

Exploring Biodiversity: The Search for New Medicines
Lecture 3 – Biodiversity at a Snail's Pace
Baldomero M. Olivera, Ph.D.

1. Begin of Lecture 3 (0:16)

[ANNOUNCER:] From the Howard Hughes Medical Institute. The 2009 Holiday Lectures on Science. This year's lectures, "Exploring Biodiversity: The Search for New Medicines," will be given by Dr. Bonnie Bassler, Howard Hughes Medical Institute investigator at Princeton University, and Dr. Baldomero Olivera, Howard Hughes Medical Institute professor at the University of Utah. The third lecture is titled, "Biodiversity at a Snail's Pace." And now, to introduce our program, the Grants Program Director of the Howard Hughes Medical Institute, Dr. Dennis Liu.

2. Welcome by HHMI Program Director Dr. Dennis Liu (1:05)

[DR. LIU:] Good morning, and welcome to the 2009 Holiday Lectures on Science. Our lecture this morning is going to be given by Toto Olivera. I was fortunate as a graduate student many years ago to hear Toto speak, and at that time, he brought... would bring a collection of his beautiful shells, and he would pass those around. And I was struck at the beauty of these shells and at the biodiversity that they represented. And I was also struck by the fact that the science done on these beautiful animals was so good and so important, teaching us fundamental things about how the nervous system works and, of course, in the end, producing medicines. It's interesting to follow a long and distinguished career like Toto's, and to see how his upbringing in the Philippines has influenced his work, and how he's now expanding his work on trying to understand how nature can lead us to new drugs. And in his next lecture, he's going to show us the importance of biodiversity and of continuing to expand that research realm. And now we have a brief video to introduce Toto.

3. Profile of Dr. Baldomero Olivera (2:23)

[DR. OLIVERA:] So, I think we're at an interesting time in biomedical science, because we've gone through many decades now of increasing specialization. And, of course, through state-of-the-art science, you want to be at the forefront of your field, and that really requires more and more technical expertise. But, I think that's come at a price. People are getting more and more narrowly trained. And, there is a need for people who have a foot in several different disciplines, particularly in education, you still have to get very specialized if you're going to make a research career. ATP to AMP... For the students who want to pursue a science career, I guess my advice is two-fold. On the one hand, you should pursue what you are really most interested in and what, at a gut level, you feel you really want to do. But, on the other hand, you have to be able to be as flexible as possible in pursuing that. So, I think, if you're working on something you're not very interested in, that that isn't very fulfilling. But, you could be working on something you are very interested in, but, you know, if you're not very flexible, it's very hard sometimes to just be able to realize what you want, right? And, so by being a bit flexible, by having a variety of ways to pursue what you're really interested in, I think that helps a lot.

4. Why are there so many venom components? (4:18)

So, it's great to be back, and let me remind you what we did last time. Cone snails are venomous, predatory mollusks, and I described how a drug was developed from a cone snail venom. And, we came up with the pharmacological resource of about 140,000 different venom components, all of which are pharmacologically active. The 140,000 peptides are, of course, evolved by the snail, and what I think we

really want to understand, is what the snail is doing with all of these compounds. And, so let's discuss two central questions. First of all, why are there so many different compounds in these venoms? And, furthermore, I told you last time, that when you look at the different species, there's essentially no overlap between venom components. And, so the second question I want to address is, why do the venoms of different cone snail species differ from each other?

5. Video: *Conus striatus* strikes a fish (5:41)

So, in order to answer that, what you're going to see is the striated cone going after its prey. And, this is going to happen very fast. This is real time now. And, so what you see is a very efficient prey capture strategy, and in this case, the snail doesn't even have to leave the substrate, it remains largely buried. And, so that indeed, is how some of these snails are able to capture their fish prey.

6. Venom components disable different targets (6:26)

So, what you've seen is a paralysis sequence, and in order to understand how these snails are able to capture fish that way, the first thing we did, was to simply isolate, from the venom, all of the components that paralyzed fish. And, what we ended up with, is shown here. They all act at the synapse, and, in fact, we ended up with at least four different components in each venom for fish-hunting cone snail that would individually paralyze a fish. And, we summarized them by Greek letters called alpha, mu, psi, and omega, and these are the components of the venom that are able to immobilize a fish.

