HABITAT ANALYSIS OF A DISJUNCT POPULATION OF THE CAROLINA NORTHERN FLYING SQUIRREL (*GLAUCOMYS SABRINUS COLORATUS*)

A thesis presented to the faculty of the Graduate School of
Western Carolina University in partial fulfillment of the
requirements for the degree of Master of Science in Biology.

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Spring 2012

ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Beverly Collins, for her tremendous help. She knew when to push me when I needed it and when to let me take the reins. My other committee members, Dr. Sean O'Connell and Dr. Ron Davis, have been wonderful teachers and been incredibly helpful with my work. I'd also like to thank the Graduate School and the Biology Department at Western Carolina University for funding and support. I enjoyed my time here immensely and could not imagine a more perfect place to have done my graduate research. The North Carolina Resources Commission, including Christine Kelly and Dottie Brown, was instrumental in my work and I thank them for funding, support, and help with field work. Along those lines, I could not have completed my field work in the time I did without help from my Biology peer Jeremy Craft, my brother Jon Allman, and my now husband Chris Parrish.

Last but not least, I thank from the bottom of my heart, my family and friends who have supported me all along and made all of this possible. I could not have done this without their countless words of advice, their love, and their encouragement.

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ABSTRACT

HABITAT ANALYSIS OF A DISJUNCT POPULATION OF THE CAROLINA NORTHERN FLYING SQUIRREL (*GLAUCOMYS SABRINUS COLORATUS*)

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The northern flying squirrel *Glaucomys sabrinus* occurs in a variety of forest types over most of North America, with disjunct populations in the southern Appalachians, Black Hills, southern Rocky Mountains, and Sierra Nevada (Wells-Gosling and Heaney, 1984). A subspecies of *sabrinus*, the Carolina northern flying squirrel, *sabrinus coloratus*, is a small nocturnal flying squirrel found in the southern Appalachians. One population of Carolina northern flying squirrel occurs within hemlock-northern hardwood forests along the Cherohala Skyway in western North Carolina. This subspecies was listed as federally endangered on July 1, 1985 (U.S. Fish and Wildlife Service, 1990) largely due to declining populations from habitat loss (Loeb *et al*., 2000). I sought to determine if the larger habitat surrounding the areas of documented squirrel activity is suitable for squirrel persistence, how habitat size and quality compares between sites with squirrel activity and other sites, and what types of foods the squirrels were consuming.

Vegetation surveys of sites with documented *G. s. coloratus* activity, either den sites or capture sites, and paired random sites approximately 70 meters away were conducted. In addition to general habitat knowledge, these surveys provided information for GIS analysis of the larger habitat around the three focal areas. Using ArcGIS v. 9.3.1

(ESRI, Redlands, CA, USA), a model delineating potential Carolina northern flying squirrel habitat based on six parameters: slope, elevation, aspect, spectral signature of den sites, soil types, and tree height data was created. Denaturing gradient gel electrophoresis and sequencing was performed on *G. s. coloratus* scat samples to determine fungal or bacterial diet composition.

Vegetation surveys revealed *G. s. coloratus* were utilizing habitat from hemlock to northern hardwood forest and habitat patches that were similar to nearby areas along the Cherohala Skyway. The GIS model revealed an area of potential *G. s. coloratus* habitat to the north (Stratton Bald). Though the distance from Stratton Bald to my three study sites exceeds *G. s. coloratus* travel distance, the model also revealed an area much closer than Stratton Bald of smaller suitable patches grouped relatively close together. BLAST results of sequenced DGGE bands of squirrel scat revealed similarity to common fungi, including both ascomycetes and basidiomycetes.

INTRODUCTION

The Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*), a subspecies of northern flying squirrel (*G. sabrinus)*, occurs in the Unicoi Mountains of the southern Appalachians of North Carolina and Tennessee. Typical habitat, spruce-fir and northern hardwood forests, occurs at elevations above 1350 meters in naturally fragmented landscapes commonly referred to as "montane islands" (Loeb *et al*., 2000). Along the Cherohala Skyway near the North Carolina-Tennessee border, a unique population of *G. s. coloratus* resides in atypical hemlock-northern hardwood forest (Weigl *et al*., 2002).

Logging, development, and the introduction of the balsam woolly adelgid (*Adelges piceae*) and the hemlock woolly adelgid (*Adelges tsugae*) have drastically reduced the amount of habitat available to *G. sabrinus coloratus*. So much so that in 1985 their small populations led to their inclusion on the endangered species list (U.S. Fish and Wildlife Service, 1990). Wear and Greis (2002) state that spruce-fir forests may be the most threatened forest type in the southeast due to air pollution and *Adelges piceae*. They also point to the southern Appalachians as an area of particular concern. With the loss of the conifer component of their habitat, due in part to the destructive actions of two adelgids, the squirrels potentially face a decrease in available nesting sites and food sources (Weigl, 2007).

Research into the foraging ecology of *G. s. coloratus* is imperative due to their role in sustaining the forests in which they reside. *G. s. coloratus* ingest hypogeous fungi, but are unable to digest the fungal spores; these undigested spores are deposited

along the forest floor via their fecal droppings and the consequential mycorrhizal fungi growth results in greater efficiency of water and nutrient uptake by trees (Weigl, 2007). The potential exists for a negative feedback: declining *G. s. coloratus* habitat may lead to reduced numbers of squirrels, which results in reduced forest growth and further habitat decline.

My research focused on the foraging habitat of *G. s. coloratus* in the hemlock – northern hardwood forest along the Cherohala Parkway. I hoped to contribute to the knowledge required to make appropriate management decisions regarding the persistence of this threatened population. I asked: 1) Is the larger habitat surrounding the areas of documented squirrel activity suitable for squirrel persistence? 2) How does habitat size, quality, and connectivity compare between sites with squirrel activity and other sites? and 3) What types of foods are the squirrels consuming?

I focused on three areas along the Cherohala with documented *G. s. coloratus* activity: Hooper Bald, Big Junction, and Whigg Branch. To determine the lichen and fungi components of the squirrels' diet, I sequenced rDNA amplified from fecal samples collected from squirrels either trapped or captured in nest boxes, and compared them with sequences within the Gen Bank database using the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To determine if the surrounding forest might be suitable for squirrels, I compared vegetation composition, canopy openness, and litter depth in plots where squirrels were documented as having denned or been caught with random points about 70 meters away. Landscape analyses were completed to delineate *G. s. coloratus* habitat patches and patch quality measured by the dominate vegetation and land cover data. I created a model of potential prime *G. s. coloratus* habitat based on

six variables: elevation, slope, aspect, tree height, spectral signature of den sites, and soil type.

Glaucomys sabrinus coloratus ecology:

Previously thought to be a habitat specialist, *G. sabrinus coloratus* is now known as a "plastic opportunist" due to its ability to adjust to variation in its environment (Weigl, 2007). These squirrels nest in tree dens and even underground areas. Although Hackett and Pagels (2003) observed underground dens for males only, Weigl et al. (2002) and the North Carolina Wildlife Resources Commission have documented underground den use by females as well. A female squirrel was tracked to an underground den under a hemlock eighteen times between March and July 2008 (Chris Kelly, Annual Performance Report). Hackett and Pagels (2003) suggested the use of underground dens may be due to competition with other tree-denning species such as red squirrels (*Tamiascurus hudsonicus*) or southern flying squirrels (*Glaucomys volans*).

Glaucomys sabrinus coloratus tend to reside on north-facing slopes in high elevation mixed-conifer forests, but will also inhabit hardwood forests (Loeb et al., 2000). They utilize old-growth forests as well as second-growth forests. When comparing nest site characteristics between mixed-conifer-deciduous second-growth and old growth red spruce forests at Mount Rogers National Recreation Area in southwestern Virginia, Hackett and Pagels (2003) found that *G. s. coloratus* is not limited to old-growth forests as long as their habitat in second-growth forests contains older trees or is close to old-growth stands. Some studies have shown the squirrels do not prefer conifers to hardwoods, but are forced into conifer habitat by the hardwood-dwelling *G. volans.*

Other studies indicate the opposite; that a preference exists due to their supplemental diet of staminate cones and seeds (Payne et al., 1989).

Glaucomys sabrinus coloratus may choose habitat less on species composition than on structural characteristics. For example, they may choose stands that contain a high amount of coarse woody debris due to the association of the debris and hypogeous fungi (Carey *et al*., 1999), a major constituent of their diet (Loeb et al., 2000). In particular, woody debris in the later stages of decay may serve as an important visual cue in truffle detection (Pyare and Longland, 2001). Hypogeous and epigeous fungal growth is dependent upon old growth, decaying wood, large surface areas, and cool, moist forest conditions (Weigl, 2007). Old growth forests tend to promote these conditions and it was originally thought the squirrel is dependent on this type of forest.

Glaucomys sabrinus have been shown to be opportunistic with regard to diet as well as habitat. Fecal pellet analysis (Mitchell 2001) revealed the West Virginia northern flying squirrel (*Glaucomys sabrinus fuscus*), consumed lichens, hypogeous fungi, vascular plants, mast, and various other plant materials. Fungi were found more often in pellets collected in the fall, and the presence of mast in pellets depended on how good mast production was that year. The staminate cones of conifers also make up part of the diet of *G. sabrinus,* with changes in their diet occurring with changing seasons and food availability (Weigl *et al*, 1999). Fungal spore analyses performed on 116 fecal pellet samples of both *G. sabrinus* and *G. volans* in North Carolina, Tennessee, Virginia, and West Virginia showed consumption of six genera of hypogeous fungi by *G. sabrinus* (Weigl, 1999). In the Pacific Northwest, Thysell *et al*. (1997) noted that *G. sabrinus* foraged on non-truffle items in 34 of 63 foraging observations. They hypothesized that

non-truffle items may be important supplemental foods in the absence of hypogeous fungi, and that squirrels can utilize more hardwood and brush habitat as foraging areas by consuming both non-truffle and truffle items. Their diet is considered to be advantageous to *G. sabrinus* because few other animals that co-occur with them consume these food items, resulting in reduced competition (Weigl, 1978).

Contrary to the findings of Carey *et al*. (1999), *G. s. coloratus* may not be as dependent upon down woody debris for fungi as previously thought. In areas of continual substantial rainfall, conditions for hypogeous fungal fruiting may be favorable regardless of presence of woody debris. In addition, hypogeous fungi may not be an easily obtainable diet item for *G. s. coloratus*. Loeb *et al*. (2000) found low numbers of truffles at ten *G. s. coloratus* sites in the southern Appalachians. They note, however, that truffle occurrence is patchy and although the researchers may have had trouble locating truffles, the squirrels, with their keen sense of smell, probably would not. Loeb *et al*. (2000) also found a negative association between truffle abundance and hardwood species; this suggests a mixed forest habitat with access to a variety of dietary items may be preferable to a one-dimensional forest habitat with limited types of foods. If *G. s. coloratus* is indeed opportunistic with regard to diet and habitat, their home ranges could extend beyond a size restricted by specialization.

