CUSTOMER SPOTLIGHT

Exploring Redwood Genomic Complexity Through Custom NGS Panels

Redwood Genomics Spotlight with Sasha Nikolaeva and Lydia Smith

Researchers studying organisms that are not traditional model systems face an uphill battle. These organisms often lack the specially designed research foundation, tools, and reagents that improve the efficiency and quality of experiments in species like humans and rodents. Still, studies into diverse organisms can have far-reaching impacts, thanks to the intrepid researchers willing to blaze new trails despite less infrastructure, commercial products, and funding.

In this interview, Twist chatted with **Alexandra "Sasha" Nikolaeva** (SN) and **Lydia Smith** (LS), two researchers studying one such organism: the coast redwood tree. Sasha (the graduate student leading the project) and Lydia (the Evolutionary Genetics Lab Manager) are part of a research team at UC Berkeley studying the world's tallest tree species. Together, they have applied a custom NGS panel and target enrichment reagents from Twist to explore the connection between the redwood genome, niche survival, and climate change.

TWIST BIOSCIENCE

Tell us how you got into your current research.

SN: I would say it was a lucky coincidence. It's a surprising story even to me because I never thought I was going to end up studying redwoods or even doing science. Originally, I was trained as a journalist and wrote about the environment. My entry into environmental journalism began when I covered forest fires in Russia. In 2010, Russia had absolutely massive fires, and from that beat, I got really interested in forestry.

When I came to the US, I wanted to know more, so I started as a master's student in forestry. I was interested in forest management, timber flows from Russia to China to the US, and forest certification.

Since I started as a student late in the academic year, all the graduate student instructor positions were taken except one, and I really needed one to support myself in my program. The only available position was for a genetics class taught by Richard Dodd, who's now one of my current advisors. I jumped at the opportunity, even though I didn't even know what the word “allele” meant at that point.

Through this very lucky coincidence and working with Richard, I learned about redwood genetics. I had never heard of a plant that was so complicated and interesting. As a journalist, I knew right away that it was a cool story.

Tell us a bit about redwood trees. What makes them so fascinating?

SN: If you talk to any plant scientist, silviculturist, or ecologist, they'll tell you how ecosystems work and then say, "Well, there's an exception." Redwoods are often that exception, both genetically and ecologically. They're like living dinosaurs.

For one, redwoods are only found in a narrow strip of the California coast and they actually depend on fog as a key water source. One of the reasons the community thinks they can survive in that region is their genome’s unique structure. Redwoods are polyploid conifers with six sets of chromosomes. Though polyploidy is not uncommon in plants, it is very rare for conifers, which are mostly diploid. Moreover, redwoods are largely a clonal species because it is very difficult for them to reproduce by seeds.

Redwood is also a great timber species as long as you manage it correctly since it grows fairly fast. That said, there are questions about how to best manage it, and that's where much of the conversation and research is focused right now.

What are the primary research questions you and your team are seeking to answer?

SN: I was really interested in polyploidy, which is a heritable condition where there are more than two chromosomes in a homologous set, and how polyploid plants survive, given the looming threat of climate change. How are redwoods (and other polyploid plants) going to do when faced with higher temperatures? Are they going to adapt? And what will this adaptation look like?

As a new population geneticist, I’ve learned to start with population structure as the first question: What is the species' genetic diversity? Beyond that, I was fascinated by and focused on whether or not there are ploidy differences across the range. Though we are still investigating ploidy variation, we did answer another related question that will be the focus of the study we plan to publish soon: Are there aneuploidy differences across the redwood range?

Aneuploidy describes a change in the number of total chromosomes within a genome. Occasionally, there will be a change where a chromosome is lost or added, leading to either seven or five copies of that chromosome in a redwood. However, it's a lot harder to study aneuploidy in polyploids compared to diploid organisms.

