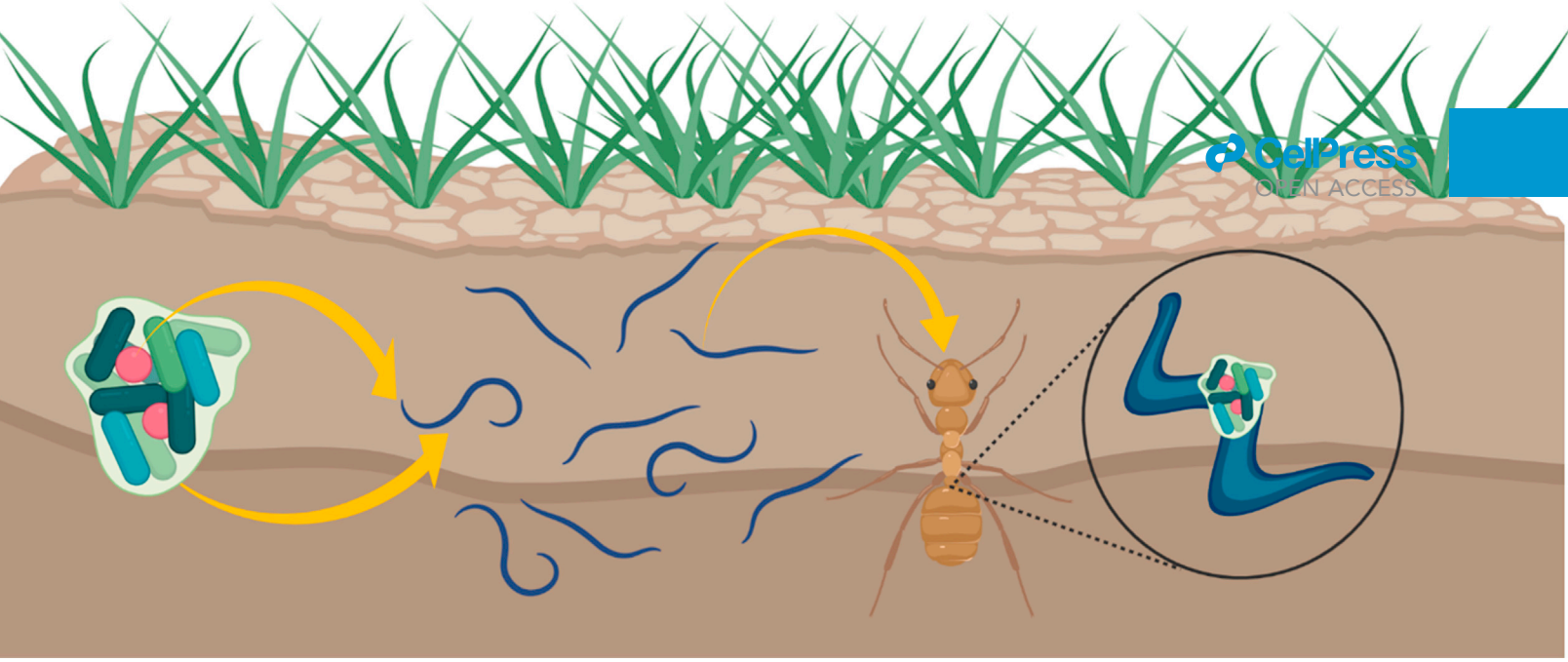

Backstory

It began on an ant hill in Maine: A story in multidisciplinary research

Suzanne L. Ishaq[1] and Eleanor Groden[2]

Science journeys don't always proceed in a timely fashion. Sometimes it takes years to complete a thorough inquiry based on the pace of what one is trying to study, but the project timeline can also be determined by the availability of resources, such as funding, technology, researcher expertise, and time.

That's how my part in this story came about: I had free time. This story actually begins on an ant hill in Maine, which led to a lab in Arizona, and ends with a trans-national collaboration on the transfer of environmental bacteria from soil to ants via nematode infection. In this backstory, I will discuss the journey leading toward our recent publication in iScience (Ishaq et al., 2021). Ours is a story of slow advances over time, made through the commitment of faculty inspired by what each other brings to the table and by working with curious, enthusiastic students.

