Transcript: Plant Breeding Stories Podcast
S2E8 Plant Breeding Stories - Dr Paul Matthews and Dr Katherine Easterling, Hopsteiner

[Theme music plays]

Hannah Senior: Welcome to this episode of the Plant Breeding Stories Podcast, where I talk to leading lights in plant breeding, asking what they do, what makes them tick and what fascinates them about the world of plants. I'm your host Hannah Senior of PBS International, world leaders in pollination control. We design and produce specialist pollination bags and tents used by plant breeders and seed producers all around the world. And through this, I've been privileged to get a unique perspective on how plant breeding globally affects our diets, farming systems and the environment. I'm excited to share a little of this with you as we meet some of the amazing people who make plant breeding their life's work.

Hannah Senior: In this episode, the last in the second series of Plant Breeding Stories, we're raising a glass to the world of hops, a key ingredient in brewing. I'm talking to Dr. Paul Matthews, and Dr. Katherine Easterling, both of whom work for Hopsteiner, a major international breeder, trader and grower of hops. In this episode we learn about hops 101. How it grows and what it's used for, before diving into some more recently discovered aspects of it's biology and why it's so darned complex when it comes to patterns of inheritance. The conversation also goes from Kazakhstan to the Sky Islands of Arizona in pursuit of wild hops and genetic diversity and touches on the many opportunities to use the species' complex phytochemistry. I hope you enjoy it.

Hannah Senior: Thank you for joining me today, Dr. Paul Matthews, Senior Research Scientist, and Dr. Katherine Easterling, Lead Research Scientist with Hopsteiner. So a good place to start is always to just ask you to introduce yourself. So perhaps Catherine, let's start with you. Could you introduce yourself?

Dr Katherine Easterling: I'm Katherine Easterling, I'm currently in Tallahassee, Florida. I finished my PhD at Florida State University about a year and a half ago and I'll be joining the team out in Yakima very soon.

Dr Paul Matthews: Hi, yes, I'm Paul Matthews. I'm the founder of the molecular breeding program at Hopsteiner. I've been here for about 20 years. I'm out in Yakima, Washington. But Hopsteiner is global; we have breeding offshoots in various other parts of the globe.

Hannah Senior: One of the things I'm always curious about is how you came to be in plant breeding. So perhaps you could tell me a little bit about your background, maybe where you grew up or went to school or what your early interest was in plants and what the journey was that led you into plant breeding?

Dr Katherine Easterling: I actually came to plant breeding very late in life. I was a musician and a ballet dancer as a child, and then wound up going to music school my first go round in college and nothing kind of stuck. But when I was younger, I loved the outdoors. I spent a lot of time in the forest and so plants were just kind of a personal interest for me for a long time. And then when I returned to college years and years later in my early thirties, I came back actually for nursing school and kept going in the direction of biological sciences. I just found that my questions, there were more questions and answers and so I kept going and going. And joined Hank Bass's lab and he studied corn. I then got into cytogenetics - so that's microscopies. So I worked a lot on the microscope and with DNA, So I did some research in the maize community and then Hopsteiner eventually reached out to us to do some collaborative work with them. And so I sort of fell into plant breeding, just out of sort of a natural curiosity for plants.

Hannah Senior: That's brilliant. It's always good to have a roundabout story that gets you in because you bring all those background experiences in with you.

Dr Katherine Easterling: It's been an odd road!

[Katherine and Hannah laugh]

Hannah Senior: And Paul, tell me about your backstory.

Dr Paul Matthews: I was born a biologist I think - to a biology family. Both my parents were bacteriologists and med techs. And so I sort of grew up in a laboratory. And then when I went to college I went pre-med just because it offered some advantages. But I became interested in evolutionary biology. And then as I finished college, decided I wanted to go to the New York botanical garden and followed that interest. I was drawn into the more functional side of molecular biology. So I did a PhD with Eleanore Wurtzel and I worked on the golden rice project where we looked at the functional biochemistry - basically gene isolation and characterization - figuring out the metabolic pathway in both rice and maize with with the goal of engineering rice that's got beta carotene content.

