Biologists and Graph Interpretation Network

Biodiversity Counts!
Making sense of diversity metrics and graphs

A BioGraphI Module Lesson Guide for Instructors

Original Authors

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Course Information

Department/Program: Ecology and Evolutionary Biology/ Introductory Biology course (BIO 94)
Level: Introductory
Course type: Lecture / Dry lab
Delivery mode: In person, synchronous
Students: First year, mostly majors
Number of students: 20 - 80 students
Estimated duration of activity: 90 min

Expected date or dates of implementation

1st implementation: February 2023
Future implementations: Winter quarters

Purpose/Background

This activity was developed as part of a dry lab research course where students analyze real metabarcoding data and explore diversity patterns across experimental treatments.
The purpose of the lesson is to 1) have students become familiar with various graphs that are used to study diversity patterns within and across sites, and 2) introduce some of the questions that can be addressed by metabarcoding studies.

List of materials needed for the lesson

- Link to interview on YouTube (total running time, 54:18)
    - https://youtu.be/5LtU_oRJsjo
- Link to presentation slides
    - P 2_Presentation slides_Biodiversity counts
- Link to 1 page Instructor Guide
    - 3_Instructor Guide_Biodiversity counts

- Link to Basics video and quiz
    - Video: https://youtu.be/kfhDh1vb4IYb
    - 5_Basics Quiz_Biodiversity counts.docx
- Link to worksheets
    - 6_Worksheet_VennDiagram_Biodiversity counts.docx
      7_Worksheet_AlphaDiversity_Biodiversity counts.docx
    - 8_Worksheet_BetaDiversity_Biodiversity counts.docx
    - 9_Worksheet_PercentStackedBarplot_Biodiversity counts.docx

About BioGraphI modules

This lesson is a BioGraphI module. BioGraphI modules address data literacy while fostering diversity in undergraduate biology classrooms. They are lessons about graph and data interpretation, featuring the scientific contributions of biologists who are members of historically excluded groups (HEGs). They include video interviews with these biologists, allowing students to hear directly from HEGs about their discoveries. For more information about how the BioGraphI project is advancing inclusion in biology and improving data literacy, visit our webpage.

Student Learning Objectives

The BioGraphI Student Learning Outcomes (LOs) describe what students can expect to gain by the end of the BioGraphI lesson. They are written in a format that can be shared directly with students.

Quantitative learning objective(s)

1. Analyze data and/or create graphs/figures related to the concepts from this lesson.
2. Reflect on your perceptions about using graphs or figures in biology.

Diversity/equity/inclusion learning objective(s)

3. Reflect on your perceptions of people who do biology.
4. Compare your own interests and/or identities to those of people who do biology.

Content learning objective(s)

5. Interpret diversity metrics (e.g. alpha and beta diversity).
6. Use metabarcoding data to compare communities.
7. Identify the research question being addressed in an experiment.

Assessments

To help the BioGraphI Project to measure the effectiveness of our modules in improving data literacy and fostering diversity in biology classrooms, we invite your students to participate in a voluntary, anonymous pre-/post-lesson survey (Geneseo IRB #202021048). This survey is designed as an opportunity for reflecting on the Quantitative and D/E/I learning objectives above and administered via LimeSurvey. Click Instructions for access to BioGraphI PrePost-Lesson Student Survey to request a survey to be set up for your students, at least 7 to 10 days in advance of your class meeting date.

Objective(s)	**Formative Assessment**	**Summative Assessments**
1	Think-pair-share and group discussion prompts	Optional: After completing this lesson, students could be given metabarcoding data to work with. They can create and interpret graphs to answer questions about patterns in diversity. Students summarize their findings in a final report or presentation.
2	BioGraphI Student Pre-Lesson Survey (link for instructions to access survey)	BioGraphI Student Post-Lesson Survey (link for instructions to access survey)
3 and 4	BioGraphI Student Pre-Lesson Survey (link for instructions to access survey)	BioGraphI Student Post-Lesson Survey (link for instructions to access survey)
5, 6 and 7	Pre-activity quiz on basics video. Think-pair-share, group discussion prompts, and jigsaw activity.	Optional: After completing this lesson, students could be given metabarcoding data to work with. They can create and interpret graphs to answer questions about patterns in diversity. Students summarize their findings in a final report or presentation.

Lesson context

Learning goals of unit

The overarching goals of the research course that are addressed by this lesson include:
- Create and interpret graphs to demonstrate an understanding of concepts covered in class
- Foster a sense of community
- Reflect on connections between science and society
- Effectively communicate an understanding of ecological concepts and experimental outcomes, using relevant terminology.

Prerequisite skills or knowledge

- Conceptual understanding of diversity metrics (e.g . alpha diversity, beta diversity, abundance, richness, evenness; these topics are covered in the basics video).
- Working understanding of the scientific method (e.g. question, prediction, hypothesis, treatment, control, dependent and independent variable, etc)
- Skills practiced prior to this lesson include interpreting graphs and working in groups.

Preparation for lesson

- Assign BioGraphI Student Pre-Lesson Survey as homework for students to complete before this in-class activity.
- If plan to assign social media activity as homework, contact the interviewee ahead of time, as a courtesy.
- Make sure students have the conceptual knowledge needed to complete this activity. To achieve this students could either watch a video (such as this basics video), read a textbook chapter or sections of community ecology chapter that focus on diversity metrics, or have the topic covered in a previous lesson.
- This basics quiz (W 5_Basics Quiz_Biodiversity counts.docx) can be used to assess whether students have the conceptual knowledge needed for the activity.

Lesson sequence

Each row of this table is a step of the activity. Column headings reveal what the instructor, interviewee, and students do at each step.