MY BEGINNING, IN THE MIDDLE

Who were the players in this project, and how did you bring everyone together?

I'm Dr. Sue Ishaq, and I started working at the University of Maine (UMaine) in September 2019 as an Assistant Professor of Animal and Veterinary Science, which means I'm a junior-level faculty responsible for research and teaching.In my first year of employment as a new faculty, my time was protected to allow me the extra weeks and months required to establish a new research laboratory. In addition to buying specialized equipment, training students in laboratory work, and developing experimental designs for new projects, establishing a lab also means meeting your new colleagues and developing ideas together. It was in meeting other UMaine faculty that I started working with Dr. Jean MacRae, an Associate Professor of Civil and Environmental Engineering.

My background is in microbial communities, mostly those that live in or on human and animal hosts, and how the microbes and the host interact, but I also have some experience with microbes in soil and inside buildings and also bioinformatics, or using statistical methods to understand microbes by studying their DNA. Jean had been studying microbial communities, too, but mostly in waste systems and pollution management. She had gotten involved with several projects at UMaine that had employed DNA sequencing to identify microbes in various experiments, and we connected over our excitement about microbial communities and analysis thereof.

Necromenic nematode *Pristionchus entomophagus* has been frequently found in nests of the invasive European ant *Myrmica rubra* in coastal Maine, United States, and may contribute to ant mortality and collapse of colonies by transferring environmental bacteria.

Sue Ishaq, Assistant Professor of Animal and Veterinary Sciences, at UMaine.

BORROWING A CUP OF DATA FROM THE NEIGHBORS

During our (Jean and myself) initial meeting while brainstorming over coffee, I mentioned that I was searching for small, unpublished datasets to use in a university course I was developing to teach students how to analyze microbial communities' DNA and develop into scientific manuscripts. Jean shared a dataset of bacterial 16S rRNA genes to use in my data analysis class in spring 2020, as well as some basic information about the samples (https://sueishaqlab.org/teaching/avs-454-554-dna-sequencing-analysis-lab/). Each file of data contained a small portion of the DNA code from all the bacteria in a different ant, nematode worm, or wax worm moth larva sample. The ants had been collected from nest colonies that were experiencing a population collapse, and the nematodes had been infecting the ants and emerged once the ants had died. The nematodes were then co-housed with larvae in the laboratory to see if they would transfer their bacteria to the larvae.

When the semester began, I assigned the data to UMaine graduate students Alice Hotopp and Sam Silverbrand, and I mentored them through the analysis, statistical comparisons of the samples to determine if the bacterial communities in ants, nematodes, and wax worm larvae were similar, generation of figures and graphs to illustrate our scientific findings, and writing up our ideas, methods, and results into a draft manuscript. It takes a massive amount of work to generate a scientific manuscript like this, and even more so when you are just learning how to do it, and *especially* when your entire life has been upended because of a global pandemic. Alice and Sam showed incredible resiliency and dedication to the project as the semester wore on. At the completion of the course, we shared the draft manuscript with Jean, as well as the more of the original research team that she had introduced us to by email.

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It was here that I learned that Alice, Sam, and I were very recent additions to a party that had been on this particular science journey for quite some time.

THE ACTUAL BEGINNING OF THIS SCIENCE JOURNEY

This work began a decade ago in the labs of Dr. Ellie Groden, Professor of Entomology in the School of Biology and Ecology at the University of Maine who has recently retired, and Dr. Patricia Stock, a Professor in the School of Animal and Comparative Biomedical Sciences at the University of Arizona. Ellie and Patricia

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Alice Hotopp, graduate student who worked on the project at UMaine.

were investigating mortality in European fire ants (*Myrmica rubra*) nests in coastal Maine, where they are presumed to have been transported accidentally and are considered invasive. The ants have a nasty sting, which is why they were named locally 'fire ants', although more in-depth investigation revealed that they are not closely related to true first ants (*Soleopsis* species). The ants disturb the local insect wildlife in coastal Maine by competing with them, and this in turn can change the local plant life if native species aren't around to pollinate or spread seeds.

