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Composition, Diversity, Abundance, and Spatial Variation in the Floral Nectar Mycobiome of Rhododendron catawbiense and Lobelia cardinalis

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Composition, Diversity, Abundance, and Spatial Variation in the Floral Nectar Mycobiome of *Rhododendron catawbiense* and *Lobelia cardinalis*

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ABSTRACT

Plant nectar microbiome, primarily consisting of fungi and bacteria, can qualitatively and quantitively alter the floral rewards by exploiting the chemical composition of the nectar which can thus impact plant-pollinator interactions, and ultimately affect plant reproductive success. Evidence suggests that changes in the microenvironmental conditions across various spatial gradients can induce changes in the floral nectar microbiome, which can account for microbial variation not only across plant species, but also within. Furthermore, nectar microbiome is also contingent on the pollinator systems and nectar quality. In this study, we used *Rhododendron catawbiense* (bee-pollinated; highly toxic nectar) and *Lobelia cardinalis* (hummingbird-pollinated; mildly toxic nectar) to explore the so-far unknown composition, diversity, and abundance of the nectar mycobiome across the spatial gradients of elevation and distance, respectively. It was done in the native Appalachian region of East Tennessee to improve the understanding of the microbiome-pollinator dynamics so that biodiversity can be better preserved. Through field sample collection, nectar harvesting, plating, isolation, DNA extraction, and Sanger Sequencing, we found that there were significant differences in terms of fungal abundance within the *R. catawbiense* and *L. cardinalis* species. Across plant species, *R. catawbiense* had more fungal abundance, attributable to being bee pollinated. *L. cardinalis* had more fungal diversity, attributable to its relatively low nectar toxicity. The results of Species Richness and Simpson's Diversity, however, were found to be not significantly different in every instance. Furthermore, the plant species only shared two fungal species in common, with others also being unique to either an elevation or location, suggesting a high fungal species-plant species-spatial variation specificity.

INTRODUCTION

The discovery of the human microbiome is considered one of the most significant recent scientific advances which has led to an explosion of studies evaluating its function and importance for human health (e.g. Bleich and Fox, 2008; Johnson and Versalovic, 2012; Relman, 2012; Samuni-Blank et al., 2014; Basu et al., 2015). Since then, microbiomes have been documented in a wide range of aquatic and terrestrial organisms including algae, fish, amphibians, birds, and reptiles among others (Keenan et al., 2013; Maeda et al., 2013; Jani and Briggs, 2014; Merrifield and Rodiles, 2015; Cirri and Pohnert, 2019; Goor et al., 2020; Mittal et al., 2020). Microbiomes have been shown to be abundant, diverse, and able to establish commensalistic, mutualistic, or pathogenic associations with their hosts (Bleich and Fox, 2008; Relman, 2012; Rebolleda-Gómez et al., 2019; Rodriguez et al., 2019). Specifically, microbiomes have been shown to aid in maintaining homeostasis, protecting against pathogens, imparting immunity, and in metabolic processes (Bleich and Fox, 2008; Johnson and Versalovic, 2012; Relman, 2012). In return, microorganisms can manipulate their hosts to obtain resources, increase survival, and enhance their dispersal (Relman, 2012). Although the presence of a microbiome has been documented in a wide range of organisms, outside of humans the diversity, variation, and function of the microbiome remains largely unexplored (Pozo et al., 2011; Alvarez-Pérez et al., 2012; Samuni-Blank et al., 2014; Rebolleda-Gómez et al., 2019; Rodriguez et al., 2019).

