The Effect of Proximity to Roads on the Biodiversity of Nitrogen-Tolerant Lichen Species

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Abstract

Our project involved determining the effects of the proximity of the road onto lichen by looking at nitrogen-tolerant versus nitrogen-intolerant lichen species. Our hypothesis states that trees in areas with closer proximity to a busy road (Friends Academy) will have more nitrogen-tolerant lichen species than in forest areas (Shu Swamp Nature Preserve). The aim of our study is to identify different lichen species in a nature preserve versus populated areas, and to see which area has more biodiversity of lichens. We cut 10 quarter sized lichen samples from trees in each of our locations and stored them in paper bags before transferring them to ethanol for better preservation. We found more foliose species of lichen at Friends Academy than we did at our local nature preserve. The species of lichen we found significantly differed between locations. Crustose species were only found at the preserve. Foliose lichen, which we found more prominently at the Friends Academy location is the most nitrogen-sensitive type of lichen, where crustose species are incredibly nitrogen-tolerant. Those crustose species were found solely at the preserve. When looking at the effects of proximity of the road on lichen species, we discovered that being near the road has little to no effect on the growth of lichen in the area.

Introduction

Car emissions release nitrogen dioxide, and car emissions can damage certain types of lichen because lichen absorbs nutrients from the air. Regardless, in areas with a lot of nitrogen dioxide, one may find species of lichen that are more resistant to nitrogen dioxide. If we sample lichens on various trees from areas closer to a busy road, we anticipated that we would locate more nitrogen-tolerant lichen species than we would in more remote areas. We carefully collected ten samples of lichens from two different locations with different proximity to a busy road and found which site has more nitrogen-tolerant lichen species.

Materials & Methods

- Collect 10 unique-looking quarter-sized samples, while avoiding bark. Store samples in paper bags to dry and keep them from degrading before transferring them to ethanol.
- We extracted and isolated DNA from our samples using the silica protocol
- We put the samples in the PCR machine so that millions of copies of a portion of each ITS gene, identified by the ITS primer, would be copied.
- We put the samples through gel electrophoresis, to show whether the PCR had successfully amplified the ITS sequence.
- The DNA was then sent to Genewiz for sequencing. Once we got the sequences back, we used DNA Subway to analyze the sequences and identify the specimens.

Results

We were only able to get results from six samples collected from FA and eight samples from Shu swamp due to not getting good quality sequencing data (many sequences were too short, sequencing didn't work for them, or were too short once edits were made during the editing consensus step). Our percentages of similarity ranged from 94.16 percent to 100.00 percent, though most were above 97 percent. Using our matches from DNA subway we found that Crustose species of lichen were unique to the nature preserve. Foliose lichen; however, was more common at Friends Academy and the only type of lichen present.

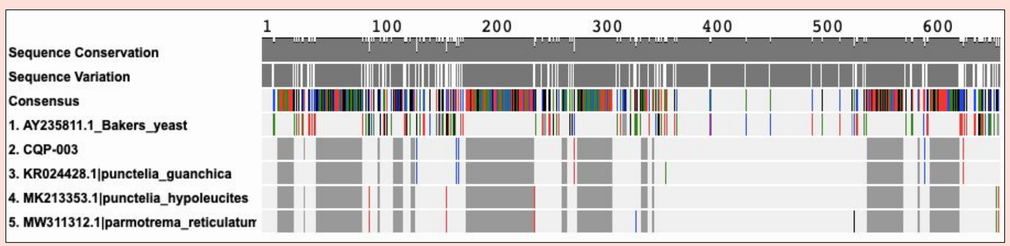

Figure 1: MUSCLE results of our third sample (foliose) determined to be *Punctelia guanicha*

Sample ID	% Similarity to closest match	Genus and Species of closest match(es)
CQP-001[1]	99.81%	*Punctelia guanchica* (foliose)
CQP-002[1]	99.52%	*Physcia stellaris* (foliose)
CQP-003[1]	99.81%	*Punctelia guanchica* (foliose)
CQP-005[1]	99.04%	*Physcia stellaris* (foliose)
CQP-006[1]	100.00%	*Punctelia rudecta* (foliose)
CQP-007[1]	100.00%	*Parmotrema hypotropum* (foliose)
CQP-008[2]	99.81%	*Punctelia guanchica* (foliose)
CQP-009[2]	100.00%	*Parmelia squarrosa* (foliose)
CQP-011[2]	99.83%	*Parmotrema hypotropum* (foliose)
CQP-012[2]	97.06%	*Graphis scripta* (crustose)
CQP-016[2]	94.16%	*Phaeophyscia endococcina* (foliose)
CQP-017[2]	99.41%	*Parmotrema hypotropum* (foliose)
CQP-018[2]	100.00%	*Cladonia caespiticia* (fruticose)
CQP-019[2]	100.00%	*Lepraria lobificans* (crustose)

Table 1: Our samples and their closest matches exhibiting similarities in the genera of the lichens as well as differences between the two collecting locations, [1] Friends Academy and [2]Shu Swamp, our local nature preserve

Figure 2: Crustose (left) and foliose (right) lichens on trees

Discussion

We found that while some species of lichen are more tolerant to high nitrogen levels than others, the proximity to roads had little to no effect on the types of lichen growing nearby. Our findings have allowed us to realize the effect that nitrogen can have on lichens because even far away from the road, nitrogen-tolerant species are still abundant, and vice versa. Despite the proximity of cars, the nitrogen-sensitive lichens were still able to grow, exhibiting how resilient lichen can be even if it has some sensitivity to nitrogen. Of course, if we did not have a cap on the number of samples we could collect and sequence, we would have been better able to assess the biodiversity of lichens in each area. We did measure the nitrogen levels in each location to see if there was a significant difference to determine if the locations had different nitrogen levels, thus explaining why there would be more nitrogen-tolerant species or not. However, we had technical difficulties with the meter and decided that the results from the meter were not reliable and would not be used in considering our thesis. If this project was to be done again we would get a better nitrogen meter to ensure we were comparing lichens from areas with different levels of nitrogen.

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