Episode Transcript

SPEAKERS

Matt Barbour, Art Woods, Marty Martin

Marty Martin: One of the foundational concepts in ecology is the idea of 'keystone species.'

Art Woods: Keystone species are species whose presence or absence have disproportionately large effects on their communities – keystone species really matter.

Marty Martin: The idea was first developed by marine ecologist Bob Paine just after he started his first job at the University of Washington in the mid 1960s.

Art Woods: On Tatoosh Island, off the NW tip of the Olympic Peninsula, Paine did what is now considered a classic experiment demonstrating the keystone effect.

Marty Martin: Major predators in intertidal zones there are Pisaster sea stars. Pisaster is a true intertidal nightmare, killing and eating many different kinds of invertebrates including snails, limpets, and chitons.

Art Woods: Its absolute favorite, though, is the California mussel, which in turn has its own pervasive effects.

Marty Martin: In intertidal zones, a limiting resource for many species is often just plain old space, a spot in which to settle down and make a living – and California mussels turn out to be really great competitors for space. Without anything stopping them, they take over most or all the space and exclude pretty much everyone else.

Art Woods: A kind of ecological monopolist; the Fords of the filter feeders

Marty Martin: Which is where Pisaster comes in. In his classic experiment, Paine removed Pisaster from some intertidal areas and, in those sections, the diversity of the other species plummeted – the mussels muscled their way in and occupied all the space.

Art Woods: In other sections with Pisaster left untouched, the sea stars mowed down the mussels and overall diversity remained much higher.

Marty Martin: These effects make Pisaster a keystone species. Its presence disproportionately affects the community.

Art Woods: OK, but important question – can entities at other levels of biological organization –

Marty Martin: not just at the level of species –

Art Woods: also be keystones?

Marty Martin: On the show today, we talk with Dr. Matt Barbour, a postdoc at the University of Zurich who will soon be starting his own lab at the University of Sherbrooke in Quebec.

Art Woods: In April of this year, Matt and colleagues published a really interesting paper in the journal Science in which they argued that allelic variation at one genetic locus can have profound effects on ecosystems, making those loci what they call 'keystone genes.'

Art Woods: Matt and colleagues arrived at this conclusion from an epic set of experiments using a simplified food web in the lab – consisting of Arabidopsis plants at the base, two species of aphids feeding on the plants, and parasitoid wasp that lays its eggs inside the aphids. Got that? Four species in total arranged in a diamond-shaped food web.

Marty Martin: The group focused on allelic variation in a set of three genes involved in producing aliphatic glucosinolates. These compounds likely evolved as defenses against insect herbivores, although they seem not to deter all herbivores including the aphids that Matt used in his experiments.

Art Woods: And you in fact are already familiar with aliphatic glucosinolates – they're the molecules that give cabbage its cabbage-y smell and mustard its pungency. Arabidopsis has these compounds because its in the mustard family of plants, which we nerds know as the Brassicaceae.

Marty Martin: Matt and his team constructed different versions of experimental food web using strains of Arabidopsis that had different underlying genotypes at three loci.

Art Woods: Remarkably, allelic variation at one of the loci – called AOP2 – strongly predicted the fate of the experimental food webs.

Marty Martin: When Arabidopsis had a functional allele of AOP2, typically the food webs collapsed into simpler forms - meaning that one, two, or all of the insect species disappeared from the system.

Art Woods: By contrast, when the plants had a nonfunctional allele of AOP2, the food webs were much more stable and often retained all of the insect species.

Marty Martin: Today, we talk with Matt about his experiments and more broadly about the idea of keystone genes.

Art Woods: We cover inferential aspects of how he got to the idea of keystone genes from the dataset that they collected and why AOP2 appears to be so important.

Marty Martin: We also discuss whether keystone genes identified in simplified lab settings will also play outsized roles in more complicated natural communities, and more generally how one might identify other keystone genes in a broader set of species for which the genetics are less tractable.

Art Woods: We also discuss the basic idea of 'keystone'-ness, as this kind of organization appears to 'crop up' in many other biological contexts.

Art Woods: Stick with us just for the thale of it!

Marty Martin: I'm Marty Martin

Art Woods: and I'm Art Woods

Marty Martin: You're listening to Big Biology

[Music break]

Art Woods 00:00

Matt Barber, thanks so much for joining us on big biology. It's really great to have you on the show. Why don't you first tell us about sort of where you are professionally and sort of what your trajectory is that led you to Zurich, and you know how you got to where you are.