The plant microbiome, for instance, can be highly complex and be composed of bacteria, fungi, archaea, viruses, and oomycetes (Compant et al., 2019; Rodriguez et al., 2019). The microbiome can also differ across plant species, plant structures, and even across time within the same individuals (Compant et al., 2019, Rebolleda-Gómez et al., 2019). The plant microbiome

can help tolerate stress (e.g. salinity, herbivory, temperature, etc.) and provide resistance against pathogens, thus impacting overall plant growth and fitness (Ortiz-Castro et al., 2009; Jia et al., 2016; Zeilinger et al., 2016; Zhang et al., 2016; Begum et al., 2019; Gaube et al., 2020). However, most plant microbiome studies have focused on the roots and vegetative structures (Alvarez-Pérez et al., 2012), leaving other plant structures relatively unexplored. Recent studies, for instance, have shown that flowers can have complex microbiomes (Rebolleda-Gómez et al., 2019). Particularly, the nutrient-rich nectar has been shown to host a variety of fungi and bacteria that have the potential to impact the success of plant-pollinator interactions by altering the quantity and quality of floral rewards (nectar and floral scent, etc.; Pozo et al., 2011; Alvarez-Pérez et al., 2012; Rebolleda-Gómez et al., 2019 and references therein). Thus, these effects can have important impacts on the plant reproductive success; studies however remain scarce and limited to a few model plant species (Pozo et al., 2011; Alvarez-Pérez et al., 2012; Samuni-Blank et al., 2014; Rebolleda-Gómez et al., 2019; Rodriguez et al., 2019).

Furthermore, most nectar microbiome studies have focused on evaluating differences among plant species (e.g. Pozo et al., 2011), and we know much less about how microbiome composition varies at smaller biological scales, i.e. within a single species. For instance, nectar microbiome composition can vary spatially among populations of the same species (Alvarez-Pérez et al., 2012; Samuni-Blank et al., 2014; Liu et al., 2020). Among-population variation in nectar microbiome composition and diversity may arise due to variation in resource availability and in micro-environmental conditions such as temperature, soil characteristics (pH, water content), and precipitation (Nichols et al., 2008; Samuni-Blank et al., 2014). For instance, the abundance and diversity of microorganisms in the nectar may decrease with increasing elevation. This is because lower temperatures and decreased oxygen levels at higher elevations may restrict

their growth (Martiny et al., 2006; Liu et al., 2020). Further evidence suggests that the nectar microbiome similarity can decrease with increasing geographic distance (Martiny et al., 2006; Samuni-Blank et al., 2014) and with increasing latitude and elevation (Liu et al., 2020). Some nectar-inhabiting microorganisms, however, are common even across very large spatial scales, i.e. continents (e.g. *Metschnikowia*) (Rebolleda-Gómez et al., 2019). Nonetheless, how the nectar microbiome composition and diversity varies spatially across different populations and across elevation gradients remains largely unexplored.

The nectar microbiome may also vary amongst species with different pollinator systems. For instance, invertebrate (bees) and vertebrate (birds) pollinators have widely different resource needs and foraging behaviors that can influence the nectar microbiome composition (Pozo et al., 2011; Alvarez-Pérez et al., 2012; Rebolleda-Gómez et al., 2019 and references therein). Differences in the nectar chemistry among plant species can mediate differences in the microbiome composition by acting as a selective filter determining the presence of specific microbial taxa (Pozo et al., 2011; Alvarez-Pérez et al., 2012; Rebolleda-Gómez et al., 2019 and references therein). Thus, the nectar microbiome could play a so-far unappreciated role in shaping floral evolution (Rebolleda-Gómez et al., 2019 and references therein). Here, we evaluate spatial variation in the composition, diversity, and abundance of the floral nectar microbiome in two native Eastern Appalachian species (*Rhododendron catawbiense* and *Lobelia cardinalis*) with distinct pollinator systems (bee vs. hummingbird pollinated). Specifically, we ask the following questions: (1) What is the composition, diversity, and abundance of the nectar microbiome in *R. catawbiense* and *L. cardinalis*? (2) Are there any differences in the composition, diversity, and abundance of the nectar microbiome between populations of the

same species? (3) Are there any differences in the composition, diversity, and abundance of the nectar microbiome between *R. catawbiense* and *L. cardinalis* ?