Hannah Senior: Oh wow, great. And I know that from there you dug further into metabolic engineering and took a postdoc at the University of Minnesota, and then Hopsteiner recruited you soon after. Was hops something you were specifically interested in?

Dr Paul Matthews: I didn't really know what hops were at the time as a plant. But they flew me out and I saw this wonderful, beautiful plant, 18 foot tall, and importantly for me a twining plant. I was interested in peas at the time and thigmotropism, and hops is not a thigmotrope, but it's a twining vine. And so I was just really intrigued by the vining habit. And so I decided to join the nascent breeding team out at Hopsteiner, really on a whim. I just thought hops were cool. And then I had also had a lot of experience in building laboratories and putting systems together and seemed to have the right tools from my previous experience. So I came out and started up a molecular biology based breeding program.

Hannah Senior: And you said something there, which I think is also a good jumping off point. You said, "At the time I didn't really know very much about hops." So let's assume that some of our listeners don't know very much about hops. Why don't you just tell us a little bit about what are hops? What are they used for, where do they grow and so on?

Dr Paul Matthews: Hops are an herbaceous vining plant they're dioecious, and they're grown as a specialty crop as a horticultural crop really. And the product of hops is the hop cone. And the hop cone is a leafy organ that is used in the brewing process in flavoring beer and also originally imparting antimicrobial properties to beer and the curing of fermentations. As an agricultural plant, it has limited economics. There's only a few hundred thousand acres worldwide but it has a big sociological and a big impact, and people are enamored with hops because of its relation to beer. Hops also has some biomedical properties and some compounds, and that's what drew me to really also study hops. The fact that it makes an anticancer compound called Xanthohumol. That is also related to a compound in hops that is a phytoestrogen. And in fact, it's the most powerful phytoestrogen known to man.

Dr Paul Matthews: And so it offered to me this idea that hops could be improved for medical benefit. Also, one of the medical benefits of phytoestrogens is post menopausal hormone replacement therapy. So hops has this combination of interesting values, both in, perhaps, world health, but also health in beer and flavoring of beer and then all of the popular culture that goes along with that. Hops is a dioecious plant, so male and female obligate outcrosser, it's not self-able. So this also makes it a sort of different breeding system.

Hannah Senior: And hops is in the same family as the cannabis plant - which is also very phytochemically rich isn’t it?

Dr Paul Matthews: Both hops and cannabis are in the family *Cannabaceae* and there's a couple of other species, but there's not too many members of the *Cannabaceae.* They have strikingly similar biochemistries in that they both make terpene phenolics and tetrahydrocannabinol as a terpene phenolic - as is humulone, which is the bittering substance in beer. So the same class of compounds are also what these plants are prized for. And so, because they're in the same family, because the biochemistry that we're interested in... Also the flavor biochemistry, the terpene biochemistry is very similar and terpenes are used to flavor beer. And are part of the hop contribution to the flavor of beer. And then in cannabis, marijuana users are very fond of their many flavorful varieties. And so there's just these great overlapping chemistries and use in flavour in both species.

Hannah Senior: Why breeding hops is difficult. I understand Katherine, you've made some really interesting discoveries in this area recently so can you tell me about that?

Dr Katherine Easterling: Normally when you're breeding plants, you have this predictable outcome. Like with corn you can make a cross with one plant to another plant and you have predictable progeny, predictable phenotypes, predictable flavor, predictable appearance. With hops that's just not the case. Not only that but hops has a very long generation time. So two years to flower, which creates another challenge. What we've been finding with hops is that the structure of the genome isn't always predictable. So we think that that is the underlying reason that we have these non-Mendelian or unpredictable inheritance patterns in hops which is for us one thing that makes it really fun to study, but also makes it quite a bit of a challenge.

Hannah Senior: So can you just expand on that for me? Give me an example of how that shows up.