7. Each toxin blocks a different synaptic event (7:24)

So, how do these things act? At synapses in general, electrical signals arrive, calcium channels open, that causes vesicles to release their neurotransmitter. And, as a result of that, neurotransmitter binds and opens the receptor, and you get an electrical signal initiated by the opening of those receptors. So, what you see is that there is one venom component that basically inhibits the electrical signals from spreading on the muscle. There's a second venom component that, together with a third venom component, completely blocks the receptor on the muscle side, so that, in this case, even though the neurotransmitter is released, it doesn't do anything. And, the fourth venom component, which blocks the calcium channels on the nerve side. Four different venom components, any one of which is sufficient to block the synapse. And,

8. Animation: Motor canal toxins block motor neurons synapses (8:37)

what we're going to show you is the next animation, which illustrates how these different components are able to paralyze a fish. So, here is the electrical signal coming down the nerve and you're getting the electrical signal on the muscle, as you've seen previously. Now we zero in on the synapse. It is at the synapse, that all of these venom components are active. So what you see is, normally, calcium enters. You get vesicle fusion, and the release of neurotransmitter, and that opens up the receptors of the muscle end. Once the electrical signal is initiated, it's spread throughout the muscle membrane by sodium channels that open up and let sodium in. So, the first venom component, is omega-conotoxin, that will plug these calcium channels. So, in fact, Prialt is a type of omega-conotoxin. The second venom component are the alpha-conotoxins that bind to the receptor on the muscle side, and they're accompanied by a second venom component called the psi-conotoxin, which basically, plugs the ion channel of the receptor. And so, even though neurotransmitter is released, it can't do anything, because these two toxins essentially inhibit the receptor on the muscle side. And finally, there is a fourth component and that fourth component will plug the sodium channels on the muscle side, so that, even though you get everything occurring on the nerve side — the release of neurotransmitter, the binding of neurotransmitter to the muscle side, and the electrical impulse initiated — when you have this

fourth component, it plugs all the sodium channels. And so, now that electrical impulse can't spread through the membrane of the muscle. That alone is able to inhibit muscle contraction. So, what you see is that the snail doesn't just make one thing to inhibit the transmission of the synapse, it makes four different things. And all of these different venom components act together to really do a number on the synapse. So, you have four different components acting at different sites, and as a result, the snail is making absolutely certain that the electrical signal doesn't get from the nerve to the muscle. And, it is blocking, not just with one toxin, but with four.

9. Snails discovered multiple drug therapy (12:04)

So, what are these snails doing here? Essentially, the snails are doing combination drug therapy. They're not just using one pharmacological agent for a particular physiological purpose, they're using four in combination. Let us contrast that with what we do when we want to use a drug to alleviate pain. We take Prialt, a single drug, and that's enough to block the whole synapse. So, I think the lesson here is, that in the real world, it's not enough to have one pharmacological component. I think the snails, due to natural selection, have, essentially, evolved a combination drug therapy, because, that's what works in the real world. It's not enough just to have one pharmacological agent. And, what's interesting is, we're beginning to find just that, in medical practice, because the most difficult problems that we're trying to alleviate by a single drug, don't work. And so, for solving cancer, for solving AIDS, one drug doesn't work very well. And so, the modern pharmacological industry is essentially, discovering what cone snails discovered many tens of millions of years ago, that things work better when you have a combination.

10. The motor cabal is like a poison blow-dart (13:38)

However, although this group of peptides is very efficient at paralyzing a fish, and we call groups of peptides like this cabals, because cabals are secret societies out to overthrow existing authority. And so, we thought that was a good name for a group of toxins that were all acting together. In a way, the cabal acts very much like an Amazonian Indian, who's trying to stop an enemy by using a blow dart, okay? And, in fact, the mechanism of the poison arrows that are used by South American Indians, is completely analogous to what the snails do. Because at the tip of the blow dart is a poisonous plant substance called curare. And curare actually binds to exactly the same site on the receptor as alpha-conotoxin that the snails make. And so, when you get hit by a blow dart, your synaptic transmission at the neuromuscular junction is inhibited, and that's why blow darts work to paralyze the enemies, if you need to use them.

