

# Episode 181\_mixdown PROOFED

Thu, Sep 05, 2024 7:29PM • 52:01

## SUMMARY KEYWORDS

bees, queen, colony, hive, question, honey bee, work, colonies, brood, lab, observation, research, bee, honey bees, rearing, nanos, field, beekeeping, nano, put

## SPEAKERS

Stump The Chump, Amy, Guest, Jamie

### Jamie 00:10

Welcome to Two Bees in a Podcast brought to you by the Honey Bee Research Extension Laboratory at the University of Florida's Institute of Food and Agricultural Sciences. It is our goal to advance the understanding of honey bees and beekeeping, grow the beekeeping community and improve the health of honey bees everywhere. In this podcast, you'll hear research updates, beekeeping management practices discussed and advice on beekeeping from our resident experts, beekeepers, scientists and other program guests. Join us for today's program. And thank you for listening to Two Bees in a Podcast.

### Amy 00:49

Hello everybody, and welcome to this segment of Two Bees in a Podcast. Today, I am joined by Dr. Zac Lamas, a NIFA Postdoctoral Fellow with Dr. Jay Evans' Laboratory at the USDA ARS Bee Research Lab in Beltsville, Maryland. Today, we are bringing Zac on to talk about his project called Nano Colonies: Rearing honey bee queens and their offspring and small laboratory arenas. So I'm really excited to talk to Zac about it today. Thank you so much, Zac, for joining us.

### Guest 01:18

Yeah, no problem. It's fun to be on here with you and Jamie.

### Amy 01:21

All right. Zac, so the first thing we always ask our guests, just tell us a little bit about yourself. Our listeners would love to hear how you got into the honey bee world.

### Guest 01:29

Yeah. Man, that was so many years ago now, and I wasn't really even doing honey bees at the time that I got into it. My partner at the time, we had been farming for years in New Hampshire while we were going to college, but we bought our first farm property, and we were transitioning over to that. We really wanted to kind of grow the farm so we could both stop working off the farm. So we decided to do a pumpkin patch because we thought it'd be an amazing way to advertise the farm in the fall and sell more raw milk and winter vegetables. And so I planted a pumpkin patch, and when I got off my tractor

after I finished plowing and harrowing and planting, I was like, oh, man, that's going to be a lot of flowers. I should probably buy a beehive. And, yeah, it was 2009 and essentially rained all summer, and the pumpkin patch kind of went to hell, and the bees lived. And that was kind of the start of it all.

**Jamie 01:33**

I love asking people how they got into bees because it's always something different. It's really neat to hear that introduction through pumpkins. Zac, I'm sorry about my voice. I'm recovering from an illness and just now getting my voice back, but I'll do my best to push through with some of the questions. I am excited that you're joining us, and so I do want to just jump straight into the research. You and some colleagues just completed a project entitled Nano Colonies: Rearing honey bee queens and their offspring in small laboratory arenas. So for our listeners out there, could you start from the beginning? Can you tell us the background, the motivation, the purpose, the objectives of this research project?

**Guest 02:51**

Yeah, Jamie, this is actually probably the most exciting thing to me, and what I like talking about the most about the project. Early on in my PhD, so this is the first year of my PhD when I was at Maryland, I was studying Varroa behavior. We had this kind of interesting finding, which was that mites not just move from one bee to another, we knew that they did host switching, but they showed a large heterogeneity in that behavior. They actively switched from one bee to another at different frequencies. But of course, I'm doing this in the laboratory, and I'm sure a lot of your listeners are already familiar with the cage trial. It's essentially a group of bees and either a Petri dish or a plastic cup inside of an incubator in the lab, and it's a way that researchers can do pretty rapid research on bees. The problem is those cage trials are so far removed from colony settings, especially field realistic settings, that we can't always extrapolate results that we get in the lab in the field. So of course, when I'm studying Varroa behavior in these arenas where there's no brood, there's no queen, there's no comb, we're really curious what we're observing here would also stand true out in the field. And I had quickly tried to do something in my queen catching little mini nucs. But no matter how small you make a colony out in the field, you can't track individual Varroa and those things. So there was this problem that I had to fix, which is, how do I observe mites moving on adult bees in the laboratory and still maintain real colony conditions. That fall, I got six queens, and I started tinkering with little different setups. That was the start of it. And it was kind of amazing how it ended up working out, because it was kind of rapid fire, partially because we're up in Maryland. Even though we have mild winters, we do kind of have a bee season and a research season that comes to an end. So I'm trying to get some of this work done so I could either finish the project that fall or start it really early the next spring. Long story short, we had a couple failures right away, but inside of ventilated plastic cups, I'd put a queen in with some workers, and the queen would lay eggs. They'd make a retinue around her, they'd consume and store the honey in the little comb that I had cut up on a table saw and put in there, and I was getting really excited. A little over three days later, the eggs would hatch, and I'd get really excited, and there'd be these L1 larvae, and I'd be like, woohoo, it's gonna work. And then I'd come back the next day, and the bees cannibalized all the larvae, and it was like, no. I'm pretty resilient, so I would keep them going, and I'd have the same pattern over and over again, where the bees would support a laying queen, but then they would cannibalize the young larvae. When I get into a situation like this, I always go to people that have a lot of research experience, and I kind of work with, and I ask them a broad question. Jay Evans is actually perfect for this. I was doing most of my PhD out of USDA at that time, anyway. And I walked up to Jay and I was like, "Hey, if you were trying to get these to rear brood in the lab, but you were

