# This Week in Microbiology

*With Vincent Racaniello, Michael Schmidt, Elio Schaechter, and Michele Swanson*

## Episode 183: Two symbioses

Aired August 24, 2018

http://www.microbe.tv/twim/twim-183/

Vincent: This Week in Microbiology is brought to you by the American Society for Microbiology at asm.org/twim.

(music)

Vincent: This is TWIM, This Week in Microbiology, episode 183, recorded on August 9th 2018. I'm Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaechter.

Elio: Hello there, hi everybody.

Vincent: How are you doing?

Elio: Okay.

Vincent: Okay. Can't ask for more.

Elio: Getting younger by the minute.

Vincent: You're reversing the normal progression.

Elio: I know, oh boy, if only.

Vincent: There was a movie about that.

Michael: Benjamin Button.

Vincent: That's right.

Elio: I was not in it.

Vincent: (laughs) Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Hello, everyone.

Vincent: How is South Carolina treating you?

Michael: Well, we finally got out of the rain period. It was raining 3 times a day and it was raining about an inch every time it rained, so we were canoeing to work there for a while (laughter).

Vincent: Better raining than burning.

Michael: I tell ya, my tomato plants in the backyard had mushrooms I had never seen before. It was a weird mushroom in that it grew up---

Elio: Take a picture and send it to me!

Michael: I should have. It melted, the mushroom melted. I've never seen a melted--

Elio: Oh, was it a coprinus? An inky cap?

Michael: It was a something. It was orange, it was orange.

Vincent: Oh yeah orange, those orange guys melt, yeah. I've had those.

Elio: Not an inky cap.

Vincent: Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello.

Vincent: Great state, the ladies have taken over politics, I understand.

Michele: (laughs) We made a pretty good showing.

Elio: About time.

Vincent: I think it's great.

Michele: So addition to unseen life on Earth, I am thinking about the sun because as some of our listeners may know, NASA is about to launch the Parker Solar Probe that is going to go within the sun's corona, and my brother in law Andy Driesman is a program manager on that project and that means my whole family is having a reunion down at the Kennedy Space Center where we are going to be able to watch it live.

Elio: No kidding.

Vincent: Nice.

Michael: That's very cool.

Michele: Yes, so in the wee hours of Saturday morning off the probe goes to collect data from the sun, the sun's corona, solar winds and all this cool stuff.

Vincent: That's very exciting. How long does it take to get to the sun?

Michele: The whole mission is about 7 years but I think they are going to start getting data back in November.

Vincent: Nice. 7 years, that's about the length of a legionella experiment, right? (laughter)

Michele: Feels that way.

Michael: Or PhD students' initial foray into graduate school.

Vincent: That's right.

Michele: The NASA website has put out a lot of information, so if anybody is curious about how it is designed, how they are gonna use Venus to slingshot and get closer and closer to the sun, they have got some great graphics to describe it.

Vincent: Neat, we will put it in the show notes. Very nice. I just want to remind everyone we are going to give away a book today later in the show, so stay tuned. Never know when it is gonna pop up so just keep listening. Today we have two very cool stories from you, for you, and from some of you, actually, who are listening. The first one was sent by Chris who writes:

Dear Vincent and TWIM gang, after the great coverage on our pre print of the Bodo saltans virus on TWIV, I thought I would send you another pre print from my PhD for consideration. This time it is a parasitoid bacterium of a heterotrophic protist, hence TWIM, that has no metabolism on its own and instead relies exclusively on the host by remodeling its mitochondrion to scavenge everything, even ATP. If that wasn't strange enough, it doesn't do binary fission, either.

Elio: Well, okay. We'll talk about that.

Vincent: Yeah, Elio objects to that, and we'll discuss that later. Thought it would make a fun little snippet, thanks for all your great podcasts.

Michele: This would be perfect for Halloween but I guess we'll do it (laughter) close to Labor Day instead.

Elio: Absolutely.

Vincent: So this is Chris's second paper. The first one he published Bodo saltans virus, which is a virus that infects, it's a mimivirus, a giant virus that infects aquatic protists and has very interesting features. Now he's got this very interesting, and this are both bioRxiv preprints, this one is called "Chromulinavorax destructans, a pathogenic TM6 bacterium with an unusual replication strategy targeting protist mitochondrion." And so Chris Deague is the first author, and this comes from the laboratory of Curtis Suttle at the University of British Columbia. Chromulinavorax destructans.

This study, the discovery of this interesting bacterium, started as an effort to identify pathogens that infect protist zooplankton. What they did, they sampled fresh water habitats in southwestern British Columbia for particles smaller than 0.8 microns. They filtered the water and they want things smaller than 0.8 microns. Then they take that filtrate and they have cultures of protists growing in the lab and they infect and they see which one gets infected. This screen gave rise to a pathogen that infects a host called Spumella elongata, that's the host.

Michael: Sounds like pasta.

Vincent: I know, it's very Italian.

Michele: Italian.