11. Motor cabal takes 20-30 seconds to paralyze (14:59)

And, in a way, the snails, as communities got more and more complicated, also were under pressure. Because, with this cocktail that I just told you about, the motor cabal, it takes 20-30 seconds to immobilize a fish. Why? Because, from the site of injection, where the snail injects it, for these components to act, they have to spread through the body of the fish, and they have to get to the neuromuscular synapses, where they're going to act. That takes some time. It takes a minimal of 20 or 30 seconds. And, that's why I showed you the video, because it's always important to return to the biology that you want to understand. When you looked at what that snail did, it took about a second to completely immobilize that fish. And yet, when we tried to reconstitute that by injecting these different components of the venom, either singly or all together, even in massive amounts, it takes 20-30 seconds, 'cause there's a transit time from the moment of injection to getting to all of these synapses where these things are going to act.

12. Video: A Taser causes rigid paralysis (16:16)

So, what's going on here? Now, as you know, people don't use blow darts anymore. And, in modern society, if you want to stop somebody from doing something, to protect yourself, a blow dart just wouldn't do, because our societies have gotten too complex to be able to take something as inefficient as a blow dart. Well, what's going on here is very analogous to a taser. As all of you know, when you need to really put somebody down fast, we have invented a new device for our more complex society. And, that is, we use tasers instead of blow darts.

13. The snail's lightning strike-cabal (17:06)

And so, in effect, what this snail has been able to do is the equivalent of a taser. And, we call that the lightning-strike cabal, okay? So, it has the components that are analogous to a blow dart, but it also has components that are analogous to a taser. And, we know that, because if you inject all of the components of the motor cabal, the fish has very relaxed musculature, all its fins are flaccid, and we call this a flaccid paralysis. But, if you look carefully at the video, you may have noticed that the fish has very, very stiff fins. And so,

14. Animation: Lightning-strike cabal acts like a Taser (17:56)

Let's look at what's going on here. Electrical signals are coming down a nerve. They're being carried by sodium channels which open up, and they're being terminated by potassium channels, the brown disks. So, when sodium channels open, you start the electrical signal, but the potassium channel ends it. And so, that's why we have an excitation moving in one direction, and transient. Because, it's started by the sodium channel, ended by the potassium channel, which lets potassium out. And so, that's the normal way in which electrical signals come down a nerve. What happens when the snail injects its venom? Well, there's a venom component that binds to the sodium channels, and normally sodium channels open and close quickly, but when a sodium channel is bound by delta-conotoxin, what happens is, that it opens, and it remains open, it doesn't close quickly. Furthermore, a second venom component, called kappa-conotoxin, plugs the potassium channels. And so, what this does, is the same as a taser, it's as if we've tasered this little bit of nerve, so that now, it remains excited, it keeps on firing, and what happens is, electrical signals are going all through the nervous system of the fish, you get this very stiff fish. So, in contrast to the normal situation where we have just a few electrical signals, now we have this massive hyperactivity, the pharmacological equivalent of a taser. So, what you can see, is that the snail, therefore has a way of immobilizing the fish, so that it ends up with very stiff fins. And really, the components of that that are important are the delta-conotoxin that keeps sodium channels open, and the kappa-conotoxin that blocks potassium channels.

15. Video: *Conus bullatus* "lightning strike" (20:05)

And so, what I'm going to show you next, is a video that really records the fastest immobilization we've ever seen, we've even been able to record. And so, this is real time, now. And, this is the bubble cone, *Conus bullatus* — and you'll have to watch carefully. It's now extending its proboscis, which is barely out. And, it's going to sting really quickly. Notice how quickly immobilized that fish is. So, from the injection site, you have this massive excitation, it's as if the fish had been tasered. Look at all of its fins, they're very, very stiff, and now, that allows the snail to completely engulf it. But, what's remarkable is, the fish is going to recover here. But, it's too late, of course. And now, you'll notice the fish became paralyzed again, and that's the second set of venom components kicking in the motor cabal.

16. Multiple venom components provide speed and safety factor (21:15)

So, in the real world, right, in order for a slow-moving snail to capture a fish, it's a rather sophisticated chemical strategy. So, the snail has evolved all of these different components that attack different molecules in the nervous system, have different kinds of effects. And, in combination, you have the lightning strike cabal that causes a very rapid immobilization. But, that doesn't work all the time. Sometimes the fish will recover, and to cover their bets, the snails also have a motor cabal that takes a little longer. But, once it kicks in, you get this irreversible paralysis, because of inhibiting synaptic transmission. And so, what you see is that, essentially, these snails have evolved both the equivalent of a blow gun, and the equivalent of a taser, but with many, many different molecular components. And so, the prey capture cocktail of the snail is very complex, indeed, as far as we understand it.