METHODS AND METHODOLOGY

Study Species:

Left: *Rhododendron catawbiense*, Right: *Lobelia cardinalis*

This study was done using *Rhododendron catawbiense* and *Lobelia cardinalis* as our focal plant species. *R. catawbiense,* commonly known as the Catawba or purple rhododendron, is native to North America. It grows from West Virginia and Virginia south to Georgia and Alabama (Blazich et al., 1991) and is known for its spring flower display, particularly in the Roan Mountain region where it stretches on for miles at high elevations. It is the largest member of the Ericaceae family (Peng et al., 2007) and is usually a dense, spreading, multi-stemmed 3-5 m tall evergreen shrub (Blazich et al., 1991). Its large, five-lobed bell-shaped purple-violet flowers are approximately 3-4.5 cm in diameter and usually have small streaks or spots. It blooms from April to June. It grows the best in acidic, organically rich, and moisture-retentive

soil (Blazich et al., 1991; Peng et al., 2007). Flowers from *R. catawbiense* are primarily visited by bees and butterflies (Blazich et al., 1991; Peng et al., 2007).

Lobelia cardinalis, commonly known as cardinal flower, is an herbaceous, short-lived perennial belonging to the Campanulaceae family and grows alongside lakes and streams throughout most of eastern North America (Devlin et al., 1987; Johnston 1991). *L. cardinalis* has erect terminal spikes containing large, cardinal red flowers – deep, tubular, five-lobed, approximately 4 cm across – on unbranched, alternate-leafed stalks, with a usual height of 1.2 m, and has a characteristic 'lip' petal near the flower opening (Devlin et al., 1987; Caruso et al., 2003). It blooms from July to September. Flowers from *L. cardinalis* attract hummingbirds and butterflies (Baker, 1975).

Study Sites:

In this study, *R. catawbiense* was collected from Roan Mountain State Park. It is situated on TN Hwy 143 at the foot of Roan Mountain which soars 6,285 ft high and contains more than 2,000 acres of rich hardwood forest intertwined with the Doe River. The elevations used in this study were 6,285 ft as the high elevation (lat: 36.1017 and long: -82.1354) at Roan High Knob and 5,500 ft as the low elevation (lat: 36.1062 and long: -82.1099) at Carver's Gap.

L. cardinalis was collected from Lamar Alexander Rocky Fork State Park and its vicinity. It is situated in the southern Appalachian Mountains of East Tennessee in the Unicoi County and encompasses 2,076 acres. The sites used in this study were a population of cardinal flowers within the state park as site 1 (lat: 36.0454 and long. -82.5601) and a population of cardinal flowers 1.2 miles away from the state park outside a Rusty Barn as site 2 (lat: 36.0569 and long: -82.5554).

Additionally, Roan Mountain State Park and Lamar Alexander Rocky Fork State Park are approximately 41 miles apart.

Field Collection:

This study was conducted during June-August. During this time, *R. catawbiense* was in bloom at Roan Mountain State Park and available for nectar sampling. The samples were collected from two elevations with an elevation difference of 786 ft. Samples were aseptically taken from ten individual plants at each elevation for a total of 20 samples with ten flowers being collected per individual plant. They were stored in sterile plastic jars in a portable cooler until taken to the lab (Alvarez-Pérez, et al., 2011). *L. cardinalis* was also sampled during the same time at Lamar Alexander Rocky Fork State Park and its vicinity. Two sites located 1.2 miles apart were selected for sampling based on abundance of *L. cardinalis.* Sampling procedure followed that of the *R. catawbiense* with ten individuals being selected at random from each site and ten flowers being sampled from each individual plant. For this study, we will only be assessing the mycobiome – fungal nectar microbiome – in *R. catawbiense* and *L. cardinalis*.

Nectar Extraction, Dilution, and Plating in the Lab:

Floral samples were then returned to a lab setting and nectar from each species was harvested within 12 hours of field collection under sterile conditions. For this, sterile and calibrated Sigma-Aldrich microcapillaries were used to remove and quantify nectar (Alvarez-Pérez, et al., 2011) before storage in sterile Eppendorf tubes. All the nectar from the ten flowers per sample was combined into one representative sample (Pozo, et al., 2010) and its quantity varied slightly between samples with a range of 0.01-2.5 µl. These were immediately diluted in varying amounts of water (Alvarez-Pérez, et al., 2011) to bring the final volume up to 100 µl.