Matt Barbour 00:12

Let's see. So right now, yeah, I'm a postdoc in Dr. Jordi Bascompte's lab at the University of Zurich. And I guess the way I mean, maybe I'll kind of start back a little bit before, like during my master's So during my master's, I studied rattlesnake - ground squirrel behavioral interactions. So I had always had a long interest in trophic interactions, but I became, started to become really interested in it because one of the things I noticed was that individual variation seemed to be a key driver, kind of in influencing how these rattlesnakes and squirrels were interacting. But of course, it's hard to study the effects of individual variation in relatively large vertebrates.

Marty Martin 00:51

Tell me about it.

Matt Barbour 00:52

So I ended up kind of moving towards studying plant-insect systems, because there'd been a lot of work showing that interspecific variation in plants could have large effects on communities. At the end of the day, I'm kind of like a food web guy. I'm interested in trophic interactions, how they affect the structure and dynamics of communities. And yeah, I ended up collaborating with Jordi on some work during my PhD looking at the effects of genetic variation on plant-insect food webs, the structure of these food webs. And yeah, because of that collaboration, Jordi was interested in doing some experimental work, and kind of give me free rein in terms of like developing a model system to explore some interesting questions about how ecological and evolutionary processes shape the structure and dynamics of food webs. And that's kind of what came to the development of this model system that kind of formed the basis for this.

Art Woods 01:53

Hey, I want to just linger for a moment on snakes. So what is it about snakes that caused them to, you know, be one way or the other, you're talking about individual variation? So what are the traits you're talking about that make a difference?

Matt Barbour 02:05

I mean, I think behavior is a trait. So in this case, it was, like, there's just this one snake that stood out to me like because usually when these rattlesnakes were hunting for the ground squirrels, they often set up in these like ambush sites right next to a squirrel burrow and wait for the squirrels to come out. Mainly they're hunting the pups. So they kind of most of the snakes a lot of them did it in a more covert fashion, like trying to kind of, you know, find a hiding spot and like relying on stealth, the catch the squirrels. But some of the snakes, especially one of them just kind of like to mix it up and just seem to like to just move around a lot and kind of almost cause like, a lot of commotion, and then get into some of these areas where it was still pretty hidden. And then the squirrel pups would end up running around, and then it seemed to be able to take advantage of them. So that was just something that I just found super interesting. But then also like, yeah, these squirrels, you know, they're attacked by other predators, you know, Bob Cats, eagles, and so I became interested in more of that foodweb context.

Marty Martin 03:10

So how does all this come together? You mentioned starting your own lab fairly soon. Is it all squirrels and rattlesnakes? Or is it Arabidopsis or yes to both? Okay. Is there a particular part of the world that you're targeting or?

Matt Barbour 03:18

No, it's definitely no more squirrels and rattlesnakes. That was, yeah, that was more of the history. Definitely plant-insect food webs, and the Arabidopsis kind of model system that we use in the paper will be kind of a core part to start to explore these fundamental questions about how genetic variation affects food web structure, and in turn, how that community context could feed back to shape genetic variation in populations. But Arabidopsis is a model system that I'm using so I'm also planning to get going with some other more field relevant, I guess, systems, I would say, and that's something I'm kind of currently exploring right now be I'm writing my first grant to try to kind of, so still brainstorming what those systems might be. So I'll be based at the University of Sherbrooke in Quebec, Canada. And so I've become recently fascinated by the Eastern spruce budworm. It's got a hyper diverse food web of parasitoids. And I think it's economically very important, it's one of the most damaging defoliating insects. So thinking about its role in a food web context and how eco evolutionary processes unfold. Also, apparently lettuce is a big deal in Quebec, Quebec produces like the majority of Canada's lettuce, and they have problems with aphids. And they've kind of created these new resistant varieties of lettuce and they're trying, they're curious about how that genetic variation might affect a parasitoid community. So still kind of brainstorming ideas related to that.

Art Woods 04:55

We're really psyched to talk about this mind-blowing paper that you published just a few months ago in science called "A keystone gene underlies the persistence of an experimental foodweb". So it's you and Daniel Kliebenstein, and Jordi Bascompte. Let's maybe just start with this phrase "Keystone gene". And I would say, you know, as biologists, many of us have heard about this idea of keystone species, right. That's the context in which it all is originated. So what is a keystone species? And why are you applying this idea to what you call Keystone genes?

Matt Barbour 05:30

Yeah. So keystone species, I would say are ones that have a disproportionate effect on communities or ecosystem processes relative to their abundance, or biomass. So this concept has been really influential at the species level, it's come to kind of define much of our thinking sometimes in community ecology. But the same concept can apply at lower levels of organization. And that's where in this paper, we find evidence for this at the genetic level, where this particular gene

has a disproportionate influence on the persistence of its associated community of interacting species.

Art Woods 06:14

And maybe let's just stick on species just for a second. So what are some examples of keystone species?

