HERE'S LOOKING AT YOU, SQUID

Margaret McFall-Ngai *has dissected the relationship between a beautiful squid and its live-in bacteria — and found lessons for microbiome research on the way.*

BY ED YONG

The aquarium looks empty, but there is something in it. A pair of eyes stick out from the sandy floor, and their owner is easily scooped up into a glass bowl. At first, the creature looks like a hazelnut truffle — small, round and covered in tiny flecks. But with a gentle shake, the flecks of sand fall off to reveal a female Hawaiian bobtail squid (*Euprymna scolopes*), about the size of a thumb. As she jets furiously around the bowl, discs of pigment bloom and fade over her skin like a living pointillist painting.

There are no other animals in the bowl, but the squid is not alone. Its undersides contain a two-chambered light organ that is full of glowing bacteria called *Vibrio fischeri*. In the wild, their luminescence is thought to match the moonlight welling down from above and cancel out the squid's shadow, hiding the animal from predators. From below, the squid is invisible. From above, it is adorable. "They're just so beautiful,"

© 2015 Macmillan Publishers Limited. All rights reserved

KENT NISHIMURA

says Margaret McFall-Ngai, a zoologist at the University of Wisconsin–Madison. "They're phenomenal lab animals."

Few things excite McFall-Ngai more than the partnership between the bobtail squid and *V. fischeri* — and that is after studying it for more than 26 years. Over that time, she has shown that this symbiotic relationship is more intimate than anyone had imagined. She has found that the bacterium out-competes other microbes to establish an entirely faithful relationship with one host. It interacts with the squid's immune system, guides its body clock and shapes its early development by transforming its body.

Some of these discoveries have helped to shape her field. When McFall-Ngai started her career in 1978, microbiologists were focused almost entirely on pathogens and disease. But in the past decade, advances in genetic sequencing have allowed scientists to identify the trillions of microbes in the bodies of humans and other animals, and to show how they support development, digestion and even behaviour. The study of these communities, collectively known as the microbiome, is now one of the hottest areas in biology, and some of the discoveries made by McFall-Ngai have paved the way. "She pioneered work on animal–microbe interactions well before everyone caught up and the microbiome became such a sexy topic," says Dianne Newman, a geobiologist at the California Institute of Technology in Pasadena.

The microbiome boom is both a blessing and a curse. Attention and funding has focused heavily on projects to sequence microbes en masse, particularly in the human body, and on efforts to understand how they affect health. The squid and its luminous partner risk being eclipsed, at a time when funding is increasingly tight. But even the most prominent microbiome researchers say that they have time for McFall-Ngai and her squid–bacteria symbiosis, because understanding this simple relationship could help to make sense of more complex microbial communities, which are, by their nature, harder to study. "I'd argue that it's important to take advantage of the lessons emerging from such systems," says Jeff Gordon at Washington University in St. Louis, one of the leading figures in human microbiome research. "Their importance isn't diminished."

The squid may represent the road less travelled — but McFall-Ngai has always been drawn by such paths. "When I first met her, we were both in LA, driving a lot," recalls her partner, Ned Ruby. "If she was driving from A to B, even if there was one obvious way, she'd try all these routes. Most would be longer. I'd say, 'Why are we doing this?' She'd say, 'You never know when the freeway's going to be blocked. I want to scout out the ways of going round.' That's how she does science. She doesn't go down the main road and get blocked. She goes down the side roads."

LIGHT THE WAY

McFall-Ngai started down her scientific road as a graduate student, when she became fascinated by bioluminescence and started studying ponyfish, which carry a glowing bacterium. She wanted to understand how these partnerships began, but was frustrated because the fish proved impossible to raise in a lab. Then, a colleague said to her, "Hey, have you heard about this squid?" A few embryologists had been studying the creature, which swims in shallow reef flats around Hawaii and emerges at night to forage. But no one had paid attention to the relationship with its bacteria — until 1988, when McFall-Ngai flew out to Hawaii to take a look.