Vincent: Spumella, spumella is one of Earth's most abundant phagotrophic nanoflagellates. Phagotrophic means it takes up particles and eats them. It preys on bacteria, viruses, and even other microbial eukaryotes. Nanoflagellate, well, the flagellate part you get, nano, they are about 2 to 20 microns in size, so they are pretty

small. These phagotrophic nanoflagellates, together with viruses, result in lysis. They're the primary mortality agents of microbial populations in aquatic environments.

Elio: So this paper is about the revenge of the microbe.

Vincent: The revenge of the microbes, that's right. Spumella, the host, is found both in fresh and salt water. So they named this agent Cromulinavorax destructans, C. destructans, and it happens, infection seems to be specific for this strain of spumella and this is an interesting result. If they treat the cultures with ampicillin, vancomycin and rifampicin, this C. destructans is still able to grow and kill its spumella host.

Michael: But he forgot one antibiotic.

Vincent: Which one?

Michael: He forgot the trimethaprimsulfa, or the instructive class of antibiotics, and I lay you dollars to donuts that that antibiotic, the sulfur classes of antimicrobials, would likely inhibit this microbe based on the story you are about to tell us, Vincent. So keep that in the back of your mind.

Vincent: Good point. So the ampicillin and vanc, they hit the cell wall, right?

Michael: Correct. And you need actively growing cells in order for those antimicrobials to have an activity.

Vincent: Okay. So either they are not growing or there is no cell wall, right?

Elio: That's one mechanism of resistance to antibiotics, lose the targets.

Vincent: Right.

Michael: Yes.

Vincent: The rifampicin hits RNA polymerase, in which case you would say that this C. destructans must not be, it must not have an RNA polymerase, otherwise it would be inhibited, right? Maybe it is the host cell pol that is being used. So these, what are these C. destructans? They are cocci, 350-400 nm, they have a lipid double layer, there is some electron dense material in the periplasm, there is also a nucleoid, and when you stain these particles for DNA they have a homogeneous staining profile. In other words, they have the same amount of DNA, which the authors say suggest that they don't replicate outside of the host, because if you just take the cocci themselves they all have the same amount of DNA. What happens is you add this C. destructans to the host cell, to the spumella (laughs), the C. destructans are taken up, these are phagotrophic hosts, right, so they grab the C. destructans. They take it into a food vacuole and then by 3 hours there is a spherical mass in the cytoplasm and the mitochondrion of the host cell begins to wrap itself around these C. destructans. By 9 hours the mitochondria completely surrounds what they call a replication body, which is the C. destructans that have been taken up, the mitochondria appears to be intact. There is a single mitochondria that surrounds this replication body. By 12 hours, the mitochondria takes up 2/3 of the spumella cytoplasm.

Michele: These electron micrographs that they provide are really spectacular.

Vincent: Yes, and this is a bioRxiv paper so everyone can see it, it is open access. This replication body begins to divide and make mature cocci and eventually the host cell bursts open and they come out. This is a lytic bacterium and it is kind of behaving a little like a virus. It is infecting a cell, it is duplicating, as you will see it is a highly reduced bacterium as well.

Michele: But dramatic impact on the mitochondria, the powerhouse of the host cell.

Vincent: Amazing, it's amazing.

Michael: But it is still intact, because the cell needs its energy. It is a true parasite.

Elio: It's also not the only bacterium that infects mitochondria.

Michael: That is true.

Elio: I forgot the name of it, there is a guy who in cockroaches lives in the mitochondria of the cockroaches. I'll look it up.

Vincent: Let me ask you, they call this a lytic bacterium, what other examples are there of lytic bacteria? They get inside another cell and cause lysis.

Michael: Bdellovibrio?

Elio: Rickettsia, chlamydia.

Michele: Legionella also ends up being, the host cell lyses, it's not clear whether that's a host defense or it's a bacterial program.

Vincent: They sequence the genome, it is 1,174,272 base pairs of circular double stranded DNA. So it is pretty small and it encodes 1,081 open reading frames. Most of these encode proteins involved in DNA replication, protein synthesis, as you will see transmembrane transporters and host manipulation. But a lot of these open reading frames, they don't know what they do. There are all 20 aminoacyl tRNAs. There are some ribosomal RNAs but that does not make a complete ribosome, so it doesn't have a complete protein synthesis system.

Elio: It does have RNA polymerase.

Vincent: It does have RNA polymerase.

Elio: So it is resistant to rifampicin.

Vincent: Yes that is absolutely right, it has to be resistant. It has a DNA replication and repair machinery, there is a single origin of DNA replication on the genome, but it is clearly highly dependent on the host cell. It uses nucleoside salvage for purine and pyrimidine biosynthesis, it can phosphorylate the products into the required nucleotides, but it needs the precursors from the host. Amino acid biosynthesis is restricted to conversions between related amino acids as a rudimentary cell division machinery, it has no lipopolysaccharide biosynthesis capability but it can make peptidoglycan and they wonder if that is the electron dense material that you see in the periplasm. And no complete metabolic pathways are encoded in this genome, no complete metabolic pathways. Just bits. There are lots of transporters to import peptides, amino acids, lipids, elements like iron and zinc, so obviously it is getting these from its host.