Matt Barbour 06:19

I mean, the classic example comes from Bob Payne's experiment where he showed that sea stars in intertidal communities function as a keystone species, because he found when he removed them from these intertidal ecosystems, it kind of altered the structure of feeding interactions in such a way that just resulted in just a few species out-competing the others for limited space. So it kind of originally came from this idea that a keystone species kind of maintains species diversity in a system. And I mean, that's also true, maybe for some other iconic examples maybe the sea otters and kelp forests, being one of them. Sea otters prey on urchins. Urchins are kind of key herbivores of kelp. And so they maintain - keep the urchin populations at bay and allow these kelp forests to flourish, which kind of maintain diverse ecosystems.

Marty Martin 07:13

Right. So the key function of Keystone is not key necessarily, not exclusive, but it's a stability force. Right? I mean, the follow on from lots and lots of urchins, or lots and lots of mussels is that the rest of the system ceases to function in the way that it used to, but in a way that's sort of sustainable and facilitates biodiversity persistence, had that Sea star or, you know, other keystone species been there.

Matt Barbour 07:41

Yeah, I think you bring up a good point that it is kind of related to this idea of stability or persistence, because I think people have started to rethink more that instead of necessarily maintaining diversity, it could just be a big change in the composition of species in the community, even though kind of Bob Payne's experiment originally showed that, we could also think about, it's kind of been more recently, the perspective is that it's just a large disproportionate effect on community. So perhaps diversity can be maintained, but the composition of the community flips. But which still aligns with very much what you said, this idea of persistence, stability of a particular community.

Art Woods 08:21

Great. So if we move now to the genetic level, so you mentioned particular loci or particular genes that act as Keystone genes, maybe let's just kind of take that apart a little bit, too. So is it something about the allelic, you know, the frequency of different alleles at that loci or is it the locus itself that functions as a keystone? And like, how does that happen?

Matt Barbour 08:45

In the case of our experiment, what we found was that for this one gene, if there was a particular allele present, in this case is nonfunctional form of this gene, that if it was present, that it played a key role in increasing the persistence of the interacting species. So I guess to kind of get to your point, it wasn't necessarily variation at this gene that was maintaining diversity, it was just that literally the presence of this one allele -

Art Woods 09:15

Presence or absence of a particular allele.

Matt Barbour 09:16

Yeah. And so if you kind of move from a plant population, where this allele is present to where it's absent, we see a reduction in the diversity or stability of the ecosystem.

Marty Martin 09:16

So that's a good 30,000-foot view, maybe. I mean, I think it'll be useful to try to walk through a little bit of the background, the key pieces of your system and the experimental design, and then tying it all back to you know, what you're talking about that specific, powerful allele?

Matt Barbour 09:44

Certainly. So I guess maybe just a bit of a bit of background and maybe I should talk a bit more about the system.

Marty Martin 09:54

Yeah, that'd be great.

Art Woods 09:54

Just put names on everything.

Matt Barbour 09:56

So the plant we use, so we have this diamond shaped foodweb. The basal species in this foodweb is the genetic model plant, Arabidopsis thaliana. And then we have two different species of aphids that feed on the plant, and then a parasitic wasp that attacks both of these aphids. So these insects are naturally associated with Arabidopsis. And one of the things we know about the insects in the system is that they seem to be influenced by these chemical compounds called aliphatic glucosinolates. So these chemical compounds, you might not be kind of familiar with them by name, but they're the same compounds that give cabbage bitter taste, or that kind of spicy flavor to wasabi. And fortunately, the genotype-to-phenotype map of these aliphatic glucosinolates has been pretty well characterized. And that's where we kind of leverage the fact that Arabidopsis is a genetic model plant where we knew a lot about the genetic basis of these chemical compounds. And for much of the natural variation is determined by three key genes. So these genes are MAM1, AOP2 and JSOH. And so these genes, depending on whether they have functional, or null alleles, determines whether the biosynthesis of these chemical compounds kind of proceeds down. So it's kind of this hierarchical kind of pathway.

Art Woods 11:28

So if I can restate, so these are, these genes encode enzymes that are sort of gatekeepers on a metabolic pathway. And so if one or more of them is missing, then that pathway comes to a stop, and compounds accumulate in some way.

Matt Barbour 11:43

Exactly. Yeah, that's exactly right. And I think the key point, though, the key thing, and this is something I discuss a lot with one of my collaborators, Dan Kliebenstein on the paper, who's really the expert in these glucosinolates and their genetic basis, is that these genes, it's better to kind of think of them as like a decision switch, like kind of for altering the flavor of these chemical defenses rather than necessarily kind of like turning on or off like, oh, we have defense/no defense.

Art Woods 12:13

So they're not faucets. They're switches.

Matt Barbour 12:14

Yeah, exactly.

Marty Martin 12:16

And just one other thing that I want to make sure we get, I think this will become important in a minute, you said quickly there, that there's a null

alternative versus a functional alternative. So can you wrap that into what we've been talking about?

