DISTRIBUTION AND POPULATION GENETICS OF NORTHERN SAW-WHET OWLS IN THE SOUTHERN APPALACHIANS

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ABSTRACT

My study looked at the distribution and abundance of Northern saw-whet owls (*Aegolius acadicus*) in the Southern Cherokee and Nantahala National Forests. Pennsylvania Toot Route Protocol (Larzone and Mulvihill 2006) was used to survey routes in the spring/summer of 2013 – 14. Nine birds were found, primarily above 1067 m.

I studied the population dynamics of *A. acadicus* in the Southern Appalachians to determine a subspecies was present. Samples from Pennsylvania, New York, Tennessee and North Carolina were obtained; cytochrome *b* and NADH dehydrogenase subunit 2 were sequenced and analyzed using AMOVAs and network analyses. The Southern Appalachian population was not a subspecies but may be a recent colonization with a small population.

Further research into habitat requirements in the Southern Appalachians as well as fecundity and general ecology can help conservation efforts. Current forest management plans do factor in the needs of this species but there are data gaps.

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I would like to thank my family for putting up with my “dangerous” habits of going into the woods at night. I would also like to thank those that came with me into the dangerous wild lands of Cherokee National Forest and Nantahala National Forest (to the wild ghost fairies). Dr. David Aborn for accepting me and helping with my time at UTC. Scott Somershoe, state bird biologist formerly of TWRA for being really excited to have someone survey for saw-whets and was able to get me the grant to do my work. Dr. Eric O’Neill and Dr. Yukie Kajita for helping me, a lot, with my genetics research. Definitely could not have done this without you guys. Dr. Thomas Wilson for giving extremely tough critique that only helped make this a better paper. Mark Hopey, Liza, and Krista of Southern Appalachian Raptor Research for letting me use samples that they worked extremely hard to collect. Dr. Glenn Proudfoot of Poughkeepsie, NY for letting me use his blood samples, once again that took a lot of time and effort to collect. Dr. Karl Kleiner of York College, who gave me my first out of state samples and recommended that I try different extraction protocols. Drs. Barbosa and Kovach for helping me with making reagents and letting me use their labs. To the biology office, for letting me use the truck and getting me supplies. All in all a big heartfelt thank you to everyone that made this possible.

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LIST OF ABBREVIATIONS

AMOVA, Analysis of Molecular Variance
BMP, Best Management Practice
ESRI, Environmental Systems Research Institute
LMP, Land Management Plan
MLRC, Multi-Resolution Land Characteristics Consortium
NAD 83, North American Datum of 1983
NLCD, National Land Cover Database
SNP, Single Nucleotide Polymorphism
TWRA, Tennessee Wildlife Resources Agency
USFS, United States Forest Service
USGS, United States Geological Survey

LIST OF SYMBOLS

Φ_{st}, equivalent of ANOVA's F-statistic. It is used in AMOVA calculations.

CHAPTER I

INTRODUCTION

Natural History

Northern saw-whet owls (*Aegolius acadicus* Gmelin; NSWO), are common in the northern forests of North America (Marks and Doremus 2000); 100,000 – 300,000 individuals estimated Canada is thought to have between 50,000 and 150,000 breeding pairs (Ruyck et al. 2012). Despite being common, they are very secretive; there is little known about its life history, population dynamics, distribution, breeding ecology, and behavior. Breeding range of *A. acadicus* includes forested regions from the Pacific Coast to the Atlantic Coast, from Alaska and Canada to Mexican Highlands (Stock et al. 2006). Some wintering birds have been found as far south as Florida and the Gulf Coast (Stock et al. 2006). They are considered to be a habitat generalist (Johnson and Anderson 2003), though the diversity of vegetation in riparian corridors is an important part of their habitats in the western breeding populations (Marks and Doremus 2000). Densities are highest in coniferous forests at moderate elevation and latitude (Rasmussen et al. 2008).

The *A. acadicus* are considered to be short-distance migrants, migrating with the seasons or nomadic, moving in response to resource availability (Marks and Doremus 2000) depending on geographic regions (Stock et al. 2006). In eastern North America, migration during the fall has been described as “irruptive, sporadic and unpredictable” (Stock et al. 2006). During migration, *A.acadicus* travels at night with powered flight. Powered flight is energy intensive and

during irruptive years, the energetic and body conditions of owls caught was below average, especially in the juveniles, suggesting that migration may be partially density dependent (Whalen and Watts 2002); however, there is bias toward female captures because of the male call (Whalen and Watts 1999) . Large migration events may result in increased food competition in stopover points and shorter time at stopover points to avoid competition (Whalen and Watts 2002). Sex-biased migration has been observed in *A. acadicus*, with males wintering closer to breeding sites and females or young migrating in larger numbers to southern regions with more stable food populations (Ruyck et al. 2012).

Genetics and Taxonomy

Mitochondrial DNA (mtDNA) evolves faster than nuclear DNA (Voigt et al. 2012). Cytochrome b is one of 37 genes in the mitochondrial genome (Cainé et al. 2006). It contains codon positions that evolve both slowly and rapidly as well as regions that are variable and conservative (Farias et al. 2001). The rate of replacement substitutions in expressed sites is lower in birds than in mammals while the rate silent substitutions were similar, thus posing a functional restraint on molecular evolution. (Stanley and Harrison 1999). In the study done by Farias et al. (2001), they determined that cytochrome b is useful in assessing recent relationships in phylogeny. It has good resolution at the genus level (Wink et al. 2004) and is informative for taxa whose speciation events have occurred within the last 20 million years, especially birds (Heidrich et al. 1995).

Cytochrome b is a transmembrane protein that makes up the central catalytic subunit of ubiquinol cytochrome c reductase (Esposti et al. 1993). This is a redox enzyme that is found in mitochondria's respiratory chain. It is involved in redox reaction of the electron transport chain,

catalyzing the transfer of electrons from ubiquinol to cytochrome c and translocates protons from inside the mitochondrial membrane to the outside (Weiss et al. 1987). Cytochrome b is highly conserved because a mutation can lead to muscle atrophy. Most mutations are not expressed as they occur at the third base of the codon (Espinosa de los Monteros 2000).

NADH dehydrogenase subunit 2 is also a part of the mitochondrial respiratory chains (Complex I) located in the mitochondrial membrane (Nosek and Fukuhara 1994). It functions as one of the electron entry points (Bandeiras et al. 2002). Complex I catalyzes electron transfer from NADH to ubiquinone (Nosek and Fukuhara 1994). The function of NADH dehydrogenase subunit 2 is still not fully understood (Uddin et al. 2015). It is also a well-known bird mtDNA marker with a neutral rate of evolution (Withrow et al. 2014).

The *A. acadicus* has two known subspecies, *Aegolius acadicus acadicus* (Gmelin, 1788), and *A. a. brooksi* (J.H. Fleming, 1916). *A. a. brooksi* are a non-migratory, endemic subspecies, restricted to the Haida Gwaii archipelago (Holschuh 2006). Previous genetic analysis of cytochrome b revealed little differentiation between the *A. a. brooksi* and the main population of *A. a. acadicus* and effectively no gene flow (Topp and Winker 2008; Withrow et al. 2014). There is speculation that the *A. a. brooksi* descended from a population of owls that migrated to the archipelago after the glaciers retreated (Holschuh 2006). Individuals of *A. a. brooksi* are darker in coloration and have unique feeding habits (Withrow et al. 2014), including feeding on marine invertebrates (Holschuh 2006).

Withrow et al. (2014) analyzed the genetic divergence of *A. acadicus* on the Haida Gwaii archipelago, where *A. a. acadicus* occur in small numbers throughout fall and winter migrations. Genetic markers used in this study showed significant divergence between *A. a. acadicus* and *A.*

a. brooksi and very low mitochondrial DNA gene flow between the two (<1 individual per generation). Sampling from Eastern North America was very limited (n = 3), with amplified fragment length polymorphisms (AFLPs) in the northeast analyzed (Withrow et al. 2014). Sampling locations were more numerous in Western North America and more samples were obtained for cytochrome b (n = 35), NADH dehydrogenase subunit 2 (n = 44), and AFLPs (n = 45).

Heidrich et al. (1995) studied molecular phylogeny of South American screech owls using the cytochrome b gene found in the mitochondria. Researchers compared six New World and two Old World species of screech owls. They tested the validity of the ecological and acoustic approach to define a species, since these species have similar morphology. Four of the six species were determined to be distinct species based on cytochrome b. These species were monophyletic Previous work has supported the hypothesis that the vocalizations and ecological characteristics are more important when classifying owls than the morphology (Heidrich et al. 1995).