Home range data for *Glaucomys sabrinus coloratus* are limited; however, we can surmise *G. s. coloratus* home range sizes may be similar to those of *G. sabrinus*. Home range sizes of *G. sabrinus* may depend on habitat quality and food resources (Smith 2007). Weigl (2007) describes *G. sabrinus* home range sizes of three to fifteen hectares. Studies by Weigl & Osgood (1974) determined *G. sabrinus* had a home range of 100-150

meters at Roan Mountain, in the southern Appalachians, and a slightly larger range of 125-200 meters in Pennsylvania where the topography was more gradual and the forest more open. Home range is determined in part by the sex of the individual and the season. Males tend to have a larger home range than females who may be caring for young. Home range sizes also tend to be larger during mating season when mate search is high. Linear travel of more than one kilometer has been reported, possibly for food or mates, and indicates dependence upon a larger habitat may be more critical than previously realized.

Size and connectivity of suitable habitat, including foraging habitat, could be key factors in persistence of populations of *G. s. coloratus*. Major contributors to population declines of *G. s. coloratus* include competition with other species of animals, especially *G. volans*; low genetic diversity in relatively small isolated populations; and perhaps most importantly, human impact on the size, quality, and connectedness of their habitat (Weigl, 2007). Stated in an Annual Performance Report from the North Carolina Wildlife Resources Commission, "The Unicoi Mountains Geographic Recovery Area is perhaps the most threatened in western North Carolina due to (1) absence of remnant spruce-fir stands, (2) impending loss of the existing dominant conifer, eastern hemlock, to hemlock wooly adelgid (*Adelges tsugae*), (3) greatest isolation from other northern flying squirrel populations, and (4) further isolation within the mountain massif as a result of the Cherohala Skyway road corridor bisecting the population, preventing dispersal and genetic mixing."

Competition with *Glaucomys volans* has been suggested to play a role in *G. s. coloratus* population decline. *G. volans* is slightly smaller and more aggressive than *G. s.*

coloratus. They often carry the intestinal nematode *Strongyloides robustus* and, while pathology is not commonly manifested in *G. volans*, infection of *G. s. coloratus* often proves fatal. *G. volans* tend to occupy hardwood forests; *G. s. coloratus* may occupy these forests as well. However, while *G. volans* seems to prefer tree cavity nests, *G. s. coloratus* also den in underground areas and dreys. The diet of *G. volans* consists mostly of mast while the diet of *G. s. coloratus* consists of items few other animals consume. Thus, there is little evidence to suggest that competition with *G. volans* for food resources or den sites is a major contributor to current *G. s. coloratus* population decline (Weigl, 2007).

Loss of genetic diversity in isolated populations of *G. s. coloratus* may be a major contributor to their declining numbers. Although little is known about the genetics of this subspecies specifically, a study performed by Browne *et al*. (1999) showed populations of *G. sabrinus* in the southern Appalachians have a decreased level of genetic variation compared to that of western populations. There is some disagreement as to how detrimental low genetic variability is to a species; many species have persisted with low variability and contrarily, many species have been adversely affected by low heterozygosity (Browne *et al*., 1999). Weigl (2007) argues there is also no solid evidence that points to genetic isolation as a major contributor to *G. s. coloratus* decline.

Climate change is thought to contribute to the decline of *G. s. coloratus* in several ways. As temperatures become warmer, diet items such as staminate cones and hypogeous fungi that are dependent upon cool, moist conditions of high elevation conifer and mixed conifer-hardwood forests can be lost as the hardwood component increases. In turn, there may be a more favorable habitat for the hardwood-loving *G. volans* and the

increased possibility of *S. robustus* transmission with fatalities to the susceptible *G. s. coloratus* (Weigl, 2007).

Perhaps the greatest threat to *G. s. coloratus* populations is human impact on size, quality, and connectedness of their habitat (Weigl, 2007). One example is the 36 mile Cherohala Skyway, with widths ranging from 38 to 100 meters. This scenic byway is a sizeable barrier to *G. s. coloratus* access to additional habitat and even provides prime tree species for its major competitor, *G. volans*, in disturbed areas (Weigl, 2007). In an 18-month telemetry study of the Unicoi population, Weigl *et al*. (2002) documented *G. s. coloratus* activity near the Cherohala Skyway, but no radio-collared squirrel attempted to cross the byway. Disturbances such as blast and fill construction along the Cherohala Skyway could also have affected the food supply of *G. s. coloratus*. Bird and McCleneghan (2005) found that low diversity of hypogeous fungi on Roan Mountain in the southern Appalachians could be explained, in part, by past habitat disturbance. Along the Cherohala Skyway, these disturbances could lead to low diversity of hypogeous fungi in *G. s. coloratus* habitats. Fortunately, measures have been taken by the North Carolina Wildlife Resources Commission with help from Duke Energy to counteract the effects of the road. Three sets of crossing structures have been erected along the scenic byway with positive results.

Another example of the human impact on *sabrinus* habitat is the introduction of pests that have had a tremendous impact on the conifer component of their habitat. Two adelgids, *Adelges piceae* and *Adelges tsugae* are killing Fraser fir and hemlock tree species throughout the habitat of *G. s. coloratus*. With the loss of these species, the squirrels incur a loss of food items and den sites. According to Weigl (2007), they may

also lose a source of natural medicine against *S. robustus* from the oils ingested from staminate cones.

G. s. coloratus decline is important and of particular concern due to the squirrels' role in perpetuating its forest habitat. My research, in which I investigated habitat trends and food consumption, lends important information for managing *G. s. coloratus* habitat and maintaining populations, and contributes to general scientific information on this secretive squirrel.

METHODS

Site Descriptions

My studies were focused between mile markers eight and three of the Cherohala Skyway, a scenic byway connecting Robbinsville, North Carolina and Tellico Plains, Tennessee. With widths ranging from 38.1 to 99.97 meters, the road is a partial barrier to squirrel dispersal. Three areas along the Cherohala Skyway: Big Junction, Hooper Bald, and Whigg Branch, were chosen for the study because these areas, as demonstrated by previous research, were considered more likely than others to show signs of *G. s. coloratus* activity.

Big Junction, N 35.301472 and W -84.021299, is one of the highest points along the Cherohala Skyway at about 1597 meters elevation. The forest in this area has an overstory composed primarily of *Fagus grandifolia* (American Beech). The slope at Big Junction ranges from gentle to steep; aspect is northerly, with springs and seeps present.

Hooper Bald, N 35.305918 and W -83.994076, is similar to Big Junction in elevation and overstory. The dominate canopy tree is *F. grandifolia*. The slope at Hooper Bald also ranges from gentle to steep with a northerly aspect and interspersed drainages and seeps.

Whigg Branch, N 35.316448 and W -84.029742, differs from the other areas; it is lower in elevation at about 1427 meters and has different overstory composition in *Tsuga canadensis* (Eastern Hemlock), *Acer rubrum* (Red Maple) and *Betula alleghaniensis* (Yellow Birch). Whigg Branch also has a shrub layer of Rhododendron (*Rhododendron sp*.), which is not present at either Big Junction or Hooper Bald. The slope at Whigg

Branch is gentle to moderate with a northerly aspect. It is an altogether wetter area than Big Junction and Hooper Bald with constant flowing water, intermittent streams, and rocky drainages.

My research relied on *Glaucomys sabrinus coloratus* trapping performed by the North Carolina Wildlife Resources Commission (NCWRC) as well as myself. Documented squirrel locations were required for vegetation surveys to address questions about habitat size and quality and whether the larger habitat surrounding these areas is suitable for squirrel persistence. Additionally, scat samples from as many squirrels as possible were vital in the molecular analyses to identify presence of lichen or fungi dietary components. A total of 1,843 trap nights resulted in three successful flying squirrel captures: two *G. s. coloratus* and one southern flying squirrel (*G. volans*). No fecal pellets, however, were expelled within the handling bags. Thus, all *G. s. coloratus* captures resulting in fecal samples for molecular analysis occurred by way of nest box checks performed by NCWRC in July 2007, January 2008, and March 2008.

Vegetation Surveys

Vegetation surveys of sites with documented *G. s. coloratus* activity, either den sites or capture sites (referred to as *known activity* plots for the remainder of this paper), and paired random sites (referred to as *random* plots for the remainder of this paper) were conducted to determine how the foraging habitat size, quality, and connectivity compare between the *known activity* and *random* plots averaging 68 meters away. These surveys also provided information for GIS analysis of the larger habitat around the three focal areas.

A nested plot design was used. A 10 m x 10 m "tree" plot was centered on a tree at *known activity* plots and *random* plots. *Random* plots were chosen by selecting a tree in an area about 50 large steps (average distance was 70 meters) in an arbitrarily chosen direction down-slope, up-slope, or across-slope from the known point. Up-slope was only chosen if 50 steps would not put the point across the Cherohala Skyway. This decision was made based on the unlikely scenario that *G. s. coloratus* would cross from the north side of the road to the south. Within each 10 m x 10 m plot, a 5 m x 5 m "understory" plot was centered on the point tree and a 1 m x 1 m "herb" plot was established in each corner (Figure 1).

Eighteen tree plots were sampled at Big Junction (9 *known activity* and 9 *random*), 17 plots were sampled at Hooper Bald (9 *known* and 8 *random*), and 18 plots were sampled at Whigg Branch (9 *known* and 9 *random*). Twenty understory plots were sampled at Big Junction (10 *known*, 10 *random*), 15 plots were sampled at Hooper Bald (7 *known*, 8 *random*), and 19 plots were sampled at Whigg Branch (10 *known*, 9 *random*). Eighty-four herb plots were sampled a Big Junction (44 *known*, 40 *random*), 60 plots were sampled at Hooper Bald (28 *known*, 32 *random*), and 72 plots were sampled at Whigg Branch (36 *known*, 36 *random*).