having a problem with cannibalization of young larvae, where's the first one or two places your brain would go?" I always think it's better to ask broad questions instead of super specific because your brain's a supercomputer, and you're asking that person to kind of tap their supercomputer. And Jay said, "I'd either think microbial or nutritional. Hey, Shane and Miguel are doing this cool nutrition experiment. Why don't you go ask them for their diet?" And long story short, Miguel Corona had been producing this supplement, and when we give it to these nano colonies, they stop cannibalizing the young larva, and they will continue to rear that larvae until completion. Sometimes, you get unlucky in life. Sometimes you get lucky. This is a point where we got lucky because we didn't necessarily know why it worked, but it worked. But at this point, it's later in the season, it's harder to get really young bees. I'm digging out bee bread from old combs that I have just to get enough pollen to get these loaded. And this was an unfunded project anyway, but I had this roommate from Turkey that was living with me. His name's Serhat Solmaz, and he has a behavioral lab in Turkey now, a really brilliant young bee scientist, and we actually converted an incubator out of a refrigerator in our living room. And we both tinkered on these over the winter for a few months. Serhat kind of helped with the ventilation part. I kept working on what type of bee composition. And, long story short, by that early spring, no, it wasn't even by that early spring, sheesh, it was sometime December, January, we had emerging brood from these things. That was the start of the experiment and the project. If we knew how cool it was, what we were doing at the time, we would have taken a lot more pictures, but that was kind of the start of it all.

**Amy 07:32**

Very cool. So you kind of answered this, but I'm going to ask you anyway. So why would you want to rear bees in the laboratory? And again, you already kind of answered this too, but what types of challenges -- so you said that there was cannibalism going on, but what other types of challenges did you have with your project at home? But also, I assume that the methodology of the paper, the project that you're working on, is different from what you were doing at home with your roommate, right?

**Guest 07:57**

Yeah, it is, Amy. Actually, to each of those points, so none of the data from what we were doing in the living room ended up making the paper. That was all be what we call preliminary work, preliminary trials. I call it the tinkering stage. Right? We have parameters that are either unknown to us and or we haven't figured out, so we're just kind of quick fire trying to work through those. But I only partially answered why you would want to rear bees in the laboratory. And to be clear, especially your lab, your labs were known for in vitro rearing of bees in a laboratory. And there's a lot of utility for in vitro work. We believe there's also a lot of utility for in vivo work, which is what our project can do. And one of them, we did partially answer, both in terms of trying to study something in a more natural environment, but the other is, we have the ability to create a lot of colonies with very little variability between replicates. So to give everyone an idea, I first started with pesticide work, and it was a pretty large field trial. I think we have 32 colonies in the trial, which, believe it or not, for a lot of pesticide work, that's a large trial. And the unfortunate thing is, when you start with a trial like that, you're always going to have a lot of variability between one colony and another. Now, that variability is good because it's something that we would see that's feel realistic in any operation. So any commercial operation is going to have a lot of variability from one colony to another, but it can be bad if you're trying to identify how something is affecting honey bee colonies because you might get a lot of noise in your data. We can take away a lot of that noise with this nano setup. For example, we did a trial that's not in this paper, and we ended up making 15 replicates. Well, those 15 nano replicates, it's roughly about 130 bees now with a small section of

comb. The comb for all of those replicates came from one deep brood frame, empty brood frame. So an old brood comb. You just cut it up on a table saw, and you can cut it up into 15 pieces. All of the bees in every single replicate came from one colony, from two frames of emerged bees. So there should be really little variability between one replicate and another. The comb is all the same, the nutritional source is all the same, the diet is the same, the source bees are the same. We use sister queens that I produce in-house, so they're not instrumentally inseminated. We leave that natural to bees. But they're all sister queens mated at the same time of the year, and so the only real difference is we are giving an exposure to one group and not the other. Whereas, if I was to do this work in the field, I'm going to have a lot of variability between my colonies. Also, I can't control some of the exposure that each colony is going to experience in the field. For example, some might go to a tree that was injected with a systemic insecticide, or some might go to one floral source versus another. Now, again, that's totally fine and field realistic, but if I'm trying to understand how a certain pesticide affects honey bees, it's a lot of background noise, and we can start to eliminate that. So that's one reason that we really think that people might want to use nano colonies and rear bees in the laboratory. There are some challenges that come with this, but they're pretty minute. It can seem tedious setting these up and doing the work to maintain them, but it's nothing compared to the amount of setup that it takes me to do a proper field trial. I would say the biggest issue with them is they are super susceptible to nutritional stress, and anyone that's done cage trials knows you need to make sure your cage trials don't get nutritionally stressed and there's no problem with the feed that you're giving them. Same thing here, I would say what we don't know is their resilience to nutritional stress. So that might be a parameter that's difficult to study in these little nano colonies.

**Jamie 11:40**

So Zac, I have three questions for you. Unfortunately, only one of those three is scripted, but I was listening to you talk, so I have two more that have developed. So I'll ask them not scripted, scripted and not scripted, kind of in that order. So from the first unscripted question, could you describe very specifically the makeup of this nano colony, like, what's in it? How many bees, how much brood? You're talking small sections of comb, of queen. What composes this nano colony?