Dr Katherine Easterling: So for example, one route to non-Mendelian inheritance patterns is what happens during meiosis. Normally in meiosis, you have parental chromosomes that have to pair and segregate. So meiosis is of course the sexually reproductive cycle that produce sperm and eggs, in all sexually reproducing organisms. So that also happens in plants. What we see during meiosis and hop plants is that the progeny or the daughter cells that come out of these myotic cycles don't have balanced genetic loci. So you'll have the daughter cells that come out of one round of meiosis appear different cytologically. And the way that translates to genotype or phenotype or flavor profiles even is that you'll have different numbers of genetic loci maybe that comes out in the daughter cells. It's this really interesting pattern that is often not balanced, and we believe could be one of the reasons for other issues in hops like low germination rates or non-Mendelian segregation of sex ratios - say we have a lot more females than males in hop plants and that's also an interesting. That's probably the most obvious non-Mendelian inheritance pattern that we see and hops.

Hannah Senior: How did you identify this?

Dr Paul Matthews: We spent quite a few years developing molecular markers and hops in various systems. We tried what was available at the time. We worked on SSRS, and other PCR based markers. But with the advent of next generation sequencing, we started producing much larger numbers of markers. We noticed that most of the markers were in segregation distortion patterns. There was hints of this when we had lower marker density, and many people thought this was because of the ascertainment bias and problems in the production of the molecular markers, or the method of making and looking at molecular markers. But I was a firm believer that there was some biological underpinning.

Dr Paul Matthews: So I took this problem, having a background in maize genetics and having studied Barbara McClintock's work, and I had in mind her papers where she showed aberrations in meiosis in maize. And I took these problems to Edward Buckler's lab at Cornell, and he had also had experience and published on maize myotic abnormalities. And so we studied the situation in the markers with Dong Zhang at Cornell for a number of years and developed this hypothesis that meiosis was anomalous and the products of the myotic segregation were also anomalous. And we started to think there's translocation phenomenon. In fact, translocations that may lead to permanent translocation heterozygosity, which could be a mode of evolution, but also limit the recombination that generates diversity among hop families. And so Katherine seemed to have the tools that could bring the physical reality to what we had discovered in molecular markers. And so Katherine really proved the physical reality of what we saw in statistical analysis of molecular markers.

Hannah Senior: So the markers you were identifying weren't showing up in the ratios that you were expecting, and then in order to understand why that is, you dug in and found that there as this anomalous miosis going on, plus the translocations - so in nutshell we are facing really complex patterns of inheritance when it comes to hops. Is that just in hops or are there any other species that do this?

Dr Katherine Easterling: Primrose is probably the most famous one. It occurs in lots of different plants, but especially given the diecious and out-crossing nature of hops. I think that it exacerbates the situation of genomic complexity or structural variability. But Primrose has been the plant that a lot of the literature points to as seeing this kind of thing, translocation heterozygosity, permanent translocation heterozygosity where instead of each homologous chromosome from both parents coming together individually and paring you've got, sometimes, the entire nucleus of chromosomes coming together in a single complex. And so there's a whole spectrum of the way that chromosomes can pair and then segregate and hops has a pattern that we haven't yet figured out! So it's sort of somewhere in that spectrum and it could be different from plant family to plant family. It's still a little bit mysterious, but what we do know is that that myotic inheritance pattern is not normal.

Dr Paul Matthews: Because hops are known to be the product in part of an interspecific hybridization, a couple of the subspecies or species of hops - a wild hop from Canada was used back in the day. We thought the phenomenon might be an aspect of interspecific hybridization, but we also collect wild hops as part of a far ranging conservation biology program and a will to intergress wild species of hops into the domestic breeding program. Katherine looked at wild hops that she had collected and found that the wild hop showed the same phenomenon. And these hops are 10,000 feet or so in the mountains, in isolated places so they were certainly not interspecific hybridizations. And so Katherine showed that it's a general phenomenon, and we don't know if it's true in cannabis.

Hannah Senior: Does it provide an evolutionary advantage? How does this come about or why does it persist? Maybe, maybe we don't know yet? [Hannah laughs]

Dr Katherine Easterling: I'll try to answer this, I'm not an evolutionary biologist, but they're people that I've spoken with and I asked this question. There can be an advantage in that if a hop shows up in a new place, there could be a phenotype that works for that area, that gets then fixed in that population, in that it can find its own niche. The fact that hops are wind pollinated and obligate out crossers, there's a whole lot of opportunity for the genome to become new. And so then in new places, even if there are low germination rates, there's a high potential for a new phenotype that does well in that area.