Matt Barbour 12:27

Yeah. So I guess the important thing I want to emphasize there is that these functional, and null variants of this gene kind of reflect natural variation. So these aren't, like I'm not turning on or off genes to create variation that doesn't exist in nature. So there's naturally these functional and null alleles. But yeah, so pretty much if one of these if, for example, this AOP2 gene is turned off, we get the accumulation of a particular glucosinolate compound, which is kind of one of the most common Arabidopsis genotype, this Colombia genotype called Zero, it has a natural knockout mutation of this. So it produces, because of that it produces a particular chemical phenotype.

Art Woods 13:14

Let me ask you about the distribution of the allelic variation in nature. So is it the case that all of these alleles occur in all populations of Arabidopsis? Or is there some kind of like spatial variation in the frequencies and, you know, presence/absence in some parts of the world?

Matt Barbour 13:29

That's a good question. There have been, so I actually haven't looked as much into the details of the within population variation, just because most of the genetic mapping of these Arabidopsis populations, they just, they're based on actual like a particular accession representing a population rather than really trying to dissect the chemical variation, even though there has been some recent work suggesting that there can be variation within these populations. But for the most part, there has been some examples showing that there could be longitudinal variation in these genes. For example, this MAM1 gene, which is actually the gene that I thought would be the most important in this in the system, because these aphids, the different aphid species impose different selection on alleles at these genes. So I thought that this would actually be the key player. And so there is geographic variation.

Art Woods 14:25

So maybe just getting back to your experimental setup. So you have these different genotypes of Arabidopsis, you have this kind of simplified foodweb of aphids and a parasitoid wasp, then what do you do you put them all in a box and let it go? Is that what happens?

Matt Barbour 14:41

Yeah, more or less.

Art Woods 14:42

You know, not to undermine the effort here. I understand.

Matt Barbour 14:45

Exactly. Yeah. So this is pretty much what I would do is, I grew the plants in the greenhouse for a few weeks before adding them to these experimental - to the community. And so at first I just started by adding aphids, allowed their population to grow, and then I added the parasitoids. And then every week, we would pull out the cages from, that we kind of maintained in these growth chambers. And we would count the abundance of all of the insects in the foodweb. And we would replace plants. So we kind of maintained resource availability for these. So we are constantly growing plants and counting insects for basically for four months.

Marty Martin 15:24

Okay. And you're measuring along the whole time. I mean, this is the experimental design. I don't know if now is the right time to mention the temperature manipulation. That was also part of this gargantuan, to me incredibly intimidating study design. So say something about that if you'd like or we can push that aside, and instead just talk about, you know, the kinds of things that you're measuring to get the concept of Keystone?

Matt Barbour 15:45

Yeah, yeah. So I definitely happy to talk about it, because it didn't really shine through much in the paper. So we did this temperature manipulation, basically trying to kind of replicate what these insects, which we had locally collected from Zurich might experience, yeah, 30 years or so in the future. So but just kind of a mean temperature manipulation of three degrees Celsius. So 20 and 23 degrees Celsius. And the main thing that we kind of found from that is that it really just accelerated the extinction of one of the aphid species. So there's one aphid species that always went extinct, and basically increase in temperature accelerated the extinction of that species. But at the end of the day, kind of the net effect on the persistence of the foodweb was more influenced by this by the genetic variation at AOP2 than necessarily our temperature manipulation. I think there could be some interesting complex metabolic effects changing body size and potentially the parasitoid sex ratios, but I don't have much evidence, that would be mainly kind of speculating.

Art Woods 16:54

Got it. So let's, let's maybe dive into what you just said about the result, which is that AOP2 ended up being really important and the presence or absence of this null allele. And so like, how did you arrive at that conclusion? And maybe along the way, say, like, what happened to the food webs in the different treatments, what are their dynamics over time.

Matt Barbour 17:15

So basically, we started with this diamond shape foodweb. And from this full foodweb, we ended up either having the system kind of collapse pretty soon to just to the plant only state, or it went to another transition where one of the aphids went extinct, and we just kind of had the food chain of just this one dominant aphid species and the parasitoid. And then from there, that food chain either collapsed, again, to just having the plant only or it went to only having the aphid and that was basically it, those were the foodweb transitions we observed. And what we found was that if this nonfunctional allele of AOP2 was present, is that it basically acted to prevent that food chain from collapsing to the plant only state. And the reason why we think it had this effect was because, I mean, I've been talking a lot about AOP2 having this, like being really important in influencing the glucosinolate chemistry of the plant. But AOP2 also has pleiotropic effects on phenology as effects on circadian rhythm, jasmonic acid signaling, and plant growth.