**Guest 12:06**

So what composes a nano colony? We have two different types of nano colonies, and right now the arena is a white Solo cup that has been cut into about a 2/3 section with number eight hardware cloth hot glued to the bottom of it. The lid is a lid that has been cut so there's no plastic center of the lid, and we replace that with Noseeum netting. There is a small section of comb. I don't remember the exact dimensions off the top my head. It's roughly two by two inches, and there are two feeders in the bottom. They're just Eppendorf tubes that the bottom has been cut off. And you can either pack one with pollen and you can fill the other one with our liquid diet, which is a mixture of honey and this supplement that another lab had created. The easiest way to set these up is to, well, there's two different types of nanos, by the way, and now I should mention that. So we have nanos that we call queenright nanos. These are the ones where you put in a minimum of 100 worker bees. You could put up to a couple hundred. We really like around 13 grams of bees, which is approximately 130-135 worker bees in there. Newly emerged are great for this. And then we add one queen, and that's a queen rearing nano. And they'll attend that queen, and within a few days, start to rear the brood from the eggs that that queen will lay directly into that brood comb. The other type of nano we have are queen rearing nanos. Now, these are set up slightly differently, and what you essentially do is you make a cell builder out in the

field, you graft into that cell builder, and for everyone that's not familiar with grafting, we can produce queens commercially. What you do is you make a hopelessly queenless hive. You transfer very, very young larvae into these very small plastic cups and colonies have a natural mechanism to replace queens if they're lost. So what they'll do is they'll overfeed those larvae and turn them, instead of a worker development pathway, they'll turn them into a queen development pathway. We take advantage of this and what we'll do is take that queen cell about 12 to 24 hours after it's been established, and take approximately 100 to 130 or so bees from that cell builder, and put them into that plastic cup setup that I just described, and then bring them back into the laboratory. Not in the paper. You can actually do this with just newly merged bees and kind of time amounts of three days old. By the time you give them a 12 hour larva, you can double graft into the queen cell cup. And you can also do this with a completely artificial environment, so you can raise queens on paraffin wax with nothing but pollen sub and sugar water, and it will still make a queen for you. Jamie, if I fully answered that first question, let me know.

**Jamie** 14:44

You did. That was perfect, which kind of leads to the scripted question. But I think your answer is kind of already embedded in what you mentioned. So in these systems, the bees are capable of rearing queens and worker brood.

**Guest** 14:55

That's correct, they are capable of rearing both. And the thing I will point out, because it might be a surprise to a lot of listeners, it's easier for us to rear queens than it is for us to rear worker brood. The queenright nanos and the rearing of the worker brood, they're a little bit more finicky. There seems to be a seasonality to it, where we do it in late fall, and we've really got to pay attention to these, whereas the rearing queens was far, far simpler to figure out. We can do tons of replicate, and it's pretty easy to do. I think there's also a component of why you would want to create bees in a closed system. So I don't want to do too lengthy of a response here because we can kind of get stuck in the weeds. But let me give you my favorite response of why we would want to create bees in a closed system, and that is my optimism of where honey bee research and science generally will go. I have a stretch goal that we'll eventually be using the honey bee as a model for human health far more than we are now. And there's some advances that are somewhat in their infancy right now that hopefully will continue to advance and become standard procedures in our research, and one of them is knock down lines of honey bee queens. And right now, if we have a knock down line of honey bees, we're not going to use those out in field. We don't want that germoplasm to end up in other areas. We have a very social insect. The movement of modified drones or queens is something that we don't want to release in the environment, but our nano system actually provides a really safe housing environment for someone to do that research and continue to study, maybe, the next generation of bees developed from those queens or to test, let's say, pesticides on knock down lines of bees. That's something that I think would be really exciting, and why you would want lab reared queens or worker brood. Our lab is also pretty famous for coming up with clone derived viruses and viruses with some genetic inserts. We only do this work inside the lab, and when I was doing my PhD, Eugene Ryabov, really, this molecular genius, was developing some of this work, and he was only able to do really injections on pupae, some movement with that modified virus with mites. And that was restricted into the lab in small laboratory spaces. We bring these nanos in, and suddenly we can do a lot more work, especially transmission work, with Eugene's viruses than we would otherwise. And in fact, we had a really exciting partnership while he

was here trying to tinker and investigate with this stuff, because we can study viral transmission easily and with some specificity and without the background noise that we just couldn't do in the field, and also we couldn't ethically do in the field with a modified virus. So I think there's some really great opportunities in the future with using lab brood queens or worker brood where you wouldn't want them in the field, but you definitely would want them in the lab.

**Jamie 14:58**

So Zac, then, here's the third question I wanted to ask you, and it's not necessarily in line with what you discussed, because what you've discussed has been very practical, clear applications that can come from it. But the thing that kind of came into my mind, which I've often wondered outside of the research that I do, is what actually defines a colony. When does putting enough worker honey bees together create a colony, rather than just a collection of worker honey bees, right? Two worker honey bees, that's two worker honey bees; three worker honey bees, three worker honey bees. But you're calling these systems with 100 worker bees or more nano colonies, and it sounds like, for practical purposes, they are functioning as small colonies. So maybe you haven't thought much about this, but I'm curious what this says about the definition, formation, the identity of a honey bee colony?

**Guest 18:42**

Wow, what an amazing question, Jamie. That's almost philosophical. You know, what defines a colony? And I haven't thought about that. That's such a good question. Man, you're going to distract me for, like the rest of the day as I'm doing my work.

**Amy 18:56**

Welcome to Two Bees in a Podcast.