These were then streaked onto Emerald Scientific Malt Extract Agar (Yarrow, 1998; Basu, et al., 2015) with 0.01% Chloramphenicol to exclude the growth of bacterial colonies (Yarrow, 1998). The inoculated plates were then incubated at 25°C for a week (Yarrow, 1998; Alvarez-Pérez, et al., 2012). From the resulting fungal colonies, colony counts were immediately obtained via visual inspection and recorded once for each plate (Yarrow, 1998; Pozo, et al., 2010; Alvarez-Pérez, et al., 2012), and then isolates were obtained as described in Yarrow (1998).

DNA Extraction and Sequencing:

From these isolates, DNA was extracted per QIAGEN DNeasy Blood & Tissue Handbook Spin-Column Protocol and PCR was performed under the following conditions: 95°C for 5 minutes, (95°C for 30 seconds, 50°C for 30 seconds, 72°C for 40 seconds) x 35 cycles, and 72°C for 7 minutes with a PCR mix of Fischer Scientific LongAmp Taq 2X Master Mix, Thermo Fisher Nuclease-Free water, samples, and primers ITS1-F_KYO2 (5'-TAGAGGAAGTAAAAGTCGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') fungal-specific primers to analyze the ITS region of environmental DNA extracts (Toju et al., 2012; Op De Beeck et al., 2014; Vu et al., 2016; Usyk et al., 2017; Ali et al., 2019) per the 25 µl reaction protocol. PCR products were cleaned using the QIAquick PCR Purification kit. DNA in the purified PCR product was then measured via Qubit and Nanodrop and then DNA Sanger sequenced by the ETSU Molecular Biology Core Facility. DNA sequences were identified via NCBI BLAST with 95% similarity scores.

Data Analysis:

From the previously recorded fungal colony counts for each plant sample, average colony counts (abundance), Species Richness, and Simpson's Diversity were manually calculated. For

this study, Species Richness is defined as simply a count of all the (fungal) species present whereas Simpson's Diversity is the number of (fungal) species present in addition to the relative abundance of each (fungal) species. The manually calculated average colony counts (abundance), Species Richness, and Simpson's Diversity were then verified through The SAS System.

RESULTS

A total of 6,020 fungal colonies were counted, 4,082 colonies for *R. catawbiense* and 1,938 colonies *L. cardinalis,* from which 26 visually distinct fungal isolates were obtained for *R. catawbiense* and 19 for *L. cardinalis,* for a total of 45 isolates. From these, a total of 22 fungal species were identified to be distinct in the nectar of both *R. catawbiense* and *L. cardinalis* altogether through the results of Sanger sequencing. Seven of these species belonged to *R. catawbiense* and 15 to *L. cardinalis.* A list of this has been provided in the Table 1.

Table 1: Fungal species and their relative frequencies (number of colonies) in *R. catawbiense* and *L. cardinalis.*

Fungal Species	**Frequency in *R. catawbiense***	**Frequency in *L. cardinalis***
Aspergillus	1	0
Hanseniaspora	156	0
Metschnikowia	1503	1529
Fusarium	1	0
Trichaptum	5	0
Cystobasidium	17	0
Cladospora	46	46
Papiliotrema	25	0

Peniophora	3	0
Aureobasidium	4	0
Meira	122	0
Ascochyta	7	0
Didymella	12	0
Daldinia	2	0
Coniothyrium	34	0
Sporobolomyces	0	628
Kwoniella	0	598
Alternaria	0	1
Curvibasidium	0	17
Davidiella	0	3

Average number of colonies (abundance) of nectar fungi was significantly different between high and low elevations of *R. catawbiense* populations ($F_{1,19} = 25.30$, $P < 0.0001$; Figure 1). Species richness ($F_{1,19} = 1.80$, $P = 0.1964$) and Simpson's diversity ($F_{1,19} = 0.00$, $P = 0.9612$) however were not significantly different.