Dr Paul Matthews: Hops is also a perennial plant. In agriculture it's usually asexually produced, and so fields of hops are clones. But we think perhaps the permanent translocation heterozygosity could be related to the perennial habit. And is in fact, has some function as an alternate mode of evolution.

[Theme music plays]

Hannah Senior: You're listening to Plant Breeding Stories brought to you by PBS International, world leaders in pollination control. We're exploring the personal stories behind people who've dedicated their careers to plant breeding, helping us to more productive plants, greater food security, and more sustainable agriculture. Now back to the podcast.

[Theme music fades]

Hannah Senior: You mentioned earlier that Hops is in the same family as the cannabis plant and in recent years with the cannabis boom there has been a huge amount of resources, time and money spent on cannabis breeding. Has the hops industry seen any benefits from all this research?

Dr Paul Matthews: Because hop and hemp are fortunate to serve the same family and they share, although there's some evolutionary distance between them, many aspects of their genomes. There's a huge opportunity now to do comparative genomics in hop and hemp and use one organism to help crop improvement in the other organism. For example, hemp has a simpler genome, it has some good published reference genomes, and it has a much shorter generation time. And so progress that may be made with the huge interest in hemp breeding now is perhaps progress that can be ported over and used in hop. So discoveries in hemp can condition discovery and applications in hop.

Hopsteiner fosters a comparative genomics project called PANCAN, or Pan-Cannabaceae in cooperation with John McKay at Colorado State University.

Hannah Senior: Is that something unique to the *cannabaceae* species?

Dr Paul Matthews: No. The idea of pan-genomics, right? Now people are doing pan-genomics, which are full, whole genome sequences of many members of the same species, but also the related species. For example, in *brassica* there's a lot of work on pan-genomics. And so, yeah this is a common perspective where you take an evolutionary comparative approach to genomics and use the genomics of related species to inform the genomics of your favorite organisms.

Hannah Senior: You just touched on wild varieties - and I don't have a good sense or a good mental image of how far the cultivated varieties have deviated from wild varieties. Are they as far apart as teosinte and corn or are they actually still very, very recognizable as being closely related?

Dr Katherine Easterling: It's my understanding that they're pretty similar in that the hops that are commercially used are very closely related to the European hops. I think that there is a difference in flavor profiles and disease resistance and things like that, but not anywhere near the difference of say teosinte and common corn and that we eat no. That's a little bit...Wouldn't you say, Paul? Closer than that?

Dr Paul Matthews: I feel that many domesticated species have a narrow bottleneck in domestication. Most of the hops are German landraces, or they're from Serbo-Croatia, or Russia. And so they certainly have a Eurasian origin. When pioneering explorers like Ernest Small discovered and characterized wild hops in North America some of the breeders, particularly in Wye College in England, did interspecific crosses to North American *Humulus neomexicanus* to the *Humulus lupulus* species.

Hannah Senior: Ooh I know about that! PBS International supplied the pollination bags!

Yes so that diversification of the germplasm is thought to have led to an increase in chemical content and it's responsible for the high alpha or the high humalone content and hops. And also vigor and other aspects of the American hop. But I think hops have gone through a domestication bottleneck and Hopsteiner is very interested and very active in systematically and carefully collecting hops worldwide, both in Eurasia, particularly in the caucuses, but also throughout the range of *Humulus neomexicanus* in the Rocky mountains. And then in the Southwest. From Canada down to Mexico, there's hops in the mountains.

Hannah Senior: My understanding is that one of the reasons for that, in addition to the benefits having greater diversity available for developing different sensory properties, is that it gives you greater capacity to deal with climate change. Perhaps you could just touch on that because I know that climate change is particularly conspicuous in Washington State, where Hopsteiner's Yakima site is?