17. Venom is also used for defense and competition (22:32)

But, no matter how many components you estimate, you still don't come up with 200. So, perhaps, 10, 15, components are used for prey capture, and these snails only eat fish. So, what are the other 180 components doing in the snail venom? And, the answer is, that the snails do not use their venom components only to capture prey. They also use their venom components to defend themselves against predators, and to deter their competitors. Now, the problem is, we don't know the details of these interactions very well. Amazingly little is known about the biology of these snails. And, in fact, our inference that they use their venoms defensively, is really coming from the medical literature, when you read why people get stung.

18. *Conus geographus* stings a man in defense (23:37)

And so, if you pick up one of these snails like I did yesterday, the snail is not very aggressive. Its first line of defense is just to go into its shell. However, there are few cases in the medical literature, where we can read what happened. And, this is a case in the Great Barrier Reef, in 1935, that was reported in an Australian newspaper. And, this guy picked up a geography cone from the Great Barrier Reef, he came to his boat, he said, "Hey, look at this great big snail I found." And, he showed it to everybody, and then he said, "You know, I can't see the pattern on the shell, because it's covered with this stuff." So, he took out his penknife, and he started scraping it, and then he said, "This thing just stung me." And, it was clear, and there were many witnesses that he was stung just as he was scraping the shell. And so, the snail really interpreted that as a predator, and so, it was defending itself. And so, the guy died about three hours later, because in those days, they didn't have helicopters, and they couldn't get him to a hospital in time. So, it was really as he was holding it and scraping it, that he got stung. So, that's part of the evidence that this snail doesn't think you're a fish when it stings you. It's because you've usually done something to make the snail think you're trying to break its shell. So, if you're a spear fisherman, and you see a big snail like this, do not spear it, break the shell, and then pick it up later, okay? Or, when you see the snail, another sting case, do not take the shell and put it in your swimming trunks, and swim around, because that's what happened with one other guy who got stung.

19. Venom composition and a snail's complex environment (25:31)

So, what I'd like to emphasize, is that every species uses its venom to interact with its predators, with its prey, and with its competitors. And, that's the rationale for why there's some many venom components, because the venom is used for a whole variety of different biological purposes. The rationale for why every snail makes a different set of venom components, is ecological. So, what is a species? Every species has its own ecological niche. And so, what this means is, that every species has a different spectrum of predators, a different spectrum of prey, and a different spectrum of competitors. And so, if we just do a little matrix with prey, predators, and habitat differences that determine who are your competitors, then one species might be eating fish, and might be preyed by crabs, and in fact, might be in a habitat with certain types of competitors, while another species has a different set of biotic

interactions. And, basically, the venom has to be adapted to all of these interactions between each cone snail species, and the animals that matter for its success, namely the prey, predators, and competitors. And so, this is why we feel that the venoms of these snails are extremely complex, and why they evolve so rapidly, so when you go from one species to the next, there is a different set of venom components. So now, I'll take time to answer a few questions. Yes? Back there.

20. Q&A: How is Prialt administered? (27:24)

[STUDENT:] I was wondering if you could explain how Prialt is administered, and if there's any side effects to it.

[DR. OLIVERA:] Yes. So, we talked about Prialt, or omega-conotoxin, the generic name for how Prialt acts. And, it has to be administered directly into the spinal cord. And so, there's a pump that's implanted that, essentially, flows into the intrathecal space continuously. That pump is loaded by injection every three months. So, since it's not a pill that you can pop every time you feel pain, Prialt is not something that you use whenever you feel a minor pain. It's really for major pain, and in particular, it's for those patients who are no longer being helped by morphine. Because, the trouble with morphine is, morphine is a great drug, if you only have to take it once or twice. But, if you have to take it continuously, patients develop tolerance to morphine. With Prialt, patients do not develop tolerance, and so, for continuous, very severe pain conditions, then nothing much helps many of these patients, and Prialt then becomes one of the major alternatives. But, it has to be applied directly to the central nervous system. People are working hard to find equivalents that will pass the blood-brain barrier, but so far that hasn't been successful. Over here.

21. Q&A: What effects do other conotoxins have on humans? (29:03)

[STUDENT:] I was wondering if like Prialt, delta, kappa, alpha, mu, si, omega-conotoxins, do they have a positive or negative effect on humans?