Information from instructor (live in-class)	**Information from scientist (within pre-recorded video interview)**	**Student follow-up or transition activity**
Prior to class, assign pre-lesson activities.	N/A	Students watch assigned basics video and answer the corresponding quiz. Students complete homework/pre-lesson survey for BioGraphI project (combination of reflective writing prompts and closed-ended questions) ● *Purpose*: prepare for class; LO 2 & 3 – Pre-lesson reflection on data literacy and perceptions of scientists
Provide slides with diversity reflection prompts	N/A	Students recall what they know about diversity metrics. This activity will help them reflect on 1) what information can be obtained from alpha and beta diversity metrics, and 2) why it might be important to consider both metrics when thinking of community composition. ● *Purpose:* Warm-up and icebreaker
Assign teams for jigsaw and explain activity	N/A	Students form teams of 5-6 students. ● *Purpose:* Prepare for collaborative work
Provide each team with a mystery graph and guiding questions	N/A	**Fundamental activity:** Make reasonable guesses to what the graph is about; student teams discuss the answers to the guiding questions. ● *Purpose*: Warm-up and icebreaker, rewards creativity, normalizes errors (it is nearly impossible to be correct without more information)

Plays first video segments	Explain the rationale for the study and your experimental setup.	**Fundamental activity:** Students take reflect on experimental design ● *Purpose*: LO 7: Identify the research question being addressed.
Ask students to revise answers to worksheet based on new information available about experimental setup	N/A	**Fundamental activity:** Students interpret/re-interpret the graph with the new information they've been given. ● *Purpose*: LO 1, 5 & 6 - Check student understanding of the concepts and vocabulary Students infer the question(s) being addressed by the figure they've been assigned. ● *Purpose*: LO 7: Identify the research question being addressed.
Students find the video that corresponds to the figure they were assigned and watch in groups.	Orient us to this graph we've been working on. (What do these axes mean? How did you measure stuff? What do the units mean? How did you make this graph? What question were you answering?)	**Fundamental activity:** Students interpret/re-interpret graph with the complete information. They evaluate whether or not the hypothesis was supported. Summarize main findings of the experiment and prepare to share with new team members. ● *Purpose*: LO 1, 5 & 6 - Check student understanding of the concepts and vocabulary
Students form new teams. Each team must have (at least) one expert from each figure.	N/A	**Fundamental activity:** Make reasonable guesses to how the research fits into the bigger picture; student teams discuss the answers to the guiding questions. ● *Purpose*: LO 7: Identify the research question being addressed.
Plays next video segment	Could you share now how the findings that you summarize in these graphs fit into the bigger picture?	● *Purpose:* Closes this portion of the lesson
Adds a little info about the scientist (ppt slide): a picture,	N/A	What questions do you have about Dr. Rebolleda Gomez's path to this work and where she is today? Share out.

affiliation		- *Purpose*: LO 4 - Identifying potential connections between students and scientist
Selects questions that align best to student generated questions; Must show the answers to at least the required interview Qs to students	***Required interview Qs*** **What kinds of scientific questions interest you the most?** **When and how did you know you wanted to be a scientist?** **Tell us about the paths that led you to your current job.** **What is it like being a scientist who identifies as [fill in the blank?** **Tell me about a moment when you felt like you really belonged in the field of science.** *Optional interview Qs* What do you do if you have questions about how to analyze your data?	- *Purpose*: Answers the questions identified by students
Optional: Instructor shares contact info (twitter, email, etc) of scientist so that students can ask leftover questions! #BioGraphI…	(braces themselves for media deluge)	Students ask their questions during class (or homework!) - *Purpose*: LO 4 - Identifying potential connections between students and scientist. Closes the in-class portion of the lesson.
Instructor could provide other resources on scientist (e.g., website, papers, etc.)	N/A	Students complete homework/post-lesson survey for BioGraphI project (combination of reflective writing prompts and closed-ended questions) - *Purpose*: LO 2 & 3 – Post-lesson reflection on data literacy and perceptions of scientists

Alignment to Universal Design for Learning Guidelines

UDL Guideline	**Lesson Alignment**
Multiple means of Engagement	Recruiting interest: Students have the opportunity to generate their own questions for the scientist Self-regulation: Students reflect on their dispositions and knowledge of scientists and graph interpretation before and after the lesson Foster collaboration and community: Students need to share their knowledge of the problem and consult with other experts to understand the bigger picture.
Multiple means of Representation	Perception: Captions are provided on the interview video. Google Slides offers automatic captioning for presenter. Alt text for images provided in Google Slides. Comprehension: Question prompts guide information processing for graph interpretation.
Multiple means of Action and Expression	Expression & communication: Multiple types of media are used in the lesson (visual, audio, text). Physical Action: Varied means are used to prompt students (clicker questions, verbal processing via discussion, written reflection following lesson). Executive functions: Jigsaw activity gives each student the opportunity to become an expert on one subtopic, and then share their expertise with others to answer a bigger question.

Implementation notes

Notes on our implementation

This lesson was implemented in an introductory biology discussion section at the University of California, Irvine in February 2023. The lesson was offered in-person to 14 different lab sections. Each section had ~30 students and was facilitated by one teaching assistant. Some of the classrooms were set up for active learning (large tables that could fit 5-6 students; each table had a screen that could be controlled and viewed by all team members), while others were more traditional classrooms (with individual chair desks that had to be rearranged for group work). Because the discussion sections were 50 min long, the lesson was broken up into 2 days as described in the following table (but note the updated suggested breakdown further ahead that incorporates feedback from the first implementation).

