Phylogenetic relationship of tree species among plots

BIOS 35502: Practicum in Field Biology

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Abstract

Molecular approaches have been increasingly used by community ecologists to study how community composition is influenced by evolution. This is why the field of phylogenetics has been a useful tool for understanding community composition. In this study I examined the phylogenetic relationship of tree species inside two plots and compared the mean phylogenetic distances between plots. My results showed a phylogenetic overdispersion effect between the tree species within plots and across plots. This suggests underlying non-random mechanisms of environmental filtering.

Introduction

Understanding the processes that drive and shape patterns of plant community assemblages is a critical challenge at the forefront of community ecology and global biodiversity issues. Two main questions in community ecology are (1) how species or organisms can co-exist in a given community and (2) why different areas or habitats dominated by different species, genera's or families. A lot of studies have dealt with identifying these processes that influence species co-existence and drive community assembly and structure (Cavender-Bares et al. 2009). Recently, however, the need to account for species' history and the evolutionary relationship among these co-occurring species has gained considerable attention as methodological advances in phylogenetic reconstructions become easier and more available (Webb et al. 2002; Losos et al. 2008).

The differences in the species that co-exist within a community are a result of modifications through divergence from a common recent ancestor, which all species

ultimately share (Webb et al. 2000). This concept is central to "phylogenetic community ecology", an emerging field which integrates phylogenetic approaches into community ecology in order to explain patterns of community assemblages. Species sharing similar traits which allow them to survive and exist in a given habitat are more likely to be found within a community. This pool of co-occurring species is also likely to be more phylogenetically closely relates ("phylogenetic clustering") by virtue of trait conservatism (i.e. extent to which close relatives share phenotypic similarity) (Webb et al. 2002). Phylogenetic clustering or clumping of taxa indicates that habitat-use is a conserved trait among the species pool in a community. Previous studies have shown that closely related tree species can co-exist in a given community, suggesting that this co-ocurrence of taxa in a regional species pool is non-random (Nigel et al. 2001); Kraft et al 2014). This suggests a type of environmental filtering, where the community composition of the area is delimited by biotic and abiotic factors. Only certain taxa with specific traits designed for survival in that specific environment conditions are therefore present under certain environmental conditions (Burns et al. 2011; Xin-Hui et al 2014); Brunbjberg & Cavender-Bares et al. 2014).

On the other hand, it is also possible that closely related species with similar traits can be locally excluded ("phylogenetic overdispersion"), resulting in a minimum overlap in niche among co-occurring taxa. This phylogenetic overdispersion happens when distantly related taxa or species converged on similar niche-use i.e. the species pool within a comunity, represents phylogenetically distant taxa. This has been observed in communities (Astor et al. 2014). This is usually detected when the species

present have different traits which allow them to obtain resources without competing against each other (niche partitioning) on the same local spatial scale (Astor et al. 2014)

In this study I examine patterns of relatedness among species co-occurring in a given habitat. My goal is to compare tree species using non-coding cpDNA regions and create a phylogenetic tree to determine how closely related they are within plots and use the results to compare the phylogenetic differences across different plots with different environmental characteristics. I hypothesize that species occurring in the same plot will be more phylogenetically related than those species between plots (phylogenetic clustering). This is because the characteristics needed to survive in each habitat are different. Therefore, these species should have different genotypes that give them the capability to survive in the environment where they exist. Alternatively, co-occurring species within a given plot may also be more phylogenetically distant (phylogenetic overdispersion).

Methods

Location: The plots selected in the UNDERC property were 50m x 50m long and distanced between each other to see how different the species were. These plots have been previously catalogued, and soil and land use histories described by Pecoraro (unpublished data). The two plots slightly differ in soil composition, with Plot 1 having Gogebic fine sandy loam, sandy substratum, 6-18% slopes and well drained soil. On the other hand, Plot 2 has a mixture of Karlin-Keweenaw-Sarona soil, dense substratum, with 6-25% slopes, surrounded by a vernal pond and nearby a lake. These plots had previously been studied and the tree species had been identified.

Sampling: A total of 18 leaves from 13 tree species were collected on both plots. The species present on each plot were: *Acer saccharum, Acer rubrum, Betula papyrifera, Picea glauca, and Abies Balsamea* (Plot 1); *Acer saccharum, Acer rubrum, Fraxinus americana, Tilia Americana, Populus tremuloides, Prunus serotina, Picea mariana and A. balsamea* (Plot 2).Leaves were collected from each tree species inside each plot, except for *Populus tremuloides* leaves, which were on the border right next to the second plot. The identification of the tree species was done by using a previously made list of the species on each plot (Pecoraro, unpublished).

