

Genome Insider Episode 4: The Big Deal About Short Plants

ALISON: Hey! I'm Alison Takemura, and this is Genome Insider, a podcast of the US Department of Energy Joint Genome Institute or JGI. Today, my colleague and fellow science communicator Massie Ballon will be joining me to help tell this story. Welcome, Massie!

MASSIE: Thanks, Alison! And hi everyone! So, what genome science story are we gonna talk about today?

ALISON: We're talking about the mysterious life of "short plants."

MASSIE: What exactly are "short plants"?

ALISON: Well as the name suggests, they're super short! And being 5 feet tall, I'm one to talk.

Fire moss *Ceratodon purpureus*, with stalks in which sporophytes, or moss babies, develop. Mosses have a two-stage life-cycle: gametophytes, which produce gametes, and sporophytes, which produce spores. Gametes fuse to become sporophytes. (Sarah Carey)

But think: mosses. They only get a few millimeters off the ground. And that's because they don't have a vascular system, which is like our circulatory system; it moves fluids and nutrients around. A vascular system is what has enabled other plants to get so tall; for example, the poplar tree, a potential bioenergy crop and the first tree to ever have its genome sequenced.

MASSIE: Okay, and why are we talking about short plants?

ALISON: To answer that, let's go back 470 million years..when ancestral plants, which were also very short, came out of the ocean, onto land. All of a sudden, they're in this new environment and their genes need to adapt...

JEREMY: Uh, you know as they moved onto land, they repurposed a lot of these things that were already kind of floating around in the, in their algal ancestors, and then made new use of them, and many times that involves making several new copies and then allowing those new copies to do new functional things.

ALISON: That was Jeremy Schmutz, the head of the JGI's plant program.

MASSIE: And Jeremy's saying that plants on land evolved new adaptations by diversifying their genes, right?

ALISON: Yes, and because scientists want to understand what plant genes do, it would be nice to be able to start with that one gene, before it diversified.

MASSIE: Yeah, that does sound way easier.

ALISON: Right, it's basically like wanting to go back in time. For example, imagine you're a scientist studying switchgrass, a potential biofuel feedstock that the Department of Energy is working on. And you're trying to figure out what this one weird gene does. But there are 30 copies of this gene.

JEREMY: But as you start stepping back in time, you get, you know, that goes down to 15, then that goes down to five, then it goes down to three. And these are early short plants, you know, there might be only one copy of that, or two copies of that.

ALISON: So studying today's short plants, like mosses.

JEREMY: It gives us a way of looking to see what the ancestral forms of these proteins really were in there. And that's kind of the goal of comparative genomics is to connect throughout the tree, these genes, and that allows us to flow functional knowledge of what these do one way or the other.

MASSIE: So does that mean mosses have genomes that are frozen in time?

ALISON: No, it doesn't. And this is important to clear up: mosses have been evolving for just as long as other modern plants. So, it's not like they have any genes that would be exactly the same as their ancestor's. But because short plants don't have a vascular system, and neither did early plant ancestors, scientists do think some genes might be more similar between the two. And that can help us deduce gene function across plants! So today's story focuses on the quest to elucidate one moss' genome. You ready, Massie?

MASSIE: Let's do this.

SARAH: My name is Sarah Carey, and I am a PhD student at the University of Florida.

STUART: My name is Stuart McDaniel, and I'm a, I'm an associate professor at the University of Florida in the biology department.

Stuart McDaniel, evolutionary biologist at the University of Florida, and his then-PhD student Sarah Carey. Carey is now a postdoctoral researcher in Alex Harkess' lab at Auburn University and the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama. This photo was taken

after our podcast interview at the 2020 Plant and Animal Genome conference in San Diego, CA, where the pair presented their work on fire moss evolution. (Alison Takemura)

ALISON: Sarah and Stuart study the moss *Ceratodon purpureus*. It's name means 'purple and horn-shaped' because when it reproduces, it grows these stalks with horn-like purple capsules on the ends. It's visually striking.

SARAH: If you look at it with the sun shining through it, it's this bright, bright purple red color.

ALISON: *Ceratodon purpureus* is also known as fire moss.