Activity	**Time**	**Notes**
Warm up and instructions (Slides 1-12)	10 min	Ask students to sit in teams of 4-6 people. Facilitate discussion. Encourage students to think individually before discussing with peers.
Part 1 (Slides 13-16)	8 min	Group work. Each team is assigned one graph. It is ok if two or more teams are assigned to independently work on the same graph, but make sure all graphs have been assigned to at least one team. Teams try to interpret the graphs before having any background information on the actual experimental design.
Experimental design (Slides 17-23)	15 min	Each slide has a video that must be played (total 13 min). Encourage students to take notes. Briefly answer questions (if any).
Part 2 (Slide 24)	5 min	Group work
Part 3 (Slides 25-28)	12 min	Group work. One student in each team will have to play a portion of the interview. Videos range from 3-5 min.
STOP HERE		
New groups (Slide 29)	3 min	Make new teams. Each team should have (at least) one expert on each of the figures.
Part 4 (Slides 29-30)	15 min	Group work. One student in each team will have to play a portion of the interview (2 min)

Part 5 (Slides 31-38)	5 min	If there is time, ask students to think about what questions they'd like to ask Maria. You can even play one or 2 videos if you'd like.
Total	**73 min**	

Students enjoyed the activity and engagement was high. Students engaged well with lesson activities that aligned with the Quantitative and Content LOs, but needed additional encouragement to fully engage in activities related to the DEI LOs. A common complaint was that the activity was somewhat rushed. As such, we suggest instructors follow these new suggested times (also summarized in this 3_Instructor Guide_Biodiversity counts):

Activity	**Suggested Time**	**Notes**
Warm up and instructions (Slides 1-12)	12 min	Ask students to sit in teams of 4-6 people. Facilitate discussion. Encourage students to think individually before discussing with peers.
Part 1 (Slides 13-16)	8 min	Group work. Each team is assigned one graph. It is ok if two or more teams are assigned to independently work on the same graph, but make sure all graphs have been assigned to at least one team. Teams try to interpret the graphs before having any background information on the actual experimental design.
Experimental design (Slides 17-23)	20 min	Each slide has a video that must be played (total viewing time 13 min). Encourage students to take notes. Briefly answer questions (if any).
Part 2 (Slide 24)	5 min	Group work
Part 3 (Slides 25-28)	12 min	Group work. One student in each team will have to play a portion of the interview. Videos range from 3-5 min.
New groups (Slide 29)	3 min	Make new teams. Each team should have (at least) one expert on each of the figures.
Part 4 (Slides 29-30)	15 min	Group work. One student in each team will have to play a portion of the interview (2 min)
Part 5 (Slides 31-38)	10 min	Ask students to think about what questions they'd like to ask Maria. As time allows, play some of the videos with responses to the most common questions.
Total	**85 min**	

Suggestions for adaptation

Reducing the length of activities

For instructors that wish to reduce the length of this lesson, we recommend to either:

1) Select only one graph for students to work on. In doing so the second part of the jigsaw activity could be removed or modified.
2) Watch only one or two of the interview questions in class and have students select 2 -3 other questions to watch at home. Instructors can prompt students to either turn in a reflection about the answers and/or a recording where they are giving their personal answers to the interview prompts.
3) Avoid or reduce the number of warm up slides at the beginning of the activity.

References/resources

1. Rebolleda Gómez, M., & Ashman, T. L. (2019). Floral organs act as environmental filters and interact with pollinators to structure the yellow monkeyflower (*Mimulus guttatus*) floral microbiome. Molecular Ecology, 28(23), 5155-5171. https://doi.org/10.1111/mec.15280

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Transcript of interview

https://youtu.be/5LtU_oRJsjo

0:00 - 1:11

- Ana Garcia Vedrenne (AG): Hi everyone! I am Dr. Ana Garcia Vedrenne. I am an Assistant Professor of Teaching at the University of California, Irvine, and today, I have the pleasure of talking with Dr. María Rebolleda Gómez. María is also an Assistant Professor at the University of California, Irvine. María and I are both from Mexico City, and we actually have a friend in common. That was the reason we met, I don't know, maybe like eight years ago or so, and so now it's really wonderful to be able to have her as a colleague also at the University of California, Irvine.

María is the first author on a paper that's called “Floral organs act as environmental filters and interact with pollinators to structure the yellow monkeyflower floral microbiome”. This was published in Molecular Ecology in 2019. Today, we would like to hear more about the questions and hypotheses that you addressed in this study, María, and explore some of the graphs that you made.

- María Rebolleda Gómez (MR): Of course. Thank you Ana so much!

1:11 - 6:21

- AG: Could you tell us a little bit more about the rationale for the study and your experimental setup?
- MR: My… We know microbes are really important for all organisms. That we have them in the skin. We have them in our guts. They help us digest. And we are trying to understand how these different communities of microbes (like the microbes in our gut) form, and what shapes their diversity, which can be sometimes related to function and just how they work.

  And in the case of plants, there was a lot of work on roots, and a little bit on leaves, but we didn't know much at the time about microbes in flowers- if there were many, how diverse, what kinds of communities were there. And I think these were big gaps because flowers are really… they're the reproductive organ of the plant and so they're very important for the survival and reproduction of the plant.

  But also, for me as an ecologist that studies communities of microbes, they were very interesting, because different parts of the flower- and so in this study we mostly look at petals (which are sort of pretty obvious what they are, they're the big, flashy, modified leaves of the plant, of the flower); we have the style (and in this case, we use basically stigma and style, and that is a stigma and pistil, so that's the style, and that's the, female reproductive part of the plant, and then there's… that's the part that leads directly to the ovules of the plant); and then there's the stamens (and at the top of the stamen is the anther, which is where the pollen is, so that's, what we think of as the male reproductive organs of the plant). And so, what was, those are the sort of three main structures we study.