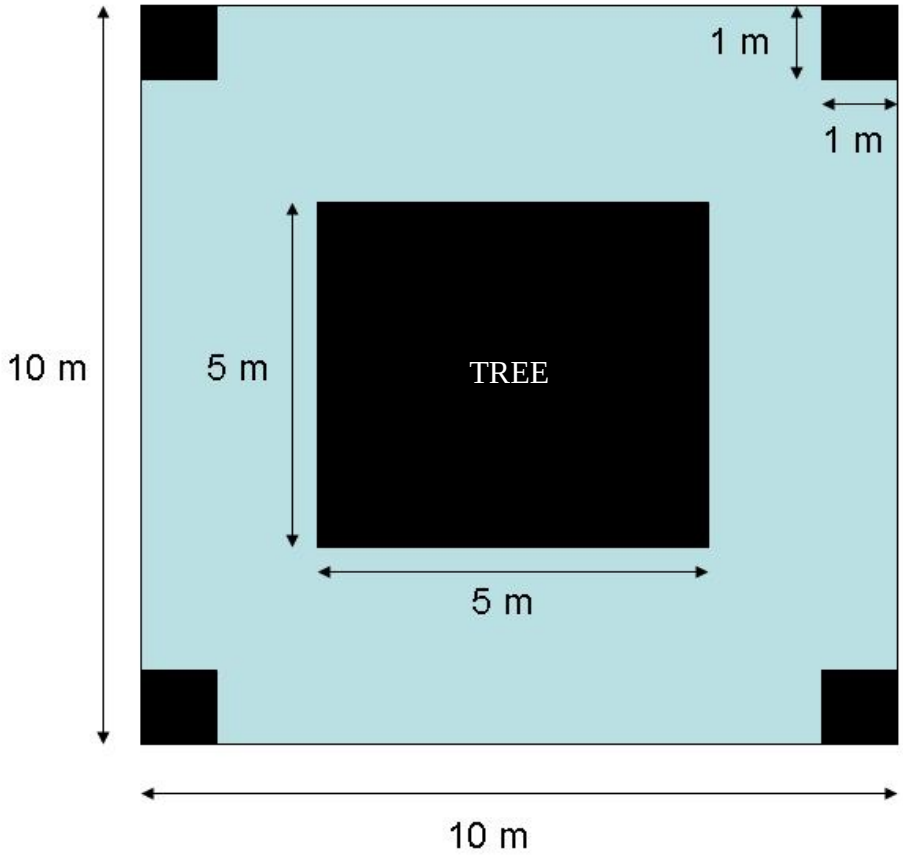

Figure 1. Nested plot design used for vegetation surveys (centered on a point tree).

Within the 10 m x 10 m tree plots, tree species composition as well as diameter at breast height was recorded. Trees were defined as woody vegetation greater than 2.5 centimeters diameter at breast height. Height of saplings (defined as less than 2.5 centimeters at breast height) taller than one meter tall was estimated and recorded within each 10 m x 10 m plot. Presence of shrubs, tree saplings less than one meter tall and less than 2.5 centimeters in diameter, tree seedlings, and herbs was recorded in each 5 m x 5 m understory plot. Percent cover of herbs and tree seedlings was estimated to the nearest one percent within each 1 m x 1 m herb plot. A sample of all unidentifiable plants was taken and pressed to be later identified in the laboratory.

Four random litter depth measurements were recorded within each 1 m x 1 m herb plot and averaged. A flag stake was placed through the litter cover until resistance from the ground was met. The length from the top of litter cover to the bottom of the stake was recorded to the nearest centimeter.

Digital hemispherical canopy photos (Canon EOS 30D Digital Camera) taken at *known activity* and *random* plots were used to describe the openness of the respective areas. One canopy photo was taken at each 1 m x 1 m herb plot. Vegetation surveys, however, had begun before the camera was purchased. For plots surveyed prior to camera purchase, two canopy photos were taken within the larger 10 m x 10 m plots, but not specifically within each 1 m x 1 m herb plot. All photos were taken one meter above the ground, for a total of 140 photos: 68 at *known activity* plots (20 at Big Junction, 18 at Hooper Bald, 30 at Whigg Branch), and 72 at *random* plots (28 at Big Junction, 14 at Hooper Bald, 30 at Whigg Branch).

Landscape Analysis

Using ArcGIS v. 9.3.1 (ESRI, Redlands, CA, USA), I created a model delineating potential Carolina northern flying squirrel habitat based on six parameters: slope, elevation, aspect, spectral signature of den sites, soil types, and tree height data.

Elevation data for the study area, a portion of Graham County, were derived from Light Detection And Ranging (LiDAR) data acquired in April 2005. These data were downloaded from the North Carolina Department of Transportation website (http://www.ncdot.gov/it/gis/DataDistribution/ContourElevationData/). Using the digital elevation models from the NC DOT, percent slope was estimated using ArcGIS's Spatial Analysis extension. Tree heights were extracted from a LiDAR derived model developed by Doug Newcomb of the U. S. Fish and Wildlife Service in Raleigh, NC. Soils for the study area were identified from the Soil Survey Geographic (SSURGO) database for Graham County (2008), U.S. Department of Agriculture, Natural Resources Conservation Service. Sites with similar spectral signature were derived from IKONOS-2 data (Geoeye, Inc.) acquired October 8, 2001 using the Feature Analyst extension (Visual Learning Systems, Inc.). For this study, the area of analysis including slope, elevation, aspect, soil types, and tree height data was determined by the extent of the available IKONOS-2 data in Graham County. Spectral signatures were determined from ten meter diameter circles centered on each of the den sites.

To identify potential *G. s. coloratus* habitat, the defining six parameters were established using GPS points of *G. s. coloratus* captures and information from northern flying squirrel researchers (Wells-Gosling and Heaney, 1984; Weigl *et al*., 1999). For elevation, suitable habitat was deemed between 1373 and 1605 meters. Squirrel sites

were consistently between zero and 27% slope. The soil types at the squirrel sites were Breakneck-Pullback complex, windswept, 15 to 30 percent slopes, very rocky (BuD); Breakneck-Pullback complex; windswept, 30 to 50 percent slopes, very rocky (BuE); Spivey-Whiteoak complex, 15 to 30 percent slopes, boulder (SvD); and Spivey-Santeetlah complex, 30 to 50 percent slopes, very boulder (SpE). Taxonomic classes for the soils were: Breakneck - loamy, isotic, frigid typic humudepts; Pullback - loamy, isotic, frigid lithic humudepts; Spivey - loamy-skeletal, isotic, mesic typic humudepts; Whiteoak - fine-loamy, isotic, mesic humic dystrudepts; Santeetlah - fine-loamy, isotic, mesic typic humudepts. Tree heights ranged from 33 to 84 feet for the sites. The aspect of the squirrel sites ranged from north to northwest (0° to 45°).

Molecular Analysis

Of 14 squirrels captured from winter 2007 through spring 2008, only nine (four from lower Whigg Branch and five from Hooper Bald) produced fecal pellets for DNA analysis (Table 1). Of the four from lower Whigg Branch, three males and one female were captured while four females and one male were captured at Hooper Bald. Pellets were stored in sterile tubes filled with ethyl alcohol and placed on ice until storage in a lab freezer was available.

Trial DNA extractions on two samples with the most amount of fecal material were performed using the PowerSoil™ DNA Isolation Kit and procedures (MO BIO Laboratories, Inc.). Once extraction procedures were verified as sound (i.e. sufficiently low level of contaminants) by successful amplification using PCR, DNA extractions were performed on the remaining samples.

Table 1. Fecal pellet samples collected summer 2007 through spring 2008 with corresponding site and nest box locations of collection.

Sample Name	**Date**	**Location**	**Box #**
1874	7/7/2007	Hooper Bald	Box 5
2017	1/29/2008	Hooper Bald	Box 21
2019	1/29/2008	Hooper Bald	Box 13
2020	1/11/2008	Lower Whigg Branch	Box 11
2021	1/29/2008	Hooper Bald	Box 13
2022	1/12/2008	Lower Whigg Branch	Box 9
2023	1/11/2008	Lower Whigg Branch	Box 11
2024	1/11/2008	Lower Whigg Branch	Box 11
2025	3/3/2008	Hooper Bald	Box 25
Lichen	From research area used as control		

PCR was performed on extracted DNA of all samples to amplify partial 16S rDNA from cyanobacteria, partial 16S rDNA from bacteria, and the internal transcribed spacer (ITS) region from fungi. The ITS region of fungi has a higher degree of variation than other regions of rDNA and is often used for identification at the species level (Vilgalys lab, Duke University). Amplification of partial 16S rDNA from bacteria was performed utilizing the primers 341F and 907R (Casamayor *et al*. 2000). Total reaction volumes were 50µL and PCR reaction mixtures consisted of 28µL nuclease-free water, 0.5µL of each primer, 20µL Eppendorf 2.5X, and one µl of DNA solution obtained from the MoBioTM extraction. Thermal cycler condititions were as follows: initial denaturation occurred for five minutes at 94°C followed by 30 cycles of denaturation at 94°C for one minute, annealing for one minute starting at 65°C and ending at 55°C, elongation for three minutes at 72°C, with a final elongation spanning seven minutes at 72°C. The samples were held at 4°C until further processing. As predicted, successful amplification of 16S rDNA from bacteria was seen across all samples.

The primers 359F and 781R were utilized in the amplification of 16S rDNA from cyanobacteria (Nubel *et al*., 1997). Total reaction volumes were 50µL and PCR reaction mixtures consisted of 28µL nuclease-free water, 0.5µL of each primer, 20µL Eppendorf 2.5X, and one µl of DNA solution obtained from the MoBioTM extraction. Thermal cycler conditions began with an initial denaturation of four minutes at 95°C followed by 30 cycles of denaturation of one minute at 94°C, annealing for one minute at 57°C, and elongation of one minute at 72°C. Final elongation at 72°C occurred for four minutes. The samples were held at 4°C until further processing. Amplification of 16S rDNA from cyanobacteria was seen in three samples, but faintly.

Primers EF4 and ITS4 were used to amplify the ITS fungal region. Total reaction volumes were 50µL and PCR reaction mixtures consisted of 23 µl nuclease-free water, 0.5 µL of each primer, 25 µL PromegaTM Master Mix 2X and 1µl of DNA solution from the MoBioTM extraction. Thermal cycler conditions began with initial denaturation of three minutes at 94°C. Thirty cycles of denaturation for one minute at 95°C, annealing for one minute at 55°C, and elongation for two minutes at 72°C were performed. Final elongation occurred for seven minutes at 72°C and the samples were held at 4°C until they could be further processed.

Nested PCR procedures were undertaken to amplify the fungal ITS region to obtain greater yield of amplicons. This occurred utilizing the primers EF4 and ITS4 for the first round and ITS1-F with a GC clamp and ITS2 primers for the second round (Anderson *et al*., 2003 and website). Reaction chemistry and thermal cycler conditions were the same for both rounds beginning with an initial denaturation of five minutes at 94°C. Thirty cycles of denaturation of one minute at 94°C, annealing of one minute at 55°C, and elongation of two minutes at 72°C were performed. Final elongation occurred for five minutes at 72°C and the samples were held at 4°C until further processing.