DNA extraction, preparation and sequencing: The leaves from each tree were frozen in liquid nitrogen and grinded to facilitate the DNA extraction. After this, the DNA extraction and PCR reactions were performed using the E.Z.N.A. DNA Plant isolation system kit (Omega Bio-tek, Norcross, GA, USA), following the manufacturer's instructions. Primers were used to amplify two non-coding regions of chloroplast DNA; the intergenic spacer region trnL-trnH and rbcLa. The reactants concentrations for the PCR reaction were as follows: 10xStandard Taq Reactions Buffer, 25mM $MgCl_2$, 5,000 U/ml Taq DNA Polymerase, 10mM rbcLa_F primer, 10mM rbcLa_R primer, 10mM trnL primer, 10mM trnH primer, 10mM dNTP's, 2uL DNA Template. PCR cycling conditions were as follows: 92 C for 5 mins; 35 cycles of 92 C for 2 mins; 56-58 C (depending on each locus) for 10 minutes and 72 C for a minute 30 seconds; and a final elongation of 72 C for 5 minutes. PCR amplicons were then sequenced in an ABI Prism 3730 at the Notre Dame Genomics Core Facility.

Phylogenetic analysis

Sequences were manually edited using the computer program Sequencher v.5.2 (Gene Codes Corporation, Ann Arbor, MI USA) removing regions in the sequences where the primers landed. Both the trnL-trnH and rbcLa regions were analyzed. However, only the rbcLa region was used in this study because it has more variations and better sequences for this region were obtained. The final number of base pairs on each sequence was 529bp. These were later aligned using MEGA v.6 (Tamura 2013) and a phylogenetic tree was created on the same program for each plot and combined plots, using the Maximum Likelihood Tree function. After sequence alignment, haplotypes were identified by looking at Single Nucleotide Polymorphism's (SNP's). In order to examine patterns of relatedness among co-ocurring taxa, the mean pairwise phylogenetic distance (MPD) was calculated for each pairwise species or taxa (Webb et al. 2002). This measure basically reveals patterns of relatedness among species within a given community and is conventionally used to describe phylogenetic relationship among species asemblages. This was performed using the Phydist function on Phylocom (Webb et al 2008).

Statistics: In order to examine whether closely (phylogenetically) related species (i.e. lower phylogenetic distance between taxa) tend to occur more within plot than between plots, I performed a simple t-test between means of all pairwise species comparison between and within plots in R v2.3 (R DeveloplentCore Team et al 2008).

Results

Species description:

A total of five species were found on Plot 1. These were: *Acer saccharum, Acer rubrum, Betula papyrifera, Abies balsamea* and *Picea glauca.* On Plot 2, eight species were found: *Acer saccharum, Acer rubrum, Fraxinus americana, Tilia americana, Populus tremuloides, Prunus serotina, Abies balsamea* and *Picea mariana.* Only the *Acer* spp., and *Picea* spp. genera's had more than one species represented in the plots. And only the species under *Acer* spp. and *Abies* spp. were present on both plots.

Molecular Variation:

A total of 529 base pairs were analyzed for each sequence. A total of 9 different haplotypes were found. Among samples from the same species, very little intra-species variation was found, mostly across plots. As expected, sister taxa (*A. saccharum* & *A. rubrum*) had very little variation. The *Picea* spp. had no inter-genus variation on the rbcLa region, and was more closely related to the *A. balsamea*, in comparison to the other species.

Phylogenetic structure:

The overall pairwise mean phylogenetic distance within plots was 0.124, while between plots it was 0.086. Comparison of the MPD among all pairs of species within and across plots showed significant difference based on the t-test (P=0.006) (Figure 1). This results indicate a higher phylogenetic distance among tree species within plots (phylogenetic overdispersion) and a higher phylogenetic relationship between species across plots. These results are further corroborated by the evidence of potential phylogenetic overdispersion within each plot.

In the first plot the construction of a phylogenetic tree (Figure 2) showed a clear divergence between the angiosperm and gymnosperm tree species in the plot. Overall, there is evidence of phylogenetic overdispersion as distantly related taxa (e.g. *Picea* and *Betula*) Some phylogenetic clustering could also be observed on each lineage among the *Acer* genus and the *Picea/Abies* genera. Among the species in the plot, *A. rubrum* showed the highest mean phylogenetic distance between all taxa. *Betula papyrifera* on the other hand, do not have any closely related species inside the plot as it belongs to a different clade than the rest of the species.