Average number of colonies (abundance) of nectar fungi was significantly different between site 1 and site 2 of *L. cardinalis* populations ($F_{1,18} = 5.72$, $P = 0.0287$; Figure 2). Species richness ($F_{1,18} = 3.02$, $P = 0.1002$) and Simpson's diversity ($F_{1,18} = 1.75$, $P = 0.2033$) however were not significantly different.

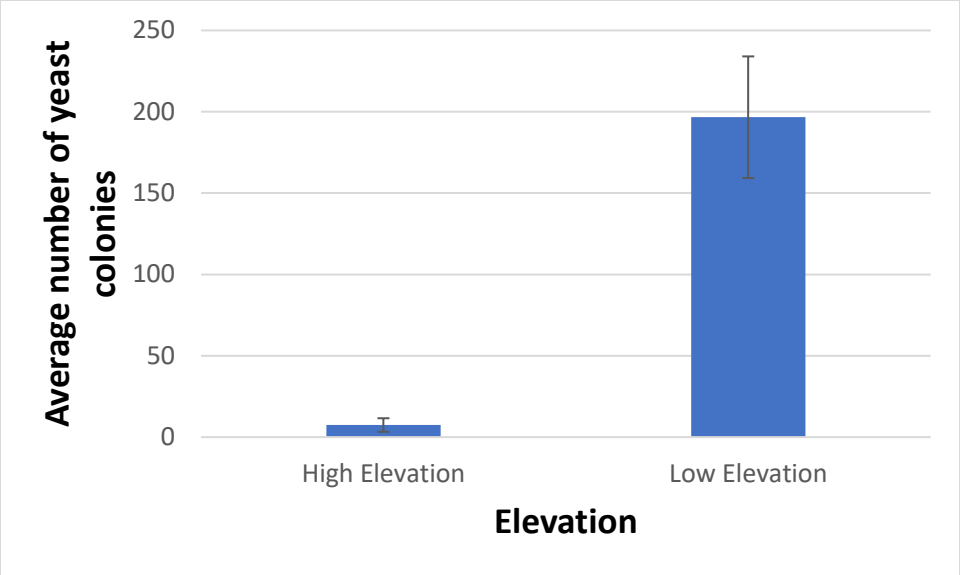

Figure 1: Average number of fungal colonies (abundance) in flower nectar in two populations (10 individuals and 100 flowers per population) of *Rhododendron catawbiense*, one at low (5500 ft) and one at high (6285 ft.) elevation.