However, we were able to develop a method capable of resolving aneuploidy, and what we found was quite surprising. We saw that there is a lot of aneuploidy in second-growth redwoods. We found that ~6% of second-growth trees were aneuploids bearing an extra chromosome.

And, it's worth mentioning that we did have one plant in the second growth data set that was missing a chromosome. My colleague, Zane Moore, found a wonderful clonal tree by looking at satellite imagery. Zane was really interested in it because it might actually be the biggest clonal plant. That clone has a missing copy of chromosome four, and every tree sampled in that clone was also missing that chromosome.

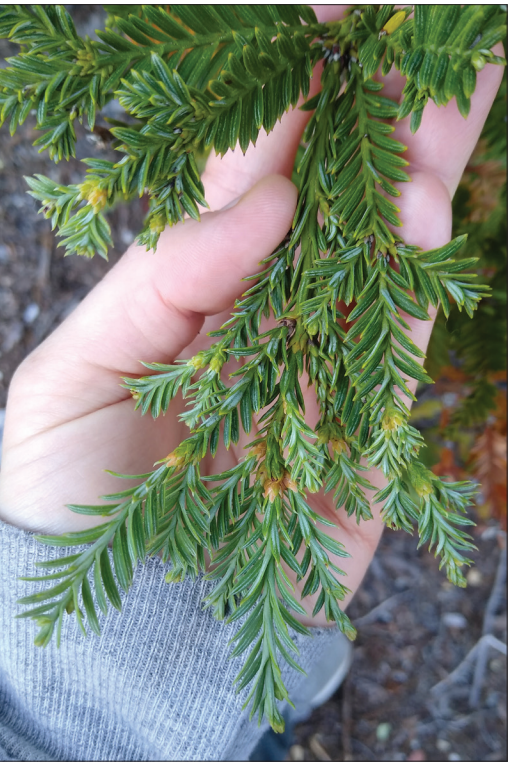
We also explored a second data set from redwood genomes sequenced from tissue culture. These are not seed plants but trees that had been vegetatively propagated from planted cuttings. In those samples, we also found a lot of aneuploids with missing chromosomes.

In contrast, not a single old-growth tree in our dataset was aneuploid. That was a very interesting finding because it suggests that there may be a selection mechanism influencing the occurrence of aneuploidy.

It seems the implication of your work is that there may be a relationship between chromosome numbers and redwood niche survival. What advantages and disadvantages does polyploidy provide redwoods and other plants?

SN: There's a great paper called Advantages and Disadvantages of Polyploidy from Luca Comai at UC Davis!

The biggest advantages are that polyploids can grow fast and may be able to occupy new niches. A lot of polyploids are found in areas where there's some climatic change, and we believe that having more genetic material to choose from is a way for plants to adapt. If there's a beneficial mutation, they can use that. If there is a detrimental mutation, there are still a number of functioning gene copies they can use.



The major disadvantage is that these plants require a lot more nutrients. Polyploidy can also slow evolution down because they often struggle to reproduce sexually, which is a big problem for generating diversity.Polyploid plants also have larger cells, including guard cells, which control evapotranspiration. These cells are basically the plants' breathing elements, helping to capture CO2 for food synthesis. In redwoods, these cells are larger than the guard cells of its closest diploid relative, giant sequoia.

We know that redwoods can absorb water through their leaves. One of the theories is that's why they can grow so tall because they don't have to pull all that water from the ground. They can reliably absorb it from fog because they're found in a major fog belt. With large guard cells and stomata, a plant can absorb more water through its leaves. However, when there is a drought event or when there's no fog, you're also losing a lot of water through these big cells.

I am personally concerned that if we have more drought events, this will be a big problem for polyploid species. It's not just about redwoods. I don't know a crop that is not polyploid. Everything that we eat is polyploid because it makes for a bigger plant.