Miranda et al. (2011) studied the phylogeny of the Philippine Lowland Scops Owls (*Otus* and *Mimizuku*) to determine if multiple Scops owl species result from multiple colonization events or if they reflect speciation events. They also looked at the patterns of DNA divergences determine if three of Philippine Lowland Scops Owl (*O. megalotis* Walden) were subspecies or distinct species. The mitochondrial genes cytochrome b and NADH dehydrogenase 2 were sequenced in three endemic species, three endemic subspecies, and an endemic genus. There are seven species in genus *Otus*, two are not endemic to the Philippines. There were two independent colonization events with diversification occurring subsequently (Miranda et al. 2011). The three endemic subspecies were determined to be distinct species (*O. megalotis*, *O. everetti*, and *O.*

nigrorum). These species represent the Lowland Philippine clade. Muscle samples were obtained from Philippine National Museum and the 1991 – 1993 Philippine Biodiversity Inventory done by the Cincinnati Museum Center, and the trees were rooted by *Bubo virginianus*.

Management Needs

A. a. acadicus is classified as S1: critically imperiled in the state with 5 or fewer occurrences statewide (Team 2015). Suitable habitat classifications include Southern and Central Appalachian cove forest, Southern Appalachian oak forest, northern hardwood forest and Appalachian (hemlock)-Northern hardwood forest. Marginal habitat includes Southern Appalachian low-elevation pine forest and montane pine forest and woodland. Preferred habitat is Central and Southern Appalachian spruce –fir forest (Team 2015).

There is little literature on the basic distribution, habitat use, and population dynamics of the Southern Appalachian population of *A. a. acadicus* outside of migration seasons. The Southern Appalachian population is morphologically different from the main population of migratory *A. a. acadicus* (Tamashiro 1996). The right and left toes are longer and the alula length, bill width and tail length are smaller than the main population and *A. a. brooksi*. The Southern Appalachian population is also smaller on average than the main population and is speculated to be a genetic "reservoir", holding some unique alleles (Tamashiro 1996). These alleles may account for the morphological differences within this population. Habitat modification in the form of incompatible forestry practices (Agency 2005) and loss in higher elevations due to climate change and anthropogenic influences (Milling et al. 1997) make the conservation of the potential genetic reservoir a high priority (Tamashiro 1996). With

conservation of their habitat and better management, the "reservoir" and potential for a new subspecies will not be lost.

There have been reports of *A. a. acadicus* found in Cherokee National Forest, however little is known about their population dynamics and abundance (Rasmussen et al. 2008). There is a need for data in order to create and implement a management plan for this species. Historical incompatible forestry practices were cited as the source of stress cited by the Agency (2005). Increased conservation efforts by can aid in future research on the fecundity of these potential breeding pairs. High-grading and short rotations of harvest can lead to fewer potential breeding sites.

In addition, there is speculation as to whether there are subspecies of *A. acadicus* in the Eastern United States, a northern subspecies and a southern subspecies. There is a possibility that the southern Appalachian population of *A. acadicus* is a distinct subspecies, because of the archipelago type distribution found throughout the Eastern United States. *A. a. acadicus* is distributed throughout the boreal forests and the temperate forests of eastern North America (Bowman et al. 2009). There appears to be two disjunct populations in the south with a larger main population in the northern regions.

Goals

1) To determine the distribution and abundance of *A. a. acadicus* in the Southern Cherokee National Forest, specifically in elevations above 1066.7 m.
2) I hypothesize that the population found in the Southern Appalachian is a subspecies based on sequence variation in cytochrome *b* and NADH dehydrogenase genes.

CHAPTER II

STUDY AREA

My study area was the Tellico district of the Cherokee National Forest in eastern Tennessee and the Cheoah district of Nantahala National Forest in western North Carolina. The Tellico district is 499.27 km^2 while the Cheoah district is 487.65 km^2. Mountain ranges in this area are the Unicoi Mountains and the Unaka Mountains. Within these mountains, elevation ranges from 335.28 m to 1667.86 m and is characterized by steep narrow valleys with steep to very steep slopes and sharp crests (Scott 2002). Annual precipitation is between 152.4 and 193.04 cm; temperatures from -6.1 to 27.7°C (Scott 2002).

Forest Type

Forest types in this area include rich cove forest, oak-hardwood forest, montane pine forest, Southern Appalachian northern hardwood forest, and low-elevation pine forest. Dominant forest cover consists of sugar maple (*Acer saccharum*), mountain maple (*A. spicatum*), striped maple (*A. pensylvanicum*), yellow birch (*Betula alleghaniensis*), cherry birch (*B. lenta*), American beech (*Fagus grandifolia*), white ash (*Fraxinus americana*), blue magnolia (*Magnolia acuminata*), Fraser magnolia (*M. fraseri*), black cherry (*Prunus serotina*), red oak (*Quercus rubra*), American basswood (*Tilia americana*), and eastern hemlock (*Tsuga canadensis*) (NatureServe 2015).

Land Management Plans

Cherokee National Forest's land management plan (LMP) went into effect in 2004. This is a 15-year plan. My study area falls in management area 6; there were three management prescriptions (9.F, 9.H, and 12.B). There are four goals within the management prescription 9.F: rare communities (Office) 2004). These four goals were 1. To restore and maintain rare communities; 2. Develop and test methods for restore spruce-fir forests to historical locations; 3. Try to minimize effects of acid precipitation and balsam woolly adelgid (*Adelges piceae* Ratzeburg) on the spruce-fir forest; 4. Develop relationships with private landowners to maintain or establish habitat corridors between patches of spruce-fir habitat (Office) 2004).

Management prescription 9.H is management, maintenance, and restoration of plant associations to their ecological potential where there are few to no constraints on vegetation management and prescribed burns. The goal of this management strategy is to create mid- and late-successional forests with 4 to 10% of the forests in early succession (Office) 2004). Prescription 12.B is remote backcountry recreation - nonmotorized. There is no timber management, limited vegetation management and few to constraints on prescribed burns. The single goal of this prescription is to discourage new construction and require landscape management for any improvements to existing utility corridors and communication sites (Office) 2004).

Nantahala National Forest's LMP went into effect in 1987 with amendments throughout years. Currently Nantahala and Pisgah National Forests are creating a new forest plan that complies with the 2012 Planning Rule, Agriculture Act of 2014 and the 2004 Tribal Forests

Protection Act (Service 2014). Based on the 1994 amendments to the 1987 forest plan, there were five management areas: 2A, 2C, 4A, 4C, and 4D (Service 1994), within my study area. Management area 2A is designed to provide scenery along roadways and lakeshores and timber management is designed to maintain these scenes. 2C is designed the same way with the exception of no timber production (Service 1994). Management areas 4A, 4C, and 4D are designed to provide a remote forest setting that is mostly closed to motorized vehicles. In addition to remote forest setting, 4A is also designed to have high quality scenery and timber management must meet these conditions. 4C does not allow for timber management and is managed primarily for black bear (*Ursus americanus* Pallas). 4D is managed to maintain high quality wildlife habitat primarily for *U. americanus (Service 1994).* In 4D, timber is harvested based on site-specific analysis before using either shelterwood or two-aged harvest methods. In a two-aged harvest, a mature stand is partially cut and a new age class is established either naturally or artificially (Service 1994). Shelterwood harvesting involves the removal of most mature trees to allow for new age class development (Service 2006). Clear cutting is allowed only after a thorough analysis (Service 1994). Vegetation management in 2A, 4A and 4D is to provide habitat conditions for species such as *A. a. acadicus,* the pileated woodpecker (*Dryocopus pileatus* Linneaus), gray squirrel (*Sciurus carolinensis* Gmelin) and golden-crowned kinglet (*Regulus satrapa* Lichenstein), by allowing up to 10% of the forests to be early successional.

Best Management Practices

Cherokee National Forest's LMP is more restrictive than Tennessee's best management practice (BMP) to minimize non-point source pollution (Office) 2004). Tennessee's BMPs are

implemented as a minimum and if the standards are exceeded, then the LMP will take precedence. North Carolina's BMP was amended in 2006 (Brogan et al. 2006) and similar to Tennessee's BMP.