  But I was interested because these structures are very different. And, and it's hard, I mean, they look different to us if we, if you grab a flower, they look different. But for microbes- it's hard to imagine- but, because microbes are so, so, so tiny, for them it's like a completely different world. It's like different planets all together, And so the petals have these cells that have these, like, big mountainous shapes, and so it's like being in the Sierras or something like that. And they're pretty dry, and so maybe the Eastern Sierra- so it’s really dry. There’s, in the style, in this case, the stigma has these like, secretions that the plant produces, to… both to attract pollinators, and have the pollen stick to it. They serve a lot of functions during reproduction. But these secretions are things that microbes might be able to eat and so it's more humid, there's more food, but it also… there are more antibiotics, it's harder to access. And the same with the stamens. That's where the pollen is and the pollen is the sort of main source of protein in the flower, but it's also sort of highly protected. Pollen is really hard to break. And so, there are all these different sort of micro-environments within a flower. And we were interested if those were actually affecting which microbes were there, and how many, and how diverse, were there.

  And so we decided to work with the yellow monkeyflower, because it's a fairly well studied plant in ecology and evolution. So we knew something about where it exists, how it's pollinated. It has this really cool thing where the stigma (so that's the top, at the top of the style. That's where the pollen kind of lands). The stigma in the, in many of the monkeyflowers closes. It's open and it closes when it's been pollinated, and if the pollination was not successful, it opens again. If it was successful, it kind of stays closed. And so this was a great way to know if the plant had been visited by pollinators. And we cared about that because one of the main reasons microbes might get to flowers is because they have all these insects going to them. In this case these flowers are not, like bird- visited flowers. There's, there's very rarely hummingbirds or something bigger going to them. But there's tons of insects that visit them and so we wanted to know, also, who was visiting them, how their insects were interacting with the plant, and how that was maybe affecting the diversity of microbes that were being moved around. So yeah, I think that was the overall motivation.

AG: That's fascinating!

6:21 - 7:56

- AG: So what's… how did you go about studying this?
- MR: Yeah, so these yellow monkeyflowers have a very broad range. So they exist in basically most of the West Coast of the US and Canada. But they really like these micro-environments where there's like, they really like to grow near water, near the… like my co-author… or one of co-authors in the paper, Tia-Lynn, my co-author said that, when we were growing them, she used to say that they "like to have their feet wet", meaning the roots.

  But in these… we went to Northern California, to the McLaughlin Natural Reserve, which is part of the UC System of Natural Reserves. And there, there's this… it's, it's serpentine soil. So the soil is really, it has a lot of heavy metals and there's these sort of outcrops of serpentine soil. So these are places where there's a lot of heavy metals like… and, and many organisms cannot grow there, so the ones that do are kind of specialized in growing this… in these places. But the yellow monkeyflower can grow there just fine. Actually, it kind of tends to grow there more.

7:56 - 11:07

- MR: And so there's these little creeks along the serpentine soil, where if you see a picture of it, it's like full of yellow patches. And those patches are, for the most part, the yellow monkeyflower. So it's really common and dominant in these areas, and so that means we had tons of flowers we could study.

  And we set either four or five locations depending on the length of each of these kind of rivers in different locations. And the idea to do there was, one of the things we wanted to know is: are plants that are close… are flowers that are closer to each other, do they have more similar communities than flowers that are sort of further apart? And so we wanted to have multiple distance… nested distances. So some that were very close, some that were a little farther, and some that were really far apart. And then in each of these locations, to sort of see how pollinators were affecting diversity of microbes, we put these cages, these pollinator cages (which was our exclusion treatment). And this treatment is basically like a little box with some, sort of mesh fabric that doesn't let the pollinators in, but it lets air and other things. And then we constructed our control, because, of course, this cage blocks a little bit of sun, it does other things that might have also affected the microbial community. And so to know that, we made a control that, we tried to mimic the conditions, but it allowed for pollinators to go in.

  And then I carefully marked the flowers when they were, like, little buds, so when they haven't opened. I marked them so that I could sample them all at the same time, and so I knew like, oh this one I marked. So three days later I would show up, and it would have opened, and then I carefully, in the field, dissected each of the organs. So I took the petals, and put them in a little tube. To all these with, like these sterile forceps, so that they might… I was not the thing moving the bacteria or bringing bacteria in. And then, we… something that's hard about bacteria is like, it's not like with trees or something, where I can go and just count the trees, and see what they are. These things are really small, and they all look alike. And so what we need to do is use DNA. And so we extracted DNA from this community, and there's a piece of DNA that all bacteria have, uh, but it's different for different species. That's this 16s region of the ribosomal gene. And so, we sequenced that, and with that we are able to tell, like, “oh this one is this species, this one is this other species”. And that allows us to know who is there, how diverse the community is, etc… in each of those different organs.

- AG: That's fascinating! So many things you need to think about when you're planning these experiments.

11:07 - 12:45

- AG: Can you tell us a little bit about the pollinator part of the experiment with the exclusion cages and the ones where the pollinators could come? What were you trying to see there?
- MR: So these plants get visited by tons of different pollinators and so we also wanted to know who actually was visiting because maybe we would see that, yes the pollinated ones were different, but only if bees had been who had visited the flower or something like that. And so the other thing we did is, we spent a lot of hours every day sitting in front of the flowers and just like, sitting there for 20 minutes at a time, but different flowers every time, and seeing who was coming and annotated it. And so we had an idea of, in each of the locations, what pollinators were the ones that were visiting our flowers. And what they were doing when they got there, right? Because these organs are different in terms of micro-environments, but pollinators also interact with them differently. So you can think of a butterfly, which will mostly just like, lay on the petal and kind of stay there. Maybe bring its… drink the nectar in, but that's it. Whereas the bumblebee- they like, come with their big fuzzy bodies, and just like, get in, and you know, touch everything. And so that's a very different kind of contact. And, and so we were interested in that.
- AG: Very interesting! Thank you.