Dr Paul Matthews: So we looked to introgression of wild hop germplasm from various regions for various reasons. We look to hops in Georgia and Kazakhstan, for example, for new sources of disease resistance. Hop is susceptible to powdery and downy mildews and we believe the Eurasian *Humulus lupulus* has resistances and new resistances from the wet growing regions of Eurasia. And so we look for that trait. With regard to climate change, we purposely sought the Southern range of *Humulus neomexicanus,* in the sky islands of Arizona near the Tucson area. And we collected across the Sky Islands because they're at the Southern end of the range, thinking these hops would afford drought stress resistance. Also *neomexicanus* has a really amazing and drastically different leaf form. It has very long pinnate leaves more like marijuana, which is thought to be a response to heat and convective cooling of leaves and an adaptation to dry and hot climates. And so we hope these traits when we bring them into the domestic germplasm will help mitigate changes in climate.

Hannah Senior: So what I'm now getting a picture of is that there is complexity and opportunity in terms of lots of different wild types that can be introgressed or properties could be derived from plus there's this genetic complexity. And then in addition to that, there is also the influence of terroir with hops. Is that correct? So tell me a little bit about how that overlays on top of all those other characteristics when you're breeding for particular sensory properties - for brewing?

Dr Paul Matthews: So in hops, we've experienced aspects of terroir. Terroir is the location, terroir means location, and includes the soil microbiome. Includes the other crops that are grown nearby, includes the soil content and then many other factors. And in fact, by looking at the flavor chemistry of hops... Anecdotally, hops grown in different locations, hops grown in Northern Idaho versus Oregon versus Yakima Valley have their particular flavors and brewers are attuned to the particular flavors of hops. For example, Cascade is the most commonly grown American variety and brewers are very careful to choose hops from Oregon from the Willamette valley or from Washington from the Yakima valley, and have their preferences. Chemically we've looked at hops in a formal experiment and we detected chemical differences in hops with aroma glycosides. Aroma glycosides are flavors that are commonly known to be a component of terroir in wine.

Dr Paul Matthews: So we specifically looked at aroma glycosides in hops and found most of the aroma glycosides in hops grown in replicant blocks in the Kootenay valley up near the Canadian border versus the Yakima valley were very similar and across genotypes follow the same pattern. And so most of the aroma glycosides we looked at were genetic, but one of them was very different and seven to eight times higher in Yakima valley. And this chemical component was hexanol glucoside. And we believe perhaps that this...Our working hypothesis now is that this is an aspect of the insect pressure, namely spider mites and aphids in Yakima, which is dry and arid and has greater infestations than the Northern climate growing regions

Hannah Senior: So a really interesting interplay of biology there! That's fascinating! You touched on, earlier, some conservation work that Hopsteiner is involved with - can you tell me more about that?

Dr Paul Matthews: Part of our conservation program for hop conservation biology is to preserve in-situ a hop species that may be in danger due to agroforestry, due to human encroachment on habitat. Hops seem to be quite particular and quite rare. It's a riparian species. It lives near streams. It lives in watersheds. Some of the ecology of hops is being destroyed. So hour hop conservation biology project, we always work with ecologists and botanists at local institutions, wherever we are, whether it's Kazakhstan, or Georgia or the United States. We work under a set of principles and policy. We have conservation ethics. We're very careful about collection. And while we want these hops for breeding in the Hopseteiner program, also we collect under the auspices of, for example, the National Forest Service and the hops that we collect are destined for public repositories. For example, the national germplasm repository of the United States or Genbank in Europe.

Hannah Senior: You both sort of took different routes into the hops world and I'm curious whether there's anything that you have brought from your former life that you have found particularly useful, perhaps in the way that you do things or the way that sheds light on the way the hops world works.

Dr Katherine Easterling: Having an artistic background and being able to observe and have a really curious approach to science, and also the ability to be creative in thinking about science. Like an approach to science that is not so linear in thinking, a way to consider all kinds of possibilities and sort of think outside the box to answer questions I think has actually really helped my approach to science. Having my background in science as a cytogeneticist and having this whole genome visualization approach has been really interesting. And now being able to dive into the specific genes and things like that has given me a perspective that I think a lot of bioinformaticians or genetics people don't really have. So I feel like I had this way of visualizing what might be going on on a more holistic level than on an individual gene level that I think helps the team sort of understand things that way.