The phylogenetic tree for the second plot (Figure 3) indicates phylogenetic overdispersion among tree species. Even though a close relationship between *A. saccharum* and *A. rubrum* was shown, as well as *P. mariana* and *A. balsamea*, the other four species present on the plot were in a considerable distant position from the other species.Similar to the first plot, the *Acer* spp. showed the same haplotypes, as well as the *Picea/Abies spp*. Out of all the species, *Picea mariana* demonstrated to have the highest mean of phylogenetic distance to the rest of the species.

Discussion

Community structure can be influenced by a variety of biotic and abiotic factors, as well as random and non-random occurrences. Understanding the processes and mechanisms defining community assembly have been subject of research for decades (Losos et al. 2008)**.** Recently, the importance of accounting for the evolutionary history of species in determining the patterns of co-existence among species has gained considerable attention (Webb et al. 2002).

My results suggest an overall phylogenetic dispersion among tree species within each plot and between plots. And the calculated MPD's for distance within plots (0.124) and between plots (0.086) indicate that there is more phylogenetic distance between the species inside each plot, rather than the species across plots. These results strongly support that there has been Phylogenetic overdispertion inside each plot, even though the phylogenetic relationships across plots are closer.

The Phylogenetic overdispersion present on these communities is evident when looking at the positions in the phylogenetic tree for each plot. The presence of different taxa that are from long divergent lineages (angiosperms & gymnosperms) within the same plot shows the amount of evolutionary distance between these. It is possible that the phylogenetic overdispersion present within plots is caused by non-random community assembly processes that specifically select species that are phylogenetically distant amongst each other. One explanation for the lack of similar co-occurring species could possibly a type of competitive exclusion, where closely related species cannot co-exist because of the competition for resources, among other mechanisms. Cardillo (2011) proposed in the so-called competition-relatedness hypothesis that closely related species can be expected to compete more strongly if traits mediating competition are highly conserved, thus causing local communities to be evenly dispersed to limit trait and phylogenetic similarity. It is possible that these mechanisms could explain the patterns I observed in each plot, however more analysis should be done to support it.

Another possible explanation for the biodiversity present within plots, which also accounts for the closer relationship between plots is environmental filtering. The environment selects for particular traits that confer adaptability and survival of species

under particular environmental conditions. The reason why species are able to survive in a place is purely because of the traits they possess that have either been : a) acquired from an ancestor; or b) obtained through convergence (development of superficially similar traits that have not been passed down from a common ancestor). The simple presence of these species in the northwoods indicates a possible environmental filtering effect. Therefore, the co-occurrence of phylogenetically overdispersed taxa points to a convergence of traits that allow survival of different species from distant lineages in these forests.

These results are in contrast with some studies (i.e. Qian et al. 2014; Machac et al. 2011; Wang et al. 2012; Graham et al. 2009) which have demonstrated that species tend to be more phylogenetically clustered, the higher in a latitudinal position they are. These studies tend to support the hypothesis that environmental filtering is driving phylogenetic clustering in communities over phylogenetic overdispersion. On the other hand, my results are supported by other studies (i.e. Bryant et al. 2008); Kluge & Kessler et al. 2011; Astor et al. 2012) that suggest that phylogenetic overdispersion is common in community assembly, especially among angiosperm lineages. Therefore, phylogenetic overdispersion on our plots must be attributed to some other assembly process that allows for the co-existence of phylogenetically distant species.

Study Limitations and Future Recommendations

The results of this study shed light into how tree communities look like here in UNDERC. However, the low sample and species number was probably a limiting factor in obtaining a solid representation of the broader phylogenetic community of trees on the entire area. This is why I believe that for future research a higher number of

samples, representing more species , should be attained while sampling. Another suggestion would be to sample more plots in order to better comprehend the community structure at UNDERC. One final suggestion would be to incorporate species traits (i.e. habitat requirements) in order to study niche conservatism.

Acknowledgements

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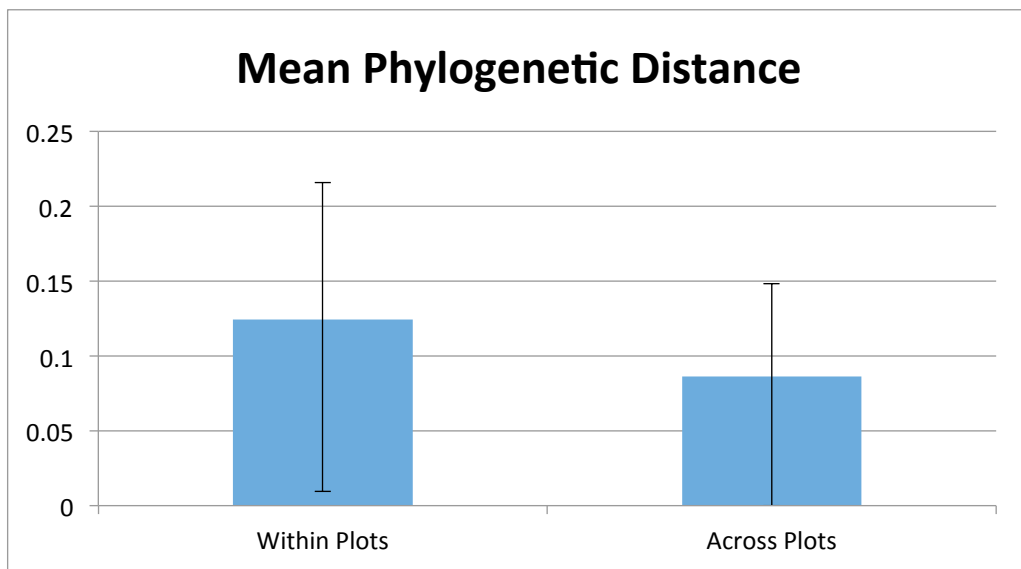
Figures