[DR. OLIVERA:] Do they have a positive or negative effect? Certainly delta-conotoxins, although we haven't tested it directly, you would expect, would excite the nervous system, and especially sensory fibers. So, they would cause pain, and the kinds of snails that I showed you, although they're not deadly, a snail like this, if it stings you, you'll feel immediate pain. And, we presume that's because of delta-conotoxins. So, those are certainly not comfortable for humans, although they won't kill you. The kappa-conotoxins, however, have had an interesting preclinical application. It turns out, if you have a heart attack, your heart is actually a great survivor, and you don't really get too much cardiac damage from the heart attack, itself, for a long time. But, people who've had heart attacks, the damage to their heart comes when you restore the blood flow. So, this is called reperfusion injury. And so, what happens is, that you have a heart attack they start the blood flow again, and for whatever reason, that people don't completely understand, it's this restoration of blood flow that causes a lot of the damage. And, it turns out that those kappa-conotoxins will protect the heart from damage. And so, that's in preclinical development. So, that's one that's a potential useful compound for therapeutics. Yes?

22. What effects do the non-predation peptides have? (31:10)

[STUDENT:] You said that the snails have, like, an extra 180 peptides for their predators and competitors?

[DR. OLIVERA:] Yes.

[STUDENT:] What are the effects on their predators and competitors of the other peptides?

[DR. OLIVERA:] We don't completely understand all of the effects of these peptides. And, one interesting thing we've observed in the aquarium, in a worm-hunting cone snail, was that one *Conus* species began to eat the worm, and the second *Conus* species then stung the worm. And, what we saw, was that the first one let go, okay? And so, we suspect that the types of compounds that the worm hunters made to deter their competitors, is something that tastes awful to their competitors, so that immediately their competitors let go of the prey. So, if you're eating a worm, you know, you don't want to be sucking up your worm after you've captured it, and find a predator on the other end, right? And so, these are the sorts of competitive interactions that we have seen. Okay,

23. Fish hunters not limited to hook-and-line strategy (32:28)

so what you've seen is the hook and line hunting strategy. And, I've described two cabals -- the lightning strike cabal and the motor cabal — that are necessary for prey capture using that strategy. What I'd like to emphasize, however, is that even for fish-hunting cone snails, this is the group of fish-hunting cone snails that we understand the best. But, the different species that hunt fish, have a whole variety of strategies to capture their fish. And, the biodiversity of just the venomous fish-hunting cone snails, has an amazing variety.

24. Video: *Conus tulipa* hunts fish by net (33:11)

And, let me show you, in the next video, one of the species that uses a different strategy. This species, the tulip cone, approaches fish by opening its mouth, and engulfing as many fish as it can. And then, only after it's engulfed the fish, will it sting the fish that are inside its mouth. And so, there are a number of *Conus* species that use this strategy, and we call this the net-hunting strategy. So, typically... and *Conus geographus* itself, is a net hunter. So, typically, what these snails will do, is they'll come out at night, and they'll go into crevices of reefs where there are schools of small fish that are trying to hide from predators. And, they'll approach the school, and if they're lucky, they can bag the whole school, okay? And then, they pick them off one by one when the fish are already in their mouth.

25. Net hunters use nirvana cabal toxins (34:17)

So, what we've found is that since they begin to inject their venom into the fish, only, after the fish is in their mouth, it's not a good idea to have a lightning strike cabal. First of all, the fish is already in your mouth, so there's no need to immobilize it in one second. It's not going anywhere if you've closed your mouth, right? And, if you had a lightning strike cabal and you harpooned the fish, and the fish started to twirl with very stiff fins, you might get wounded every time you tried to swallow your fish. And so, in fact, snails of this type do not have a lightning strike cabal. Instead, they release a little bit of their venom, even before they sting the fish, and that seems to make the fish sedated, quiet, hypoactive, okay? And so, instead of having a lightning strike cabal, what these snails do is, they also have a motor cabal, but to substitute for the lightning strike cabal, they have what we call the nirvana cabal, okay? And so, the nirvana cabal has components that really make the fish act as if they're in an opium den, they're all, kind of, pretty sedated, pretty quiescent.