Michele: It is scavenging instead of building.

Vincent: There are two copies of an ATP ADP antiporter, so this would be a membrane complex that would exchange ADP for ATP.

Michael: And they bring stuff in.

Vincent: That seems to be getting it from the host. That is why the bacteria are probably right next to the mitochondrion, they get a steady supply of ATP and this antiporter allows it to get into the C. destructans.

Elio: If I may interrupt, I just happened to look and found a paper with a tantalizing title, it is a review paper in the Journal of Biochemical Pharmacology, it is called "Mitochondria, a target for bacteria" by Lobet, Letesson, and Arnould, which are investigators in Belgium. I'm going to send you the reference so you can put it in the notes, okay?

Vincent: Very good, yes. Great. No genes for lipid metabolism, so it gets its lipids from the host. Many of its genes encode proteases, nucleases, and hydrolases, again breaking down host cell structures and importing them. The genome encodes 98 copies of what are called anchorin repeat domain proteins, and these have unclear functions. They are implicated in membrane modification and counteracting the host immune system. They have a secretion system, probably used to export proteins into the periplasm, and the last thing I want to tell you is that these cells, C. destructans, remain infectious after being stored for 4 years at 4C. (laughter)

Michael: Not frozen, not frozen!

Michele: Wow.

Vincent: I wonder how they, I understand that was planned.

Michael: They're not burning anything. Remember, the thing that ages all cellular life is metabolism. So if they are not effectively doing any metabolism, they are not taking all the oxidative hits that is a consequence of normal life, and they are sort of cheating. They are sort of cheating in that they are using the host mitochondrion to take all of the, if you will, free radical hits from metabolism. So this cell is really very clever and the author goes into the discussion about why the, what is the evolutionary rationale for it being so long lived? And it really begins to make sense when you read those last snippets of his discussion. He points out that the life in water is not easy. What is the likelihood that you are actually gonna find the spumella in order to infect? You have to be ready in order to be engulfed by the host and then take advantage of that. If you have to tool everything--

Elio: Wait wait wait wait wait, you are going to a different subject. You made a hell of a good point about not having metabolism and therefore being not affected by oxidative, don't go to another point (laughs)

Michael: Well, you know, its the way they describe this candidate phylum, it is dependent. It is Dependentiae. And it is really dependent upon the host and being able to find a host and it has a very narrow host range. This poor organism has to wait for the right match.com event to happen for it in order to replicate itself.

Vincent: Many viruses face the same problem, finding a host.

Michael: That's absolutely true.

Vincent: But they degrade, they don't last, at 4C especially, they don't last forever.

Michael: That's right.

Vincent: So there is something different about this that is preserving it, maybe its structure is such, first of all, it has DNA which helps a lot, because if you have RNA you degrade very quickly, but the DNA may be protected in interesting ways. I think that is a good point that they are just sitting there, but also the other point is they have to last a long time to encounter a host. That is pretty cool. This phylum, by the way, candidate phylum TM6 or Dependentiae, what else do we know about it? Their biology is known only from reduced genomes recovered from metagenomes, so people sequence different environments and they find these in them. There are only two isolates that have been described and they infect amoebae, and so this is the first one that does not infect an amoeba. The third isolate which infects this spumella (laughs). So I was attracted because it has got viral characteristics.

Michael: That's what I wanted to ask you, do you think this is not a bacterium but could be a virus? Does the cell need to make energy and have a metabolic system in order to be alive? Or is this a true bacterium?

Vincent: Elio, you were talking about this before, what are your thoughts?

Elio: Okay, my point of view is that what makes a virus a virus is that it loses its bodily integrity.

Michael: Oh.

Elio: That's the definition of a virus to me. And it is reconstituted using host machinery. This guy doesn't do that. Although they are highly dependent on the host for its metabolism, it doesn't lose its bodily integrity. It looks, in fact, the schematic diagram which they have in the paper which is gorgeous, makes it look almost exactly like Bdellovibrio, except instead of pouring into the periplasm the bacterium goes into the mitochondria to flagellate. But otherwise it looks almost exactly like Bdellovibrio. So I don't think that losing metabolism alone makes a bacterium into a virus. It makes it into something that is degraded, which is very weak, if you wish, but to be a virus you have to really fly apart in the replication. That's my view.

Vincent: The only problem with your view is there are some viruses that do not lose their bodily integrity.

Elio: Biology is that way. (laughter)

Vincent: I would say that the definition is, for a virus, is that you make new viruses by assembling individual components, whereas bacteria you make more by binary fission. So the question is, does this organism, this C. destructans, does it do fission? And I think probably it does, right Elio?

Elio: It does, it does! It just doesn't do it, this is a strange form of binary fission in the sense that instead of dividing right away it first makes filaments and chains and then they break up. But it is the equivalent of binary fission. I mean, it is not, a filamentous bacterium does the same thing. It is not that different.