STUART: So, one of the reasons it's called fire moss is because after a forest fire happens, this is one of the very first plants to recolonize the burned ash and it will cover whole acres and you'll see nothing but this moss and a couple other species of so-called fire mosses.

ALISON: This fire moss is cosmopolitan.

SARAH: We have lines from Alaska, all over North America. We have ones from Ecuador, southern parts of Chile, Antarctica. This species of moss grows everywhere

ALISON: It even grows on disturbed sites, like next to mines, around heavy metals, and even around houses.

SARAH: It grows on rooftops. So some of the isolates we have in the lab are ones from one of our colleagues. He just reached outside of his office window and just grabbed a bunch of it and sent it to us. And that's one of our populations that we've been studying.

ALISON: JGI scientist Jeremy thinks he might actually have an infestation of fire moss. Though, it could be a close relative - he says they're hard to tell apart. He showed us his backyard on our video call.

JEREMY: Here you go. You can see It's invading and it's really kind of, really annoying.

ALISON: Massie, I thought it looked great. It added texture!

JEREMY: I think this is actually sporing here.. Like getting .. This is our sexy moss time here. And they're putting out their structures.

MASSIE: Sexy moss time!

ALISON: Yes, Massie. Jeremy's talking about the purple stalks with the horn-shaped capsules. Back to Sarah and Stuart. They're evolutionary biologists, and I asked Sarah why she chose to study mosses.

An unidentified moss proliferates among the grass in the Alabama yard of Jeremy Schmutz, head of JGI's Plant Program. (Alison Takemura, Zoom video call footage)

SARAH: People don't really think that much about plants. It kind of makes me care about it a little bit more. There's so much of an animal bias in the field we kind of work in, that we're the people who are like, no plants are cool, too. I think is what drives me to studying them.

STUART: Sarah's a ferocious defender of the underdog.

MASSIE: I gotta agree with Sarah; plants are cool! They can make their own food, they are food, and some are potential bioenergy sources.

ALISON: Yeah, and one way we can better understand how plants do all those things is by sequencing short plants, including fire moss.

Sarah and Stuart are especially interested in the fire moss' sex chromosomes. These are the DNA molecules that encode the sex-specific genes: If you're a male moss, you get one set of genes. If you're a female moss, you get these other genes. We don't know exactly what those genes will turn out to be, but we can expect to find genes that are related to the way mosses reproduce. For example, mosses send out mysterious volatile compounds to attract critters that are like pollinators.

MASSIE: The same way flowers do?

ALISON: Yes, really similar, only mosses don't have flowers, that's a separate group of plants called the angiosperms, and instead of attracting insects like bees, mosses attract -- shorter animals?

MASSIE: What?

ALISON: They're micro-arthropods. These wee animals with exoskeletons are the size of a pencil tip, and can be found trundling along in a moss. We're talking moss mites, springtails... tardigrades.

MASSIE: NO. Tardigrades as in the adorable water bears? I've seen those electron microscopy images!

ALISON: Me, too. We'll link them in the show notes.

So these micro-arthropods are the sex shuttles for the mosses. They can visit a male moss, get some sperm on them, and then take it over to a female moss.

MASSIE: Oh, sperm delivery!

ALISON: Yeah, and since it's analogous to the way flowering plants interact with pollinators maybe there's a shared genetic mechanism.

MASSIE: Ok, and you're saying that that could be encoded in the moss sex chromosomes?

ALISON: Yeah, and since it's analogous to the way flowering plants interact with pollinators maybe there's a shared genetic mechanism.

MASSIE: So Sarah and Stuart got the sequences?

ALISON: Eventually, but it was a rough road! As Stuart can attest to.

STUART: Part of the issue was I was extremely naive about how challenging sequencing a genome was, in particular, the sex chromosomes. And I had naive ideas, that using classical

genetics or relatively simple population genetic approaches that we would relatively easily be able to identify chunks of the genome that were sex-linked. And that turned out to be just far more challenging than I had anticipated.

MASSIE: Wow. Can we just stop here for a minute and just talk about Stuart's comments,

ALISON: Sure.

MASSIE: I mean, at the JGI, we now routinely generate billions of bases a day. And it feels like genome sequences are being announced every day. So sequencing just the sex chromosomes of a plant, a short plant, like Stuart said, should've been easy, right?