26. Nirvana cabal toxins may yield drugs to treat epilepsy (35:43)

And, we have begun to characterize the components of the nirvana cabal. And so, some of the components of the nirvana cabal, which causes this hypoactivity of the nervous system, are shown here. These were all mostly isolated by undergraduates, the sleeper peptide that I told you about earlier, a peptide that makes mice sluggish, and some other components that we've more recently characterized. But, it occurred to us, if in fact the snail uses these components to quiet down the nervous system, can

we use these same components in pathological conditions where the nervous system is too active? And, what's the first thing you can think of that is a pathology of the nervous system, where they're too active? Epilepsy. Right, epilepsy is clearly a pathological condition where, in fact, the nervous system is too active. And, what we've found is that, yes indeed, some of the components of the nirvana cabal, do have promise for these conditions, and two of these have reached human clinical trials. One of them, which we originally called the sluggish peptide, for conditions where your pain circuitry fires uncontrollably, and so, that's a condition called intractable pain. And one of these has reached human clinical trials for that purpose. And, the sleeper peptide, has actually reached phase one clinical trials for epilepsy. So, these have really shown some therapeutic promise.

27. 3D structure of toxin peptides (37:38)

Now, what I'd like to do, is to do a little bit of chemistry, so that you have some idea of the chemical basis for all of these pharmacological activities that I've been telling you about. So, if we look at the different venom components, all of these peptides have structures, and you can determine those structures experimentally, you can use techniques like x-ray crystallography, or NMR. And so, many of these peptides, we know exactly what their structures are, and a few whose structures have been solved, are shown here. And, as you can see, different peptides have different shapes. But, what gives a small peptide its structure? Well, it turns out that the structure is largely determined by a scaffold. And so, this scaffold holds together the structure of the peptide, and stabilizes it. So, if we look at a peptide, just from its linear sequence, you see a string of amino acids that are all strung together with peptide bonds, and so you have a linear array of amino acids.

28. Cysteine cross-links form peptide scaffolds (38:52)

But, if you compare two peptides in the same, what we call superfamily, what you see is that, in fact, most of the amino acids aren't the same. But, in these two peptides, you can tell that the C residues, which stands for the amino acid cysteine, are the same, and those are conserved. And so, we represent this diagrammatically, by a bar like this where the white regions are any amino acid can be put in there. And so, those are hypervariable regions. But, the cysteine residues, are conserved. And, the reason they're conserved, is because they're special residues for structure, because cysteine has sulfur. And, it turns out that under the right conditions, you can form a crosslink between two cysteine residues, and this is called a disulfide crosslink. And so, the sulfur residues form a crosslink, and by making crosslinks, and so what you can see in this example, is you have three crosslinks. Then, in fact, those crosslinks hold the structure together, and are critically important for these peptides to have a particular structure.

29. Toxins in a superfamily with similar shapes but different functions (40:17)

And so, basically, the different peptides in a superfamily all have the same scaffold, the same arrangements of cysteines, and of disulfide crosslinks. But, because all of the amino acids in between are different, you have all of these side chains sticking out that, essentially, give the surface a different shape. And so, although they have similar structures, they can have different functions. And, in this example, what you can see is that, although you have two peptides with the same scaffold, one blocks sodium channels, and one blocks potassium channels. And, of course, those have very different physiological effects. So, the one that blocks potassium channels is part of the lightning strike cabal, and the one that blocks sodium channels is part of the motor cabal. So, in any particular superfamily, you have a particular pattern of crosslinks, and a conserved scaffold, and they're many superfamilies. Here's one with only two crosslinks, we call this the A superfamily. And so, the three most common superfamilies that we find in cone snail venoms, are the A, O, and M superfamily, each one having a different set of crosslinks. So, when you look at any particular venom, what you find is that the majority

of the peptides belong to these three superfamilies. So, in a fish-hunting cone snail with, say, 200 different peptide components, there might be 150 that belong to these three superfamilies. So, maybe, 60 belong to the O superfamily, and 40 belong to the A superfamily, and so forth. There are minor superfamilies. But,

30. Hypermutation in non-cysteine peptide sequences (42:12)

it appears that the chemical basis of these venom components is a few gene superfamilies that are expressed in all of the species of cone snails. And, every time two species diverge from each other, there's a hypermutation, so all of those white regions in the bars that I showed you diagrammatically, those amino acids evolve very quickly, diverge from each other. And so, what happens is, because those things diverge so quickly as speciation occurs, then what you end up with in the venom, is different peptides in every species. However, once a species gets established, that process of hypermutation is, apparently, suppressed. And so, all the individuals in the species will, pretty much, have the same peptides with a very, kind of, average, sort of, rates of mutation. But, when speciation occurs, then the hypermutation kicks in. And, as a result, you can get, suddenly, many, many different peptides generated. And so, in these different superfamilies, the white bars are amino acids that keep on changing, and so, you might have some that hit calcium channels, some that hit sodium channels, some that hit potassium channels.