Michele: Another approach to the debate is to look at the phylogenetic relationships of the proteins it does encode and see whether they are more bacteria like or virus like. It loos like based on the nomenclature that they are bacterial origin.

Vincent: It does, yeah.

Michele: There's an FTSA, FTSZ, for example, cell division machinery. That really smells like bacteria. Peptidoglycan.

Vincent: But this may be on the way to becoming a virus, right? One of the theories--

Elio: That's possible. I think what Michael said earlier makes a lot of sense, this is really a solution to an ecological problem, and this guy has a particular solution to it, namely surviving by not having metabolism (laughs) I like that.

Michele: But then it is pretty aggressive when it does get a host.

Michael: The fact that it can divide within 12, I mean, from start to finish, 12 hours post infection, which is pretty remarkable considering how long it can remain dormant.

Elio: This guy belongs in the scheme of parasites, strict intracellular parasites, and I don't have any question that there is gonna be a lot of phylogeny and a lot of that kind of study being done. It is being done now, I mean, rickettsia, chlamydia, etc. There are a lot of strict intracellular parasites in the bacterial world. They are fascinating. Each one has its own specialty.

Vincent: Anyway, thank you Chris for sending it, it is really cool and presumably it is submitted somewhere, so good luck with that.

Michael: He's got a typo in his material methods that says see chapter 2.

Vincent: I saw that too.

Michael: That's in sampling, so we are--

Vincent: Oh, that's from his thesis, probably, right?

Michael: That's right, it's a direct lift. (laughter) So we are doing a little editing for bioRxivs. He's gotta fix that in the sampling section of his material methods.

Vincent: Alright, thanks everybody. Elio, what do you have for us?

Elio: I have a paper which is related to the subject. We don't often have two things that are related. The title is " Recurrent symbiont recruitment from fungal parasites in cicadas" it is by several Japanese and Chinese authors, one from University of Montana, two from University of Montana. The authors are Yu Matsuura, Minoru Moriyama, Piotr Lukasik, Dan Vanderpool, Masahiko Tanahashi, Xian-Ying Meng, John P. McCutcheon, and Takema Fukatsu.

And this is a fun paper because cicadas are interesting in that they are insects which make a lot of noise when it is time to mate. That's their thing. By the way, I looked it up, the noise they make is actually more like drumming than stridulation in other things like in crickets. They rub legs one against the other, it is called stridulation. Here it is more like they have like beating on a drum. Anyhow, they make a lot of noise at night. They have one interesting problem, their sole food is the xylem of plants. There is a sap which is very poor in everything except sugar, it is essentially a sugar solution. These guys are drinking Coke all day long (laughter)

Michael: So they're computer programmers. They just drink sugared beverages all day. We'll get letters for that.

Elio: So anyhow, where do they get their amino acids? Where do they get the vitamins? The answer is they get it from bacterial symbionts! And this is a long story with a lot of plant feeding insects, sap feeding insects have to have this, and Wolbachia comes into the picture and so do two organisms, two bacterial symbionts, Sulcia and Hodgkinia, named for some ecologican. Anyhow, that's the story, basically, is that the symbionts provide

the amino acids and vitamins to the cicadas and the cicadas are very unhappy they don't have them because they starve to death. However, like many bacterial symbionts and just like the one we just discussed, Sulcia and Hodgkinia have tiny little genomes. So much so that the typical genome of a Sulcia is something like 200,000 bases. That is nothing, I mean it is less than some viruses have. But it is stable, Sulcia does the job, it has the genes for a bunch of the missing amino acids. By the way, the cicadas, the insects can make certain, it's like us. We have essential amino acids which we need to import from our food and nonessential which is the ones we will make. Same thing with the cicadas and other insects.

Anyhow, Sulcia does the job for some of them and Hodgkinia does the job for others. However, these very reduced genomes, I mean they are tiny, they really call for maybe 200 genes which is considered less than what is essential for independent life, and sure enough these bacteria do not live by themselves. They have to live inside, they are endosymbionts, they have to live inside of host cells which provide them with necessary food. The story is very similar to the one we just talked about in the case of the…

Vincent: Destructans.

Elio: Right. Anyhow, the problem here is the Sulcia does fine but Hodgkinia does not. If you look at Hodgkinias in various cicadas, these are Japanese cicadas they look at, you find that the genome of Hodgkinia is not in a single molecule but it is broken up in various mini circles. It reminded me of the kinetoplast of trypanosomes. That's another story we can talk about some time. Anyhow so they have many circles instead of having a single molecule, a single chromosome as a single molecule, and these get lost.

So Hodgkinia is on the way out, it is not, it is possible that Sulcia will be on the way out, too but much more slowly because it is still there. But the Hodgkinia are fragmented and differ from regular endosymbionts, from other Hodgkinias found in other cicadas. So this is characteristic of Japanese cicadas. Okay. So missing a gene for essential nutrients, namely histidine, methionine, the other amino acids being supplied by the Sulcia, cobalamin and riboflavin, in other words, vitamins. So how do the cicadas cope with the dire need to acquire nutrients that are essential to them when one of the partners is bugging out? (laughter)

Michael: No puns.