CHAPTER III

METHODOLOGY

Distribution and Abundance

Protocol

I surveyed *A. a. acadicus* during the breeding season, from March through June 2013 – 14. Six surveys were conducted in 2013 (4/10, 4/24, 5/08, 5/24, 6/19, and 6/27) and five surveys in 2014 (3/27, 4/03, 4/21, 5/01, and 5/08) lasting for 2 hours.

The survey followed the Pennsylvania Project Toot Route Protocol (Larzone and Mulvihill 2006) which is an eight-point survey with points spaced 0.8 km apart, spanning 4 km. Points were spaced to minimize potential overlap of territories. At each point, we broadcasted *A. acadicus* calls for 11 minutes after waiting 3 to 5 minutes. The track starts with a listening period of 2 minutes, followed by a calling period of 2 minutes and 55 seconds, another listening period of 2 minutes, another calling period of 2 minutes and 5 seconds and a final listening period of 2 minutes. For the duration of the track, any vocalizations heard and any birds seen were recorded for each period of the track. I surveyed elevations of 1066.8 m and greater where accessible, with a concentration in areas above 1219.2 m. Surveys started an hour after sunset, between 21:00 and 22:26.

Routes

The survey routes used in 2013 were the Cherohala Skyway/Route 165, Sycamore Road/Whigg Meadow, Beaver Dam Bald, and North River Road. The routes surveyed in 2014 included the Whigg Meadow area, Fire Road 81, and Wolf Laurel Ridge (Table 1). North River Road and Fire Road 81 were discarded after being surveyed once due to excessive noise. John McCormick of East Tennessee State University surveyed the northern range of Cherokee National Forest in the spring and summer of 2013 – 14.

Table 1

Coordinates of routes surveyed and habitat type in Spring/Summer 2013 – 14 (TCS = Tennessee Cherohala Skyway; BBD= Beaver Dam Bald; SYWH= Sycamore road/Whigg Meadow; FR81= Fire Road 81; WLR= Wolf Laurel Ridge; NRR= North River Road). Each route is 4km long. Habitat type and elevation were taken at survey point and verified within ArcGIS® v. 10. 3.1 (Institute 2015). North American Datum of 1983 (NAD 83) was used with this dataset Elevation data is from the National Elevation Database (NED) and land cover data is from National Land Cover Database (NLCD) 2011. Land cover classifications are used by Multi-Resolution Land Characteristics Consortium (MRLC) and modified from the Anderson land cover classification system (Survey 2014a).

Route Name	Ele (m)	Y	X	Habitat Type
TCS01	1095	35.36408	-84.1067	Developed, Open Space
TCS02	1091	35.35707	-84.0925	Deciduous Forest
TCS03	1068	35.35693	-84.0849	Developed, Open Space
TCS04	1135	35.3535	-84.0777	Developed, Open Space
TCS05	1145	35.35021	-84.0678	Deciduous Forest
TCS06	1204	35.34636	-84.0646	Deciduous Forest
TCS07	1248	35.3471	-84.0561	Developed, Open Space
TCS08	1296	35.34714	-84.0487	Deciduous Forest
BBD01	991	35.25256	-84.0842	Deciduous Forest
BBD02	1013	35.24643	-84.0849	Deciduous Forest
BBD03	1110	35.2494	-84.0891	Deciduous Forest
BBD04	1163	35.248	-84.0972	Deciduous Forest
BBD05	1186	35.25013	-84.1036	Developed, Open Space
BBD06	1167	35.24984	-84.1097	Developed, Open Space
BBD07	1176	35.25077	-84.1158	Developed, Open Space
BBD08	1255	35.25071	-84.1211	Deciduous Forest
SYWH01	1158	35.32407	-84.0466	Deciduous Forest
SYWH02	1155	35.31879	-84.0481	Deciduous Forest

SYWH03	1172	35.31506	-84.5323	Deciduous Forest
SYWH04	1245	35.31019	-84.0598	Deciduous Forest
SYWH05	1313	35.30977	-84.0519	Developed, Open Space
SYWH06	1360	35.31128	-84.0498	50% Developed, Open Space, 50% Deciduous Forest
SYWH07	1438	35.30986	-84.0421	Deciduous Forest
SYWH08	1514	35.30988	-84.0367	Shrub/Scrub
FR8101	1301	35.33709	-84.031	Developed, Open Space
FR8102	1318	35.34085	-84.0265	Developed, Open Space
FR8103	1294	35.34532	-84.0232	Deciduous Forest
FR8104	1238	35.34769	-84.0252	Developed, Open Space
FR8105	1219	35.34615	-84.0168	Developed, Open Space
FR8106	1158	35.34475	-84.0083	Deciduous Forest
FR8107	1098	35.34209	-84.0002	Deciduous Forest
FR8108	1012	35.34359	-83.992	Deciduous Forest
WLR01	1390	35.36414	-83.9784	Deciduous Forest
WLR02	1379	35.36144	-83.9817	Deciduous Forest
WLR03	1347	35.35954	-83.9878	Deciduous Forest
WLR04	1333	35.35946	-83.9938	Deciduous Forest
WLR05	1329	35.35977	-83.997	Deciduous Forest
WLR06	1321	35.35444	-83.9994	Deciduous Forest
WLR07	1263	35.3099	-84.0421	Deciduous Forest
WLR08	1191	35.30988	-84.0367	Deciduous Forest
NRR1	863	35.32827	-84.0674	Deciduous Forest
NRR2	880	35.32182	-84.066	Deciduous Forest
NRR3	946	35.31969	-84.0608	Deciduous Forest
NRR4	1017	35.323	-84.0546	Developed, Open Space
NRR5	1087	35.32816	-84.0468	Deciduous Forest
NRR6	1145	35.33043	-84.0414	Deciduous Forest
NRR7	1189	35.32673	-84.0412	Deciduous Forest
NRR8	1257	35.33125	-84.0361	Deciduous Forest

Study Area along Routes

My study area along the routes was defined as 1.61 km on either side of each route (Figure 1). In ArcGIS 10.3.1 (Institute 2015), a buffer of 3.22 km was created around each route (1.61 km on either side) using the analysis tool: buffer. I consolidated the buffered routes into a single polygon using the data management tool: merge. I used the spatial analyst tool: extract by

mask in ArcGIS to extract the land cover data from layer N33W081 (Survey 2014b) obtained from United State Geological Survey (USGS) National Map Viewer Basic (v1.0) that overlapped with elevation data.