Next, denaturing gradient gel electrophoresis (DGGE) was performed on samples at 65V and 60°C for 15 hours using D-Code System (BioRad). Bands were excised from the DGGE gels and reamplified using the ITS2 and ITS4 primers with thermal cycler conditions of an initial denaturation for one minute at 96°C with 25 cycles of denaturation for ten seconds at 96°C, five seconds of annealing at 50°C, and four minutes of elongation at 60°C. Sequencing was performed using primer 907R with BigDye 3.1 and analyzed by a 3130 automated capillary DNA sequencer (Applied Biosystems).

DGGE analysis was not performed on samples 2022 and 2024 due to amplification products not produced during the second round of nested PCR.

DATA ANALYSIS

Vegetation Surveys:

Non-metric multidimensional scaling (NMDS) ordinations were performed on the vegetation data to visualize patterns in species composition between *known activity* plots and *random* plots and among the three sites (Hooper Bald, Big Junction, Whigg Branch). The NMDS ordinations were performed with the program PC-ORD (McCune and Mefford, 1995) and were based on Bray-Curtis dissimilarities.

Multiresponse permutation procedure (MRPP; McCune and Mefford, 1995) was used to determine if species composition differed among the three sites or between *known activity* and *random* plots. Indicator analysis (McCune and Mefford, 1995) was used to determine the species that distinguished *known activity* and *random* plots as well as the three sites.

The Gap Light Analyzer (GLA) software application was used to analyze the digital hemispherical canopy photographs taken at each site (Frazer *et al*., 1999). Analysis of variance (ANOVA) was used to test the null hypothesis that means of canopy openness did not differ among plots or sites.

Analysis of variance was also used to test the null hypothesis that means of sapling height, ground litter, and downed woody debris did not differ among plots or sites.

GIS Analysis:

Using ArcGIS v. 9.3.1 (ESRI, Redlands, CA, USA), a model to delineate suitable *G. s. coloratus* habitat areas around the Cherohala Skyway was created by converting six parameters (slope, elevation, aspect, spectral signature of den sites, soil types, and tree height data) to 10 m x 10 m cells and assigning a value. Using ArcGIS Spatial Analysis Raster Calculator, the six parameters were added across the study area. Cells across the study area ranged from zero, with no parameter present to six, with all parameters present. Cells with scores of five and six were deemed potential habitat for squirrels.

Molecular Analysis:

Sequences of rDNA amplified from *Glaucomys sabrinus coloratus* fecal samples were analyzed utilizing the Genbank database search tool Basic Local Alignment Search Tool (BLAST) and results with the highest similarity (at least 90%) and relevance were recorded (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Only those with multiple hits were discussed in detail.

RESULTS

VEGETATION SURVEYS

Tree Layer:

Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities (final stress 15.6; Figure 2) revealed a gradient in tree composition along the X and Y axes from the Big Junction and Hooper Bald sites to Whigg Branch. Greater distance separating Whigg Branch from the other two sites suggests a more dissimilar tree composition at Whigg Branch. In addition, greater distances among plots suggests greater variability in tree species composition at Whigg Branch plot types (*known activity* and *random*).

Verifying the ordination results, MRPP revealed abundance-weighted tree composition of *known activity* plots at Whigg Branch differed from that at Big Junction ($P < 0.0001$) and Hooper Bald ($P < 0.00002$). Similarly, *random* plots at Whigg Branch differ from those at Big Junction ($P < 0.0002$) and Hooper Bald ($P < 0.0002$) (Figure 3). Species composition also differed between *known activity* and *random* plots at Whigg Branch ($P < 0.006$). Species composition between *known activity* and *random* plots at Big Junction ($P < 0.52$) and Hooper Bald ($P < 0.41$), however, did not significantly differ (Figure 3).

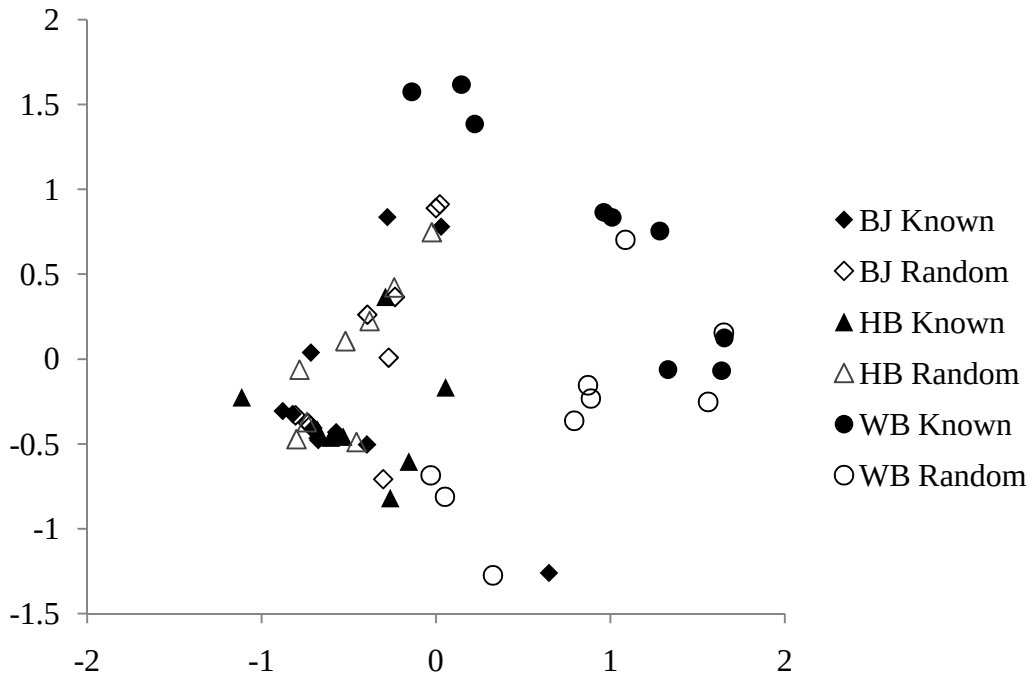

Figure 2. NMDS ordination based on relative abundance of tree layer species in Carolina northern flying squirrel *known* and paired *random* plots at Big Junction (BJ), Hooper Bald (HB), and Whigg Branch (WB).

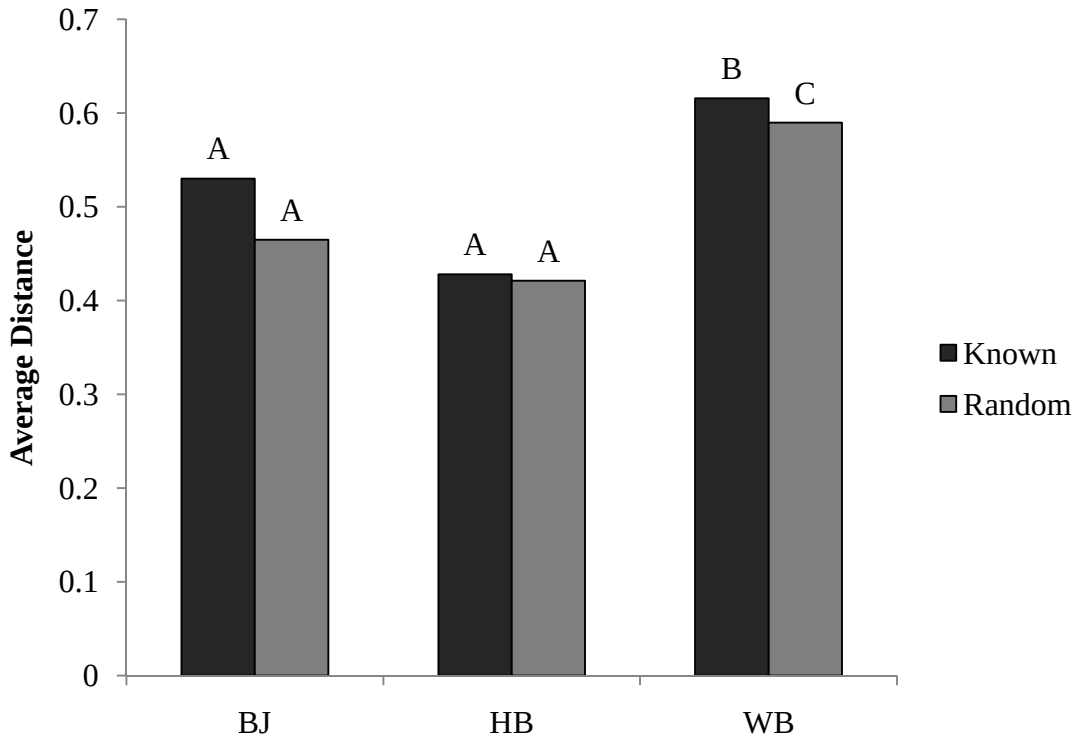

Figure 3. Mean dissimilarity of abundance-weighted tree species composition within *known activity* plots and *random* plots at Whigg Branch (WB), Big Junction (BJ), and Hooper Bald (HB). Means with the same letter do not differ significantly at $P = 0.05$.

Table 2. Tree species abundances (basal area in square centimeters) summed over *known activity* and *random* plots at Big Junction, Hooper Bald, and Whigg Branch. Indicator tree species ($P < 0.051$) for *known activity* and *random* plots within each site are indicated in bold. A minimum of the top five greatest abundances are shown for each site and plot type.

	Big Junction		Hooper Bald		Whigg Branch	
	Known	Random	Known	Random	Known	Random
Acer pensylvanicum	222	13	128	29	5	9
Acer rubrum	0	0	0	40	5341	0
Acer saccharum	918	3064	66	1755	0	3265
Aesculus sp.	1459	36	3111	122	0	0
Betula alleghaniensis	6472	**10153**	2707	**5458**	**16535**	1301
Fagus grandifolia	**11499**	**6430**	**8867**	**6760**	277	**4403**
Magnolia sp.	0	0	0	0	444	0
Prunus pennsylvanica	769	0	0	0	1018	0
Tsuga canadensis	0	0	0	12	**39141**	**10818**

Indicator analysis revealed that tree composition for Whigg Branch was unlike the other two sites due to its considerable *Tsuga canadensis* (Eastern Hemlock) component in both *known activity* and *random* plots (Table 2). *Fagus grandifolia* was an indicator species in *known activity* and *random* plots at both Big Junction and Hooper Bald and in *random* plots at Whigg Branch. *Betula alleghaniensis* was an indicator at the Big Junction and Hooper Bald *random* plots as well as *known activity* plots at Whigg Branch.

Understory Layer:

Ordination (NMDS) based on Bray-Curtis dissimiliarities (final stress = 25.5; Figure 4) revealed shrub composition overlaps between *known activity* and *random* plots at both Big Junction and Hooper Bald. Little overlap among Big Junction, Hooper Bald, and Whigg Branch suggests each site has distinct shrub composition. Further, separation of known and random plots within Whigg Branch suggests small-scale (70 m) pattern in shrub species composition at this site.