These invasive fire ant colonies would sometimes collapse, and for unknown reasons. Ellie was researching the ecology and management of these ants for Acadia National Park in coastal Maine and wanted to know what was causing the ants to die, as it might be something like a microbial infection or a parasite that could be used as a biological control strategy. She isolated the nematode and found that it could reinfect and cause mortality in the worker ants. At this point she contacted her colleague, Patricia, at University of Arizona, a nematode specialist, who agreed to identify the species. Patricia was working on a program that brought aspiring high school students into her laboratory. Two of these students worked

with Patricia to complete the morphological measurements and initial DNA isolation needed for identification.

After their initial work, Ellie was able to join Patricia's efforts, and during a sabbatical they confirmed that all of the nematodes isolated from ants collected from different locations in Acadia National Park, which exhibited different levels of virulence, were the same species, *Pristionchus entomophagus*. This species had previously been described as necromenic, in that the infection doesn't kill the ants, but the juvenile just hangs out in their body and waits for its host to die so it can feed and grow inside the cadaver. Inspired by conversations with many scientists that Ellie met at a NemaSym conference, organized by Patricia as part of a project of her's funded by NSF which brought together nematologists from varying fields of study to foster research on nematode-bacteria symbiosis, they developed hypotheses about the virulence of these nematodes. These particular nematodes can ingest bacteria whole from the soil, and researchers wondered if worms could transfer soil bacteria to ants once the worms invaded ant tissues and the bacteria could be causing ant colonies to die.

To test this idea, Ellie's lab at UMaine, with consultation from Patricia, began investigating the bacteria associated with the ants collected from different colony sites, but soon Ellie handed the project over to two UMaine undergraduate honors students, Jonathan Dumont and Amy Michaud, whom she mentored through the process. This work was completed several years ago, but Ellie teamed up with Jean MacRae, and with her microbial expertise and help from a part-time technician and several more eager undergraduate assistants, they completed the lab work that generated the sequence data that was analyzed in my class.

THINKING ABOUT SCIENCE FROM A NEW PERSPECTIVE

Did you encounter any challenges or any benefits of working with people from different backgrounds and expertise?

One of the benefits to working with multiple researchers, and in particular, with researchers from different specialties, is that you can look at the same problem from multiple angles. My experience has mostly related to how mammals interact with microbes, which is largely governed by our immune systems, diet, and connection with other mammals. The opportunity to study host-microbial interactions in insects allowed me to look at something that appears similar on the surface but is actually governed by different factors, such as proximity to the soil, morphology of the mouth and ability to ingest whole microbes, and vectored transmission.

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FINAL THOUGHTS

What did you learn about interdisciplinary research from the project and what tips would you give to anyone considering undertaking such work? Are there any other challenges you encountered that aren't discussed here?

We spent summer 2020 combining the three papers into one massive draft - weighing in at 60 pages! Because the research had spanned so many experiments, researchers, and years, it was critical that excellently detailed notes had been taken along the way to allow us to fully describe what had been done and how. This was particularly crucial for me, as I was new to entomology and I needed to understand the biology and ecology of these ants and nematodes, as well as how the experiments had been conducted, in order to blend in my knowledge and results of the microbial communities.

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I've got a long history of working with diverse research backgrounds, because a team can look at a problem from different perspectives. It requires you to be an excellent communicator, and more challengingly, learning this new perspective takes time - something which is in short supply as researchers are asked to squeeze more research productivity out of less funding.

This scientific journey concluded with a scientific article, several presentations, two graduate students trained in microbial DNA analysis, and has launched some new collaborations for me. Although my lab doesn't focus on insect microbes, this work has given me experience studying bacterial transfer due to a biological vector, a mechanism which can affect humans and animals as well.

REFERENCES

Ishaq, S.L., Hotopp, A., Silverbrand, S., Dumont, J.E., Michaud, A., MacRae, J.D., Stock, S.P., and Groden, E. (2021). Bacterial transfer from Pristionchus entomophagus nematodes to the invasive ant Myrmica rubra and the potential for colony mortality in coastal Maine. iScience *24*, 102663.