First, she had to learn how to catch the animals; in knee-deep water, she could snag dozens with just torches and nets. She began breeding them in 1989, when she started her own lab at the University of Southern California in Los Angeles. She found that just 8–10 pairs could produce 60,000 juveniles a year. And unlike animals whose symbionts provide essential nutrients, the squid can survive without *V. fischeri*. This meant that McFall-Ngai could raise the partners separately, introduce them, and watch their first dates.

But first, she needed a collaborator — someone who understood the bacterium. "I think I was the third microbiologist she came to and the first who said yes," says Ruby. The two had met when they were taking courses in Los Angeles. They have been professional partners ever since she started working with the squid, and romantic ones for most of that time. "I think it's a real symbiosis the two of them have," says Nicole Dubilier from the Max Planck Institute for Marine Microbiology in Bremen, Germany.

McFall-Ngai and Ruby embarked on a journey to unpick every aspect of the squid–bacterium symbiosis, at first in separate institutions, then on adjacent floors at the University of Hawaii at Manoa in Honolulu, and finally in adjoining rooms at the University of Wisconsin–Madison. They knew that the squid are colonized by *V. fischeri* within hours of hatching. But how does the bacterium infiltrate the light organ? And why is it the only species to do so, when other ocean bacteria collectively outnumber it 1,000-fold? To find out, McFall-Ngai carefully dissected the light organ, and Ruby loaded the bacteria with fluorescent proteins to track the microbes' movements.

Some details of the symbiosis are still falling into place. But the pair now know that the relationship begins on the underside of the newborn squid, when mucus-lined fields of beating hairs called cilia create a current that draws bacteria close. Physics then gives way to chemistry. When *V. fischeri* first touches the squid, it changes the expression of scores of squid genes — a finding[1] made in 2013 by former postdoc Natacha Kremer. Some of these genes produce a cocktail of antimicrobial chemicals that create an inhospitable environment for most microbes while leaving *V. fischeri* unharmed. Others release an enzyme that breaks down the squid's mucus to produce chitobiose, a substance that attracts more of the bacterium. It takes just five *V. fischeri* cells to trigger these changes, and the microbe soon dominates the fields of cilia (see 'What the squid hid').

Chitobiose also stimulates the bacteria to start migrating into three blind-ended crypts in the squid's light organ. Once they reach their destination, they cause the pillar-like cells that line the crypts to become bigger and denser, enveloping the microbes in a tight embrace[2]. The crypts close off, sealing *V. fischeri* inside for the rest of the squid's 3–10-month life[3].

In 2004, McFall-Ngai's team showed that two molecules carried by the bacteria — peptidoglycan and lipopolysaccharide — are responsible for these changes[4]. That was a surprise. At the time, these chemicals were known only in the context of disease — they were described as pathogen-associated molecular patterns, or PAMPs, tell-tale substances that alert animal immune systems to burgeoning infections. McFall-Ngai took the acronym, swapped the pathogenic P for a microbial M, and rebranded them as MAMPs. These molecules, she proposed, can trigger debilitating inflammation but they can also start a friendship: without them, the squid's light organ never reaches its mature form.