MRPP confirmed species composition of both *known activity* and *random* plots differed among all sites ($P < 0.05$ for all pairwise combinations of plots). *Known* and *random* plots within Whigg Branch were also significantly different ($P < 0.005$). MRPP showed no difference at both Big Junction and Hooper Bald between *known* plots and *randomly* chosen plots ($P = 0.85$ Big Junction, $P < 0.25$ Hooper Bald) (Figure 5).

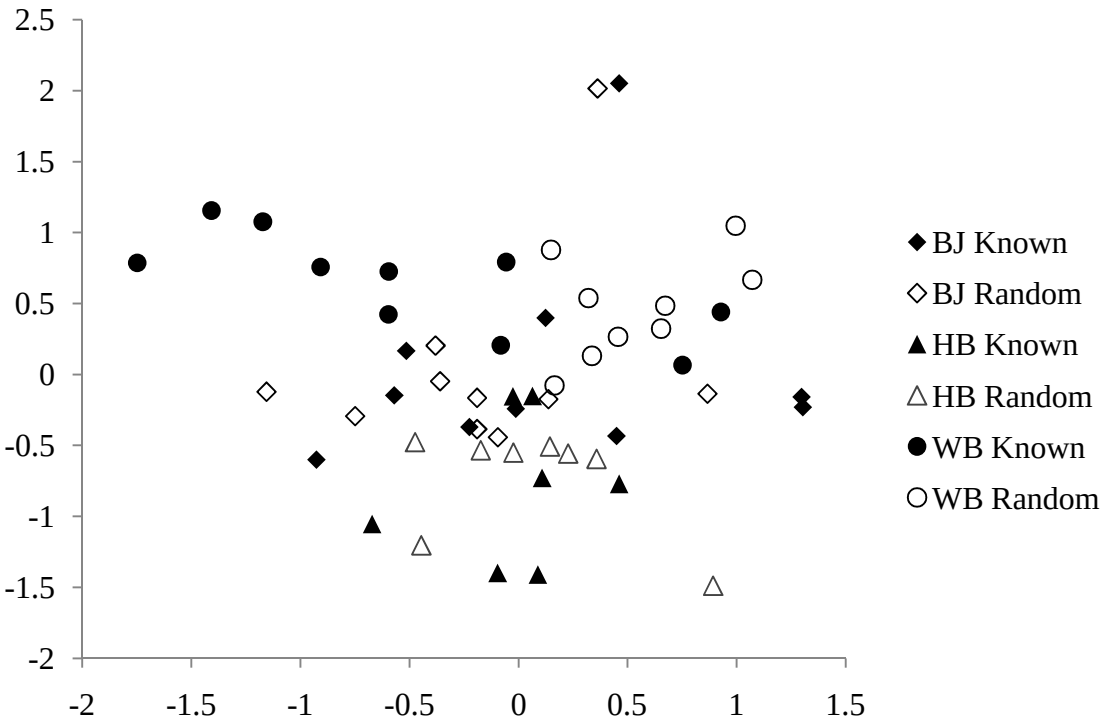

Figure 4. NMDS ordination of understory layer species composition at Carolina northern flying squirrel *known activity* plots and paired *random* plots at three sites: Big Junction (BJ), Hooper Bald (HB), and Whigg Branch (WB) on the Cherohala Skyway. Final stress = 25.5

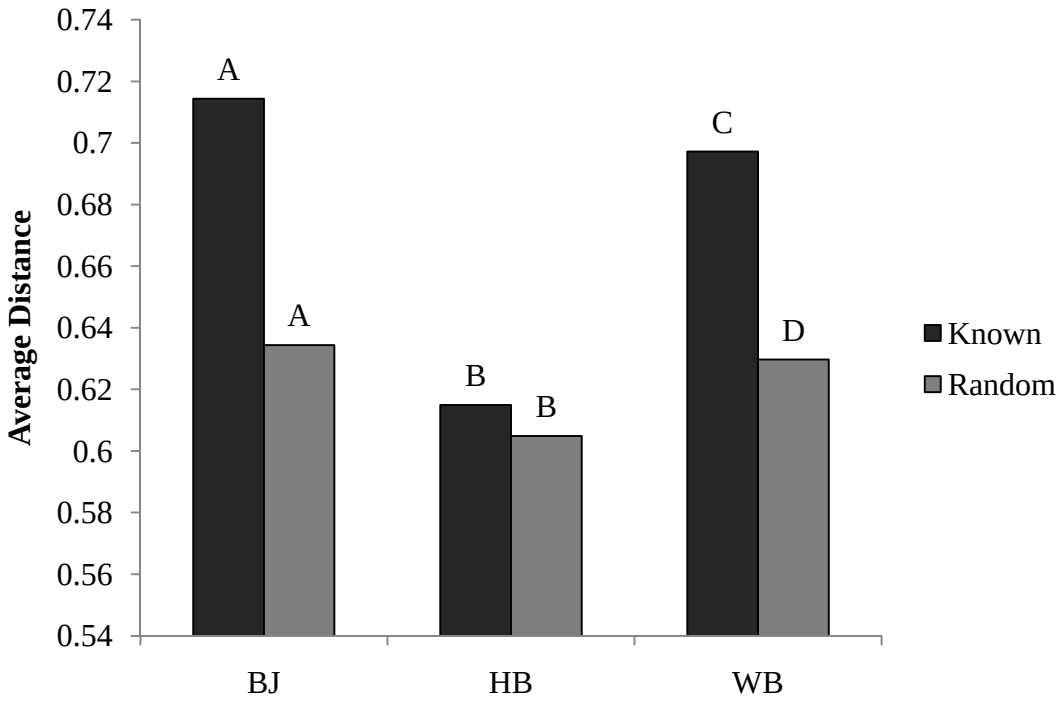

Figure 5. Mean dissimilarity of understory layer species composition between *known activity* plots and *random* plots at Big Junction (BJ), Hooper Bald (HB), and Whigg Branch (WB). Means with the same letter do not differ at $P = 0.05$.

Table 3. Frequency (presence summed across plots) of understory species for *known activity* and *random* plots within Big Junction, Hooper Bald, and Whigg Branch. Indicator understory species ($P < 0.05$) for *known activity* and *random* plots are underlined and indicated in bold. Only the top ten species are shown for each plot type.

	Big Junction		Hooper Bald		Whigg Branch	
	Known	Random	Known	Random	Known	Random
Dryopteris intermedia	10	10	6	6	9	10
moss	7	9	5	8	9	9
Tiarella cordifolia	6	7	4	-	3	6
lichen	6	8	-	4	6	-
sedge	**6**	-	3	-	-	-
Angelica triquinata	5	6	-	-	-	-
Viburnum sp.	5	5	6	**8**	4	6
Eurybia divaricata	5	8	**7**	**7**	-	6
Oxalis sp.	5	5	-	-	4	-
grass	4	6	**7**	**8**	-	-
Viola sp.	-	**5**	-	-	-	-
Trautvetteria caroliniensis	-	-	**5**	**6**	-	-
Huperzia lucidula	-	-	3	4	**7**	**9**
Stellaria media	-	-	**3**	-	-	-
Veratrum sp.	-	-	-	**4**	-	**5**
Maianthemum canadense	-	-	-	**4**	-	-
Rhododendron sp.	-	-	-	-	**7**	-
fungus	-	-	-	-	4	-
Medeola virginiana	-	-	-	-	3	-
Mitchella repens	-	-	-	-	-	**8**
Streptopus lancelatus	-	-	-	-	-	**5**
Acer pensylvanicum seedling	-	-	-	-	-	**5**

Indicator analysis revealed only two species characterized Big Junction (sedge $P = 0.01$, *Viola sp*. $P < 0.04$) (Table 3). Hooper Bald and Whigg Branch shared one indicator species (*Veratrum sp.* $P = 0.002$ and $P = 0.01$ respectively). Whigg Branch shared *Lycopodium sp*. as an indicator between its *known activity* ($P < 0.02$) and *random* plots ($P < 0.001$). Three indicator species identified and shared between Hooper Bald's plot types included *Eurybia divaricata* ($P < 0.01$ *known activity*, $P < 0.03$ *random*), grass ($P = 0.01$ *known activity*, $P < 0.002$ *random*), and *Trautvetteria caroliniensis* ($P < 0.01$ *known activity*, $P = 0.002$ *random*).

Herbaceous Layer:

NMDS ordination based on Bray-Curtis dissimilarities (final stress = 29.2; Figure 6) revealed gradients in herb composition along the x-axis from Big Junction to Whigg Branch and along the y-axis from Whigg Branch to Hooper Bald.

MRPP revealed significant differences in herb layer composition between *known activity* plots and *random* plots both within each site and among all sites (Figure 7; $P < 0.05$ for all pairwise combinations of plots).