ALISON: Yeah, you'd think, Massie. But sex chromosomes, in general, are tough nuts to crack. They have lots of repeated sequences, which makes it confusing to figure out how to line up the pieces in the right order after you sequence them.

MASSIE: Ohhh.. So how did Sarah, Stuart, and the JGI get this done?

ALISON: Well, they needed time and perseverance. They started back in 2010, and they tried a litany of technologies. These included 454 sequencing, making a bacterial artificial chromosome library, and long-read sequencing with PacBio technology. But it wasn't until HiC sequencing came around that they were able to claim victory.

MASSIE: HiC? Like the drink?

ALISON: Yup, just like that: H-i-C. But this has nothing to do with the drink, I promise. It's just a really cool technology that lets you predict which pieces of DNA are near each other in physical space — like on the same chromosome.

MASSIE: Oh, OK and that would help you to order the pieces, even if they had repeats.

ALISON: Exactly. Using HiC technology, the sex chromosomes were finally sequenced. But before we get to what Sarah and Stuart found, let's just quickly reflect on how this kind of science gets done. It's a team effort, right? And Sarah and Stuart and their collaborators worked really closely with JGI.

STUART: Our experience with JGI has been fantastic. JGI said we would like to do this next thing that we think is, is gonna solve the problem and 'send us this kind of DNA.'

SARAH: I've been the person who's often growing the tissue or extracted the DNA or extracted the RNA and sent it to them. And and so what I did was I, I had these nucleotides and I send it to JGI. And what I'd get back is an extremely high quality resource to be able to address these, these questions that we have in our lab, they basically just magic together everything that I want to use to study the system...

ALISON: I love that. JGI magics everything together! It probably wasn't actually magic, though; Sarah and Stuart worked with Jeremy, JGI's Plant Program head.

STUART: Jeremy Schmutz has been a terrific resource. And very much a straight shooter. And I really appreciate that. He tells us that something's not gonna work. And then he makes things work that we didn't think would work.

ALISON: Sarah and Stuart also worked with a member of Jeremy's team, genomics researcher Jerry Jenkins.

STUART: And Jerry Jenkins has been a tremendous resource as well. And actually sort of a

secret advocate for us, I think, although I don't have confirmation of that.

MASSIE: Oooh, how mysterious!

ALISON: Oh, totally. So, JGI completed the sequencing and Sarah and Stuart found in the moss's sex chromosomes some nifty surprises. For example, the sex chromosomes were massive, which is part of what had made sequencing them so challenging.

SARAH: The sex chromosomes are a third of the genome!

ALISON: So are there only three chromosomes?

SARAH: No, there's 13. There's 13.

ALISON: So, do you mean that they're, they're just really big?

SARAH: Yeah.

ALISON: Wow.

SARAH: Yes.

ALISON: Crazy.

SARAH: Oh yeah, they're the biggest chromosomes in both males and females.

One work day morning, Sarah Carey inadvertently selected an outfit that really matched her data analysis of *Ceratodon purpureus* sex chromosomes (red and blue lines on the computer screen). (Leslie Kollar)

ALISON: Sarah and Stuart estimate each of the fire moss sex chromosomes has 3,400 genes. Animal Y chromosomes, on the other hand, have less than 100 genes! That 34x difference suggests that the moss' sex chromosomes could have a much bigger influence on their biology than we're used to.

MASSIE: So that 34x difference is unexpected.

ALISON: Yeah, right? And the sex chromosomes are also full of transposons or jumping DNA. These are stretches of DNA that can copy and insert themselves around the genome.

MASSIE: Does anyone know why there are so many transposons??

ALISON: Yeah, scientists think it's because of sex chromosome biology. Sex chromosomes don't have a partner chromosome to recombine with and shuffle the DNA around. For example, let's say you have a really helpful gene next to a transposon that's more like dead weight. Because you don't have recombination between chromosomes, you can't break up gene combinations like this..So the transposon gets to stay, just because it's cozied up next to this really helpful gene. That's how DNA can get clogged with repeats.