Figure 1. Graphic representation of mean phylogenetic distance among tree species within and across plots (P-value = 0.006)
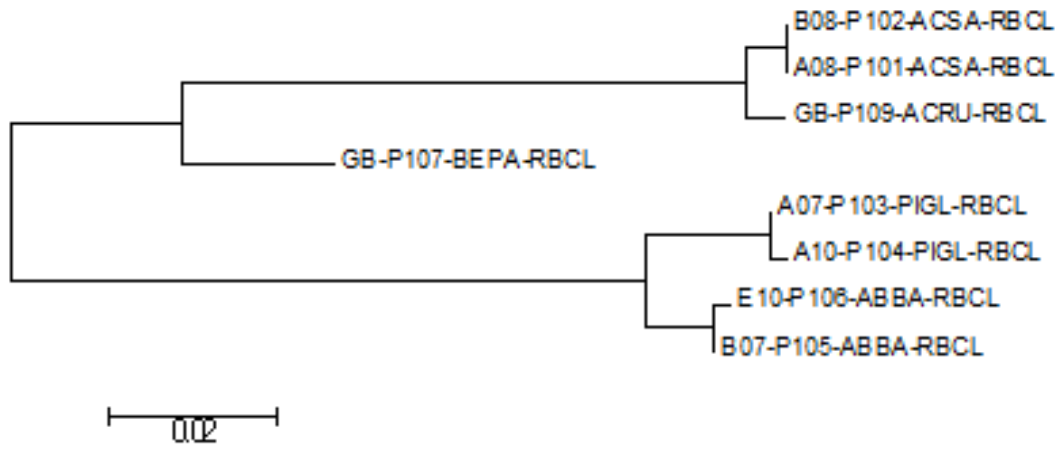
Figure 2. Cladogram representing phylogenetic relationship among tree species in Plot 1
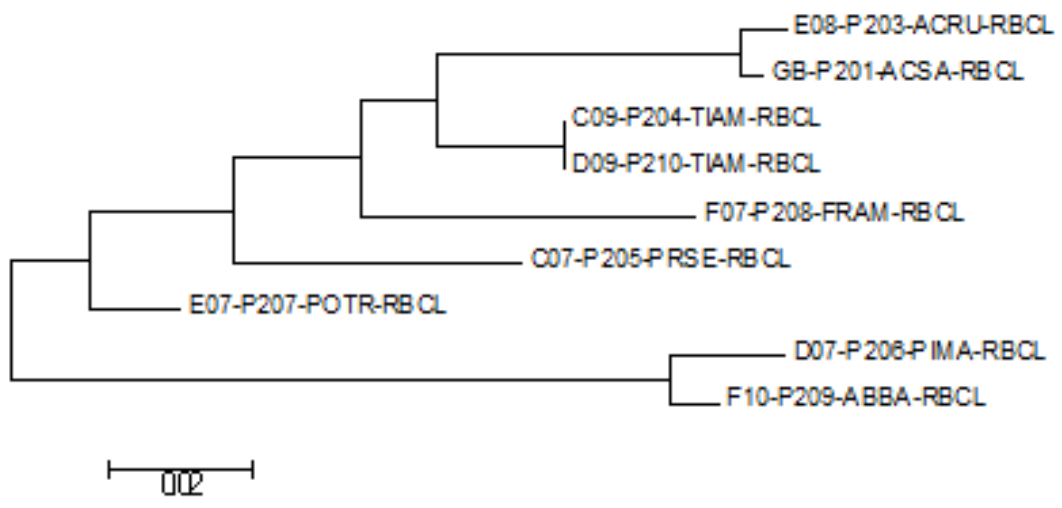

Figure 3. Cladogram representing phylogenetic relationship among tree species in Plot 2