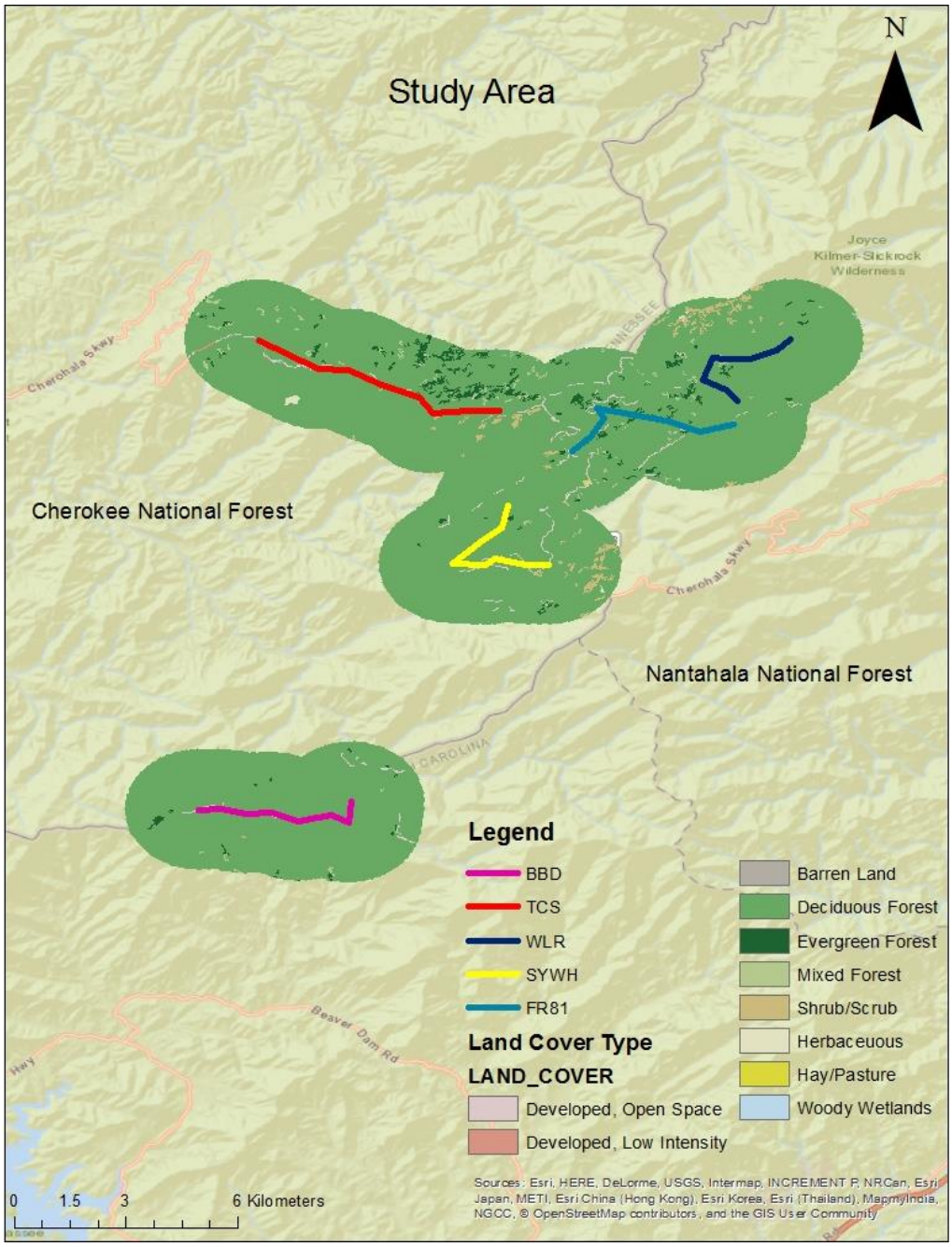

Figure 1

Map of study area in South Cherokee National Forest, Tennessee and Nantahala National Forest, North Carolina. Map was created in ArcGIS v.3.5.1.1. (ESRI 2015). Land cover data is from United States Geological Survey. Basemap was obtained through ArcGIS online. Routes surveyed are color-coded (Wolf Laurel Ridge = dark navy; Sycamore Road/Whigg Meadow = yellow; Cherohala Skyway = red; Fire Road 81 =delft blue; Beaver Bald Dam = pink). Land cover types are color-coded (Shrub/scrub = beige, Mixed Forest = light green, Evergreen Forest = dark green, Deciduous Forest = medium green, Developed, open space = peach).

In order to gain percentages of land cover type, I calculated proportions of pixels in each land cover category in relation to the total number of pixels in the selected area. The study area was 1x 10^3 km^2 and classified as deciduous forest (95.3%), evergreen forest (1.7%), developed land, open space (1.1%), shrub/scrub (1.0%), mixed forest (0.7%), pasture/hay (0.015%), barren (0.011%), and wetlands (0.004%). Classifications were used by the Multi-Resolution Land Characteristics Consortium (MRLC; Table 2) and modified from the Anderson land cover classification system (Survey 2014a). National Land Cover Database 2011 (NLCD 2011) is considered provisional because the formal accuracy assessment has not yet been published.

Table 2

Definition of land cover classification These land classification are used by the MRLC and are modified from the Anderson land cover classification system (Survey 2014a).

Land Cover Classification	Description
Developed, Open Space	Mixture of some constructed materials but mostly vegetation. < 20% impervious surfaces.
Deciduous Forest	Trees are greater than 5 m tall and more than 20% of total vegetation cover. > 75% of the trees undergo leaf abscission.
Evergreen Forest	Trees are greater than 5 m tall and more than 20% of total vegetation cover. >75% of the trees do not undergo leaf abscission.
Mixed Forest	Trees are greater than 5 m tall and more than 20% of total vegetation cover. Neither evergreen or deciduous species are greater than 75% of total tree cover.
Shrub/Scrub	Vegetation is less than 5 m tall and shrub canopy is more

	than 20% of total vegetation.
Barren Land (Rock/Sand/Clay)	Areas of bedrock, scarps, talus, slides, strip mines, gravel pits and other earthen materials. < 15% vegetation cover.
Pasture	Areas of grasses, legumes, or mixtures of the two for seed or hay crop production. This vegetation accounts for more than 20% of total vegetation cover.
Wetlands	Soil or substrate is periodically covered or saturated with water

DNA Testing

Sample Collection

Obtaining DNA from feathers is a non-invasive technique that is less stressful for the bird (Bello et al. 2001) that also shortens the sample collection time compared with other techniques, such as blood sampling. Feathers are a good source of mitochondrial DNA (Maurer et al. 2010). There is a possibility that molted feathers or samples from museum specimen may be degraded (Maurer et al. 2010), thus there is a need for fresh samples or skin samples from the museum specimen (Mundy et al. 1997). If fresh feathers are stored at room temperature, they may be viable for up to two weeks (Bello et al. 2001). If stored at 4° C, they can be viable for a month (Bello et al. 2001). There are limitations to this method; however, one being there are a small number of loci that can be sequenced from the feathers (Maurer et al. 2010). All locations of samples obtained found in Figure 2. I obtained 26 breast feather samples from Dr. Karl Kleiner's lab at York College of Pennsylvania (hereafter York), 9 of which amplified enough to use. From the Southern Appalachian Raptor Research (hereafter Southern Appalachian) in Eastern Tennessee-Western North Carolina I obtained 26 samples, 19 of which amplified enough to use.

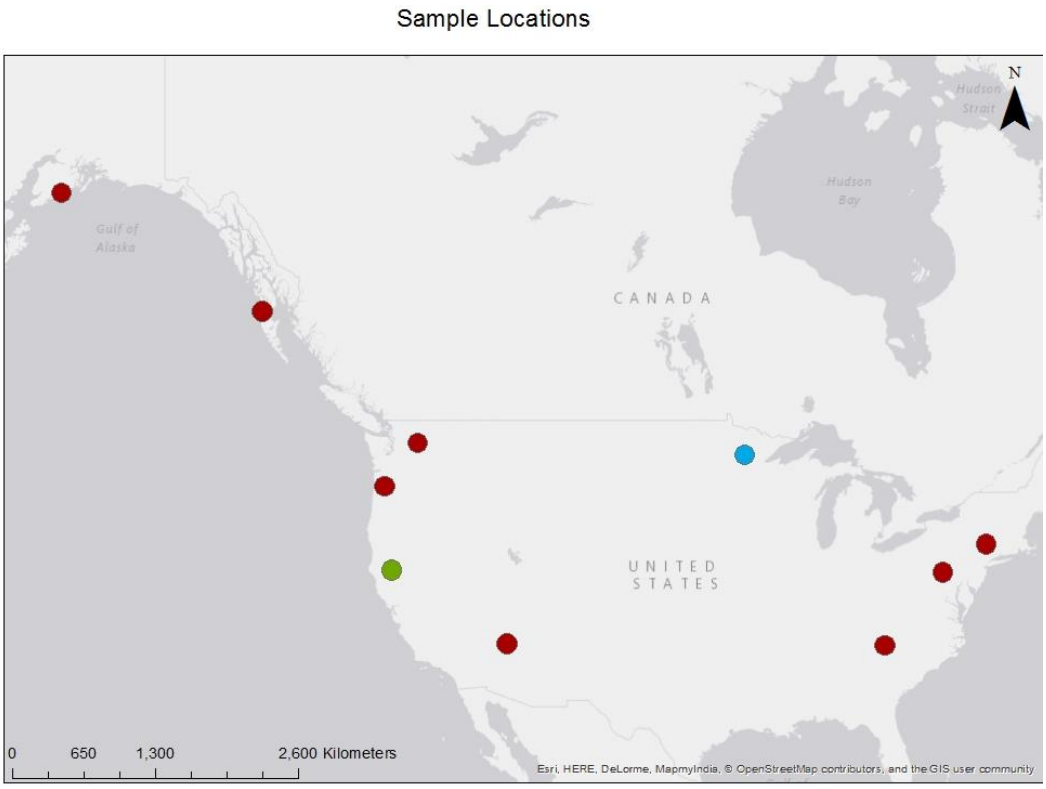

Figure 2

Locations of samples Circles represent locations of samples used in this study. Locations in eastern North America were collected in this study. Locations along the west coast and in the Sierra Nevada are from Withrow et al. (2014) and obtained through GenBank ® (Benson et al. 2013). Locations that contained samples with cytochrome b and NADH dehydrogenase subunit 2 are colored red. Locations that contained samples that only contained cytochrome b are blue. Locations that contained only NADH dehydrogenase subunit 2 are green.