**Jamie 18:57**

Yeah, well, Zac, think about it because I've often thought about it. You'd mentioned that we can in vitro rear workers. We can. And one of the things we'd like to do, although we can't yet, because of just the way royal jelly, etc, works, we'd like to rear honey bees in vitro, etc, and try to develop colonies out of these. Again, that's not something we can do now. We've got to go through permits and all that stuff. I'm just acknowledging that this has been something that's on my mind is like, well, when do bees cease to be collections of bees and actually become colonies? And so I would argue what you're describing is kind of the smallest colony unit that I've ever heard of, maybe the smallest functioning colony unit. So it's interesting. Think about it, and let me know if you want to work on it together someday.

**Guest 19:39**

Yeah, no. These are the type of questions that pique your interest, and hopefully the thought doesn't enter your brain around 11 o'clock at night when you're going to bed, because that's a good way not to sleep through the night, right? Well, let me start with, oh man, it's such a great question, because every time we get this question, it's not this question. It's usually tied to ELAP payments. What constitutes a colony? How long does it have to live? When can you make a claim? And it's very practical, it's very applied. It's economically attached, right? You're asking something completely different. And there's an older paper about worker bees finishing and capping queen cells in the laboratory. A single worker bee can cap that queen cell, and the rearing and finishing of a queen cell is definitely a colony act, right? It's

the intention that we will replace a queen and have continuity in this hive, survival going to the future. But I wouldn't say that one bee capping the queen cell represents continuity going in the future. It can't do all of the other things necessary for it to be a colony, just like with our colonies, we are their pollen and honey provisioners. We supply resource acquisition for these colonies. We haven't asked them to forage yet. So does the act of not being able to provide for themselves going into the future now not make them philosophically a colony? On the flip side, they show incredible cohesion. You can sit, I don't recommend sitting in a walk-in incubator for long periods of time, but you should, and you should sit in front of these things and watch them. And they're not just bees running around frantically inside of a Petri dish. They're engaging in antonation regularly with each other and trophallaxis. They are crowding the wax like -- this is me being subjective, but like a healthy colony in the field would. They are forming a retinue around the queen. Essentially, they're engaging in cohesion as a cooperative super organism would. To me, that is the most defining feature of a honey bee colony, that they have cohesion and that they're a cooperative organization. I can't fully answer your question here. We'd have to talk for a lot longer.

**Jamie 21:46**

That's okay. Yeah, I knew it was philosophical, but it is an intriguing question to me, and maybe your protocol can help this question be answered. Amy, over to you.

**Amy 21:56**

Yeah, definitely. I was about to say, Zac, I hope you can sleep tonight for the rest of the week.

**Guest 22:01**

Thank you.

**Amy 22:02**

I'm going to bring us back around again, back to just looking at honey bees raised in a lab versus field raised honey bees. I think many of our listeners may be listening to this, I think that they understand, yes, from a scientific side of things, they understand from a research side, you've got more control, you're able to take out some of those outside factors that may be in the way. But are there concerns about raising honey bees in the lab versus in the field? Have you ever gotten those questions? I'm wondering, because I do know that you have a lot of speaking engagements as well.

**Guest 22:37**

I don't believe I've had that question about concern about raised in the lab versus the field. If I've given a talk about these nanos before, there's usually a comment they make about the fact that this isn't going to be field practical or commercial. Honey bee queen breeders have become extremely efficient, especially in your state, producing lots of queen cells and lots of new queens, and we can do this really efficiently out in the field. So I don't necessarily see this setup as replacing that. Just from a practical standpoint, I don't think anyone should be concerned by it. I wouldn't be concerned at all about taking one of our bees that we produce in the lab and putting it in a colony and thinking of any negative consequence. It was essentially raised on a similar diet and similar feed. It's just in a more artificial environment, we should say. But I think there's a lot of opportunities to look at the difference between field raised and lab raised bees. For example, in our paper, there was no significant difference between the mating success of queens reared in our first trial, if they were reared in a traditional cell builder

versus in the lab. But a lot more of them mated being field raised in the second trial than in the lab during the second trial, and that was really interesting. And if I could just do iterations of those trials out, I would want to because if there is a significant difference in different seasonality or with different, maybe, environmental exposures between the two, then I think we have a really good opportunity to do some experimental biology and try to ask, what are some really important parameters that affect queen health, queen development, queen mating, an economically important question, a really interesting question if you're just interested in mating biology or queen reproductive health, and I think having the opportunity to do things in the lab and also in the field, you can have some comparisons that might make for some really lucrative science. That's kind of how I would think about and pose that question.

**Jamie 24:28**

So Zac, we have a really large beekeeping audience around the world, and so a lot of the beekeepers are going to want to know, how is the industry going to be able to use this? Maybe not the method directly, but how will it ultimately benefit them?

**Guest 24:40**

Well, originally, we were hoping that we could use these modified nanos to overwinter queens inside. And if we could, it might be, I just earlier said, it's not really field practical. The beekeepers are so good at rearing queens, and that's true, but there's some northern climate industries, so primarily Canada, that is putting a huge investment into trying to figure out how they can overwinter queens, because it's a bottleneck in their industry that they really need to economically fix, and it's not a problem you need to fix. But if you were to think of the economics, they do. That's something that we were thinking there might be some utility here. You would definitely need to figure out an efficient, cost effective way of feeding bees because, again, they're super susceptible to nutritional stress. I think the beekeeping industry could possibly use this as maybe a cheaper, more rapid screen for pesticide studies. And after I had started to use this for mite work, that's really where my brain wanted to go with doing some of this work. I said, I started with pesticide work at UMD, and that experience with the variability and working out -- and I moved down from Vermont to Maryland to the DC area. I was familiar with heat, but I wasn't familiar with with doing field trials in that heat. There's some huge appeal to possibly doing some of this pesticide screening in these nanos, and I kind of hope that someone adopts it for that use.