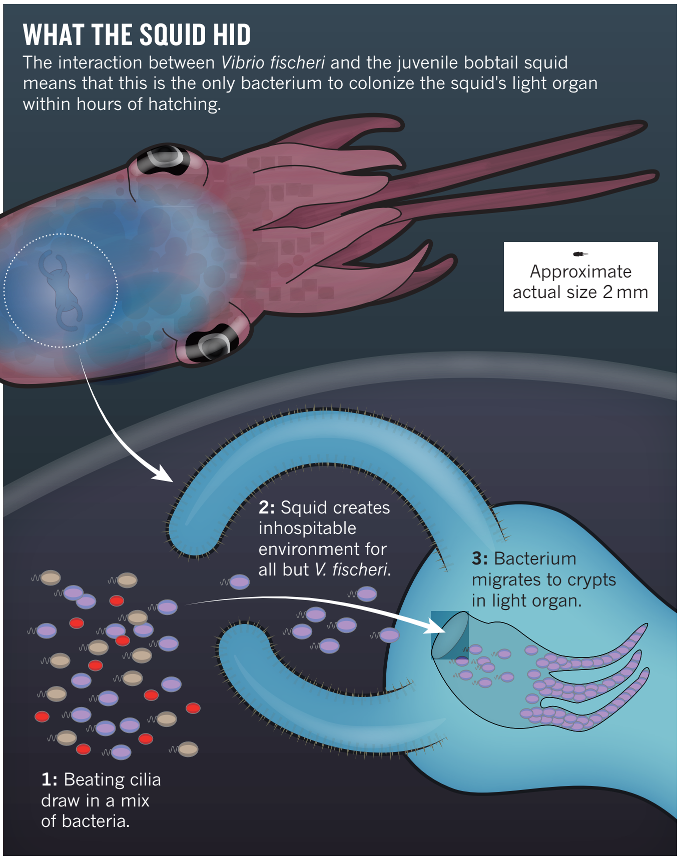
To McFall-Ngai, these results hinted at a broader theme in biology: animals grow up under the influence of their microbes, not just the blueprints encoded in their genomes. "Most of us would say: Isn't that interesting? Margaret said: That's interesting … and microbes play a role in development," says Angela Douglas, an entomologist and microbiologist at Cornell University in Ithaca, New York. "She doesn't deal in little ideas." McFall-Ngai proposed[5] the concept in 1991, and other scientists have confirmed it, finding that the bodies and immune systems of animals ranging from tsetse flies to mammals mature properly only after exposure to bacteria — sometimes in response to the same MAMPs.

Michael Hadfield, a marine biologist at the University of Hawaii, for example, has shown that the larvae of some marine worms metamorphose into adults only when they encounter bacterial molecules[6]. This made sense when he considered that the earliest animals originated in oceans that were swarming with bacteria. "They very likely evolved to 'use' those bacteria as a source of cues for developmental change," he says.

McFall-Ngai has championed other ideas, too. One of them emerged when she started thinking about the adaptive immune system, a trademark of vertebrates that targets incoming microbial threats with bespoke antibodies and retains a memory of past encounters. Invertebrates, including squid, rely on innate immunity — a simpler, short-lived and ever-present battalion of defensive cells. Many immunologists had assumed that vertebrates evolved adaptive immunity because they live longer than invertebrates, and a more complex immune system affords them better protection against pathogens across an extended lifespan.

In 2007 — just as interest in microbiomes was taking off — McFall-Ngai

© 2015 Macmillan Publishers Limited. All rights reserved



offered an alternative explanation. In a commentary for *Nature* called Care for the Community[7], she argued that adaptive immunity evolved because vertebrates need to control a more complex microbiome than invertebrates do. They use it to support beneficial microbes and to block those that pose a threat.

Not everyone buys into the hypothesis. Forest Rohwer, an immunologist at San Diego State University in California, points out that corals lack adaptive immunity but host some of the most complicated microbiomes around. Still, he agrees that adaptive immunity might allow vertebrates to fine-tune their large microbiomes, and other scientists concur. "It's a different way of thinking about the immune system," says Douglas. "People can agree or disagree with it, but it is a touchstone. If someone says, 'Remember Care for the Community?', everyone knows what they mean. It's short for a suite of ideas that challenge traditional notions in a really informed way."

TALK ABOUT A REVOLUTION

McFall-Ngai exudes a stateswoman's confidence as well as a scientist's exuberance; friends describe her as regal. And so convinced is she by the importance of animal–microbe interactions that her message can verge on evangelism. "We now know that microbes make up the vast diversity of the biosphere, and that animal biology was shaped by interacting with microbes," she says. "In my mind, this is the most significant revolution in biology since Darwin."

McFall-Ngai has broadcast that message widely. In 2005, when the American Society for Microbiology was dominated by infectious-disease researchers, she persuaded the organization to run its first meeting on beneficial microbes — a meeting that continues to be popular today. She served on a National Academy of Sciences committee convened by President Barack Obama to outline where biology in the United States will go in the 21st century. In 2012, she helped Newman to create a course that would teach undergraduates the principles of biology using microbes as the starting point of every topic — and she regularly flew from Madison to Pasadena during her holidays to teach the class.