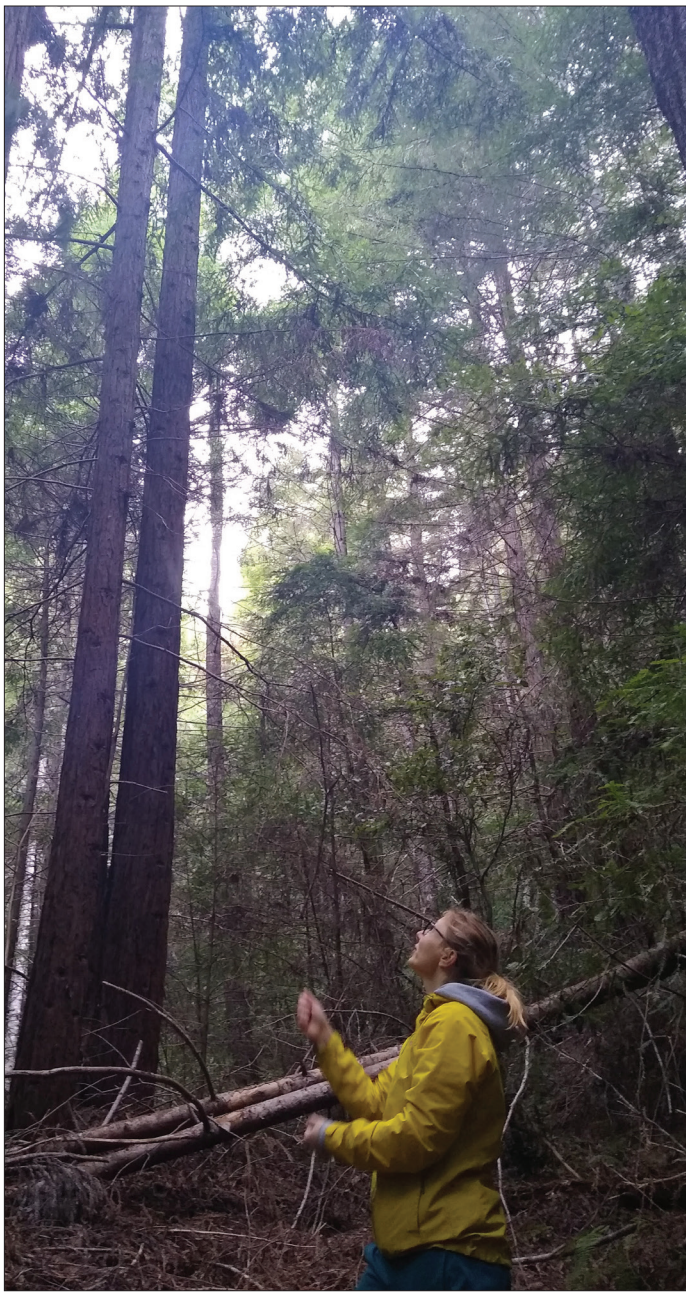
What are some potential follow-up studies and future directions for this research program?

SN: I'm looking at Lydia right now because I will need a lot of help. Lydia is awesome. I think she single-handedly saved my study.

LS: It was a team project, but you were the leader!

SN: What's next is definitely a better genome. We need to have a chromosome-resolved genome to be able to understand how redwood's structure is changing. It's necessary for everything: phylogenetic, selection, and association studies (including GWAS). Name any study, and you need a better genome. So that's the first very big and major step, which is going to be challenging.

LS: Folks at Twist can imagine the cost and complications of sequencing the redwood genome. Obviously, it's a lot less expensive today than it was even five years ago, but it's still an endeavor that needs funding.



To put this into context, I work at the Museum of Vertebrate Zoology, where we study salamanders, including many that live in redwood forests and even in the crowns of the redwood trees. Though salamander genomes can also get quite huge and can be challenging to sequence, they do not have this polyploidy complication like redwoods.

But similarly, they are also non-model organisms. So, despite this whole genomics revolution, we're still kind of grasping in the dark with a lot of our salamander species because we don't have the funding to get a chromosome-level reference genome that's 30 or 50 gigabase pairs (Gbps) or more in size. Discounting the complications of polyploidy, a genome that size requires much more data and coverage as well as longer reads to resolve.