Elio: Okay, that's right, pun intended.

Michael: Pun intended.

Elio: The answer is, of all things, they acquire new endosymbionts and these are not bacteria but fungi. Wow. This is news, that you can substitute the bacteria in the symbiont for a fungal one. I don't remember running into this in any case. Does it sound familiar to any of you?

Michael: It sounds almost like mutualism where the cicada is desperate to get the amino acids and it will take them from anyone who can contribute.

Elio: This is mutualism, all right. No question.

Michele: But to your point, Elio, the first author, Dr. Yu Matsuura, for years was looking at this interaction and trying to understand what was happening in these cicada and he too was just thinking about bacteria, bacteria, bacteria. And then by looking in the microscope he was just stunned to see these pockets of fungi which had just not been on the radar screen. So yeah, it is a surprising and fascinating new biology.

Elio: It is interesting it only took us looking at a microscope, I mean you break up a cicada and it will outpour this fungi, disease like fungi, and you've got the answer.

Michele: At first he thought it was just that particular insect had a fungal infection, but when he began to see it consistently and then do the molecular genome analysis the story becomes more clear as you will tell us.

Elio: So what are these fungi? Well, they are an interesting set of, they belong to a group called the Ophiocordyceps, piece that into two words ophio means snakelike and cordyceps are fungi that grow on insects, and they are well known, the ophiocordyceps because some of them enslave ants and other insects, teaching them to grow, to climb up plant stalks, where they reach a certain height, they impale themselves with the mandibles, and the fungi which grow in the brain which is a good place to grow if you can modify the behavior of your host (laughter) they come out of the brain as a fruiting body.

You may have seen this, this is in National Geographic all over the place, on television, you can see that you've probably, many of you listeners know what I'm talking about, you find ants and other insects, weevils for instance, impaled up on a stalk and out comes a very bizarre looking fruiting body which is a stalk and a sac of spores at the end, sort of a little, I don't know what to call it, it just looks very funny. Eventually, the sac of spores opens up and the spores are distributed and the simple thought is they do this because by being up in the air, they have a better chance of dispersing their spores than if they start on the ground. So on the ground it is going to be a problem to spread the spores around, but up in the air where the wind will take care of it and spread the spores all over and the fungi are very happy. Not so the insects.

Michael: The insect is dead. It's a bad science fiction movie.

Elio: That's right. Anyhow, here the ophiocordyceps do something different. They do not kill, they do not modify the behavior of the host, or at least it is not known, this particular ones don't seem to do that. But they provide the host with its amino acids. Sure enough, at least one of these symbiotes which can be cultivated, by the way, most of these fungi can not be cultivated and I think that that may be because they are advanced in their relationship, in the evolution, and they have evolved that they do not have to grow outside of the body of the insects, so why should they? This one symbiont of a cicada called Meimuna opalifera, whatever it is, it is a Japanese cicada, here they can cultivate the organism and when they do, imagine they find that it has a whole lot of genes for essential and non essential amino acids, vitamins for nitrogen recycling, and it includes the genes that are normally provided or used to be provided by the Hodgkinia.

So they say these results highlight metabolic versatility of the fungal symbiont that is more than sufficient to compensate for the absence of Hodgkinia. So that is pretty much the story but there are a few more points. To ensure that this fungi are past alone, mind you they don't grow outside, they may grow outside the insects but they are not known to do it, but just to ensure that this isn't a problem, the fungi are located not just in symbiont having structures which by the way are common in insects, when they have bacterial endosymbionts they have specific structures called bacteriomes or bacterial sites. But in this case the Sulcia also grow in the developing oocytes. In other words they ensure vertical transmission from insect to insect. So that is an amazing bit of evolution where you substitute the ability or the necessity to provide necessary nutrients from bacteria to, well the bacterium in this case, one of the bacterium seems to be on the way out, on the way to be obliterated, and this is now substituted by a fungus. So this is an amazing piece of evolution.

Michele: It is interesting too that this fungus which can be quite pathogenic, it can grow inside the insect and then burst outside of the brain and then kill it, and now it has been through the course of evolution tamed, if you will. Now the insect is exploiting the fungus to generate amino acids that it needs. It's amazing.

Elio: It's a nice point you make, it shows you the connection, the evolutionary connection between parasitism, mutualism if you wish, and how these things are really interrelated and they can go from one state to another. Let me read the last sentence, paragraph I think, of the summary. The authors say these findings highlight a straightforward ecological and evolutional connection between parasitism and symbiosis which may provide an evolutionary trajectory to renovate deteriorated ancient symbionts via pathogen domestication. Exactly what you just said.

Vincent: Would be interesting to know what changes occur in the fungus to do that, right?

Elio: That's right. You can cultivate at least one of the fungi, you should be able to study in more detail. I imagine that this started on this and I'm sure they are gonna do much more.

Vincent: Elio, at some point in the future the Hodgkinia will be gone I presume, right?

Elio: It's already gone from some.