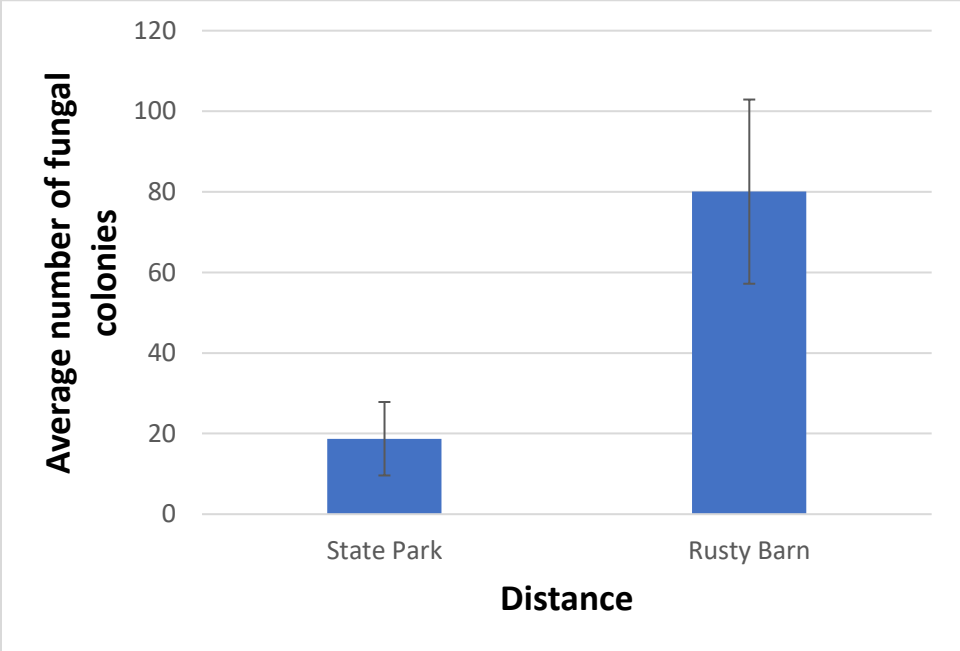

Figure 2: Average number of fungal colonies (abundance) in flower nectar in two populations (10 individuals and 100 flowers per population) of *Lobelia cardinalis,* one at State Park (site 1) and one at Rusty Barn (site 2) separated by 1.2 miles.

Average number of colonies (abundance) of nectar fungi was significantly different between *R. catawbiense* and *L. cardinalis* ($F_{1,38} = 5.24, P = 0.0283$; Figure 3). Species richness ($F_{1,38} = 0.34, P = 0.5613$) and Simpson's diversity ($F_{1,38} = 1.93, P = 0.1731$) however were not significantly different.

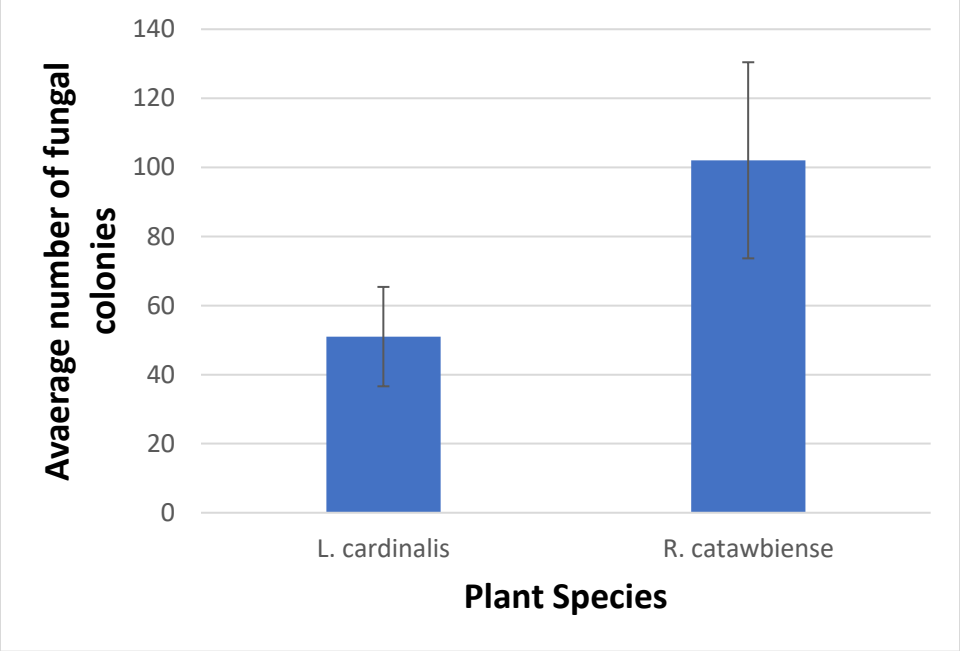

Figure 3: Average number of fungal colonies (abundance) across spatial gradients in flower nectar for *R. catawbiense* and *L. cardinalis* populations (20 individuals and 200 flowers per species population).

DISCUSSION

About 90% of all angiosperm species are thought to be animal-pollinated (Klaps et al., 2020) wherein nectar quality and pollinator behavior (Pozo et al., 2011; Alvarez-Pérez et al.,

2012; Rebolleda-Gómez et al., 2019 and references therein) can be influenced by the microbiome's exploitation of the chemical composition of nectar. Thus, the floral nectar microbiome has the potential to impact plant reproductive success which is why it is key to study the composition, diversity, abundance and factors that mediate variation in nectar-inhabiting microorganisms across plant species.