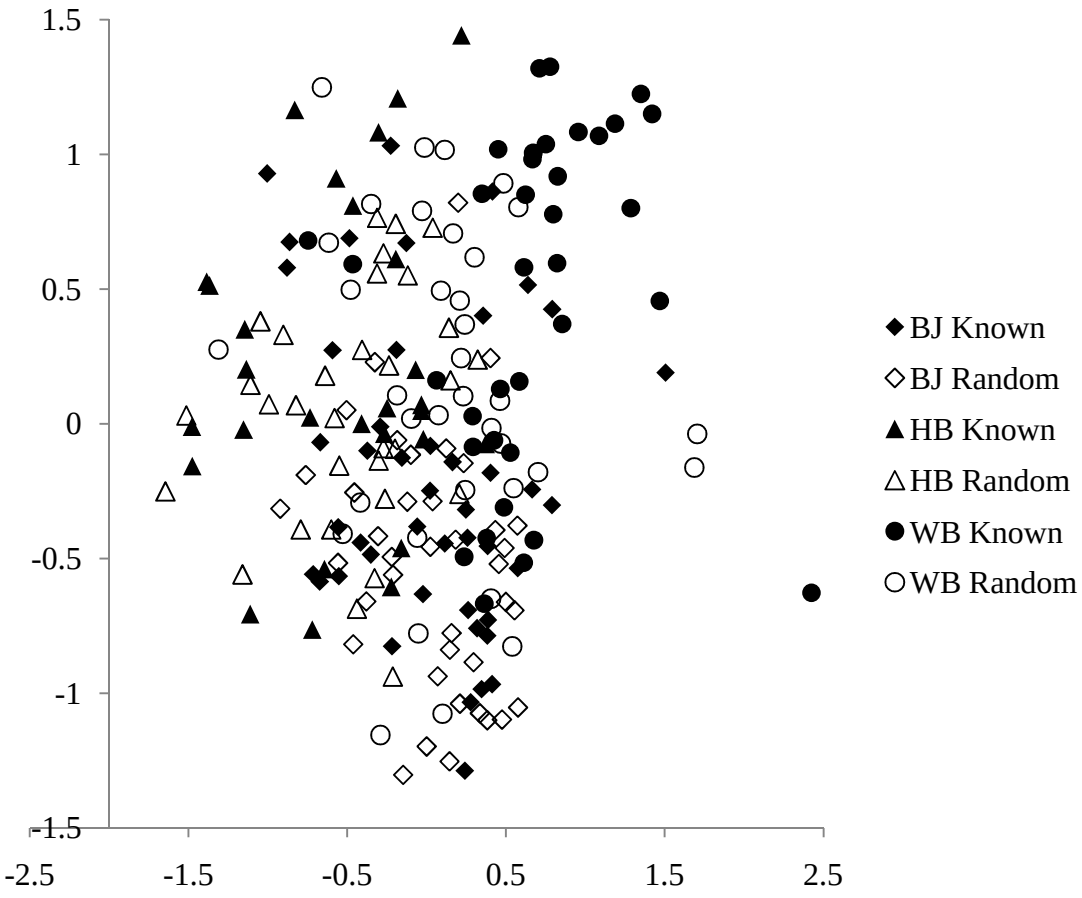

Figure 6. NMDS ordination of herbaceous layer species composition at *Glaucomys sabrinus coloratus* known activity plots and random plots at three sites: Big Junction (BJ), Hooper Bald (HB), and Whigg Branch (WB) on the Cherohala Skyway.

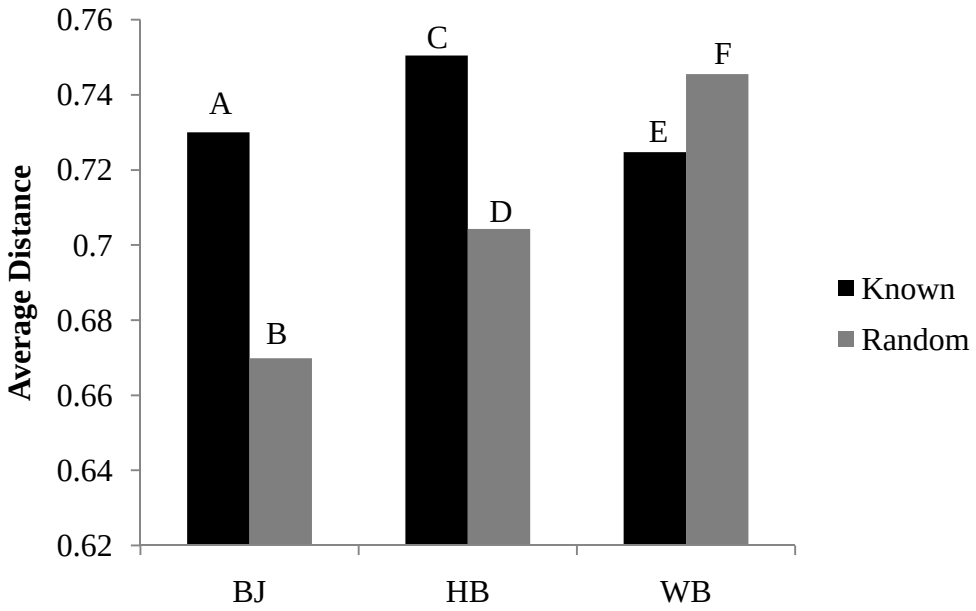

Figure 7. Mean dissimilarity in herb layer species composition, weighted by percent cover, in *known activity* and *random* plots at Big Junction (BJ), Hooper Bald (HB), and Whigg Branch (WB). Means with the same letter do not differ significantly ($P > 0.05$).

Table 4. Percent cover (averaged) of herbaceous layer composition for *known activity* and *random* plots within Big Junction, Hooper Bald, and Whigg Branch. Indicator understory species ($P < 0.05$) for *known activity* and *random* plots are underlined and indicated in bold. Only the top five species for percent cover are shown for each plot type.

	Big Junction		Hooper Bald		Whigg Branch	
	Known	Random	Known	Random	Known	Random
Dryopteris intermedia	**22**	**34**	**11**	10	12	13
grass	**15**	**17**	**17**	**17**	-	-
Viburnum sp.	**6**	-	**12**	**14**	4	**6**
Eurybia divaricata	**6**	**5**	11	**11**	-	**10**
Trautvetteria caroliniensis	**5**	-	**8**	-	-	-
sedge	-	**4**	-	-	-	-
Angelica triquinata	-	**4**	-	-	-	-
Maianthemum canadense	-	-	-	**5**	-	-
Rhododendron sp.	-	-	-	-	**21**	-
Huperzia lucidula	-	-	-	-	**6**	**6**
Oxalis sp.	-	-	-	-	**3**	-
Mitchella repens	-	-	-	-	-	**8**

Due to the large number of indicator species identified across all three sites as well as each plot type, it may be more appropriate to discuss those indicator species unique to each site and/or plot type rather than those that are common. At Big Junction the unique indicators were sedges ($P < 0.01$ *random*) and *Angelica triquinata* ($P < 0.03$ *random*). Hooper Bald contained one species, *Maianthemum canadense*, that was not identified as a top five indicator at any of the other sites ($P < 0.001$ *random*). Whigg Branch was characterized by four distinct indicator species that were not identified by indicator analyses at Hooper Bald and Big Junction: *Rhododendron sp.* ($P < 0.001$ *known activity*), *Oxalis sp.* ($P = 0.02$ *known activity*), *Mitchella repens* ($P < 0.01$ *random*), and *Huperzia lucidula* ($P < 0.002$ *known activity*, $P = 0.03$ *random*).

Site Characteristics:

Analysis of variance (ANOVA), used to test differences in mean canopy openness, bare ground, litter depth, and downed woody debris among sites (Big Junction, Hooper Bald, Whigg Branch) and plot types (*known activity, random*) revealed significant differences in only two characteristics: canopy openness ($F(5, 134) = 19.32$, $P < 0.0001$) and litter depth ($F(5, 208) = 10.96$, $P < 0.0001$). *Random* plots were more open than *known activity* plots at Big Junction and Whigg Branch. In contrast, both *random* and *known activity* plots at Hooper Bald had a relatively closed canopy (Figure 8). Litter depth measurements revealed *known activity* plots and *random* plots varied slightly and depth was the greatest at Hooper Bald followed by Whigg Branch and Big Junction (Figure 9)

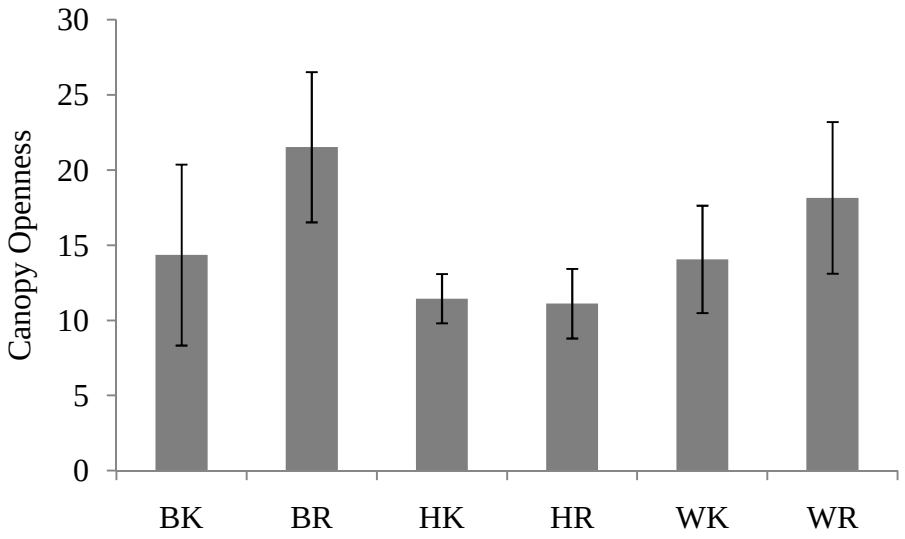

Figure 8. Means (+1 standard deviation) of percent canopy openness at Big Junction *known activity* (BK) and *random* (BR) plots, Hooper Bald *known activity* (HK) and *random* (HR) plots, and Whigg Branch *known activity* (WK) and *random* (WR) plots.

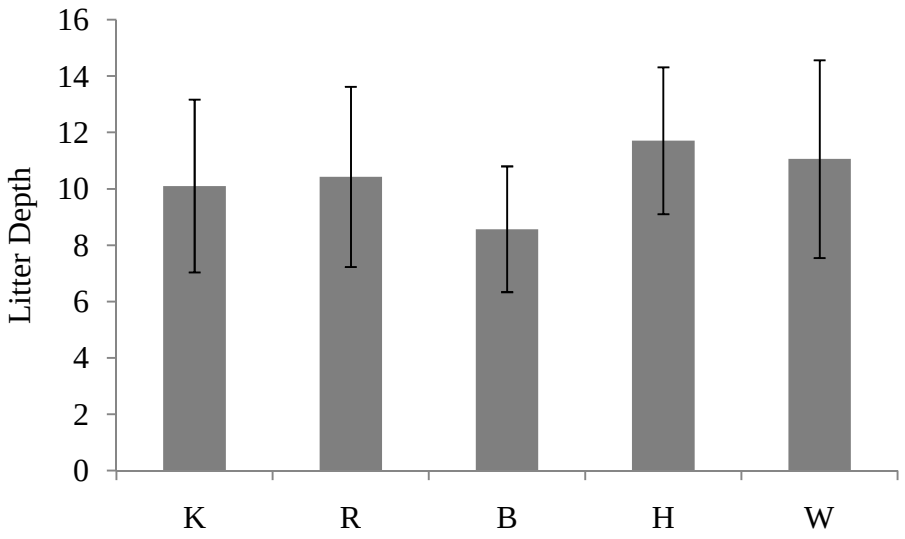

Figure 9. Means (+1 standard deviation) of litter depth in centimeters at *known activity* (K) plots, *random* (R) plots, and Big Junction (B), Hooper Bald (H), and Whigg Branch (W) sites.

LANDSCAPE ANALYSIS

Based upon habitat conditions associated with known *G. s. coloratus* locations, potential habitat required elevation between 1373 and 1605 meters, slope between zero and 27%, soil types BuD, BuE, SvD, and SpE, and aspect from north to northwest (0° to 45°). Tree heights for *known activity* and *random* plots ranged from 33 to 84 feet.