Vincent: Some of the cicadas, yeah. Any idea of how long ago these fungi came in?

Michele: So they did a phylogenetic analysis that they include in their paper and they also make the point that on 3 separate occasions fungi have been exploited, so 3 separate events in the history of this coevolution. But I didn't recall how many years ago they estimated these events occurred.

Vincent: I loved this phylogenetic tree because they have pictures of each cicada. Usually you just have the name and here you can see which one is which and I can see the ones we have in our backyard there. The big ones.

Michele: Wow. They have some beautiful photography in this paper, looking at the different sacs where the fungi are growing, it's really exquisite. The first author is very thoughtful and persistent person, again his name is Dr. Yu Matsuura, he is currently an assistant professor at the University of the Ryukyus in Okinawa, Japan. He was actually born in Osaka, his father was a math teacher who loved botany, and he himself just loved nature, collecting bugs, frogs, fish, other animals he would capture in the neighborhood and bring them into his home as pets.

He learned though after a while that his friends did not share these interests so he maybe kind of a little peer pressure, he turned to other things like sports, but especially languages and studying the world outside of Japan. So when he began college, he actually started studying foreign studies, languages, and comparative cultures. He has learned not only of course Japanese, his native language, but he speaks English, Hindu, and Spanish. In 2002, Elio, he came to University of California San Diego for a summer session.

Elio: No kidding! My goodness, I missed him.

Michele: He told his parents, I'm going to study English and culture, but it was there that he took several biology classes and he learned that evolutionary biology is the most fascinating and fundamental field if you want to explore life on Earth. So during that stay in San Diego he became determined to become a biologist. He also had been doing a fair amount of writing, or reading, rather, news, media, textbooks including molecular biology of the cell and books by the evolutionary biologist Lynn Margulis.

He also read one by Ishikawa who in Japan is a pioneer of insect symbiosis research. So he then had to return to Japan, he finished his Bachelor of Arts degree, got a job teaching English at a private language school. That helped him support himself and really began to explore this new fascination with science. Then he realized that

there are several universities in the area that would accept all of the credits that he had earned, both in Japan and in San Diego, and that could jump start him to return and get a second bachelor's degree, this time in biology. So that's what he did.

Elio: This was from where, UC San Diego?

Michele: No, he was back in Japan and now enrolled in Osaka City University where he got a second bachelor's degree, this one in science, where he was studying with Professor Numata looking at mangrove crickets, which are from his region in Okinawa, and also circadian clocks and beginning to use molecular techniques. But one day during that undergrad period he read an article by the senior author on this paper, Fukatsu, describing the pioneering work on aphids that had lost an obligate bacterial symbiont and instead had acquired a fungal symbiont. So it was kind of a precursor to the study--

Elio: Oh, I didn't know that, so there is an antecedent to this story.

Michele: That's right. So then he became very excited and knew that he wanted to study with Professor Fukatsu, so he got both a masters and a PhD with him, and pointed out that this project took more than 10 years to bring to fruition because it was technically very complicated and of course it took a while to appreciate that the fungi were playing such a key role. So he especially would like to thank his co authors on this paper, MinoruMoriyama, who was the expert in cicadas and insect histology, and also Tanahashi, who is an insect loving mycologist who is very good at culturing fungi and identifying them. He said they they could never have done any of this without their collaboration with colleagues at the University of Montana and co authors Piotr Lukasic, Dan Vanderpool, and John McCutcheon.

Elio: I know John, I like him very much. He got his training with Nancy Moran, one of the leading people, she Is now at Texas, used to be at Yale, one of the leading people in the subject of bacterial endosymbionts in insects.

Michele: I noticed that she was the editor who managed this paper for PNAS.

Elio: That's right.

Michele: He clearly has got a great attitude and a sense of humor. He said that although it took them 10 years to gather the evidence for this publication, he recognized that there is a parallel, it is just like cicadas that spend a long time underground only to emerge and sing (laughter) So they are singing now.

Michael: And he's got a PNAS paper, yes.

Michele: That's right. I asked if he had any advice and he says well first, I feel like I am just starting my career, but he does say if you want to find something new, keep looking, and again pointed out that just by using a standard light microscope and being thoughtful and with an open mind he was able to recognize those symbiotic fungi within the insects. So he says even though we have been doing microscopy for hundreds of years, it still can lead to inspiring results, things we can't see with our own eyes. He encourages people to look at things with a fresh point of view. You are going to have challenges and feel dumb, but keep pushing, and he says that we are lucky to live in this era when we have research tools and information that is openly shared. We can have collaborations and that has really made this project very rewarding and exciting.

Vincent: Thank you, Michele.

Michele: Yeah.

Vincent: I want to point out on a recent episode of This Week in Evolution we talked about a fungus that manipulates the behavior of flies similar to what Elio was saying. This was entomophthora and in this paper from Mike Eisen's group, they found a virus in the fungus in the fly.

Elio: Oh, yes.

Michele: Wow.