In this study, we identified 7 distinct fungal species (4,082 fungal colonies) in the nectar of *R. catawbiense* and 15 fungal species (1,938 fungal colonies) in *L. cardinalis* for a total of 22 fungal species (6,020 fungal colonies). This exceeds both the fungal diversity and its abundance compared to what has been reported for other species in previous studies (e.g. Pozo et al., 2011; Samuni-Blank et al., 2014). For instance, Pozo et al., found 6 fungal species with frequencies of 20 or below in 24 plant species (2011). Of the identified fungal species, some have been found to be rare, for instance, *Meira, Didymella, Daldinia* (as compared to Pozo et al., 2011; Alvarez-Pérez et al., 2013; Von Arx et al., 2019; Klaps et al., 2020, etc.) as they have not been previously reported in floral nectar microbiome studies. However, we also found some very common nectar-inhabiting fungal species such as *Metschnikowia, Alternaria, Aspergillus, Aureobasidium, Fusarium, Cladosporium* (as compared to Pozo et al., 2011; Alvarez-Pérez et al., 2013; Von Arx et al., 2019; Klaps et al., 2020, etc.) that have been reported in a multitude of previous floral nectar microbiome studies. Furthermore, we also found *Metschnikowia* to be one of the most dominant nectar-inhabiting fungal species similar to other studies (e.g. Rebolleda-Gómez et al., 2019; Klaps et al., 2020), followed by *Sporobolomyces* and *Kwoniella. Metschnikowia* is a nectar specialist yeast which has been shown to positively impact the nectar chemistry to highly attract pollinators while *Aureobasidium* and *Sporobolomyces* are generalist species shown to produce unimportant nutrients or undesirable changes in the floral nectar (Sobhy et al., 2018). Of the 22

fungal species we identified, 82% were found to be unique to either an elevation or a site, demonstrating that the spatial variation most likely played a role in dictating the composition of the microbiome (Martiny et al., 2006; Liu et al., 2020). This specificity could be due to differences in the microenvironmental conditions, for instance the temperature, pollinator visitation, and sunlight exposure at different elevations and sites (Martiny et al., 2006; Samuni-Blank et al., 2014; Liu et al., 2020).

For example, we found 4 fungal species at the high elevation compared to 6 at the low elevation (with *Davidiella/Cladosporium* being the only one unique to the high elevation). This could be due to lower temperatures and oxygen levels at the high elevation which disfavors the fungal species that require warmer temperature to grow, for example, *Metschnikowia* (Zucconi et al., 2012). *Metschnikowia* has been shown to require a slightly warmer temperature for its optimum growth as compared to *Davidiella/Cladosporium,* which could explain why the latter was only observed at the high elevation (Zucconi et al., 2012). For instance, there was a 3°C difference in the temperature between High Knob and Carver's Gap the day the *R. catawbiense* samples were collected, with the high elevation, High Knob, being colder and having limited sun exposure. These variations in micro-environmental conditions may result in differences in microbiome composition across the elevations (Zucconi et al., 2012; Sobhy et al., 2018). Additionally, pollinator visitation has been observed to be lower at the high elevation which has also been shown to impact nectar microbiome composition (Samuni-Blank et al., 2014).

In terms of abundance, we found only 94 *Metschnikowia* colonies at the high elevation for *R. catawbiense* compared to a total of 1,435 colonies at the low elevation. Also, there was only 1 colony of *Sporobolomyces* at the high elevation and 627 colonies at the low elevation. This was generally the trend of abundance for the rest of the 5 fungal species found within the *R.*

catawbiense nectar. *Metschnikowia* species has been shown to increase the nectar temperature which can further favor the growth of other fungal species and increase pollinator interactions (Sobhy et al., 2018) which can explain the difference in abundance across the elevations. Thus, these results support that the microenvironmental changes due to spatial variations can induce differences in the nectar microbiome (Martiny et al., 2006; Liu et al., 2020). This can in turn affect plant fitness by influencing the number and diversity of pollinators visiting flowers (Herrera et al., 2013; Schaeffer and Irwin, 2014; Rebolleda-Gómez et al., 2019).