**Amy 26:04**

So, Zac, that leads me to the last question that I have for you. What research do you think needs to be done next?

**Guest 26:09**

What research needs to be done next? Not needs, but what I really want to do, and I think some people are really already starting to work on it, and it's an exciting place where I think our research field can go to and will go to. So when I started this project, I was still taking classes at UMD. I was doing a lot of research for my project, trying to propose it to my advisor at the time. And I came across this amazing paper, two amazing papers, both out of University of Illinois, and one was by Julia Fine. She has this queen quantification lane set up in the lab, and then they also have that original Caranot paper with the bursty patterns where they put QR codes on thousands of bees, video record them and quantify the interactions. And I thought, independently, these papers were both amazing in different ways. But of course, I'm in the lab, and I have a nano colony in my hand, which is a plastic cup, and bees trying to



act like a colony. And I'm like, well, if we can merge these two papers together, we could do some really cool work. And there were multiple reasons for this one. I think the camera that they used in that bursty paper is like \$30,000 so it's really prohibitive for a lot of labs to use. But those arenas that Julia Fine was using are really inexpensive and you can observe them really quickly. Wouldn't it be cool if you can merge those two with a less expensive camera and have really inexpensive replicates, where you might be able to, say, study the exposure of a virus versus no virus on mixed bees inside of these nano colonies, and then at the end, freeze all of your samples? What the goal is, and what the goal of mine has been, is to be able to try to triangulate an exposure to a change in behavior of a specific individual, and then grab that individual to go to molecular targets of interest. To me, that would be a really big advancement and something kind of amazing that we could do. And I know there's some different interests in different groups on doing something like that, but that's really where I'm hoping the research could go. Again, we get a lot of noise sometimes when we do field trials, or when we sample large numbers of bees and try to extrapolate how an exposure is affecting those bees. To me, and if you look at my other research, we always look at the individual level and then start to build a bigger and bigger, bigger story about the social organism based upon interactions on the individual level, and whether that's bee to bee interactions, or mite to bee interactions, or mite to mite interactions, we've done that. That's kind of where I'm hoping this research will go. Essentially, I call them 2-D nanos. If we can get these nanos into a Petri dish with paraffin wax, you can simply monitor all of those individual bee interactions and the brood rearing, but then you can start to extrapolate how different exposures affect these within that single replicate. And again, the goal is to do it for pennies on the dollar for what other research is doing. Unfortunately, this has been kind of the red-headed stepchild of my research, where it just seems to be the thing I can't get to, can't get to, can't get to, because we have so much lucrative mite research that's been kind of done in the meantime, but that's where I'm hoping to go with this when we really pick it up again.

**Amy 29:20**

Yeah, definitely. So what's next for you? What are you hoping to continue doing?

**Guest 29:25**

I'm hoping to land a permanent job so I can do this work indefinitely. That's a postdoc joke, Amy. I think it's the thing that all postdocs kind of have on their mind.

**Amy 29:34**

I know. That's actually why I kind of asked you that question.

**Guest 29:36**

Oh, really, was it? But it's a really good question because I get two emails somewhat frequently in my inbox, and it's people asking about some of the in-house methods I have for handling and maintaining mites. And the number two thing are these nano colonies, people that are really interested in gut microbiota works, utility in this, and then people that are using any type of modified organs with bees kind of see the utility of this. Again, that thing with safe housing. But what I want to work on next is starting to combine exposures with what I just described there. But that's going to be likely a long iterative process of figuring out all the glitches.

**Amy 30:13**

Very cool. Well, I'm excited to see you continue in your career and contribute to the honey bee world. Thank you so much for all of that. I was just wondering if you had anything else to add while we are about to end this episode.

**Guest 30:23**

No, I don't think so. This is, I think, Amy, and don't let this go to your head or Jamie's too much, but I think this was the most pleasurable podcast experience I've ever had. This has been just nice. Well, it's almost like we're sitting around bee boxes, having a beer, chatting.

**Jamie 30:40**

High five, Amy.

**Amy 30:42**

Air five. Air five on the radio.

**Jamie 30:45**

Virtual air five.

**Amy 30:45**

Oh, yeah. Well, good. I'm glad to hear that, Zac. We try to make it as easy as possible. We are here for our listeners and trying to just disseminate all the information that we can. So we truly appreciate you being on the podcast, and we'll have to have you on for future papers that you publish as well. So thank you so much for joining us today.

**Guest 31:05**

Well, thanks, Jamie. Thanks, Amy. Hope you all have a great rest of the day. Feel better, Jamie.

**Amy 31:20**

So Jamie, I'm glad that we got Zac on the call today to talk a little bit about the nano colonies. I thought it was kind of funny when you had asked the question of, like, what makes a colony a colony? I mean, I don't know. Like, I probably won't be able to sleep at night because now I'm realizing, like, what does need to happen inside of a colony? But I think it's really interesting what Zac is doing with his project. It really is seeming like a lot of it is the methodology. Am I right? Am I reading it correctly? What are your thoughts on these nano colonies and rearing workers and queens in the laboratory?

