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Title: Do Fungal Communities in Trees Influence Black-capped Chickadee Nest Site Selection?

Significance

Previous studies of Black-capped Chickadee nest-site selection lead to inconsistent results. Sedgewick and Kopf (1990) reported that no single or pair of variables could differentiate Black-capped Chickadee nest sites from control sites. However, no prior studies have examined the role that fungal communities play in determining nest-site selection in Black-capped Chickadees. I will identify wood decay fungi to species level and examine the role that wood decay fungi play in nest-site selection by comparing the fungal communities in Black-capped Chickadee nest cavities to control trees. My results will increase our understanding of Black-capped Chickadee ecology, and provide insight into how wood decay fungi might affect other systems across the Upper Peninsula where nest cavities are a limited resource. The results of this study will be disseminated through publication in a peer-reviewed journal, and through presentation as a poster at an ornithological conference.

Background

Black-capped Chickadees (*Poecile atricapillus*) are primary excavating songbirds that are common across the majority of the northern United States and southern Canada, from coast to coast. Black-capped Chickadees (hereafter chickadees) live in variable habitats, including coniferous and deciduous forests, forested wetlands, riparian communities, and even suburban and urban areas. They are year-round residents, and engage in flocking behavior during the winter. Chickadees select decaying trees for nest sites. Females choose the nest site location and build the nest (Odum 1941, Smith 1974), but both sexes excavate the cavity (Smith 1991). Juvenile chickadees will disperse from territories, but adult chickadees will inhabit the same local territory for multiple generations. Their diet during winter

months includes mixed berries, seeds, and insects, and changes to a completely insectivorous diet during the breeding season (Smith 1993).

Many factors have been shown to influence chickadee nest-site selection, such as nest-site selection characteristics like tree density (Sedgewick and Knopf 1990), prey abundance (Schroeder 1990), and specific habitat selection characteristics like conspecific attraction (Ramsay et al. 1999). However, it is unknown if chickadees are influenced by the presence of fungal communities when selecting trees to excavate. Menill and Ratcliffe (2004) found that most chickadees select rotten white birch (*Betula papyrifera*), sugar maple (*Acer saccharum*), and quaking aspen (*Populus tremuloides*) trees as nest sites, but did not examine the role of fungi in nest-site selection. Chickadees also randomly oriented their nest cavity entrances, suggesting that the sun and prevailing winds do not influence nest-site selection in terms of cavity entrance direction (Menill and Ratcliffe 2004). Lorenz et al. (2015) found that six species of primary excavators (n = 259) had nest cavities with significantly softer interior wood than compared to random trees. Similarly, Martin et al. (2004) found that primary excavators selected quaking aspen trees in British Columbia despite quaking aspen only comprising 15 percent of available trees. However, Lorenz et al. (2015) and Martin et al. (2004) did not examine the role of wood decay fungi in these systems. Thus, it is possible that wood decay fungi may play a role in nest-site selection throughout many cavity excavating systems.

The purpose of this study will be to examine if chickadee nest-site selection is influenced by the presence of certain fungal communities. To test this, I will identify and compare the fungal communities in chickadee cavities to similar nearby trees without excavations (control trees). I will also record the physical measurements of trees with chickadee cavities, including diameter at breast height (DBH), diameter of the limb containing the nest cavity, aspect and direction of the cavity, wood hardness, and species of tree. I will measure the DBH, wood hardness, and record the species of tree for control trees

to compare these factors between chickadee-selected trees and control trees. I predict that chickadee excavations will contain fungal communities that are significantly different than the fungal communities in trees without excavations.

Proposed Methods

Field Methods

This study will be conducted in Marquette, MI, USA. I will search for chickadee nest sites in a 53 ha mixed deciduous-coniferous forest plot where the Lindsay Lab has a population of radio-frequency identification (RFID) banded chickadees, and opportunistically across Marquette through use of citizen reports of chickadee nesting activity. I will search for and monitor chickadee nest activity in the plot weekly from early March to late August, 2018. Upon discovery of an active chickadee excavation, I will monitor the site daily until the completion of the nesting cycle. I will aim to find at least 10 active chickadee nest sites, but 30 or more would be ideal. Once the chickadee nestlings fledge at each site, I will take fungal subsamples from the nest cavity and from two similar trees that do not contain cavities (controls) by aseptically drilling into sapwood, following the basic protocol from Jusino et al. (2014). Drilled samples will be stored in two ml sterile tubes in CTAB buffer and kept in a -80°C freezer to await further processing. I will also record the physical measurements of trees with chickadee cavities, including diameter at breast height (DBH), diameter of the limb containing the nest cavity, aspect and direction of the cavity, wood hardness, and species of tree. For control trees, I will measure the, wood hardness, and record the species of tree.

Molecular Methods

The DNA from fungal and control samples will be extracted, amplified using a polymerase chain reaction (PCR), and sequenced using Next Gen Sequencing following the procedure from Jusino et al.

(2014). DNA sequences will then be used to identify fungal species present in the samples using the BLAST (Basic Local Alignment Search Tool) algorithm from the NCBI (National Center for Biotechnology Information) website. Sequences with a similarity match of less than 97 % will not be used to for species level identification.

Data Analyses

Data analyses will be conducted using the program R (R Core Team 2013), unless mentioned otherwise. I will use the R package Species (Wang 2011) to calculate the species richness of fungi in wood samples by creating taxon accumulation curves. I will use the R package Vegan (Oksanen 2012) to preform nonparametric multidimensional scaling (NMDS) to compare the dissimilarities in fungal communities between control trees and trees with cavities. I will use a permutational multivariate analysis of variance (PERMANOVA) test to examine whether tree physical measurement variables are related to fungal community structure. Chi-squared tests will be used to see if the fungal communities differ between chickadee cavities and control trees.

Expected Outcomes

I predict that the fungal communities present in chickadee cavities will have significantly different species richness and higher number of fungal species than in control trees, which would suggest that fungal communities influence chickadee nest-site selection. If fungal communities are a significant variable in determining chickadee nest-site selection, this will provide insight for management practices to preserve trees that chickadees will select based on fungal communities found in the trees. These results could also provide insight into other cavity-excavating systems where excavators select decayed trees.

Permits

This study will not require IACUC approval, because I will not be handling any birds directly. Other activities associated with this work are covered by the correct IACUC, USFWS, and state banding permits which are already acquired.

Literature Cited

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Budget

Supplies	**Source**	**Cost**
CTAB, 100 g	Sigma Aldrich	$51.70
Tris-hydrochloride, 100 g	Sigma Aldrich	$131.90
EDTA, 100 g	Sigma Aldrich	$22.00
Primers (ITS1F and ITS4b)	Eurofins	$17.00
Offsite next-generation sequencing	USFWS	$15 x 100 samples = $1500
Total		$1728.60*

*Additional funding sources will be sought from additional grants or awards to cover expenses beyond the amount awarded from this grant. No other grants or awards have yet been received.

Timeline

January – submit grant proposals

March 2018 – begin collecting field data

Spring 2018 – defend proposal

Summer 2018 – collect field data, order supplies for molecular work

Fall 2018 – analyze data

Winter 2018/Spring 2019 – write up preliminary results, attend conferences

Summer 2019 – subsequent field data collection