Marty Martin 18:30

Never just does the one thing you want does it?

Matt Barbour 18:32

Exactly, which is I mean, so side note, the funny thing was, I thought, oh, okay, I'm going down to the genetic level that it would be...

Marty Martin 18:41

Simpler?

Matt Barbour 18:42

Somehow easier.

Art Woods 18:46

Finally, we're gonna understand all this stuff, right?

Matt Barbour 18:48

Exactly. So anyways, yeah, at the end of the day, I think one of the things we didn't quite nail is like the phenotypic mechanism like about how AOP2 is actually promoting the persistence of the food chain, but what we think is going on is that it's this pleiotropic effect on plant growth, which reduces aphid fitness. So these aphids have slower growth rates. And what I think that's probably linked to is that these aphids probably have larger body sizes on plants with a nonfunctional allele, and that the parasitoids also would then have higher fitness, because they would preferentially oviposit in aphids, larger aphids, so what we're seeing is this increase, intrinsic growth rates kind of with the plant cascading to the aphid cascading to the parasitoids. So it's not necessarily just population numbers, we see demographic effects kind of cascading,

Marty Martin 19:46

Okay. So AOP2, the null version, is causing the plants to grow faster, which means the aphids are larger, which means the parasitoids when they, I mean we haven't said parasitoid I think most people know what a parasitoid is, but they lay their eggs inside the aphids. And if, presumably, the aphid is really tiny. That's no good. That's not going to work. A bigger aphid is better for the parasitoid. So the integrity of the whole system is maintained, because AOP2 is causing the plants to grow faster. I mean, presumably, I know you're not sure about that part.

Matt Barbour 20:17

Exactly yeah, that's kind of yeah, the working hypothesis.

Art Woods 20:22

So this, for me, sort of brings up ideas about growth defense tradeoffs in plants, right. And so thinking about this in terms of selection on Arabidopsis, and what's good for the plants in the end, right, so shouldn't this whole kind of cascade of things exert selection on AOP2?

Matt Barbour 20:41

Yeah, so this result didn't make it into the paper.

Art Woods 20:45

Uh, huh.

Matt Barbour 20:45

Yeah, so happy to discuss this. And so. So we also tracked change in kind of plant biomass, like over the course of this experiment. So let's assume we can, that

plant biomass like in these populations would be a proxy for the plant fitness at the population level. At the beginning of the experiment, we see strong selection for this nonfunctional allele of AOP2. It has faster growth rates in absence of insects. And even with the insects, it still like has a bit faster growth rates. But then over time, AOP2 - the nonfunctional allele, promotes the persistence of the food chain, otherwise the food chain collapses. So then we see that selection starts to act against the nonfunctional form, because what you ended up having in some cages, because we also didn't just stop the experiment when the foodweb collapsed, we just kept going and replacing plants and measuring the foodweb. But then plant fitness ended up starting to equilibrate, even with plants that had the functional allele. So what we see is that although there is strong selection on this nonfunctional AOP2 allele at the beginning, it starts to kind of balance out at the end of the experiment. So this could be kind of this indirect effect kind of feeding back to kind of create more balancing selection. But that would be kind of at a more like metapopulation level.

Art Woods 22:12

So it's another way to say this, that the fitness effect of the AOP2 null allele appears positive at the beginning because the plants, they're growing rapidly. That's your definition of fitness here.

Matt Barbour 22:23

Yep. Insect abundances are low.

Art Woods 22:25

Yeah, but then the insects, it sounds like they really come on and their population sizes increase. And so in the end that ends up being harmful to the plants.

Matt Barbour 22:35

Yeah, it ends up being harmful to the plants and then the insects go extinct on the plants with the functional AOP2 allele. So then they lose the insects. So now they don't have this herbivore pressure. And so they ended up kind of catching up in terms of their fitness.

Art Woods 22:55

Right, right. So that's the plant using this aliphatic glucosinolate as a defense and you know, sort of successfully causing the insect to go to extinct. So it's released from herbivore pressure, right?

Matt Barbour 23:06

Yeah. But I guess, I mean, so that's one of the things we observed in this experiment. I mean, I guess I could also talk about what I think kind of probably maintains AOP2 variation, like in nature, I think...

Marty Martin 23:21

Did you get our notes Matt? That's exactly what's in the progression. What maintains genetic variation in AOP2 in nature?

Matt Barbour 23:27

Yeah, yeah. So I mean, it could be it's likely due to the fact that there is a more diverse herbivore community than just these aphids. On AOP2 there's a, it could influence herbivory from generalist herbivores, maybe other leaf chewing herbivores. So it's hard to kind of untangle what is actually maintaining AOP2 in nature, even though we do have evidence that it could still be maintained, even from this simple experimental system, just from this extinction, but in nature, so this mechanism could still be at play. But it could be other kind of more diverse herbivores, or just these other aspects related to kind of competition, phenology, because it has these pleiotropic effects, because interestingly, the functional form of the allele is more common in nature. So again, I mean, maybe that is related to the persistence mechanisms we see that I was just discussing, but it could be due to other variable selection pressures.