Vincent: They are wondering if the presence of the virus actually confers to the fungus some of its behavior manipulating abilities, so, that is another cool story. Here as well, in these cicada fungi, maybe there are viruses in them that do something.

Michele: Making the best of a bad situation.

Vincent: Yep. (laughs) And as he said, you just have to keep looking. You'll find interesting things, right?

Michele: Right.

Elio: Some people think that viruses are important.

Vincent: Some people do, yes. (laughter) That just might be. Let's read a couple of emails. Michael, this first one has your name on it, can you read that?

Michael: It says:

Hi TWiMpeeps,

We really can't win, where microbes are concerned: People are now dying from microbes splashed up from sinks due to increased attention to hand washing!

I wonder if this also ties in with the change from copper pipes to plastic? I bet it does.

Discuss:

"Drains of sinks, which can be potential reservoirs for nosocomial pathogens, have been implicated in an increasing number of outbreaks in the past decade.

Is this you or the author writing this?

Vincent: That is from the Stat News article.

Michael: Okay, so the Stat News article writes:

It has been speculated that these outbreaks might be an unexpected consequence of efforts to improve hand sanitation in hospitals. Backsplash with contaminated droplets onto nearby surfaces where medical staff prepared tubing and other equipment used in patient care is thought to be a mode of dissemination (https://www.statnews.com/2016/10/25/hospital-sinks-infections/).

They give you the reference which I assume Ray will put into the show notes on this from Stat News and this is from 2016, so it is about two years old but there is an increased frequency that we are seeing more and more about sink drains being implicated. Then the author of this note goes on:

Biofilms containing colonies of these bacteria form in the sink drain and in pipes that drain the sinks

(https://www.statnews.com/2016/10/25/hospital-sinks-infections/). Replacing contaminated sinks and their nearby plumbing may not be sufficient, as the biofilm may regrow from plumbing that remains in the wall."

http://www.promedmail.org/direct.php?id=20180627.5879225

And the author, Steve Lunton from Bedordshire, England, concludes by saying:

Time to put the traditional copper plumbing back, I think.

Well, you know, the built environment of hospitals is being carefully looked at by architects and one of the solutions that the architects have come up with is they simply change the location of the faucet and the drain, so that the faucet nozzle is not directly above the drain, which is contrary to what you see often in your normal lavatory sink in your home bathrooms. Normally the faucet is directly above the drain in case you have a drip or something running. And folks have appreciated this and people have been studying drains and the pathogens for 30+ years, trying to understand the plasmids and they have been looking at the multidrug resistant microbes and I don't think short of throwing the water out the window we are gonna be able to get around this, because sanitary sinks are a fact of life and hand washing is so important in breaking the chain of transferring microbes from patients to the environment and vice versa and I think the risk far outweighs any of the benefit that we would receive from not washing our hands with sinks.

Vincent: Michael, the drain pipes, they were never copper, weren't they? Other things besides like, iron, or something?

Michael: Probably cast iron. Most of the drains in old homes are cast iron pipes. They were rarely copper and in fact in research labs, if you remember back to ancient times, they were glass.

Vincent: Yeah, right, right.

Michael: We had a lot of glass, I'm sure Michigan over in the chemistry building still has a large number of glass piping that takes care of the organic acids and acids that are routinely dumped down the sinks.

Michele: So back to hospital sinks, something else I've learned from my legionella colleagues that are environmental microbiologists is an unintended consequence of the low flow design so that we aren't even using faucets anymore, we are stepping on a pedal and just get water on demand, that there is significantly less flow through those pipes and therefore greater opportunity for biofilms to form.

Michael: That is indeed true.

Michele: So we are saving money on water but we are, the unintended consequence is higher risk for nosocomial infection.

Michael: Given the recent current events in poor California you can understand the need to conserve water. The poor Californians are desperate for water.

Elio: You're not kidding! (laughs)

Michael: I think the whole state is gonna eventually burn.

Vincent: Michele, can you take that next one?

Michele: Sure, it is from Jonathan.

In drinking water and wastewater treatment, we routinely use Colilert by IDEXX for identification and quantification of fecal coliforms and specifically E. coli.

https://www.idexx.com/en/water/water-products-services/colilert/

At my wastewater treatment plant, we sanitize the effluent water using chlorine gas dissolved in water to produce at least 1 ppm after 20 minutes. Drinking water standards are, of course, different. Would this treatment produce VBNC bacteria, and would our EPA approved detection method fail to detect them?

I have been listening to TWIM for about a year, and I really enjoy you all, and the enlightenment your podcast provides. I did two years of undergraduate work, one in physics, and one in biochemistry, before I left school to raise children and incur consumer debt. I have been a wastewater treatment operator for seven years.

Appreciate your time and your efforts in education.

Jonathan in Waco, TX

Where at the time that he wrote was sunny and 89 but forecast to reach 102.

Vincent: Michael, this refers to your paper on VBNC, right?