**Jamie 31:53**

Yeah, essentially, what he and his colleagues have done is develop a method. And for those of you who are listening who may be unfamiliar with this, there's research -- all experiments are built on the back of methods, and a lot of those are standard methods, like PCR, polymerase chain reaction, or currently, in vitro rearing of honey bees, right? But a lot of projects need new methods, new designs, new protocols to be developed to be able to answer questions, and so Zac and his team of colleagues have done that. They've developed this method of using nano colonies to rear queens, to rear workers and allow us to test things in ways we weren't able to test before. Our team has a recent history of method development, and it's just equally important to develop these protocols that can be used repetitively, predictably and globally, almost as it is to generate the new knowledge using these

protocols. And that's really the power of what Zac and his colleagues have done as they've developed this new system that -- who knows what we're going to be capable of doing with it, and what types of questions we'll be able to answer. And so anytime you have a new tool, new method, a new protocol to answer questions, these things tend to take on a life of their own and open up new avenues of research we'd never before considered.

**Amy 33:11**

Yeah, absolutely. And I also think just from that perspective, like sometimes, and we know this, and we recognize this, research takes time, right? Research does take a while. It usually takes much longer than what people realize that goes on behind the scenes, but there is a process to it, and I think exactly what you said with just creating new methods and new tools that other researchers can use, even that takes time. This is kind of just part of the scientific process, which I really enjoy. I like that process. I like seeing how different methods work, just different projects, different objectives, and the way that they can be used in the long run. So I appreciate what he and others, Dr. Jay Evans, we've had him on the podcast before, but I appreciate all that they do.

**Stump The Chump 34:04**

It's everybody's favorite game show, Stump the Chump.

**Amy 34:14**

All right, welcome back to that question and answer segment. Jamie, I've got three questions here for you. The first one's taking us back to the very beginning of time in our podcast. I mean, this is probably from 2020, honestly. It's episode 26. So we're going back in time. I hope you can remember.

**Jamie 34:32**

The missing year. That was the missing year, right, with COVID. I did chuckle when I saw that it was episode 26. I'm like, good gracious, there's no way I remember anything from episode 26.

**Amy 34:43**

I know. I'm like, you don't remember exactly episode 26 and what we talked about? But okay, so in episode 26, we had Dr. Tammy Potter on and the questioner is asking, so Dr. Potter mentioned a couple of times about smoking bees about 30 minutes before you start an inspection. This person had not heard of this before. And so the question is, does this work? I mean, is there a time before you go into your hives that you should smoke, kind of let it sit and then go in, or is it more of like an immediate thing where you light the smoke, or you puff it in a little bit and then you open it up and do your inspection?

**Jamie 35:16**

Yeah, so I don't recall the specific quote from Dr. Potter, so I hesitate to speculate specifically on what she meant. And I've also never seen research, like, this isn't a topic that I've looked at before in the literature, research associated with how long before you work a colony should you smoke the bees? However, I will say that my mentor was kind of a firm believer in smoking the bees a few minutes before you go into the colony. And of course, the vast majority of commercial beekeepers, and myself included, I'm not a commercial beekeeper, but I've been beekeeping for decades, we never smoke, and then wait and then go into the colonies. I smoke the bees while I'm taking the lid off the hive. I might

smoke some in the nest entrance. So it might be one of those things that's beneficial, but I would say maybe it's not necessary in most situations, and only because that's anecdotal support, right? Because that's just not what most people do. Literally, when I work colonies, I'm smoking into the nest entrance of a hive while I'm removing the lid. So personally, I would slow down if removing the lid and smoking the nest entrance, I might get an unusual amount of bee attention in that process. However, I think more often than not, you can smoke bees and work them and then make the decision while you're in those early stages of working them, whether additional smoke is necessary. So I have heard people smoke hives well before you end up going to work in the colonies but I just don't think it's necessary in most cases. Again, I'm not trying to go for or against what Dr. Potter said, because I can't remember what she said. I'd have to go back to the episode. I would just say, in most context, smoking bees in the process of going into the hive is usually sufficient.

**Amy 35:16**

You know what we should do? We should do like a whole -- maybe 2025 will be the year where we go back and listen to every single episode, and then we can do commentary on what we said just to say hm...

**Jamie 35:47**

Oh gosh, the editor's cut. I don't know if I want to hear myself. Most of our listeners may not believe this, but you and I don't go back listen to the podcast because we don't like to listen to ourselves. That's why it's so hard for me to recall, really, what people say. I know I'm guilty. It sounds terrible that we would do that, but it's just because most people who ever record themselves doing anything don't want to go back and hear it or see it.

**Amy 37:23**

I definitely agree with that. Okay, so for the second question that we have, this person is asking, so we've had episodes before talking about queens piping. Do you remember the one where we did the tooting and the quacking?

**Jamie 37:35**

I do.

**Amy 37:36**

Yeah, that was a lot of fun. That was a memorable episode, for sure. So this person was asking about piping that unemerged queen's embark on. So if the queens are piping from within their cells, do you know why a queen who cannot defend herself would pipe from inside a queen cell when she would essentially be calling for execution?