Marty Martin 24:28

Do you know anything, did you guys I mean, the experiment - we've already talked about how gigantic and ambitious you were, but what about heterozygotes? Do you know anything about heterozygotes? I mean, is there an advantage or could you foresee some advantage?

Matt Barbour 24:41

Yeah, that's something like in this case, I'd have to discuss with Dan about to get his insight. Because the main thing that we know with these genes is that it seems to be kind of more this like presence/absence functional/nonfunctional allele that kind of drives most of the natural variation in these glucosinolate compounds.

Art Woods 25:04

I want to ask another question about these sort of more complicated food webs that you were alluding to in natural contexts. And so, so you did these, let's just call them mesocosm experiments, and from the patterns of change in these food webs, you can deduce that AOP2 is, you know, allelic variation in that is really important. So one question that occurred to me thinking about this paper is

whether that result depends on using this kind of simplified, experimental system. And if you go out in nature with a more complex set of things that are interacting, are we still gonna be able to find Keystone genes? Or is that natural context going to be so complicated that its going to, you know, dilute out all the effects of these things in ways that make it hard to identify them?

Matt Barbour 25:49

Yeah, so I guess I mean, that's definitely a valid point. I mean, and that's also kind of more classic question like, can we scale from these mesocosm experiments to more complex systems? So I'll take the, at least defend the potential for them. Because I do think that at least, and I'll speak a lot with the, because more of my background is with plant-insect systems, but we do know that with plant insect food webs, that there's a lot of genetic variation in plants, in chemical compounds. These chemical compounds are highly heritable. So potentially, they do have a relatively simple genetic basis, and that they kind of form the basis and influence multi trophic interactions. So I think in that context, there is scope for these effects to be present. And I think I've started to think of them as being potentially prevalent in genes kind of related to defense and immunity, primarily because they seem to have a relatively simpler genetic basis compared to kind of more quantitative traits, like body size, which are determined by the many genes of small effect. But these also these genes could kind of be tightly integrated into I mean, Art, you had mentioned about kind of tradeoffs, like growth defense tradeoffs, but I think we're starting to kind of realize that it might not be like, it's kind of, it's a good conceptual model. But things might not be as simple as that there's kind of these complex metabolic networks. And so these genes are also kind of tightly integrated in these metabolic functions. And so there is potential for these to kind of have more cascading effects on these other integrated kind of phenotypes.

Art Woods 27:29

It's never as simple as you'd like it to be for interfacing with theory. Right?

Matt Barbour 27:32

Exactly. Yeah. But I mean, I think your point is a good one and I think that's also a bit of a more general critique of the Keystone concept. And I mean, at any level, you got to kind of define what it is, what you kind of care about, or the scope of it. In this case, it was a pretty small system.

Art Woods 27:54

Yeah. And don't get me wrong. This is not a harsh criticism its more just like this, this practical issue of like, yeah, how do you look for these things out in nature?

Matt Barbour 28:02

Yeah, and I think, and I think for some of them, and then other people even Yeah, because there's a nice Trends in Ecology & Evolution paper that kind of first kind of tried to put this concept of the Keystone gene on the map. And they speculated that it would, it's likely in traits that have a simple genetic basis, ecologically important traits. But I think at the end of the day, I would even maybe go a step further and kind of think it might be more common than these defense and immunity related traits, just because of what I said they might have a simpler genetic basis, but still be integrating metabolism to have these pleiotropic effects.

Marty Martin 28:40

So I find, let's try to tie all of this up, because I think this is an incredibly cool system. But complicated nonetheless, and I want to make sure we hit it all. So to scale out and talk about the implications of Keystone genes and those sorts of things. Make sure we tie this together. So you've got one locus, that in one species of plant, you're calling Keystone, because very simple variation, or constrained variation in a simple gene, right? With simple meaning we know exactly how it works, has cascading effects such that when one flavor is there, the community sort of behaves as it should, maintains its stability, and you flip the switch and you go to the other variant of the gene and now all bets are off at least you know, as far as stability goes, and repeatability, committee integrity, that's gone. Okay. So that's the Keystone connotation. In this context, that's how Keystone genes are Keystone genes as you're portraying them. Can you help me understand why you guys chose the word Keystone? And I didn't know about the TREE paper you were just referring to so I'm not sort of saying that you're the only ones talking about that. But there's a difference to me about Keystone as you use it relative to its typical uses. Conspicuously that Keystone tends to be within a level of biological organization right, so Keystone is a species because a species relative to the others through competition, predation, whatever it is the starfish example, you were talking about, that's all in the food web. And yet there are other keystones like people think a lot about Keystone genes, but not as you've talked about them. Keystone for a regulatory network person is a hub, and a regulatory pathway. You know, how epistasis and pleiotropy all work together to produce phenotypic variation. Why did you guys choose Keystone as an across level kind of phenomenon, when Keystone is usually about within level? Does it matter?