12:45 - 17:26

- AG: Okay, so now let's talk about some of the graphs that you present in your paper. Let's start with figure two. So figure two is composed of several different panels. The figure is titled “Contribution of floral organ differences to bacterial community structure of monkeyflowers”. So, can you tell us more of what's going on in figure 2 panel D? This is the one where you have an alpha diversity plot that examines diversity across floral organs.
- MR: There's different kinds of diversity that we might measure, and so we can measure within one community. So for example, in this case within one flower, one organ, what are the microbes there? And what we might ask there is- first of all, how many species there are? and also how abundant some species are relative to each other? So there might… we think it's more diverse if there's many species and they all are sort of balanced, versus having one species that's very dominant and a few rare ones, or just having very few species. And so this is called Alpha diversity and there's different ways of measuring it that weigh- that put different weights into how many species there are and how abundant they are relative to each other. So in the y-axis we put Shannon alpha diversity, which is one that more or less weights them- not completely equivalent, but it's not biased towards one or the other too much, so it takes both into account, really- the dominance and the richness. And then in the x-axis and in the different colors we have the different organs of the flower. So we have petals, stamens, and styles. And then each, there's a ton of little points in this graph, and each of those little circles or points is one community. So that's one for all the microbes in one organ, in one flower, basically. And so we want to ask okay, for this one organ, one flower, who is there? how diverse is that community? And that's what this is measuring. But, and then we have the two treatments: so when we excluded pollinators that's the open circles, and when we did the control where we allow pollinators to visit, that's the filled circles. So in this case, that's our control, because the manipulation we did is: exclude the pollinators. We didn't add the pollinators. They’re there.

What we did was excluding them. And then… but it looked like just a ton of little dots, which is good because you see all the distribution. So you see, for example, in the petals that there's some communities that are very diverse way at the top, and there might be some that are much lower… so there's a lot of variation within each organ, and each treatment. But we wanted to compare between them, so we said what's the average? And the average is the bigger points sort of towards the middle. They're all between sort of two and three in the Shannon index. And so in those cases, what we have here is that some organs have a lot of variation, the petal is, specifically the petals… the control petals actually are some of the most diverse communities. And actually, at the top, surprisingly, all the communities that are at the top are in the control treatment, which is something we didn't… something we would expect because pollinators are bringing things, as I said, so they might bring some rare species and that might increase diversity. But there's actually not sign… not a significant difference between neither treatments, nor the different organs. So all the organs are more or less the same. And in general, they can, the controls are a little more diverse than the exclusion, but there's so much variation that this is not a significant trend.

- AG: Very interesting. Thank you!

17:26 - 21:36

- Okay now can you explain how to read figure two, panel A. This is where you have the PCoA plot, and so… can you tell us what we learned from it?
- MR: Yeah.. so as this plot… basically, the way we measure beta diversity is: we are comparing each community to each other, and we're saying how similar they are. So in this plot, each point is one community. So it's one, let's say one petal for one flower, it's all the microbes in that community. And what we're saying is, okay, if I take that community and another community, I want to know what the distance is. But what gets crazy here is that distance… we have a lot of variables going in, and so that distance is for each species… You know, you can say: Okay, species one in community one: that species is very abundant and in community two, that species is not even there, so that's a big difference. And that's just one dimension. Then you go species two, and three, and four, and there's hundreds of species, and each of those species is a distance in its own way. And so you are comparing this sort of big, multi-dimensional thing. And so these PCoA plots, what they do is basically smoosh, sorry… smoosh these multi-dimensions and so now, the distance between the points means how different they are once you account for all the species in the community. And so points very close in this plot are more similar to each other, and points that are farther away are more different in all of the areas of the plot. And so, that's why these axes are kind of hard to know what they mean. Basically what they mean is, a bunch of these distances sort of about what, what species were considered in each axis. And each axis weighs the different species like, so the axes don't matter all that much. What matters the most is where the points are in this space. And so again, we have two treatments: we had an exclusion treatment, where we block out the pollinators, and then a control where we let the pollinators visit the flowers. And then we have different floral organs: the petals, the stamens, and the styles. And our question was… we had sort of two questions here. One was: are plants from different pollinator treatments, are their communities different to each other? And then, are organs different to each other? And the answer is pollinators are not, and so for each organ… or if you see all the points, you'll see that the points that are exclusion and control, they are all over the place. They're not grouping in different sections of the plot. So that means that any difference between two exclusion treatments is as much as any difference between the control, or a control and an exclusion. Whereas organs, we do have that there were significant differences. So the petals are almost all to the right, and the styles are almost all to the left. They're in different sections of the plot altogether, and so basically all petal communities are more similar to each other than they are to any style community, and the other way around. That's what that means.

21:36 - 24:42

- AG: Okay. Now, can you tell us more about figure two, panel C, where you show the Venn diagrams of the core microbiome for each floral organ and for each pollinator treatment?
- MR: Yeah. So one question we had were… are different organs different? And if there are certain microbes that are always the microbes of the petal, let's say, or always the microbes of the style. And so what we tried to do is, okay are there microbes that are there a lot of the time? That in any petal we take, or in the majority of petals we take, they're all we find. We keep finding them. And then, from those, how much they're shared between organs? Like, are they truly just a petal? or do they exist in other? And so we did these Venn diagrams, where it's a bunch of circles nested. And the way to read these is… well, and we have one for the exclusion treatment (where we excluded the pollinators), and one for the control (where pollinators could visit the flowers). And the way to read these is, we have these circles that partially overlap. So where the circles don't… and each circle is an organ of the flower. And so where the circles don't overlap, that is just the bacteria that are very common for this organ and are in no other organ. So in the exclusion treatment, for example, there's 13 bacteria that are very common in the style, that are not in any other organ. Then there's the parts where they overlap that are shared. And so there's 13 that are shared between style and petal, 27 that's shared between all the organs, and five that are shared between stamen and style. So in total that is thirteen, plus thirteen, plus twenty seven, plus five… fifty eight bacteria, sorry, 58 bacteria that are very common in this file of the exclusion treatment, and that… but of those, of those 58, only sort of around half, 27, are shared between everyone. And there's some that are exclusive. So the petals have the most bacteria that are just theirs. And so they had, in the exclusion treatment, 36 bacteria there are just in petals, and in the control 41 that are just in petals. So they're sort of the more different organ. And we think this makes sense because it has very different conditions. It's more outside of the plant, so it has different pollinators that might visit it.
- AG: Thank you!