**Jamie 37:54**

Yeah, that's a good question, and I can only speculate, because it's not really well understood, but the questioner's exactly right. You could ask that about a lot of things in the beehive. Why would, for example, for hygienic behavior, the adult worker bees can detect a diseased or parasitized immature bee, open up the cell, abort that brood, and hopefully get rid of the Varroa, American foulbrood, or whatever. So you could ask the question, if hygienic behavior is bee pheromone mediated, ad again, that's got to be worked out, but if it is, why would brood say, I'm sick, remove me, right? That would

lead to their removal. And I would say just kind of a few things on this front. You've got to remember that selection in honey bee colonies increasingly happens at the colony level, rather than the individual bee level. We see a lot of altruistic type behavior in honey bee nest anyway. For example, worker bees die when they sting. Why would they ever sting if they're going to die? Well, the questioner saying, why would a queen pipe in her cell if it leads to her execution? Because maybe that's one of the ways that the first emerged queen can find her competitors and execute them. But remember that piping may serve another function as well, that the first emerged queen can just take advantage of. What would that function be? Well, one queen who is piping could be communicating to workers outside of the cell that, hey, I'm mature, I'm ready to emerge. And under what context might that occur? Well, there's research that shows, in some cases, when a colony swarms, as an example, and the swarm leaves behind a hive with multiple queen cells, let's just say, for the sake of argument, 10 queen cells, that workers might coalesce around those queen cells and not let the queens inside emerge, and then they do slow releases of one queen at a time. So they might allow one queen to emerge, who will then lead a secondary swarm, and then another queen to emerge, who will leave a tertiary swarm. So in those contexts, the workers would be protecting those queens that are developing from those virgin queens that are emerging so those virgin queens and gold swarms, but they still have queens developing in the nest. So it's possible that piping didn't originate from a 'here I am, come kill me' standpoint, but more of 'okay, we're ready to emerge, let me emerge, let me emerge' as a communication to the workers that virgin queens subsequently could take advantage of and use as a way to find their competition and kill them. So it becomes kind of a chicken and egg argument. Did the positive attribute of piping come before the negative attribute of piping? And it's certainly possible. And you have to remember, in this kind of social context where the colony is the important unit, you can get what looks like negative individual behaviors like hygienic behavior or stings that kill the workers, or piping that kills queens, potentially, that overall benefit the whole nest. And so this might be one of those cases as well.

**Amy 40:56**

Interesting. Yeah, I don't think I thought about the secondary and tertiary queens at that point. Very cool. Okay, so the third question. So there are a lot of questions to this third question, but I do think it's important for us to discuss all of them. This questioner is asking about recommendations or techniques on basically putting a frame into an observation hive. Jamie, we do a lot of outreach events with our lab. I mean, I feel like any beekeeper who's doing outreach events, we know that when you have an observation hive, that is what attracts people over to the table. That's always the case. This person is wanting to minimize stress on the bees that are staying behind, but also the bees that are going to be at the event. Let's just start from the beginning. So, yeah, I'm like, there's a beginning here.

**Jamie 41:43**

I heard you say that. The bee-ginning.

**Amy 41:44**

Oh, I didn't do that on purpose.

**Jamie 41:45**

You said let's start at the bee-ginning. I'm like, oh gosh, Amy, what a pun.

**Amy 41:50**

I know. Oh my gosh. Okay, the other part of it too -- Let me take a step back real quick. But the other part of it, too, is that these events where we do have observation hives, sometimes they're just a couple of hours. Sometimes they're multiple days. Really, let's start at the beginning. What is the best time to transfer frames over from your hive to your observation hive?

**Jamie 42:12**

The key is when you are putting an observation hive on display such that the bees cannot leave the nest, they can't forage. It's not like they're plugged up to a wall where the bees are coming in and out of the wall into this glass hive. When you are putting bees on display where they cannot fly, it's a stressful time for the observation hive. So leaving them in that observation hive the least amount of time possible is always more favorable for the bees. And so your first question, Amy, is, when should we put bees into the observation hive? If I get to choose the ideal time, it would be the morning of the event, right? If the event is from 10 in the morning till four in the afternoon, then I would put them in the observation hive at eight in the morning, right? Nine in the morning, in the time leading up to the event. That may not be practical. You may have to do it the afternoon or evening before, but it's always better to do it as close to the event as possible, because that minimizes the amount of time that they have to be in that hive.

**Amy 43:11**

Yeah. So that takes me to my second question, if they're going to be in the observation hive, what would you say the maximum time you could keep those frames and the observation hive would be? I know that there are events sometimes, I mean, I think that state fairs always have, the State Beekeepers Association will bring an observation hive, and that could be up to a month sometimes.

**Jamie 43:33**

Yeah, it's really tricky in those circumstances, right? Because I would argue, if you're going to completely enclose a hive where it cannot forage, and you are unable to open it up over the course of a few days and allow them to forage, I would say something like three to four days is really pushing it from a bee health perspective. And so a lot of times what people will do at these kind of long, say, State Fair events or things like that, they get permission to move in the parent hive somewhere on the property to where it's not a place where people and animals can frequent. It's kind of out of the way, maybe fenced, maybe completely hidden from other people. And daily beekeepers rotate, like at the end of the day, they'll put the frames from the observation hive back out into the hive somewhere outside that's currently hidden, allow them to do their thing. First thing in the morning, they restock the observation hive and take it back into the fair. That's the best thing. But if you can't do something like that, I would say something around three to four days max is really the maximum amount of stress that you want a fully enclosed, cannot fly, three or four day max is really what you're shooting for.

**Amy 44:44**

Now, what about water? Because I know that sometimes I've seen beekeepers either spritz some water where there are breathing holes. How often do you think that beekeepers should be giving water to the bees, and how would they give the water to the bees?