Matt Barbour 30:37

That's a good question. I was going to say one of the things about a keystone gene is that since it, because you may have this gene kind of co-occurring in multiple species. So it actually could have larger effects, at least at the aggregated level among species, there are some examples. I mean, one of the

examples that comes to mind is like in agricultural systems, for example, like, wild mustard is like an invasive weed. And if that's invasive to nearby agricultural fields, like with broccoli, or cabbage or other brassicas, there's at least I'm thinking about the AOP2 example, there would be the potential scope for genetic variation to kind of act across the species to have bigger effects. But I mean, yeah, to be honest, I hadn't necessarily thought about that distinction. But I guess I would say that that would be the one difference between, I kind of feel like, the keystone species and Keystone gene concept is that, yeah, Keystone genes could kind of pervade other levels.

Marty Martin 31:39

Yeah. It just that, to me, it comes to, you know, we all have our perspectives, we all have our training trajectories and the biases that we bring to these ideas. The system seems like, the paper seems like an unbelievably cool example of how a very particular form of genetic variation influences the phenotypic variation in that plant. And secondarily what it means. But I guess to me, that's like an unbelievably cool mechanistic dissection of how things manifest genetic to community. But Keystone? I don't know, I think it's just because I mean, there is a keystone in the Bob Payne context that we're all taught early. But I'm also biased because I think about regulatory networks, I tend to think within levels of organization, when I use the cuts of the Keystone, we haven't mentioned super spreader, for example, as epidemiologists, you know, arguably, those are keystones. But we don't usually think about it, at least in the context of say, COVID, where most of the transmission is only among humans, we think about Keystone within a level. Now, if you're talking about zoonotic disease, the parasites are getting passed among lots of different hosts, perhaps now you're moving across levels of organization. But yeah, it was just it was a different use of Keystone that I've, that I've seen in the past. I mean, Art you have another? We were talking about this offline Matt, before you joined us. Art has another take on this that I mean, I think we're coming at the same general thing. But you had a different approach, or do you want to share what that was? Are talking about my statistical question? Yeah, the statistical

Art Woods 32:13

Yeah, yeah. Okay. So I was thinking about this in terms of the number of genes that you manipulated and that you used in your experiments. So three, versus how many genes there are in Arabidopsis, which I understand is about 25,000. And so at some level, you could say, well, that's like amazing to like, look at three of them and find one that's a keystone. And how many other things might you identify as a keystone gene if you looked at all 25,000, which, like, effectively, no one's ever going to do in an experimental way, right? There's just there's like, no way. But I guess the question is, two part maybe, or there's some kinds of like genome scanning ways that you could use to try to look at total genomic variation and identify hotspots that are meaningful for the food webs

and the ecosystems that depend on things like Arabidopsis? And the other one is maybe what you already said, which is that you think, you know, the really key targets are to look at defensive compounds and things that are involved in in metabolism and growth?

Matt Barbour 34:00

So I mean, I think kind of what you just alluded to about, like almost, not quite a shortcut, but at least like scans to, like, identify these. I mean, that's something I'm very interested in plan to do, actually, with, with this system.

Art Woods 34:15

Oh, awesome.

Matt Barbour 34:15

Because I guess one of the things that I thought about at least with like, you could do genome wide association studies to look at how not only, I mean, people have done it with looking at effects of Arabidopsis on plant growth, but we could look at this on kind of testing my hypothesis that it influences the population growth rate, the fitness of the aphid and then also kind of the multi trophic effects on the parasitoids. So by scanning that, you could at least identify a hypothesis, okay, these genotypes or these genes would be associated with more kind of persistent foodweb that you could then it would kind of pinpoint those potential genes that you could then experimentally test. That's one thing that would be kind of, yeah, one way to go. But just kind of thinking back to your point Marty about like, I guess the whole Keystone genes concept is a little bit like focused on the community ecosystem level. It is almost, I think, kind of, perhaps biased by that keystone species concept about thinking about communities and ecosystems, but at least that's how I've thought about it.

Marty Martin 35:25

Yeah. And in fairness, I don't know anybody has ever called, you know, a hub gene, Keystone. So I'm attributing of word to something that, you know, maybe never happened, I'm talking about, you know, I'm using it that way, because it could function. I mean, I think it's understood to function in a comparable way. But the word hasn't been applied.