SN: The redwood genome is 51 Gbp. So you can imagine that re-sequencing and haplotyping is a very, very big challenge. For exome sequencing in this project, we only sequenced about 17.5 megabase pairs.

What led you to start using Twist's custom NGS panels and target enrichment reagents? How did Twist support the progress of your research?

SN: Lydia told me about Twist, and that was really a big game-changer for us because we didn't think we were going to be able to do this using exome sequencing because of the associated costs. We thought we would have to go with restriction site-associated DNA sequencing (RADSeq). But then my advisor, Rasmus Nielsen, strongly suggested exome capture and gave us additional funding. Plus, you guys did us a huge favor providing a discount that helped reduced our costs. That was enormously helpful because as you can imagine, grad students are often doing research on a shoestring budget.

How do you describe your experience working and communicating with the Twist team? How was your experience building a custom panel with us?

SN: In terms of working with the team, that was extremely efficient. Twist's bioinformatician, Owen [Hardy], also did us a lot of favors throughout the process.

Basically, I sent him the annotation, the sequences that were already available, and the criteria. Then I said, "Well, we have this much money, and we need to sequence the most conserved regions because these will be easier to sequence and are probably going through a lot of selection." And he just did it for us!

Knowing what I know now, I could probably do it, but I was a very, very green researcher at the time, and he just helped a lot. So that was super important for getting the project moving. From there, we talked about a lot of other experimental details of interest like, "can we do chloroplasts?" and so on. Owen walked with me through the entire process and replied to countless emails. I cannot tell you how many emails I sent him!

Everything else was very smooth. Shipping was very fast. I think the capture itself was a major success from the lab's point of view. 80% of our Illumina reads mapped to the probe sequences, and due to this we ended up with sufficient coverage while spending less money on sequencing lanes than we'd budgeted.

LS: From Sasha's preliminary mapping, I was floored by the on-target percentage, or the specificity of the capture. Our lab works with a lot of non-model organisms, and we're used to seeing maybe 40% or 50% of sequenced reads on-target in larger genomed organisms, using probes synthesized by other vendors. When you have a large genome and a relatively small target, having that many of the reads actually map back to the probes was very impressive.

Plus, it's true if Sasha had collected RADSeq data, she would have a big data set that she'd be tearing her hair out trying to analyze. Though she would have publishable data from it, being able to get actual target captured sequences makes a huge difference in the rigor of the research, the statistics that can be applied, and the number of directions the data can be taken in later.

> **"It was great working with Sasha and Lydia on this exciting project! Given that Redwoods have a huge genome with incomplete annotation it is a challenge to create a high performing custom panel. I'm thrilled I was able to help them focus on the best targets and achieve remarkable results in the first pass."**
>
> – Owen Hardy, Twist Bioscience

As Sasha said, we also appreciate that Twist is willing to work with researchers at their budget levels. The vast majority of our graduate student researchers have to get their own funding together. And there aren't as many funding sources as there used to be, so they're often cobbling together these small pockets of money. Every little bit of discount we can get really makes this work possible.

Lastly, I just want to put in an acknowledgement of the support of Twist in general. When non-model-organism and custom probe design started being available through Twist to our researchers, we very much appreciated it. Meanwhile, other companies were cutting costs and focusing more on fitting everything into one box rather than working with customers to devise custom probes that really fit their highly specific research needs. Twist bucked the trend. So, props to Twist for moving in this direction and being aware of the need for custom probes.

Thank you to Sasha and Lydia for discussing their research and redwood fascination with us.

Note: this interview was edited for length and clarity.

About

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Want to schedule 30 minutes with an NGS expert who can help jump start your unique custom panel project?
Schedule a meeting and select Scientific Discovery Call.
Schedule a meeting here