31. Convergent evolution between toxin superfamilies (43:35)

And, what we've found is that we can correlate what is going on in different lineages of cone snails. And, that if you take two different groups of fish-hunting cone snails, what you find is that very often, each branch will have used a different superfamily for the same purpose. And so, what I'm showing you are two branches of fish-hunting cone snails, and, in fact, both of these branches have the lightning strike cabal. And so, they have peptides that inhibit potassium channels, and yet, what we've found is that one branch uses the O superfamily, and the other branch uses the M superfamily. And so, what you see here is that we have an example of convergent evolution, in which all of the species in one branch have developed peptides to inhibit potassium channels that come from the O superfamily. Another branch of fish-hunting cone snails are using the M superfamily. So, you have an example, at the molecular level, of convergent evolution. And, that's because these peptide genes are evolving so fast, that every time there's a new ecological situation, essentially, the snails can evolve new peptides to be able to track the change in ecological conditions.

32. Molecular basis for venom divergence (45:15)

So, to address the two questions, I think you can see that the venom is, as was discussed yesterday in the discussion section, really the result of the history of each species. Every species has its own set of biotic interactions, and therefore, needs different venom components. And, the molecular basis for this very rapid divergence of venoms, is these gene superfamilies that evolve very rapidly, and as a result, you end up with a big difference between one species and another species. So, this is the molecular basis of the divergence in the venoms.

33. Turrids are the most numerous venomous snail (46:08)

So, what I'd like to end up with, in the last few minutes, is to tell you that we've only scratched the surface of the biodiversity of venomous mollusks. And, that the cone snails, that we've studied all these many years, are really, perhaps, only 5%, or so, of the biodiversity of venomous mollusks. And, you could divide mollusks that are venomous into three groups, the cone snails, the turrids, and the auger snails. The auger snails are a little smaller than the cone snails, and they're adapted to sandy

environments, and they have these very sharp thin shells, that probably many of you have seen. But, the real biodiversity of venomous snails is the turrids. 12,000 species is the present estimate. So, if you have this vast biodiversity, why has no one studied them at all? Why has very little been published about the turrids? And, the reason is that turrids are, first of all, rather small, rather rare, and worst of all, most of the species live in very deep water. And so, to find the average turrid, you have to dive at the limits of present scuba diving, and look around for a very, very small snail, right? And so, that's hard work, it's much easier to study a big cone snail like this. And so, in fact, we have wanted to study turrids for a long time, and what you see, are some of the big turrids, bigger turrids that we have been able to collect. But, never enough individuals to really do a good job on them, and not enough species to move our work forward.

34. Phylogeny of Turrids (48:07)

Recently, however, we've begun doing the phylogeny of turrids, and so, if we put in our phylogenetic tree, not just the cone snails, but also the auger snails and the turrids, the cones and augers are very distinctive branches, but to our surprise, the turrids are not a single branch. Rather, there are five, really, not at all closely-related groups that are all called turrids. And so, even the name turrids is a misnomer, because one's really referring to a heterogenous group.

35. Video: Deep nets to harvest Turrids (48:45)

So, how can one begin to study the turrids? Well, there's been a breakthrough. And, the breakthrough isn't scientific, it's a commercial source. And so, what you see is a fisherman in the Philippines, and what this fisherman is doing is, he's diving, and he's looking for a rope that he tied on a rock. And now, he's taking the end of the rope, and he's going to bring it up to his boat, and you can tell the other end of the rope goes way down into 50 meters of water. And now, the fishermen are, 15 minutes later, able to pull up the net, which is down at 50 meters, and you notice it's a net with very fine mesh. And so, what these fisherman do is, they take old nets that they've discarded, tie them together, and the finer the mesh the better, and this is called lumun lumun, which in the Filipino language, in the central Philippines, is called "combining things." So, they use this combination of old nets. They shake out the contents of the net, and out of the contents of the net, they're able to get an incredible biodiversity of tiny marine organisms that lived at a depth of about 50 meters. So, this is the result of two nets, okay? As you can see, not a lot. We decided to collect everything in that net and just do a census.