Michael: And I think the answer that Prof. Keevil's data suggests that any oxidative insult probably sends a significant fraction of the population into VBNC state, but for the most part it will kill the bulk, but there is always some microbes remaining, so it gets back to what the US EPA and the water standards of your community require for sewage and I know for potable water it is a much lower number. So again, the EPA is all about safety and making certain that we deliver potable water to our homes in a safe and effective manner. I think that the work of Professor Keevil from South Hampton really brings a whole new level of discussion to water and waste water treatment given his data with VBNC.

Michele: It is also the case that our EPA guidelines were formulated when we were most concerned with enteric pathogens, so things that cause diarrhea, foodborne illness, once we have largely taken care of that with our modern wastewater treatment and water treatment but now we are seeing that the legionella and the other respiratory pathogens that live in water maybe require a different set of standards, so.

Michael: Things like cryptosporidium which is much bigger than legionella.

Michele: Right, much bigger. Smaller as a cell but a bigger problem.

Michael: Yes. Poor folks in Milwaukee can attest to that from a few years back.

Vincent: Kieron writes:

I graduated in engineering, but I enjoy your lucid reviews of papers in the biological sciences, of which I know little. You people know your subject (at least I'm convinced), bring out real passion, and appreciation for the science involved in getting to the results.

I catch you on Science 360.

Great stuff!

Elio: We can fool anybody, can't we? (laughter)

Vincent: Kieron is from Wellington, New Zealand. Thank you.

Elio: Wow.

Michael: That's where we should go and do a TWIM away team.

Vincent: Sure, would love to go to New Zealand.

Michele: What time?

Vincent: Michael, can you take that last one, please?

Michael: I'll take the last one. Triett writes:

Dear TWIM hosts and TWIMers,

My name is Triet (or Treat as in Halloween trick or treat), I am currently an upcoming-to-fourth-year undergrad biology student at University of Regina, Saskatchewan, Canada. Thank you for the wonderful podcasts that had left me in awe at the end of each and every of them. I have a question for you guys. We heard a lot about mutualistic relationship between bacteria and it's host (like in the bobtail squid and human gut). However, in most of the scenario, the hosts are the ones that dictate the concentration of the microbes (purging of human microbes as fecal matter, bobtail squid getting rid of the vibrio when we knock out the lux or luminescent genes). I am just wondering if there is any example where the bacteria dictate the relationship? (Like turning on pathogenic factor to kill it's host if the host stop providing benefits to the bacterial community).

Thank you very much for the amazing podcasts, I wish you guys successful episodes to come.

Cheers from Canada,

Triet

That's a great note and we thank you for your compliments. I was thinking about what happens when the poor host gets influenza, this is a paper that we did on one of our very early TWIMs when the host gets influenza, the normal commensal Strep pneumo that is living in your upper respiratory tract effectively no longer is a biofilm and it goes deep into your lungs and effectively gives you pneumonia as a consequence of you getting of all things the flu, because you didn't get your flu shot, of all things. So that is where the bacterium is sort of revolting against a viral attack. To your specific relationship, I think quorum sensing and a lot of the complex communication that is going on between the gut flora in our system, we just don't have enough data to directly answer your question at this time. But as Elio often says, stay tuned. I'm sure we'll find a paper about that very subject coming shortly.

Vincent: Your flu example is a good one, though. I like that. You have a good memory, I forgot about that paper. Alright, stay tuned.

Michael: We haven't given away the book!

Vincent: That's right, we're gonna give away a book! Let's do it right now. I have a copy here on my desk of Antibiotics: Challenges, Mechanisms, and Opportunities. It is edited by Chris Walsh and Tim Wencewicz, published by ASM Press. Wonderful book, it is 2016, has mechanisms chapters for all different classes of antibiotics and some biosynthesis opportunities for new development. Great book, it is yours, all you have to do is send an email to twim@microbe.tv with the subject line "antibiotics". You have to do that. If you don't put it in the subject line I won't consider you. Contest closes on August 30th, after August 30th no more entries. We will take all the emails and do a random selection. You can find TWIM at Apple Podcasts at asm.org/twim, and of course most of you listen on your phone or mobile device of some kind, you use an app. In that app you can search for TWIM and please subscribe. It really helps us a lot to have subscription numbers and you get every episode as we release them, that is twice a month. Now Google Play Music also allows you to subscribe to podcasts and we have a link in our show notes for that as well. If you really like what we do consider supporting us financially. Go to microbe.tv/contribute. We have a number of ways that you can do that. Of course, send your questions and comments to twim@microbe.tv. Michele Swanson is at the University of Michigan, thank you Michele.

Michele: Thank you, see you next time.

Vincent: Elio Schaechter is at Small Things Considered, thank you Elio.

Elio: My pleasure, thank you.

Vincent: Michael Schmidt is at the Medical University of South Carolina. Thank you, Michael.

Michael: Thanks everyone.

Vincent: I'm Vincent Racaniello, you can find me at virology.ws. Thanks to ASM for their support of TWIM and Ray Ortega for his ever excellent technical help. Music on TWIM is by Ronald Jenkees. Thanks for listening everyone, see you next time on This Week in Microbiology.

(music)



Transcribed by Sarah Morgan.