Her passion for the squid has also spawned an academic dynasty. Ruby and McFall-Ngai have now trained dozens of scientists, around 16 of whom are still studying the same symbiosis, now as heads of their own labs. But the duo discourages rivalries. "I grew up watching fields that eat their young, and I didn't want that," says McFall-Ngai. The pair invites postdocs who set up their own lab to claim an aspect of the symbiosis for themselves — and every year they host a symposium-cum-party affectionately called the Pow-Wow, at which everyone gets together to share their results and plans. "If someone else says, 'I was going to do that too', they sit in a corner and talk about it," says Ruby.

Despite the conviviality, the group knows that it must compete for a limited pot of funds. "I've been told, 'We've already funded Margaret or Ned; how many more can we fund?'," says Spencer Nyholm, an early student of McFall-Ngai who now works at the University of Connecticut in Storrs. "I can't imagine they would ask this if someone proposed to work with *Drosophila* or *C. elegans* or mice."

McFall-Ngai says that she and her protégés are just getting started. In one project, she is examining an evolutionary theory predicting that every microbiome should be plagued by cheats — microbes that reap the benefits of life in their hosts but do not provide anything in return. Sure enough, the squid is sometimes colonized by strains of *V. fischeri* that do not make any light. McFall-Ngai's team has found that the squid can use light-sensitive proteins in its light organ to detect a few dark bacteria among a million brightly glowing ones, and selectively evict them[8]. The team now wants to find out more about how it does this — and the answers might help to explain how humans and other vertebrates manage more complicated microbiomes.

The team has also shown that the squid's relationship with *V. fischeri* varies over the course of a day, controlling the microbes so that they produce light only at night[9]. And in 2013, former student Elizabeth Heath-Heckman showed that *V. fischeri*, in turn, influences the squid's daily rhythms through a gene that makes a cryptochrome — a type of protein that affects circadian rhythms in many animals[10]. Cryptochromes are usually activated by environmental light, but Heath-Heckman showed that one of the squid's cryptochrome genes responds only to the blue light that *V. fischeri* emit, ramping up production of the protein.

On the basis of this work, the team predicted that interactions between people and their resident microbes might also change from day to night — and soon, the evidence was pointing that way. Last year, a group in Israel showed that a significant proportion of microbes in the human gut rise and fall in abundance in a 24-hour cycle[11], and regular jetlag, for instance, can promote weight gain by disrupting these rhythms.

"One of the things we pound into people who come to the labs is that nobody really gives a damn about the squid," says Ruby. "They care about the big questions that the squid will help to answer." To tackle more of those questions, in a few months McFall-Ngai and Ruby will move to share the squid's home. They will return to Hawaii, where McFall-Ngai will head the Pacific Biosciences Research Center in Honolulu. It is a dream job, and a chance to indulge more in her favourite pastimes — skateboarding and bodysurfing — as well as watch the squid on moonlit nights.

"This was completely backwater science," she says. "Now it's front-seat science. It's been fun to watch people realizing that microbes are the centre of the Universe, and to see the field blossom." ■

Ed Yong *is a science journalist based in London.*

1. Kremer, N. *et al. Cell Host Microbe* **14,** 183–194 (2013).
2. Montgomery, M. K. & McFall-Ngai, M. *Development* **120,** 1719–1729 (1994).
3. McFall-Ngai, M. J. & Ruby, E. G. *Science* **254,** 1491–1494 (1991).
4. Koropatnick, T. A. *et al. Science* **306,** 1186–1188 (2004).
5. McFall-Ngai, M. J. & Ruby, E. G. *Science* **254,** 1491–1494 (1991).
6. Hadfield, M. G. *Ann. Rev. Mar. Sci.* **3,** 453–470 (2011).
7. McFall-Ngai, M. *Nature* **445,** 153 (2007).
8. McFall-Ngai, M., Heath-Heckman, E. A., Gillette, A. A., Peyer, S. M. & Harvie, E. A. *Semin. Immunol.* **24,** 3–8 (2012).
9. Boettcher, K. J., Ruby, E. G. & McFall-Ngai, M. J. *J. Comp. Physiol. A* **179,** 65–73 (1996).
10. Heath-Heckman, E. A. C. *et al. mBio* **4,** e00167-13 (2013).
11. Thaiss, C. A. *et al. Cell* **159,** 514–529 (2014).

© 2015 Macmillan Publishers Limited. All rights reserved