24:42 - 28:24

- Now let's talk about Figure four, specifically about panel B. Can you tell us what this graph is about?
- MR: Yeah. So, this is one of the graphs that's the most different in the paper. So in general, and when I talk about the experimental setup, we were looking at microbes in the flowers. But in this case, we were interested to see the community, and the diversity of pollinators that are visiting the control treatment, where pollinators can visit. So in this case we don't have any of the exclusion, because no pollinators visited those flowers. And we just looked, not only if pollinators were visiting the flowers, but where, what organs were they visiting. And of course, you're seeing the pollinators. You don't want to disturb them, and so it's really hard to see exactly which organs they are touching. Especially, some of them are pretty big, and once they get in the flower, you don't know what they're touching. And so… but, what we can say is, were they mostly just in the external surface of the plant? So in the petals, especially *Mimulus guttatus,* the yellow monkey flower, has this, like, big petal that acts as a landing strip, so pollinators can land there. And so are they mostly just there? Or are they actually getting inside, and touching the internal organs of the plant? And so in the x-axis here, we have if they're external or internal, and then the percentage of pollinators. So they, these blocks are a hundred percent each one, and then they're divided in sections of different colors. And each section is a different type of pollinator. And we try to group them sort of in big groups. So the different colors are, more or less related. And so all the red and yellows are bees, the blues are flies, the sort of beige is moths and butterflies, and then, in the other colors are other things like beetles, wasps, and even other rare things that once in a while come in like, ants once in a while would get in, but they're not common pollinators. And then, what we can see is, in the external, we have a lot of flies

and butterflies and moths visiting- so that's like more than 75 percent is just flies and butterflies and other things. There's very little bees. The bees would go and almost always get inside and touch internal flowers. And they're also, in the internal flowers, they're the only ones that go, almost the only ones that actually get in the flower. And so in the internal, it's totally dominated by bees of different kinds, like bumblebees, small solitary bees, all kinds of different species of bees.
- AG: Thank you so much for explaining all of these figures.

28:24 - 30:46
- AG: I was wondering, could you share now how the findings that you summarize in these graphs, how do they fit into the bigger picture?
- MR: Yeah. So, I think overall we… this study helped us see that 1) organs are really important. They really shape, I mean some of these flowers were very separate from each other in distance, and even then, like really, the biggest difference between two microbial communities is between the different organs. It’s not where they are, it's not if the pollinators visited or not, it is the organs. It also… many studies have seen a much more contribution of pollinators and larger differences in alpha diversity between pollinators and treatments where they excluded the pollinators, like here. But us, there was a little difference, and in general, in all the organs, in our controlled treatment where the pollinators were able to visit, there was a little bit more diversity, and the most extreme points of diversity, where all, when pollinators visited and they were able to bring some new species. But this difference was not significant, and so there's tons of variation that was also interesting. Like each community is different to the next one. So actually, even finding a core microbiome, our core, like, oh these are the petal microbes, these are the anthers… there was not many of them. So there's only a few that are very common in the petal but not shared with anyone else, or very common for each organ. In some cases it's like four only in one organ and not the others and are common in that organ, right? And so that was I think another big thing. And one thing that was, I think, really cool is that the pollinators, what they mostly were doing is: bacteria arrive to the petal, it does seem like different environments are very different, and are creating sort of different communities, but then the pollinators visit and mess it all up, and just like get them all over the place.
- AG: Thank you so much for sharing this study with us!

30:46 - 33:19
- AG: If you don't mind, I'd like to ask you a little bit more about you as a scientist. So could you tell us, what kind of scientific questions interest you the most?
- MR: Yeah. So I think I'm, I'm very interested in both ecology and evolution. I work with microbes. I like bacteria, so that's what I work with. And I'm really… I… we tend to think of organisms on their own, and especially in bacteria, until very recently, we would study bacteria like one species at a time in the lab. We couldn't, we didn't have, yet, the technologies to study a lot of interactions, and in the lab, we were mostly studying, as I said, one species. And so I'm really interested in how communities form; how different communities affect the interactions between species; how they affect the ways in which bacteria evolve; and I'm, I mean, I think we're all shaped by our interactions and our context, and it shapes us in different ways. And so I was, I don't know, why not? It also affects bacteria. They don't behave the same when they're together than when they're alone. And when they’re with different bacteria they might do different things. And so I was really interested, and I think interested in that. And I think this question has a lot of implications because, there's a lot of… In the soil, for example, when we're thinking about carbon cycle for climate change, there's whole complex communities. And the whole community is required to, like for example, degrade leaf litter, or something, and produce CO2. And so how those interactions change might affect how they're going to respond to climate change, and the feedbacks that that might

cause. Or in our guts, we have tons of different bacteria, and the changes in the communities is actually what sort of can take a gut from a healthy to an unhealthy gut. And so, understanding how these communities might change, and how robust they are, and what kinds of disturbances might change them, and how their interactions shape all that is is sort of what I'm interested in.

- AG: That sounds really interesting and also really complicated.