Art Woods 35:41

The one thing and so yeah, correct me if I'm wrong, because I'm not as familiar with these details, but one of the things I would kind of highlight about Keystone genes is that they should exhibit natural variation. So like, if it's a highly conserved gene that is like core to kind of metabolic function, and if you

take it out and the organism dies, it like it doesn't have the chance to be kind of expressed in nature. So I would say that that's one of the things that, I don't know, it might be a slight distinction there. Because yeah, you could have these genes that are core to pathway like photosynthesis.

Marty Martin 36:19

No, exactly. I just find I find that the concepts like that really cool. And well, I'll see you at ESA, we could go on about this for hours.

Art Woods 36:28

So Matt, maybe tell us just for a few minutes, you're starting up your own lab here soon at University of Sherbrooke? What's your lab gonna be doing for the next five years to tackle some of these questions?

Matt Barbour 36:37

Yeah, one of the things I'm really interested in is exploring, I mean, we did a, this past paper is, just a very targeted test of a few specific genes. But we know that, you know, Arabidopsis, and other plants exhibit substantial genetic variation in multiple traits. And so I'm really interested in kind of exploring these effects of genome wide variation and how it structures foodweb interactions, because I think it could give us some really exciting and interesting insights. I mean, one of the things I've kind of thought about is in agricultural systems, even like, you know, we need to reduce pesticide use. These might be alternative ways to potentially guide communities to increase biological troll, for example, through this kind of these indirect trophic interactions. The other thing that I'm really interested in doing is starting to explore the effects of genetic variation in multiple interacting species. we're not going to be able to get to the effects of down to the genetic level like we would with Arabidopsis. But we could still start to explore these effects of genetic variation across different trophic levels. I think there's reason to expect that there might be kind of predictable asymmetries in how genetic variation is distributed among interacting species kind of based on their body size, life history traits, trophic position, I think we can make some of these predictions from population genetics. So I'm kind of interested to explore those ideas, but link them more into predictions for community ecologists. And then the other thing, which is kind of I haven't really discussed at all but, influence of environmental variation. The phenotypes aren't just determined by genes, but the environment in G by E interaction. So I've become really interested in the effects of warming just because of I mean, of course, it's a key driver of environmental change, but the temperature dependence and metabolism as key effects on trophic interactions. And so trying to potentially unravel these will be kind of the core part of my work over the next five years.

Art Woods 38:40

That sounds really exciting.

Marty Martin 38:41

Do you have sort of candidate Keystone genes in the context of crop management, pesticide resistance, anything like this? Have you gotten into that level of detail yet?

Matt Barbour 38:50

No, not yet, other than it would be, part of me would just, would think about exploring it with the Arabidopsis. Because Arabidopsis is often used kind of as a model or people think about it, you know, the genes identified there for crop improvement. So as a way, it could start to give insight to kind of other cool crops, because also these functional null alleles in these glucosinolates they actually have a very similar genetic basis in other brassicas that are common crops. But I've also I mean, kind of, like I mentioned at the beginning, I'm kind of interested in lettuce because there's a collaborator at Sherbrooke, who's starting to work, there might be a nice system too. And they've identified this gene that influences resistance to one of the key aphid pests there. But it's part of a complex multi trophic system. So really dissecting these effects will be hard and I'm interested in that.

Marty Martin 39:43

Well, this has been awesome, Matt. I really enjoyed it. I love talking about things that are far afield from anything that I do. That's a big part of the reason we do this podcast. We always give guests the chance to say anything we didn't ask you about. Is there anything else that you'd like to say about the system the future? Anything you'd like. That's my favorite conference!

Matt Barbour 40:01

I mean, one I just have to say thanks for inviting me to come on. It's been a great experience. And just also seeing the breadth of topics that you that you guys are covering. I just love to see that. Because also, I mean, I just came from the GRC Unifying Ecology Across Scales and thinking about how these topics are interconnected is, it's my jam. It's mine, too. So I was really happy to be on here, I guess. I mean, I don't know. I mean, just kind of as a side note, I guess I would just mention that, like, I didn't expect AOP2 to be special. I mean, literally, I thought it was going to be variation at this other gene that was going to be important, but I think it does kind of provide some insight to where we might detect these effects in nature, kind of these genes that have these pleiotropic

effects and just might be tightly integrated with kind of these metabolic networks.

Marty Martin 40:54

Yeah, Matt, thank you so much for the for the chat. Interesting paper. Yeah, fantastic.

Matt Barbour 40:58

No worries. Yeah, thanks again for having me.

[music break]

Marty Martin: Thanks for listening to this episode! If you like what you hear, let us know via Twitter, Facebook, Instagram, or leave a review wherever you get your podcasts. And if you don't, well we'd love to know that too. All feedback is good feedback!

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