36. Generous biodiversity in a single net (50:28)

And so, here's a picture of what came out of that net, and what we found was there were over 1,000 different animals in that net, eight different phyla, 300 different species. And, the most abundant were the snails, where there were over 600 specimens in that net, 155 species. And, it turns out, that, in fact, out of those, 36 species are venomous, and 30 of them were turrids. Okay. So, there were an average of 30 or 40 turrids in every net, and when you compare across nets, there's only about a 20% overlap. So, suddenly, we have a brand new source of an enormous biodiversity of turrids, and let me show you what these millimeter long snails look like, the venomous ones. Here is one group of related snails from one net, and most of them, we found, don't even have names, they've never been described. Because, after all, how many people are able to collect a 2 millimeter snail, crawling around in 50 meters of water, okay? However,

37. Turrid venom is hugely diverse (51:50)

with modern biotechnology, we can access all of these... these are all venomous snails, we can access all of the compounds that these snails make. And so, we took one of the unnamed ones, that one there, and

dissected as many venom ducts as we could, and did a molecular analysis. And, we now have 30 sequences of the peptides that this snail makes, and we're trying to synthesize them, and we're trying to determine what they do. So, I wanted to close with that, just to let you know that venomous snails are much more biodiverse than you might think. Instead of just 700 cone snail species, there are 13,000 venomous snails, most of them turrids, and we find that the venoms of all of these snails are almost just as complex as the cone snail, some perhaps even more complex. And so, now, instead of 140,000 compounds, we're talking about millions of compounds that are evolved to affect the predators, the prey, and the competitors of each of these species. So, who knows what innovations these guys have evolved. And, of course, we don't know anything about turrids, yet, and we're just beginning to find out. And, I hope some of you in the room, in the future, will either help in this quest, or perhaps, have a chance to use some of these compounds that come from biodiversity. Thank you very much, let me take your questions. Okay, any questions? Yes.

38. Q&A: What about toxins that interfere with clotting? (53:38)

[STUDENT:] When you say you've been working with a lot of synaptic toxins, -- inhibits channels within the synapse, in the readings that we got, there were also mentions of blood-thinning, blood-clotting toxins, and I was wondering if you've done any work with those?

[DR. OLIVERA:] Yes. So, our focus has been the nervous system. And so, in general, what you find depends on what you're looking for, right? However, it's clear that there are other components that don't act on the nervous system. And, some of these, we can rationalize. So, there are a number of peptides that appear to affect the circulatory system, and in particular, that cause blood vessels to contract. And so, you can imagine that if you inject the motor cabal into your prey, you want to get those peptides as quickly as possible to the junctions between nerves and the muscles that control the fin musculature. And so, in order to do that, if you were able to constrict the big blood vessels, then your venom would be taken up by capillaries much faster, right? And so, there are effects on other systems, but in general, we've tried to rationalize those effects as helping the major toxins that affect the nervous system. But, that's because we're very oriented towards finding things that are very useful for basic research of neuroscience. Yes.

39. Q&A: When do cone snails' prey actually die? (55:34)

[STUDENT:] You discussed, in pretty good detail, how cone snails induce paralysis in their prey. At what point do their prey actually die?

[DR. OLIVERA:] That's a good question. As far as we can tell, when they're hit by the lightning-strike cabal, they remain alive for quite a long time. And, you may have seen in the videos that this immobilized fish with stiff fins, actually its gills were still moving, and as it disappeared into the mouth of the snail, its gills were, in fact, still moving, so it clearly wasn't dead yet. I suspect that when they can no longer move their gills, because the motor cabal, essentially, has paralyzed that musculature, then they will die, right? But, death is, probably, a slow endpoint compared to immobilization. And so, I think...

[DR. LIU:] Thank you, thank you, Toto.

40. Closing by HHMI Program Director Dr. Dennis Liu (56:38)

So, thanks for another terrific talk, Toto. I think it's really gratifying that it only took 60 million years for humans to reinvent the taser. But, I think, more seriously, it's a great example of how following an interest, how much science is a tool for learning about the world. And yet, every question just uncovers

more and more questions, and that will... There's obviously, plenty of work for everyone to do. So, thank you very much.