I obtained blood samples from Dr. Glenn Proudfoot in Poughkeepsie, New York (hereafter Poughkeepsie; n = 14). A single museum specimen, originating from Minnesota, from Dr. David Aborn's lab at University of Tennessee – Chattanooga (hereafter SP) was used to obtain muscle, skin, and feather samples. The DNA collected was extracted using Qiagen QIAamp® Micro Kit, Qiagen DNeasy® Blood and Tissue kit, and an extended protocol (Volo et al. 2008). Additional samples from GenBank® (Benson et al. 2013) were used to supplement the data (Table 3). For the NADH dehydrogenase subunit 2, 79 samples were used (Poughkeepsie =

11; York = 7; Southern Appalachian = 17; GenBank® = 44). Cytochrome *b* used fewer samples (Poughkeepsie = 8; York = 7; Southern Appalachian = 10; Minnesota = 1; GenBank® = 41).

Table 3

Samples obtained from GenBank® (Benson et al. 2013) Samples were uploaded to GenBank® (Benson et al. 2013) by Withrow et al. (2014) and Topp and Winker (2008)

Species Name	**GenBank Accession Numbers**	
	Cytochrome b	**NADH dehydrogenase subunit 2**
A. a. acadicus	EU075383 - EU075387	KC620150- KC620168
	EU075398 - EU075412	EU601051
	KC620183	
A. a. brooksi	EU075388 - EU07597	KC620126- KC620149
	KC620169 - KC620182	

Extraction methodology

Modified extraction method found in Volo et al. (2008) was used to obtain DNA from the York College feather samples. I removed the calamus tip of each feather and soaked it in 70% alcohol for 30 minutes, rinsed in double distilled water, and soaked in double distilled water for 30 minutes. Each calamus tip was split in half. These halves were placed in separate 2 mL microcentrifuge tubes for different extraction protocols. One extraction method used was the protocol outlined in Volo et al. (2008). Other extraction method used was the modified Qiagen protocol (Bush 2005).

Volo et al. (2008) Protocol

For the Volo et al. (2008) protocol, each tube contained 500 µL of the digestion mix. The digestion mix was as follows: 600 µl Tris-NaCl-EDTA pH 7.5, 60 µL 1 M Tris-HCl (pH 8), 45

µl Proteinase K (25 mg/ml), 10 µl 25% sodium dodecyl sulfate, and 30 µL 1 M dithiothreitol (DTT). Samples were left to digest for six days, in a heating block (55 °C) and vortexed for 20 sec three times during this period. After the digestion, I added 233 µL of 7.5 M ammonium acetate to each extract, vortexed the samples for 20 sec each and centrifuged them at 15,000 rpm for 30 minutes. I transferred the supernatant to new centrifuge tubes that contained 600 µL of 100% isopropanol. Tubes were inverted to mix the contents and frozen overnight (-20 °C). I centrifuged the extracts at 15,000 rpm for 30 minutes. I poured off the isopropanol and added 600 µl of 70% ethanol to each tube. To complete the extractions, samples were centrifuged for 2 minutes at 15,000 rpm and air dried after the ethanol was poured off. The supernatant was resuspended in 50 µL TE (pH 8) and stored in a -20°C freezer.

Modified Qiagen Protocols

Feathers samples were extracted with the modified Qiagen protocol for plucked body and contour feathers (Bush 2005). I used the Qiagen QIAamp® DNA Micro Kit and Qiagen DNeasy® Blood and Tissue Kit. These kits were also used to extract DNA from blood, muscle, and skin from other specimen following standard protocol (Qiagen 2006; 2014).

With the Qiagen QIAamp ® DNA Micro Kit, feathers were cut into small pieces and placed into a 1.7 µL microcentrifuge tubes where 200 µL of Buffer ATL and 20 µL of Proteinase K (20 mg/ml) were added to each sample. Samples were incubated at 50 °C for two days. After the incubation period 200 µL of Buffer AL was added; the samples vortexed for 20 sec and incubated at 70 °C for 10 minutes. 200 µL of cold ethanol was added to each sample; after they were vortexed for 20 sec and incubated at room temperature for 5 minutes.

Each solution was transferred to a spin column and collection tube. These were centrifuged at 10,000 rpm for 1 minute and the digestion mix was discarded. I added 500 µL of Buffer AW1 and centrifuged the tube at 9,000 rpm for one minute. After the initial alcohol wash, I added 500 µL of Buffer AW2 and centrifuged the tube at 9,000 rpm for one minute. To dry the samples, I centrifuged the sample at 11,000 rpm for 3 minutes. After each centrifuge the collection tube was discarded and a new one was used. After the alcohol wash, a microcentrifuge tube was used instead of a collection tube. 100 µL of Buffer AE was added to each sample and they were incubated at room temperature for 5 minutes. The last centrifuge step was at 8,500 rpm for one minute after which the spin column was discarded and the extracted DNA was stored at –20 °C for future use.

Qiagen DNeasy® Blood and Tissue protocol follows the Qiagen QIAamp® Micro protocol with the exception of the alcohol wash and volume of Buffer AE. The centrifuge velocities were as follows 9,500 rpm for one minute (AW1); 10,500 rpm for three minutes (AW2). The volume of Buffer AE added was 200 µL.

Standard Qiagen Protocols for Blood Samples

Blood samples collected were subsampled. DNA was extracted using both Qiagen kits. I vortexed and centrifuged blood samples before the extractions began. After each centrifuge step, the collection tube was discarded with the exception of the final step. After the final step the spin column was discarded (Qiagen 2006; 2014).

With the Qiagen DNeasy® Blood and Tissue protocol, I added 50 µL of blood to 20 µL of Proteinase K, 200 µL of Buffer Al. Each sample was vortexed and incubated at 56 °C for 10

minutes. After incubation, I added 200 µL of ethanol and vortexed the samples for 20 sec. The solutions were placed in spin columns and collection tubes. Samples were centrifuged for one minute at 8,000 rpm. For the initial alcohol wash, 500 µL of Buffer AW1 was added and the solutions were centrifuged at 8,000 rpm for one minute. The second alcohol wash consisted of 500 µL of Buffer AW2 and a centrifuge velocity of 14,000 rpm for 3 minutes. Each spin column was transferred to a microcentrifuge tube, wiped down to remove any remaining buffer, and 200 µL of Buffer AE was added. The solutions were incubated at room temperature for one minute before they were centrifuged at 8,000 rpm for one minute.

For the Qiagen QIAamp® Micro Kit, I added 100 µL of blood, 10 µL of Proteinase K, and 100 µL of Buffer AL. Solutions were vortexed and incubated for 10 minutes at 56 °C. I added 50 µL of ethanol, vortexed the samples and transferred them to a spin column. They were centrifuged in the same steps used the Blood and Tissue protocol. However, the volume of Buffer AE used was 100 µL and the final centrifuge speed was 14,000 rpm.

Polymerase Chain Reaction (PCR)

Once the extractions were complete, separate PCR were run using the primers L0-25/H117 (cytochrome b), L15560/H15646 (cytochrome b), and L5215/Htrp (NADH dehydrogenase 2). A list of the primers and their sequences can be in Table 4 (Burg and Lauff 2012; Marthinsen et al. 2009; Topp and Winker 2008; Withrow et al. 2014). Volume of DNA used in each reaction ranged from 2 µL to 16.2 µL. The PCR were run on Eppendorf Mastercycler ® Nexus Gradient. The products were observed with an electrophoresis gel to determine if the PCR was successful.

Table 4

Primers and their sequences L0-25 and H117 were from Topp and Winker (2008). L15560 and H15646 were from Marthinsen et al. (2009). L5215 and Htrp were from Burg and Lauff (2012). Internal primers for NADH dehydrogenase subunit 2 were from Withrow et al. (2014). Mixed bases in the sequence are either labeled as one "Y", which denotes either a C or T base, or two "R", which denotes either an A or G base.