Dr Paul Matthews: I think my early interest in evolutionary biology and the stuff I read as a teenager really had a big impact on me. And then I came into an era of more reductionist, functional genomics and metabolic engineering, and the idea that crop systems and plants were engineerable. So evolution and applied evolution or crop development and variety development are similar, right? They're all evolution or co-evolution. So I think this having an evolutionary perspective, having worked with genes and functional biochemistry, has given me sort of an evolutionary perspective on applied crop evolution. And it comes together. And then finally, with my family really having worked in medicine and my early interest in medicine, and having worked on the golden rice project, which is a world health initiative, I see the same things in many crops. All food, all flavor crops and chemical crops like cannabis and hop have an impact on world health. Hop is a perfect residence for somebody who has an evolutionary point of view, but wants to do applied engineering work.

Hannah Senior: Whats next? There is some really interesting stuff going on in your research and in Hopsteiner more generally, and in the hops world more generally. So where do you see the big opportunities or the interesting opportunities for the future? Maybe, Catherine, let's start with you?

Dr Katherine Easterling: What we're really excited about getting into now is mutation breeding. So the reason that we know so much about the corn genome is because of all of the mutation breeding that was done. So if we can make general mutations throughout the genome of hop and then study what they do as this functional genetics program, we'll have better answers to how the biosynthetic pathway works and how to improve on the phenotype of hops. And that's what I'll be doing when I get out to Yakima. And I'm really excited about it.

Dr Paul Matthews: I think mutation breeding in hops now is predisposed by the fact that there is a whole genome sequence that has been published by the USDA. And this can be used as a utility to examine and understand the structure of genes and gene families involved in the flavor compounds and then other pathways that are relevant to hop breeding. So a combination of a full set of genes and the ability to manipulate hops and tissue culture and the advent of next generation sequencing can allow us to do targeted mutation breeding or a version of next generation sequencing, assisted tilling if you will.

Hannah Senior: And wrapping things up, what is it about Hops and Plant Breeding that you enjoy the most?

Dr Paul Matthews: What I really enjoy about hop breeding and hop crop improvement is really the scientists. I mean, we're a great team and we have a sort of special focus on a specialty crop. Hop is a glamorous crop and it invites glamorous people, or interesting people anyway, to come and do research. So I find that we have, you know, we're a little bit off the beaten track in terms of the species we work on. And that brings in people that are a little bit off the beaten track. And so we just really have a diverse and interesting group. I think plants and people have a beautiful co-evolutionary history and one of the best ways to influence and have an impact on human ecology and human health is to enhance and dive into and dwell in this co-evolution and speed it up and steer it. So it's just very intriguing to have the opportunity to work with plants.

Dr Katherine Easterling: I personally just really love plants. I always have. I agree with Paul completely that it's really nice to have these international collaborations and to learn about different approaches that people take. And all of us have our own specific understandings of the plant and then we come together and create this really nice way of working together and understanding things. What I also love about science in general, but I think sort of especially plants is that there's always something new. The more we learn, the more we realize that we don't know and so there's this constant sense of never being bored. The medicinal applications have always interested me. I've always been interested in medicinal plants and the chemical libraries that plants produce will always be of great interest to me, and I think humanity. And I think that we have this whole pharmacopeia available to us that we know very little about. In fact, there are bioactive metabolites in hops that could be useful for antiviral medication and those kinds of things are being looked at too.

Hannah Senior: Fantastic, Thank you very much for taking time to talk to me today. It's been really interesting - I thought I knew a bit about hops, but I have learned so much more in the course of this conversation! Dr Paul Matthews and Dr Katherine Easterling of Hopstiner, thank you for telling me your plant breeding stories.

[Theme Music Plays]

Hannah Senior: You've been listening to plant breeding stories by PBS International and I'm your host, Hannah Senior. Plant Breeding is a pretty specialist podcast topic, which can make it difficult for people who share our interest in this kind of thing to find it. So if you've enjoyed the podcast, recommend it to your friends and colleagues, and please help others in the plant science community to find it by rating this episode and subscribing to the series. I'd love to hear from you if you want to suggest people you'd like me to interview, you can contact me on twitter @PBSInt or on Instagram @PBS_Int. Until next time, stay well.

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