33:19 - 35:11

- AG: What do you do if you have questions about how to analyze your data?
- MR: Well sometimes… sometimes, the internet. So there might be, if they are very specific questions like: I'm analyzing my data, I have these very complex data, and I'm just, like writing my code in R, which is how I usually analyze my data, and have a little question, then I might go to the internet. But if it's more of a big, conceptual question of how to analyze the data, then that's why we do science in community. And so, I might go to colleagues, I might read other papers that might have done something similar, and see how they did it. Sometimes, even when you read the paper, that's not enough, and you can write them an email and say like: hey, I like these analyzes, but I have a question. And yeah, I mean sometimes I discuss it even with my students in the lab. And so there might be students, and I like present to them, like this is what I'm working on. This is what I'm thinking. How does it sound? How would you test these differences? And sometimes the students actually are the ones that have the right answer on how to analyze data. And I think the.. that one thing that's really important is just like, have a very clear question. And that question will help you know what, how to analyze the data. So yeah, it's important to frame the questions and hypotheses very well because from that comes all the rest, I think.
- AG: That's a great tip!

35:11 -38:52

- AG: When and how did you know you wanted to be a scientist?
- MR: I think I've known both all my life, and at the same time never. I don't know. And so, I think I've always, like I mean, as a kid, I really wanted to be an explorer- which now I feel very conflicted about, because it has all sorts of colonial implications. But I was, I think what I wanted was to go out to nature and try to understand what's happening there. And so, which I think is some of the things I do now, I hope not in the same colonial way. But I also have always liked history and art, and so there were many things I wanted to study at a point, and at different points they have different weights, and some I wanted to do more or less. I also didn't quite know that I could just do science, or what that would look like as a job. Like, I knew some people were scientists, but I didn't quite understand what that meant, and if it was different or not than the experiments I was doing at school or something. And so it was when I was… in Mexico you have to choose what you're gonna study, your major, you choose it when you start the university so… and then you'll almost only take classes in that major, you don't…
- AG: There are no undeclared majors.
- MR: Yeah, so when I was deciding to go to university and what to study there, I had a very great biology teacher that took us out in like a field trip. And one thing that I think is really cool about Mexico is it's one of the most diverse countries per area in the whole world. It's on the top five, and so it's very easy to… if you go from Mexico City to either coast- which are like three hours on either direction, more or less, by car- you would go through like oak forest, and oak pine, and pine forest, and then like this environment that's called the cloud forest which has… it's crazy because there's some pines, but there's ferns, and there's orchids… and then you would go to, like, more tropical rainforest, and then you're in the coast. And so there was, like, all these ecosystems. And I was like, just found it so wild that you could just cross so many ecosystems, and the organisms were so cool. And I started wondering like, you know, how

something like a jellyfish can exist. And like, why did it evolve to be what it is? And how can that be alive? All those questions. And I think that made me want to not only be a scientist, but study biology. And then, yeah, from there I then learned about bacteria and how crazy they are, and how diverse they are at the metabolic level in terms of the functions they do, and how important they are for our planet. And I was like, this is so cool. I'm gonna study bacteria! And so here I am.

- AG: Thank you.

38:52 - 45:58

- AG: So the next question is: tell us about the path that led you to your current job. So, you already talked a little bit about the motivation. How did you get from, like, knowing “I am really interested in bacteria and want to study them more” to the Assistant Professor position you have now?
- MR: Yeah. So I first, you know, I studied at university, and I think with these things it's a lot about luck, unfortunately, and taking different opportunities that happen, and a lot of weird contingencies. So I was studying, and the other thing that we have in Mexico, almost everyone, especially in the sciences, has to write a like honors thesis. And so I was writing my thesis in bacteria already, because I had thought they were really cool and I had this teacher that studied bacteria in the desert in Mexico, in a beautiful place called Cuatro Cienegas. And so I was studying there, and because it's such a cool place to study there's researchers from all over the world that come once in a while to study things. And there, I met this guy that I had read a paper of his in one of my classes. So I was like, oh my God, the person that wrote the paper is here! Mike Travisano. And we talked, and he had very cool ideas, and I really liked the work. And so I decided to apply to do a PhD with him. And at the time, I didn't… at first I didn't even know if I wanted to do a PhD, and kind of continue that route. But that got me super excited, and I actually only applied to there and a program in Germany- which I don't recommend. I think you should apply more broadly if you're going to do a PhD. I was lucky, but I could have not gotten in in those two programs. And so, I applied, I worked with Mike, and there… and there, I was studying kind of different questions to what I'm doing now. It still had to do with relationships between cells, but I was studying yeast, which is not a bacteria. It's a fungi. This is still a microbe, but it's not… and I was studying the evolution of multicellularity- so how we went from organisms that have only one cell to organisms like us that have multiple cells and their function. And I was very, I mean I still am into these very big evolutionary questions. And I wanted to see, like, at what point do you go from being a group, to being a whole entity. And so I started working with that, and around that time, I think the microbiome really started taking huge… huge sort of center in our world. We started thinking more, and more about it. And it actually got me thinking, like, well… but we're not just a bunch of cells of like, human cells, but we also have all these bacteria that also are, kind of part of us. And are we also part bacteria? And so those questions kind of got me really interested in these communities of bacteria, and what makes them more or less cohesive, and how they affect multicellular organisms like us. And so, that's when I went to work with plants, because there were these multicellular organisms that had bacteria. And I was wondering, like, you know, how plants require, or not, their bacteria to be what they are. And so I started working in a plant lab to learn how to do plants, and that's where I learned everything I know about flowers, and pollinators, and all these things. That was Tia-Lynn Ashman’s lab. So she's the co-author of the paper. And after that, I mean, getting… I knew I at that point I wanted to get a a job as a faculty position. I really like mentoring students, and so I wanted to keep doing the mentoring. I really like thinking, and designing experiments, and I still get to do all that. And so, I was really excited for that. But it is not an easy job to get, I would say, and there's a lot of things that happen. And so, the first time I applied to jobs I didn't get any. I got one interview, which is actually already kind of not easy- which made me really excited. And then, I got a second postdoc. And that was working only with microbial communities, sort of in more experimental