Gene	Primer	Sequence
Cytochrome b	L15560	5'-GYGAYAARATCCCATTCCACCC-3'
	H15646	5'-GGGGTGAAGTTTTCTGGGTCTCC-3'
	H1117	5'-GGGTGCTTGCTATTGGGAGTAGGACGAGG-3'
	L0-25	5'-ATGGCCCCAAACATCCGAAAGTCTC-3'
NADH dehydrogenase subunit 2	L5215	5'-TATCGGGCCCATACCCCGAATAT-3'
	HTrp	5'-CGGACTTTAGCAGAAACTAAGAG -3'
NADH dehydrogenase subunit 2 Internal	ND2 F	5'-TCTTGCCTCCTCCTAACAACAGCA-3'
	ND2 R	5'-TGTTGATAGGATGGCCATGGAGGT-3'

PCR protocol for L0-25/H117 was 94 °C (2 minutes); 29 cycles of 94 °C (1 min), 48 °C (2 min), 72 °C (2 min); and at 72 (5 min). For primers L15560/H15646, protocol was one cycle of 94°C (2 min), 54°C (45 sec), 72°C (1 min); 37 cycles of 94°C (30 sec), 54 °C (45 sec), 72 °C (1 min); and at 72 °C (5 min). For L52145/Htrp, protocol was 94 °C (2 min); 37 cycles of 94°C (30 sec), 45 °C (45 sec), 72 °C (1 min); and at 72 °C (5 min).

Sequencing

DNA was sequenced at the University of Kentucky HealthCare Genomics Center for medical professionals, Lexington, Kentucky. My target coding genes are NADH dehydrogenase subunit 2 and cytochrome b. Cytochrome b is well studied and has a fairly constant rate of evolution, making it ideal to study (Topp and Winker 2008). NADH dehydrogenase 2 is also highly conserved mitochondrial DNA that is used extensively in phylogenetics (Donne-Gousse et al. 2002). Internal primers for NADH dehydrogenase subunit 2 were also used for sequencing purposes (Withrow et al. 2014).

Sequences were edited in Geneious® v.6.1.7(Kearse et al. 2012). Consensus sequences were generated with Geneious® v.6.1.7 and alignments were created and edited. Sequences were found in Genbank® (Table 6). Samples from the same individual were concatenated and analyzed. Concatenated sequences consisted of the cytochrome *b* gene first followed by NADH dehydrogenase subunit 2.

ExoSAP-IT® Protocol

I cleaned the PCR products were cleaned using ExoSAP-IT ® (Corporation c2000) clean up kit. PCR protocol for Exo-SAP-IT is 37°C (15 minutes), 80°C (15 minutes). The reagents used in this protocol are Exonuclease I and Shrimp Alkaline Phosphatase (SAP) (Corporation c2000). I added 5 μl of DNA product and 2 μl of ExoSAP-IT ®.

BigDye® Protocol

I sequenced the products with BigDye® Terminator v3.1. Big Dye Generic (Biosystems c2010) protocol: 96°C (1 minute); 25 cycles of 96°C (10 seconds), 50°C (5 seconds), 60°C (4 minutes). One μl of each DNA sample and 5 μl of the sequencing cocktail were loaded into a 96

well plate. The sequencing cocktail consisted of the BigDye (glycerol and dideoxynucleotide triphosphate big dye terminators), Big dye buffer (Tris-HCL pH 9.0 and $MgCl_2$), deionized nuclease-free water, and either a forward or reverse primer.

Data Analysis

Network Analysis and AMOVA

Haplotype networks are used to show genetic diversity within a group (Prost and Anderson 2014). Networks were inferred in NETWORK (Bandelt et al. 1999) using the median-joining technique for each gene and concatenated sequences.

I obtained Φ_{st} values from AMOVA (Analysis of Molecular Variance) were used to support the networks. AMOVAs were run in Arlequin v3.5.2.2 (Excoffier et al. 2005). Transitions and transversions were given the weight of 1 while deletions were not given any weight. *A. a. acadicus* (Withrow et al. 2014), Poughkeepsie, York, and Southern Appalachian were placed in a single group. *A. a. brooksi* (Withrow et al. 2014) from GenBank® samples were omitted because they were a known subspecies.

Haplotype and Nucleotide Diversity

Haplotype and nucleotide diversity were calculated using DnaSP v5 (Librado and Rozas 2009) for each individual population and gene. Haplotype and nucleotide diversity were also calculated for concatenated sequences. DnaSP was also used to calculate average pair wise population sequence divergence. All populations were compared to Southern Appalachian. Cytochrome b haplotype of samples obtained in this study can be found in Table 5 and NADH dehydrogenase subunit 2 can be found in Table 6.

Table 5

Cytochrome b haplotype of samples collected These are haplotype of all samples collected in this study (M = Poughkeepsie; NP = York; SAP = Southern Appalachian; SP = Minnesota sample). Samples from GenBank® are not included.

Samples	Haplotype				
	48 - 57	443 – 452	557 - 566	650 - 659	680 - 689
M3	CTACTTGCCA	ACACTTCCTA	GTTCCATCCA	GGGCGACCCA	TCCACTAGTC
M4	C*********	A*********	G*********	G*********	T*********
M5	C*********	G*********	G*********	G*********	T*********
M6	C*********	A*********	G*********	G*********	T*********
M8	CA********	A*********	G*********	G*********	T*********
M9	CT********	A*********	G*********	G*********	T*********
M10	C*********	A*********	G*********	G*********	T*********
M13	C*********	A*********	G*********	G*********	T*********
NP1	C*********	A*********	G*********	A*********	T*********
NP2	C*********	A*********	G*********	G*********	T*********
NP9	C*********	A*********	A*********	G*********	T*********
NP12	C*********	A*********	G*********	G*********	C*********
NP14	C*********	A*********	G*********	G*********	T*********
NP16	C*********	A*********	G*********	G*********	T*********
NP17	C*********	A*********	G*********	G*********	T*********
SAP6	C*********	A*********	G*********	G*********	T*********
SAP7	C*********	A*********	G*********	G*********	T*********
SAP8	C*********	A*********	G*********	G*********	T*********
SAP9	C*********	A*********	G*********	G*********	T*********
SAP10	C*********	A*********	G*********	G*********	T*********
SAP11	C*********	A*********	G*********	G*********	T*********
SAP12	C*********	A*********	G*********	G*********	T*********
SAP23	C*********	A*********	G*********	G*********	T*********
SAP24	C*********	A*********	G*********	G*********	T*********
SP1	C*********	A*********	G*********	G*********	T*********
SAP26	---------A	A*********	G*********	G*********	T*********

Table 6

NADH dehydrogenase subunit 2 haplotypes of samples collected These are haplotypes of all samples collected in this study (M = Poughkeepsie; NP = York; SAP = Southern Appalachian). Samples from GenBank® are not included.

Sample	Haplotype			
	192 - 201	781 - 790	861 – 870	930 – 939
M2	CACTGCATCG	CTCCCCAAGT	CCTCAGCCTA	CCTCAGCCTA
M3	C********A	C*********	C*********	C*********
M4	C*********	C*********	C*C*******	C*C*******
M5	C*********	C*********	C*T*******	C*T*******
M6	C*********	C*********	C*********	C*********
M8	T*********	C*********	C*********	C*********
M9	C*********	C*********	C*********	C*********
M10	C*********	C*********	C*********	C*********
M11	C*********	C*********	C*********	C*********
M13	C*********	C*********	C*********	C*********
M14	C*********	C*********	C*********	C*********
NP1	C********G	C*********	C*********	C*********
NP5	C********A	C*********	C*********	C*********
NP9	C********G	C**T******	C*********	C*********
NP12	C********A	C**C******	C*********	C*********
NP14	C*********	C*********	C*********	C*********
NP16	C********G	C*********	C*********	C*********
NP18	C*********	C*********	C*********	C*********
SAP2	C********A	C*********	C*********	C*********
SAP4	C*********	C*********	C*********	C*********
SAP7	C*********	C*********	C*********	C*********
SAP8	C*********	C*********	C*********	C*********
SAP9	C*********	C*********	C*********	C*********
SAP10	C*********	C*********	C*********	C*********
SAP11	C*********	C*********	C*********	C*********
SAP12	C*********	C*********	C*********	C*********
SAP13	C*********	C*********	C*********	C*********
SAP15	C*********	C*********	C*********	C*********
SAP17	C*********	C*******A*	C*********	C*********
SAP19	C********G	C*******G*	C*********	C*********
SAP20	C********A	C*********	C*********	C*********
SAP22	C********G	C*********	C*********	C*********
SAP23	C********A	C*********	C*********	C*********
SAP24	C*********	C*********	C*********	C*********

SAP27	C********	C********	C********	C********

CHAPTER IV

RESULTS

Distribution and Abundance

In the spring of 2013 a total of five *A. a. acadicus* were heard. They were heard along Sycamore road/Whigg Meadow route. In the spring and summer of 2014 a total of four owls were heard, once again along the Sycamore Road/Whigg Meadow route and along a new route, Wolf Laurel Ridge. While surveying the Wolf Laurel Ridge, an individual NSWO flew into a tree but did not vocalize.