Small (less than one hectare) predicted optimum habitat patches occurred at a much higher frequency than large. Those patches of the size within the proposed three to fifteen hectare home range occurred from two to six times, whereas those of size 0.04 hectares occurred 1538 times (Figure 10, Figure 12). The largest patch, at 21.44 hectares occurred twice. Additionally, nearest neighbor calculations showed suitable patches were relatively close together; the highest frequency occurring with patches approximately 40 meters from one another (Figure 11, Figure 12). Stratton Bald, an area lying approximately 7,140 meters to the north of the three study sites appears to contain optimum habitat for *G. s. coloratus* populations (Figure 12).

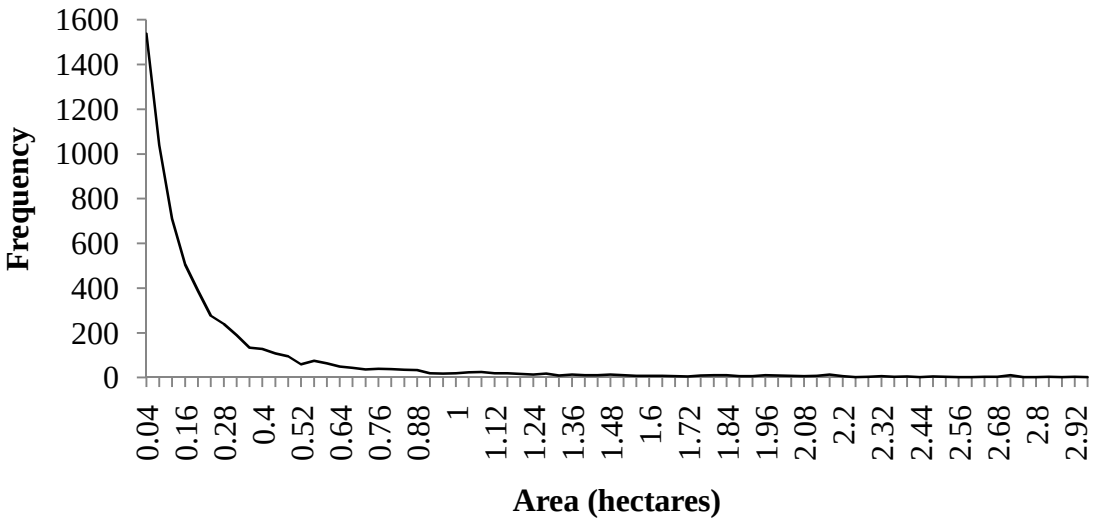

Figure 10. Patch size frequency distribution of potential *Glaucomys sabrinus coloratus* habitat as predicted by the GIS model. Larger patches have been removed from graph for ease of viewing.

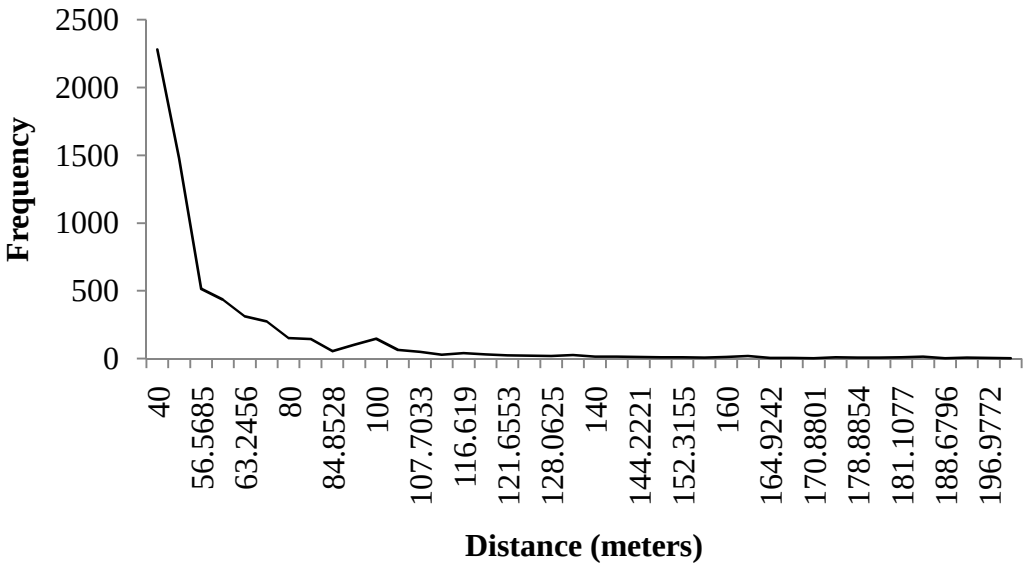

Figure 11. Frequency distribution of nearest neighbor potential habitat patches. Longer distances have been removed from graph for ease of viewing.

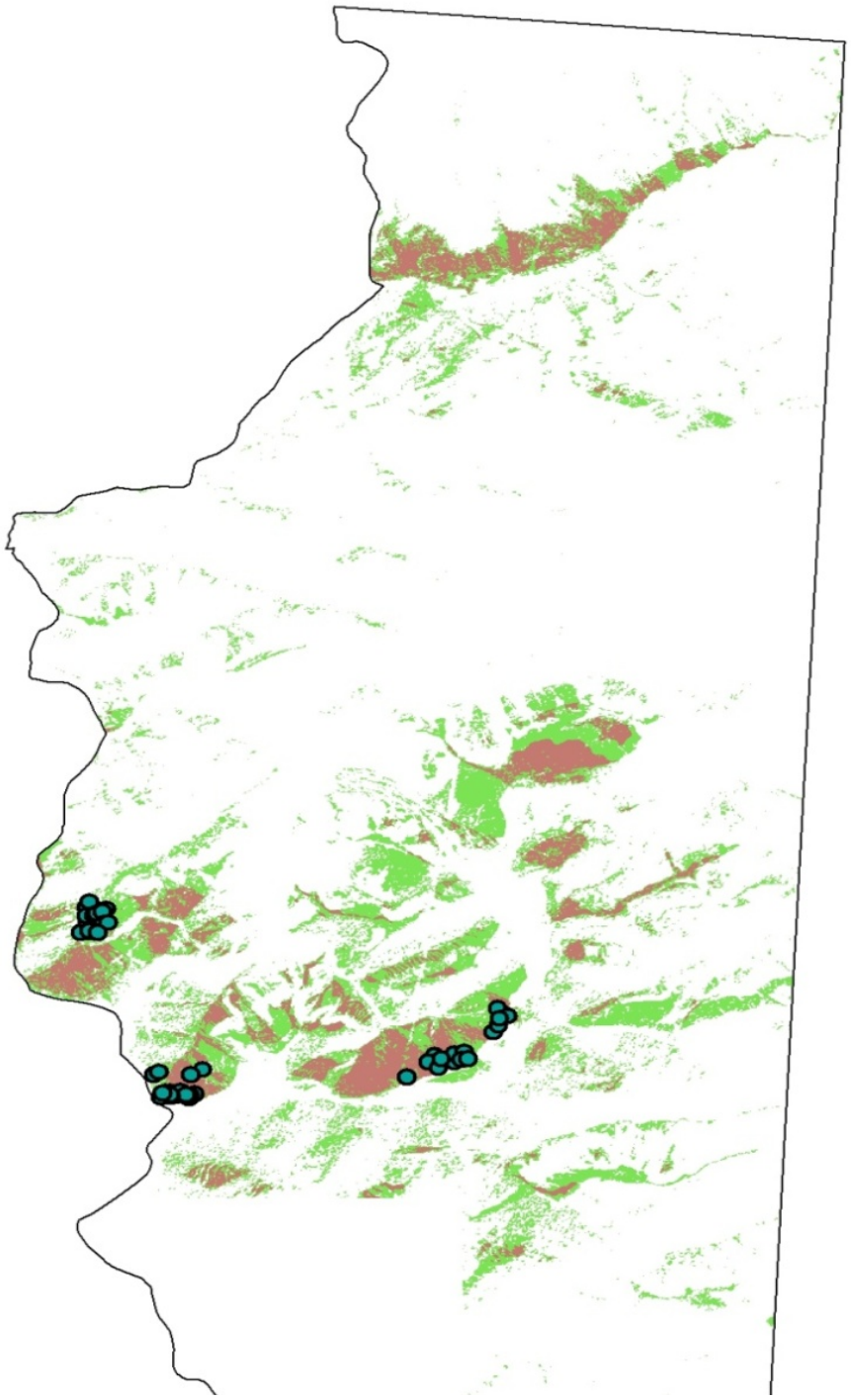

Figure 12. Areas thought to be the most suitable habitat based on elevation, slope, aspect, spectral signature, soil types, and tree height data of *known activity* plots. The areas shaded green have all six of the designated parameters while the areas shaded brown have some combination of five out of six parameters. Circles shaded green are locations of vegetation survey plots.

MOLECULAR ANALYSIS

The PowerSoil™ DNA Isolation Kit and procedures (MO BIO Laboratories, Inc.) resulted in successful extraction of DNA from *Glaucomys sabrinus coloratus* fecal pellets. PCR performed on extracted DNA of all samples yielded amplification for bacteria, four times for cyanobacteria, and eight times for fungi (Table 5). Due to the sensitivity of detection that occurs with nested PCR, fungal amplification was often more pronounced in the second round of PCR versus the first. It is important to note that cyanobacteria amplified just as well, perhaps better, than the fungi, but due to the lack of nested DGGE PCR primers, this group was not analyzed further.

The DGGE gel showed distinct bands among all fecal samples as well as for the lichen sample, which acted as a control. Thirteen bands with the most intense signal were excised for sequencing (Figure 13). Sequencing results showed that some sequences were represented by multiple bands: 3A, 3B, and 5A; 8A, 8B, 6A, and 6B; 7A and 7B.

Fungal sequences from the fecal pellets were analyzed utilizing the Genbank database search tool Basic Local Alignment Search Tool (BLAST) and yielded several *Trametes sp.* and *Ganoderma sp*. matches (Table 6). Of the twelve sequence matches yielding phylogenetic information at the level of genus, five were basidiomycetes and seven were ascomycetes.

Table 5. PCR product amplification presence (+) or absence (-) for 16S rDNA from cyanobacterica and bacteria and the ITS region from fungi for fecal samples collected from flying squirrel next boxes (Fungal Nested I = first round of PCR and Fungal Nested II = products of first round targeted with internal primers in a second round of PCR).