**Jamie 44:57**

Yeah, I worry less about water mainly because, usually when bees are in an observation hive, they are in some sort of climate controlled room, right? You're inside of something that people are walking around. If, on the other hand, you happen to be stationed outside, you wouldn't necessarily have to spritz them with water. You could just feed them sugar water, and they can get enough water, potentially, from that to satisfy their water needs. I mean, spritzing them with water requires you to have some sort of screen mesh somewhere in the hive, and it's just not something I've worried much about unless, again, you're outside and it's really, really hot where they are, in which case you could spritz them through an opening at the top just a couple of times a day. And when I say an opening at the top, I mean a screened opening at the top.

**Amy** 45:42

Not opening the frame.

**Jamie** 45:43

That's right.

**Amy** 45:44

Yeah. Okay, there are two more questions to this one question. It's like question three, but A, B, C, D, E, F. Okay. So the question is, there are different types of observation hives, so there is the observation hive with the one observation hive on top, and it's basically over a nuc, and then you also have just a single frame hive. Are the nuc size observation ones less stress-inducing on the bees than a single framed hive?

**Jamie** 46:08

Yeah, a few comments about this. So if we broaden the question to say, what type of hive design is best? Well, it would be all under different circumstances. The single frame hive, which is usually a single deep frame, is really good. If you're just going somewhere for the day, you got a few hours, you got to go to a school class, a 4-H class, Boy Scouts, a church social, something like that, where you just have a few hours, but it's less than a day, you could pop in a single frame. No big deal. If the bees are going to be in there, unable to fly for successive days, you really want probably two or more frames, because you're going to want one of those frames to be predominantly resources, honey and pollen, so that the bees aren't going to starve to death or experience nutritional stress while they're in that observation hive. A single frame usually isn't sufficient for multiple days because that's pretty stressful, because they don't have enough resources. Now, the questioner specifically mentions that nuc observation hive, there are a couple of equipment manufacturers that essentially make a five or four or three frame nuc body that has a lid with a slit in it, and that slit is a queen excluder. And over that lid with the queen excluder is mounted a glass observation hive. So you can move a frame from the nuc base into the glass part of the observation hive above the excluder. So you can put the queen on the frame above the excluder in the glass observation hive part, and bees can come and go through the excluder material down into the nuc body where there are other frames of bees and resources and brood. This is a good design for multiple days, where it's essentially a functioning colony that has had one frame pulled out above the hive that you can see through glass. Even still, the bees in the nest below, they can't carry out their dead, they can't forage, but it's still a better way to have resources available to a single frame over time. It even lends itself, Amy, to, at the end of the day, you can go put the nuc outside, let it fly, then you come first thing in the morning, close up the nuc, bring it back inside

and start all over again. So it's a really nifty design to kind of have your cake and eat it too, where the bees have enough resources it can survive multiple days, etc. So there are kind of pluses and minuses to those different observation hive styles.

**Amy 48:34**

Yeah, on that note, those nuc styled observation hives, they have that queen excluder so that the queen can stay above in the observation area. And that's what people are really attracted to. They love playing find the queen. That in itself, I feel like, is a risk, just as far as you having your colony at home and donating your colony to put into an observation hive, which leads me to my last question. Are there risks that the home colony, so the colony that you left at home, could they start replacing a queen if she's away for a long period of time? Is that a concern that a beekeeper should have?

**Jamie 49:08**

Absolutely. Bees can detect that they are queenless. A colony can detect queenless in about 12 to 24 hours. So obviously, if the queen is outside the hive past that threshold, the colony begins to believe itself to be queenless, and can start the requeening behavior. I don't worry about that at all. If it's just a single day observation hive, you take her with you in the morning, and then you put her back in the evening. If I do it for a second day, probably when I put her back into the original hive at the end of the second day, I might check to see if there's been an effort to start making queen cells. If so, I might remove them. But days three onward, I might consider introducing that frame with the queen back into the hive as if I were introducing a new queen. I might cage that queen for a few days when I put her back into her original hive and then manually release her after 48 hours to ensure that the bees have reaccepted their former queen. So a day, wouldn't worry about it all. Two days, I might check for the development of cells. Days three onward, especially if it's a strong hive and a weak frame that you took out with the queen, I might approach it as if I was introducing a new queen back into that hive.

**Amy 50:20**

This is a totally random thing, but could you put a queen pheromone in there while you have the queen out of it? Is that a crazy idea?

**Jamie 50:27**

You certainly can. That can stabilize the hive in the absence of the queen. So maybe they wouldn't initiate the queen production process, but that could smell just different enough from their original queen that they still might not recognize her. So it's certainly something that might stabilize them for 24, 48, 72 hours, but maybe over the long term, you still might have to take that reintroduction step just to make sure they don't kill that original queen.

**Amy 50:52**

Sounds good. All right, listeners, so those were the questions for our question and answers for today. You got a little bit of extra questions with that observation hive, but keep the questions coming. Send us an email or write to us on one of our social media pages. Thanks for listening to today's episode. This episode was edited and produced by our podcast coordinator, Mitra Hamzavi. Thanks, Mitra.

**Jamie 51:24**



Visit the UF/IFAS Honey Bee Research and Extension Laboratory's website, UFhoneybee.com, for additional information and resources for today's episode. Email any questions that you want answered on air to honeybee@ifas.ufl.edu. You can also submit questions to us on X, Instagram, or Facebook @UFhoneybeelab. Don't forget to follow us while you're visiting our social media sites. Thank you for listening to Two Bees in a Podcast.