Network Analysis and AMOVA

Network analyses showed one major haplotype shared among the populations of *A. a. acadicus* (Figures 3 – 5) with few variations within individual populations. AMOVA analysis supported these networks.

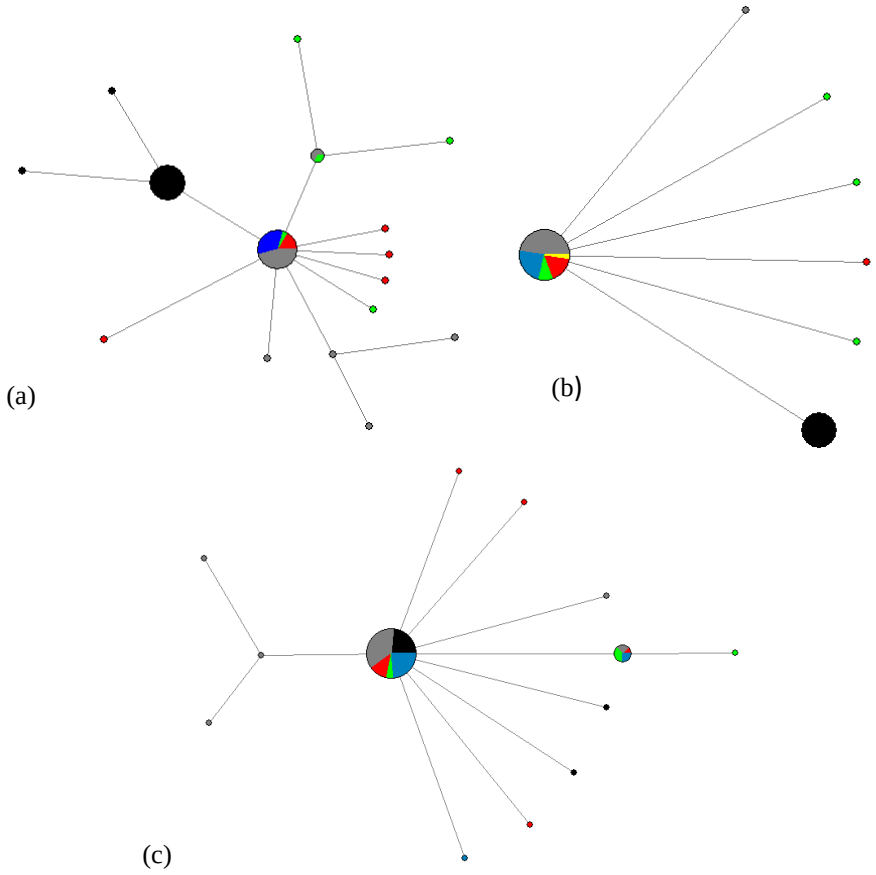

Figure 3

Network analysis In clockwise order starting from the top left: (a) Concatenated sequences; (b) cytochrome b; (c) NADH dehydrogenase subunit 2. Poughkeepsie is red, York College is green, Southern Appalachia is blue, *A. a. acadicus* (Withrow et al. 2014) is grey and *A. a. brooksi* (Withrow et al. 2014) is black. The circles are proportional to the frequency of haplotypes.

For the concatenated samples, variation among the populations (Φ_{st} = 0.10463, p = 0.00391 ± 0.00233) accounted for 10.46% of total variation (Table 7). Most variation occurred within populations (89.54%), though it was not statistically significant. This trend was seen when the genes were analyzed individually as well.

Table 7

AMOVA results for concatenated sequences AMOVA calculations were done in Arlequin v3.5.22(Excoffier et al. 2005). Statistically significant variation is denoted with an asterisk (*). Major sources of variations appear only to be biologically significant. York, Poughkeepsie, Southern Appalachian, and *A. a. acadicus* (Withrow et al. 2014) from GenBank® were grouped together. *A. a. brooksi* samples (Withrow et al. 2014) from GenBank® were omitted.

Source of Variation	d.f.	Variance Components	Variation (%)	P-Value	Fixation Index (Φ_{st})
Among populations	3	0.05535	10.46	0.00391 ± 0.00233	0.10463
Within populations	34	0.47367	89.54		
Total	37	0.52901			

Significant variation among the NADH dehydrogenase subunit 2 samples existed among the populations (Φ_{st} = 0.08514, p = 0.00978 ± 0.00326). This accounted for 8.51% of the total variation; the rest was within populations (Table 8). There was no significant variation among the cytochrome b samples (Φ_{st} = 0.02459, p = 0.17698 ± 0.01258); among population variation accounted for 2.46% of total variation.

Table 8

AMOVA results for NADH dehydrogenase subunit 2 sequences AMOVA was run in Arlequin v.3.5.2.2 (Excoffier et al. 2005). Statistically significant variation is denoted with an asterisk (*). Major sources of variations appear only to be biologically significant. York, Poughkeepsie, Southern Appalachian, and *A. a. acadicus* (Withrow et al. 2014) from GenBank® were grouped together. *A. a. brooksi* (Withrow et al. 2014) samples from GenBank® were omitted.

Source of Variation	d.f.	Variance Components	Variation (%)	P-Value	Fixation Index (Φ_{st})
Among populations	3	0.02943	8.51	0.00978 ± 0.00326	0.08514
Within populations	51	0.31625	91.49		
Total	54	0.34568			

Haplotype and Nucleotide Diversity

In general, there appeared to be no more than one single nucleotide polymorphism (SNP) per sample. For both genes, all *A. a. acadicus* populations shared a single haplotype. There were additional haplotype within individual populations (Table 9). Within the cytochrome *b* gene there was one additional haplotype among the samples from Poughkeepsie (n = 8, H_d = 0.250), three additional haplotype among the samples from York (n=7, H_d = 0.714), and no additional haplotype among the samples from the Southern Appalachian population (n = 1, H_d = 0.00). The *A. a. acadicus* (Withrow et al. 2014) samples had a single additional haplotype. It appeared that the Southern Appalachian population was more closely related to *A. a. acadicus*.

Table 9

Haplotype and average sequence divergence among populations Number of haplotype in each populations and average pair wise sequence divergence for each population. Average pair wise sequence divergence was calculated in DnaSP v. 5 (Librado and Rozas 2009) for each gene and concatenated sequence. For average pair wise sequence divergence, all populations are compared to Southern Appalachian. NADH dehydrogenase subunit 2 abbreviated as "ND2". Cytochrome b abbreviated as "cyt. b". Concatenated sequences abbreviated as "con".

Population	**Haplotype**			**DNA Divergence**		
	Cyt. b	ND2	Con	Cyt. b	ND2	Con
Southern Appalachian	1	3	1	-	-	-
York	4	3	5	0.00053	0.00072	0.00071
Poughkeepsie	2	5	5	0.00015	0.00050	0.00032
***A. a. acadicus* (Withrow et al. 2014)**	2	6	6	0.00006	0.00053	0.00024
***A. a. brooksi* (Withrow et al. 2014)**	1	3	2	0.00124	0.00025	0.00056

A total of 60 sequences were concatenated. Samples from York had the highest haplotype diversity (n=5; H_d=1.000), while Southern Appalachian samples had the lowest haplotype diversity (n=8; H_d=0.0000). The nucleotide diversity within the NADH dehydrogenase subunit 2

gene was lowest among the Southern Appalachian samples, while York College had the highest (π_{SAP} =0.0000; π_{NAP} = 0.00112). Poughkeepsie samples fell in the middle (π_{POU} =0.00064).

For NADH dehydrogenase subunit 2, *A. a. acadicus* (Withrow et al. 2014) had five additional haplotype. There were two additional haplotype among the samples from Southern Appalachian (n=17, H_d= 0.324), three additional haplotype from York (n=7, H_d = 0.714), and five from Poughkeepsie (n=11, H_d = 0.618).