context. And it was really fun. It was with this Spanish guy, Alvaro Sanchez, that had this really cool system to look at how communities assemble. Because after this paper, I was really interested in like, okay, what makes a community form? And how? But it's really hard to ask some of these questions with plants, and as you saw there's tons of variation. There's tons of factors. And sometimes it's hard to just break them all apart. And so these are, this was an experiment where they would basically grab microbes from soil- which is a super, super diverse community. Like thousands, upon thousands of species. And then put them in an environment that was much more constrained. So like, only one carbon source, so the food is very… it's only one type of food, and very kind of limited. And if I do that many, many times, can I predict which microbes will form the community? and can I understand that? And so I started getting into these questions. That is the very long wait, but then I applied to this job, and I really like UC Irvine because it's… I really wanted to work in a public university. Our students are from very diverse populations. I wanted to be between… I'm a queer woman, and my wife's family lives in the Bay Area, and I wanted to be somewhere in between my family in Mexico, and her family in the Bay Area, so this area was good. So these are all the things that one considers, and that is weighing when deciding where to go. And I really wanted… I mean a lot of our students, like around 30% are Latinx, and I really wanted… I felt like it was more meaningful for me to be in a school where a lot of the students would also come from a similar background, and so, yeah.

45:58 - 50:32

- AG: Tell me about a moment when you felt like you really belonged in the field of science.
- MR: Yeah. I think there's been a couple moments both where I felt like I really belonged, and both when I felt I really didn't belong. And so I, I really felt like I belonged… I was in this project called “Market Science” at some point where we would go to farmers markets in… first in Minneapolis, and then I started it in Pittsburgh. And in this Market Science we're like, very diverse, and it was like PhD students sort of highlighting our… and postdocs highlighting our work, and doing these small hands-on activities in the market. And I think doing that… there was one day that we went to this market in a part of Minneapolis where there's a lot of people that speak Spanish. And I was actually with a friend we have in common, with Gaby, and we were talking about fermentation. And there was another student. She grew up in Texas, but she also speaks Spanish. And, and at some point we actually decided, like, why? Like a lot of people in this market speak Spanish, we all speak Spanish, we could do this in both languages. And so we put a sign that said, like, “Se habla español” (“we speak Spanish”). And we started doing the explanations in both languages- in English and Spanish. And I don't know, seeing other people like me doing science, and also, it just kind of belonging in that space felt really good. Another time I really felt like I belonged is… I'm part of the committee, the Diversity Committee in the Society for… in the American, the Society…
- AG: ASN?
- MR: We call it ASN, American Society of Naturalists. That's what it is. And in that… first of all, it's the people I work with in the committee are all great, and, and kind of… I really fell, feel like I belonged there, but also, we organize with two other societies and their diversity committees in this big conference on Evolution. Last year, we organized this event called “Evolution’s Rainbow”, based on a book that's called the same way, that was about, like sort of the sexual diversity of organisms. Like just… we were talking about flowers, and just like flowers reproduce in the craziest ways. They have tons of sexual diversity, like some flowers only have male parts, some have male and female parts, some only have female, some in the same plant have male flowers and female flowers, some need different plants… like there's all that. Some mate with it, themselves, some mate across. You know, they do all these crazy things to attract pollinators, and there's all this crazy diversity. And we also have learned that, in animals there's a lot of sexual diversity that we had not explored, there's a lot of same-sex behavior that people had ignored just

because we thought that, we had these preconceptions of what organisms should be doing. And we thought that animals only have sex for reproductive purposes… and so they were doing all these other things, and so we organized this diverse… this event that was also open to the community, which was really fun. And so we had a famous drag queen from the Cleveland area be the MC, and then we had talks by scientists talking about, sort of the diversity in, and how… I mean how these strategies have evolved, and so cool! And so that was another moment that I really… felt like I was part of something really cool. And then I think in my department people have been really welcoming, and there's a bunch of new professors, and we have our little community, and that has also felt like a very- a moment of belonging in some ways.

50:32 - 53:45

- AG: So, you shared just now that you identify as a queer Mexican. What is it like being a scientist with those identities?
- MR: I think there's… there are things like, as I described, in which it's really exciting, and I think it helps me think in different ways. It helps me, you know, we all, we all come from our own backgrounds, and that makes us think of problems and things in our own particular ways. At times, it can… I think less and less there's more and more like queer scientists. There's starting to be more Latinx scientists in the US, and there's definitely great science happening all over Latin America. And that makes me feel, sort of more understood and welcome. There's definitely times that, either because of political reasons, like at some point, I had my green card… for the first time, I was applying for my green card. And for those of you that haven't had to deal with immigration in this way, visas can be really restrictive. They can really limit where you go, and when I was applying for the green card… which it's still pretty lucky, because there's many Latinos in the US that don't have access to that kind of immigration privilege, but when I applied to the green card- you cannot travel for a while until it gets approved. And I got invited to a conference in Australia. They were gonna pay my flight, and I've never been to Australia. I would love to go, and I just couldn't! I just couldn't go! And so there's, there's these moments in which you're like agh! So those are the more extreme. I think there is… at times a feeling that… most people I interact with, even though I know a lot of queer scientists, and the internet has made it great, and in conferences there's often a, like, meet event like, most of the time it does feel like there so few of us, and, and the culture in the department and other things is very different. And so there are moments of sort of feeling lonely, but at the same time I look around to our students and other people and I'm, like, you know, I see that the future is, looks different, and, and it feels good to be able to be there for that.
- AG: It's hard for you, but you being here makes it easier for others that are coming up later.
- MR: Thank you.

53:45 - 54:19

- AG: María, thank you so much for chatting with us today. If anybody wants to learn more about this work you can read María's paper. The name is “Floral organs act as environmental filters and interact with pollinators to structure the yellow monkeyflower floral microbiome”. It was published in Molecular Ecology in 2019, and we include the link to the paper on this slide.
  Thank you for watching the video!
- MR: Thank you!