For *L. cardinalis*, 7 fungal species were found at State Park (site 1) compared to 10 at Rusty Barn (site 2). The two sites were separated by 1.2 miles, with site 1 being in a relatively undisturbed damp and shaded environment and surrounded by other shrubs compared to site 2 which was in an open, clear field with maximum sun exposure, and was slightly disturbed. In terms of abundance, we found only 148 *Metschnikowia* colonies at site 1 compared to a total of 1,355 colonies at site 2. Also, there was only 1 colony of *Cystobasidium* at site 1 and 16 at site 2. Other than *Metschnikowia* and *Cystobasidium*, however, all the other fungal species were unique to either site 1 (e.g. *Aspergillus, Hanseniaspora, Fusarium*, etc.) or site 2 (e.g. *Cladosporium, Meira, Coniothyrium*, etc.). Again, a high abundance of *Metschnikowia* at site 2 can explain the overall high abundance of fungi in comparison to site 1 (Sobhy et al., 2018). The higher mycobiome diversity and abundance observed at site 2 may be due to the higher temperatures and potentially higher levels of visitation favoring the growth of more microbial taxa in open and more exposed sites (Yarrow, 1998). This result, however, contradicts the notion that undisturbed plant populations contain more diverse microbiomes (Mendes et al., 2015).

Additionally, only 2 out of the 22 identified fungal species were common between the plant species (*Metschnikowia* and *Cladosporium*). Even though a greater fungal diversity (7

fungal species versus 15) was observed in *L. cardinalis*, *R. catawbiense* was found to have more fungal abundance. However, the differences between the most abundant mycobiome members across the species were found to be insignificant, for instance, a total of 1,529 *Metschnikowia* colonies in *R. catawbiense* vs. 1,503 colonies in *L. cardinalis*, and exactly 46 colonies of *Cladosporium* in both the plant species. The differences in diversity and abundance could be attributed to the vastly different pollination systems and differences in nectar chemistry (Pozo et al., 2011; Alvarez-Pérez et al., 2012; Rebolleda-Gómez et al., 2019 and references therein). *R. catawbiense* is chiefly bee pollinated (invertebrate) whereas *L. cardinalis* is hummingbird pollinated (vertebrate) (Baker, 1975; Blazich et al., 1991; Peng et al., 2007). Previous studies have shown bees to carry more microorganisms (Klaps et al., 2020) which could be why *R. catawbiense* had more fungal abundance despite restricted fungal diversity. Additionally, floral nectar of *R. catawbiense* contains andromedotoxin and grayantoxin and is thus, highly toxic to pollinators and humans (Stout et al., 2006). On the other hand, floral nectar of *L. cardinalis* contains alkaloids such as lobelamine and lobeline and is mildly toxic to humans and other animals (Kelley et al., 2019). This high toxicity may be restricting the diversity of microbial taxa within the *R. catawbiense* nectar directly or by affecting pollinator visitation (Pozo et al., 2011; Alvarez-Pérez et al., 2012; Rebolleda-Gómez et al., 2019 and references therein). For instance, the chemistry of the nectar microbiome in wild plants was found to have an impact on its appeal to the pollinators that visited it, thus, dictating specificity in plant-pollinator-microbiome interactions (Sobhy et al., 2018). Furthermore, the differences in fungal diversity and abundance between the plant species could also be attributed to spatial variation as both the state parks where the samples were collected from are situated about 41 miles apart. Compared to the 1.2-mile separation for the two sites for *L. cardinalis*, markedly increased dissimilarity in the

composition, diversity, and abundance of floral nectar mycobiome was observed between the plant species. However, more extensive, and perhaps experimental research is needed to fully uncover the mediators of the patterns observed in this study. For instance, studying the whole microbiome (and not just the mycobiome) within and across different plant species, manipulating the said microbiome and observing its consequences on pollination and such.

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