In general, cytochrome b appeared to be less variable than NADH dehydrogenase subunit 2 and more structured in its variable sites. There was a fixed polymorphism site at base 823 in the *A. a. brooksi*, however in other sample populations it was more variable. Nucleotide diversity within the cytochrome b gene was lowest among the Southern Appalachian samples while York had the highest and Poughkeepsie is lower than York (π_{SAP} =0.0000; π_{NAP} = 0.00106; π_{POU} =0.00031 respectively).

NADH dehydrogenase subunit 2 was less structured in its variable sites. There appeared to be a fixed polymorphism site at base 201, found in 8 individuals from *A. a. acadicus* (Withrow et al. 2014), York, Poughkeepsie, and Southern Appalachian. Nucleotide diversity within the NADH dehydrogenase subunit 2 gene was lowest among the Southern Appalachian samples while York College had the highest and Poughkeepsie is lower than York College (π_{SAP} =0.00032; π_{NAP} = 0.00082; π_{POU} =0.00069 respectively).

CHAPTER V

DISCUSSION

Distribution and Abundance

I detected *A. a. acadicus* primarily in deciduous forest above 1066 m. They were mostly in areas with an observed dense forest cover (<60 m^2 of forest surrounding point) with a few detected near scrub at Whigg Meadow.

With the survey, there is possibly a gender bias as a male's advertising call is being used. As part of the survey, any vocalization and sighting were recorded; however, females are less likely to vocalize and owl wings in general are designed to be silent. There could have been individuals that flew in to investigate the calls that were being played as part of this survey but did not vocalize and were not seen, so remained undetected - due to the possibility of *A. a. acadicus* not vocalizing my results could be underestimating the number of individuals in the area.

The forest type where I detected owls does not match the hypothesis of Tamashiro (1996), who found owls predominately in forests that were spruce-fir dominated. However, my results do match some of the findings of Rasmussen et al. (2008), which suggests that *A. a. acadicus* rarely breeds this far south, occurring more along higher elevations, and is more likely to breed as far south as Maryland and Pennsylvania.

However, they are known to over winter in the South (Rasmussen et al. 2008) because of the milder climate, increased forest cover and prey availability. An earlier study on autumn migrants along the Atlantic coast by Brinker et al. (1997) supports this finding. Coastal banding stations in the Mid- Atlantic states of Virginia, Maryland, and New Jersey caught 2,596 *A. a. acadicus*, many of them females, with some direct inter-station recoveries, birds caught at one station and then at another within the same season. The *A. a. acadicus* were recovered in the region and in the Southern Appalachians were from outside the Mid-Atlantic coastal region; however, none of the southbound migrants were recaptured near the Great Lakes in the spring of 1996.

Composition of the Southern Appalachian breeding grounds differs in regards to known breeding grounds of *A. a. acadicus* in the northern reaches of North America because of the decreased range of firs (*Abies* sp.), spruces (*Picea* sp.) and birches (*Betula* sp.). Pockets of the northern coniferous forest are found along the higher elevation of the Southern Appalachians. Along those patches in the Cherokee and Nantahala National Forests are larger patches of northern hardwood forest. Species such as the magnolias (*Magnolia* sp.), laurels (*Kalmia* sp.), and other broadleaf evergreen plants are more abundant (Brinker et al. 1997).

Milling et al. (1997) found the majority of *A. a. acadicus* breeding territories in the Southern Appalachians were located in boreal forest and boreal forest/northern hardwood eco-tones. Around 12% of the territories observed in the study were found in pure hardwood stands and there were separate reports of *A. a. acadicus* in hemlock (*Tsuga canadensis* L. Carrière) at lower elevations and in hardwood forests. My study area coincides qualitatively with this finding.

Brinker et al. (1997) also suggests that the breeding range does include deciduous forests and dense thickets where potential for predation is less in addition to being closely tied to the northern coniferous forest (Buidin et al. 2006). Fall migration from the breeding grounds tends to begin during or after the last leaf fall (Brinker et al. 1997), suggesting that *A. a. acadicus* could be present in deciduous forests during breeding season.

Genetic Analyses

My data suggests that the Southern Appalachian population is a recent colonization. Most of the population has a haplotype that seen in other populations around North America. There was little variation among cytochrome b sequences. Any SNP present was not shared among all individuals within a population except among *A. a. brooksi*. Among the NADH dehydrogenase subunit 2 samples, there was more variation as expected. As seen with cytochrome b, any SNP was not present among all individuals within a population.

Samples from York were significantly different from all sample populations in both genes. Natal origin is unknown among the samples from York since they were caught during fall migration. Since the natal origin is unknown for these specimens, I was unable to assign a specific geographic population to the samples. It is likely that they are from the main population of *A. a. acadicus*. York samples could represent a potential subpopulation that is using this migratory route. All samples collected in this study contained unique haplotype. York samples could represent a potential subpopulation that is using this migratory route.

My data support Withrow et al. (2014) as well as Topp and Winker (2008), who found that there were three haplotype, two of which were in *A. a. acadicus* (n = 45) and that there is significant difference between *A. a. brooksi* and *A. a. acadicus* (Φ_{st} = 0.933, p < 0.0001), but not among the populations of *A. a. acadicus*. According to Topp and Winker (2008), there was low genetic diversity among the *A. a. acadicus*, with three haplotype separated by a single base change.

Management Implications

With an unknown population size within the Southern Appalachians, genetic drift is a concern and the loss of the variability of haplotype in the Southern Appalachian would be a loss for this species. Low detection rates in the field suggest a small population of *A. a. acadicus* within Cherokee and Nantahala National Forest. Milling et al. (1997) also suggests that the southern Appalachian population is relatively small (~ 500 breeding pairs). These low numbers can make the population vulnerable to major environmental stochasticity and habitat degradation. Ecosystems in higher elevations of the Southern Appalachians are vulnerable to climate change (Commission 2010a). Changing temperatures allow for expansion of native and exotic invasive species such as the hemlock woolly adelgid (*A. tsugae* Annand) and beech scale (*Cryptococcus fagisuga* Lindinger). Human foot traffic on trails can also lead to the spread of invasive species because spores and seeds of invasive species get stuck on hikers' shoes.

Reduced snowfall due to rising temperatures affect the moisture content of soil and acid precipitation can lead to increased heavy metal toxicity in soil (Commission 2015). These changes in soil chemistry affect nutrient availability and negatively impact plant growth. Northern hardwood forests may be easier to manage and restore than boreal forest due to

distribution. Pure spruce-fir forests have disjunct distribution but may also be subdominant in northern hardwood forests (Commission 2010b) which have a more extensive and continuous range.

Current LMPs for Cherokee and Nantahala aim for a mid- to late- successional forest with blocks of early successional forest. Nantahala's 1994 LMP does mention managing areas for *A. a. acadicus* in addition to other species that require mature forests. Providing restoration of plant communities to their previous range and protecting rare plant communities are also priorities for Cherokee National Forest. Through these efforts, conservation for the Southern Appalachian population seems relatively straightforward. Hinam and Clair (2008) suggest that *A. a. acadicus* benefit from low levels of fragmentation and habitat in the form of increased prey abundance. Two-aged and shelterwood harvest methods could be advantageous for *A. a. acadicus* in the area. Conserving land for *A. a. acadicus* could also benefit game species such as wild turkey (*Meleagris gallopavo* Linnaeus) in addition to non-game species such as pileated woodpecker (*D. pileatus*), Carolina northern flying squirrel (*Glaucomys sabrinus coloratus* Handley; a federally listed endangered species), and Weller's Salamander (*Plethodon welleri* Walker).

My data do not support my hypothesis that the Southern Appalachian *A. a. acadicus* are a subspecies. While the majority of samples shared a haplotype with other populations around North America, a few unique haplotype were found in the Southern Appalachian population. These data suggest that they are migrating at some point because there is high gene flow with both genes. It has been suggested by Tamashiro (1996), that there has not been enough time for divergence to occur since the possible rapid colonization of the area after the last glacial retreat.

Based on low DNA divergence and the shared haplotype, my results support this suggestion. It appears that the Southern Appalachian population is a recent colonization. More testing would be needed to estimate when they colonized the area. Other future research topics include fecundity, influence of human interference, climate change, predation risks and general ecology could be researched.

Hardwood forests appear to be capable of supporting *A. a. acadicus*, thus conservation of this habitat in addition to spruce-fir forest is a priority. In doing so, haplotype present within this population can persist and could increase the evolutionary potential by helping the species persist and increasing adaptability to environmental changes.

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VITA

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