SARAH: And in fact, in our analyses, we found that there about 80% repeats on the sex chromosomes. And so that's one of the large reasons why it was so hard to sequence it without some of these longer read technologies, is lots and lots of repeats on there.

ALISON: Now, Sarah, Stuart, and their team can compare the fire moss genome to the sequenced genomes of other plants — and thus, refine our understanding of how plants have evolved.

SARAH: We're taking a snapshot and making these comparisons of effectively what's happened in the past. We don't work on kind of making evolution happen within the lab, we look at what has happened from nature, these are all collected from the wild, and we see what has driven changes in the genome.

ALISON: Yeah, I hadn't realized how similar it is to archaeology and paleontology, you know, you have these snapshots and then you try to infer this rich past.

SARAH: Oh yeah, that's entirely true. Yeah. So in evolution.. we would from like a phylogenetic perspective, we can see when something arose. So when did, when did mosses arrive on land? When did we start having flowers instead? And when did the volatiles or the odors in mosses arrive to attract micro arthropods? And so yeah, it's a snapshot back into the past.

MASSIE: It's almost like Sarah and Stuart are history detectives. They're looking for clues that made the genome what it is now. So what are they finding?

SARAH: We've been finding that moss sex chromosomes are really, really old. The oldest genes that we have suggest at least 300 million years ago, these arose in mosses, which is — it's really cool.

ALISON: For comparison, Massie, that means fire moss's sex chromosomes are 290 million

years older than the oldest sex chromosomes in flowering plants. Mosses have had so much longer to accumulate genes that help them reproduce. And insights from those genes could ultimately help scientists improve plant breeding programs. Of course, before we get there, there's still a crucial next step.

SARAH: And now that we have this awesome genomic research ... the next thing is to actually see what these things do. So even if genes are annotated, in a lot of cases, we don't actually know what they do. If we take this gene and we knock it out of the species, what happens? And in particular on the evolution of the sex chromosomes and seeing, what happens if I take it out, or if I swap it, this gene copy from males to females.

The sequencing kind of opens up the ability to then start doing that functional work, right? What do these genes really do?

ALISON: One of those "functions" or attributes that Sarah and Stuart will be looking at more closely is that perfume that fire moss uses to attract micro-arthropods — like we talked about earlier. And maybe uncovering those genes will shed light on the sex lives of other plants.

MASSIE: Yeah, Alison. And all of data are publicly available for free on our plant portal, Phytozome. And then other researchers around the world can look at this fire moss genome and compare it to their genomes of interest. And maybe, Alison, just maybe, they can uncover genes to improve bioenergy feedstocks.

ALISON: Yeah! I think it's amazing that deciphering a tiny genetic blueprint in one very short plant can have an impact on our understanding of the way all plants work.

This episode was directed by me, Alison Takemura.

MASSIE: With production help from me, Massie Ballon.

ALISON: Thanks Massie!

MASSIE: David Gilbert helped edit the show.

ALISON: Genome Insider is a production of the Joint Genome Institute, a user facility of the US Department of Energy Office of Science, and we're located at Lawrence Berkeley National Lab in beautiful Berkeley, California.

MASSIE: Thanks to our guests Sarah Carey, Stuart McDaniel, and Jeremy Schmutz for sharing their research.

ALISON: If you enjoyed the podcast and want to help others find us, leave us a review on Apple Podcasts, Google Podcasts, or wherever you get your podcasts. If you have a question or want to give us feedback, Tweet us @JGI, or record a voice memo and email us at jgi dash comms at L-B-L.gov. That's jgi dash c-o-m-m-s at l-b-l dot g-o-v.

MASSIE: Check out our website for a transcript of this episode, and a screenshot of Jeremy's sexy backyard moss.

ALISON: And because we're a user facility, if you're interested in partnering with us, we want to hear from you! We have projects in genome sequencing, synthesis, transcriptomics, metabolomics, and natural products in plants, fungi, algae, and microorganisms. If you want to collaborate, let us know!

MASSIE: Find out more at jgi.doe.gov forward slash user dash programs.

ALISON: And if you're interested in hearing about cutting edge research in secondary metabolites, also known as natural products, then check out JGI's other podcast, Natural Prodcast. It's hosted by Dan Udvary and me. That's it for now. See you next time!