Sample Name	Bacteria	Cyanobacteria	Fungal Nested I	Fungal Nested II
1874	+	-	-	+
2017	+	-	-	+
2019	+	+	-	+
2020	+	+	-	+
2021	+	-	+	+
2022	+	-	-	-
2023	+	+	+	+
2024	+	+	-	-
2025	+	-	+	+
Lichen	+	-	+	+

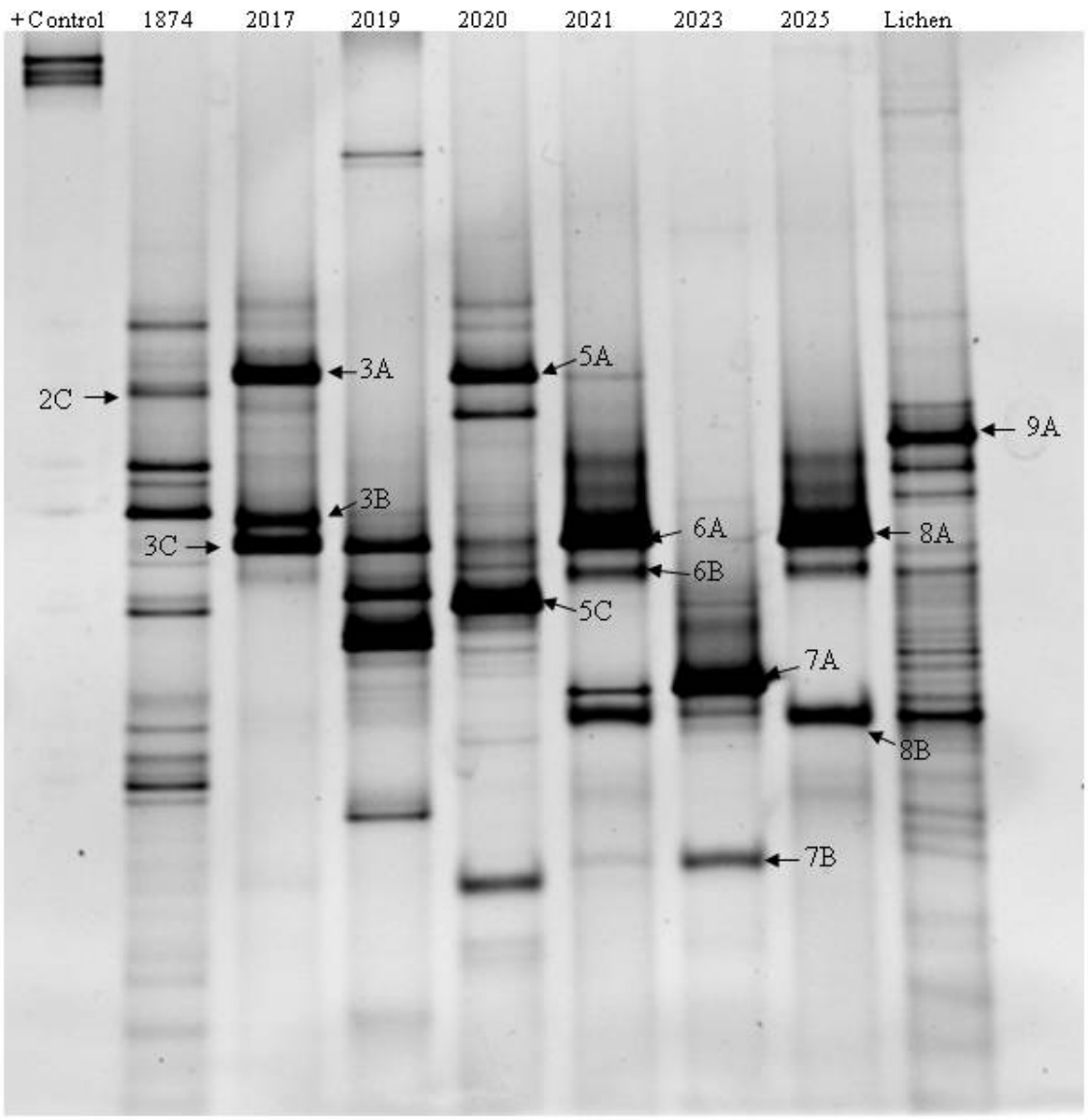

Figure 13. Denaturing gradient gel electrophoresis (DGGE) banding pattern for PCR products of the ITS region from fungi in *Glaucomys sabrinus coloratus* fecal pellets. Labeled bands were those that yielded DNA sequences (as reported in Table 5). All fecal samples were collected in January except those from squirrel 1874 (July) and squirrel 2025 (March).

Table 6. Percent similarity of sequenced *G. s. coloratus* fecal samples to top BLAST hits. Results with the highest similarity as well as relevance are shown. Sequence names are named in the format "ear tag_DGGE band".

Sequence Name	**Top Cultured BLAST Hits**		**Top Uncultured BLAST Hits**		**Accession #**
1874_2C	*Gandoderma applanatum*	100%	Uncultured soil fungus clone	100%	GQ388264.1
	Trametes sp.	100%	*Ganoderma applanatum*	100%	
	Ganoderma lipsiense isolate	100%	*Trametes sp.*	100%	
2017_3A/B; 2020_5A	*Trichosporon pullulans*	100%	Uncultured fungus clone	100%	GQ388265.1; GQ388267.1
	Guehomyces pullulans	100%	Uncultured eukaryote clone	100%	
2017_3C	*Ganoderma sp.*	92%	*Ganoderma sp.*	92%	GQ388266.1
	Dichomitus squalens	92%	Uncultured soil fungus clone	92%	
2020_5C	*Cladosporium sp.*	100%	*Cladosporium* sp.	100%	GQ388268.1
	Dothideomycete sp.	100%	Uncultured fungus clone	100%	
2025_8A/B; 2021_6A/B	*Ganoderma sp.*	92%	Uncultured soil fungus clone	92%	GQ388270.1
	Dichomitus squalens	92%	Uncultured soil fungus clone	92%	
2023_7A/B	*Sigmoidea sp.*	100%	Uncultured soil fungus clone	100%	GQ388269.1
	Hypocreales sp.	100%	*Nectria mauritiicola*	100%	
	Acremonium sp.	100%	*Sigmoidea sp.*	100%	
Lichen_9A	*Capronia sp.*	96%	*Capronia sp.*	96%	GQ411538.1
	Ascomycota sp.	90%	Uncultured fungus clone	90%	

DISCUSSION

Collective results from the vegetation surveys, GIS-based landscape analysis, and molecular techniques indicate *Glaucomys sabrinus coloratus* forages on common fungi, utilizes habitat patches that are similar to nearby areas along the Cherohala Skyway, and may persist in hardwood forest as the hemlocks in its habitat die.

Diet:

Comparison of fungal nucleotide sequences in *G. s. coloratus* scat to sequences within the BLAST database resulted in several 100% similarity matches to various ascomycete and basidiomycete fungi, primarily *Trametes* and *Ganoderma* (both basidiomycetes). Most fungi with large fruiting bodies such as mushrooms, polypores, puffballs, boletes, and chanterelles belong to the Phylum Basidiomycota (Stephenson, 2010). *Ganoderma tsugae* is a shelf fungus commonly found on *Tsuga canadensis* (eastern hemlock), a component of *G. s. coloratus* habitat (Kuo, 2004). This epigeous fungus may occur at Whigg Branch due to the presence of *Tsuga canadensis* at this site. The results of the DNA analysis suggest further research into this fungus and its possible role in the diet of *G. s. coloratus* may be warranted. *Trametes versicolor* is a common North American fungus often found on decaying wood and occasionally on conifers (Kuo, 2005).

Morels and truffles are examples of fungi with conspicuous fruiting bodies belonging to the Phylum Ascomycota (Stephenson, 2010). Truffles are the fruiting bodies of hypogeous, mycorrhizal fungi and have been documented as a component of the diet of *G. sabrinus* (Carey et al., 1999; Maser et al. 1978; Pyare and Longland, 2001).

These fungi form sporocarps that mature underground and as the squirrels ingest the hypogeous fungi, they in turn ingest the sporocarps. The fungal spores are then deposited elsewhere along the forest floor via the squirrel scat, providing fungal inoculum of tree seedlings whose seeds were previously dispersed elsewhere by animals (Maser et al., 1978). By foraging on hypogeous fungi, the squirrels aid in the perpetuation, restoration, and regeneration of forest communities. Truffle sampling (Loeb et al., 2000) at ten northern flying squirrel sites in North Carolina revealed *Elaphomyces granulatus* as the most common species. Bird and McCleneghan (2005) sampled hypogeous fungi in *G. s. coloratus* habitat on Roan Mountain and found *Elaphomyces muricatus* to be the most common species. My BLAST results did not reveal any matches to *Elaphomyces* species because *Elaphomyces muricatus* and *granulatus* sequences are not currently in the BLAST database. We should therefore not rule out the possibility of *granulatus* or *muricatus* as components of *G. s. coloratus* diet. Further, all except one of our *G. s. coloratus* scat samples were retrieved from early and late winter foraging squirrels (January and March). Research supporting underground foraging in winter by *G. sabrinus* is limited, but matches to sequences within the Phylum Ascomycota suggest that it is a possibility for the Cherohala population.

Habitat:

At the landscape scale, a large patch of potential *G. s. coloratus* habitat occurs at Stratton Bald, to the north of Whigg Branch, Hooper Bald, and Big Junction. At 7,140 meters (the average distance from the three sites to Stratton Bald), this patch exceeds the squirrels' travel distance. Although it is unlikely the squirrels at the three study sites are travelling to and from Stratton Bald, another population of *G. s. coloratus* may occur

there and future surveys for *G. s. coloratus* might concentrate on this area. Another area of potential habitat, composed of smaller patches grouped relatively close together, occurs to the north-east of the three sites, much closer than Stratton Bald and within the three to fifteen hectare home range postulated by Weigl (2007). These results suggest that at the microhabitat level, *G. s. coloratus* habitat is relatively uniform and unfragmented. When considering the larger area surrounding the Cherohala Skyway, however, some fragmentation exists between known suitable habitat and Stratton Bald to the north. These smaller patches may be acting as "stepping stones" to larger, more suitable habitats as demonstrated by British red squirrels (Hale et al., 2001). Furthermore, Hale et al. (2001) suggest rapid gene flow with the occurrence of stepping stones in a fragmented landscape. According to Wilson (2003), northern flying squirrels in the Pacific Northwest "...residing in a highly fragmented forest had markedly lower genetic variation, a reduced gene pool, and less variety in genetic form than did populations within relatively continuous forests with few physical barriers to squirrel movement." If these potential *G. s. coloratus* habitat patches are indeed acting as stepping stones, we may see an effect on the genetic structure of this population.

In the southern Appalachians, the squirrels more commonly reside in mixed hardwood, spruce-fir forests (Weigl, 2007). Along the Cherohala Skyway, however, *G. s. coloratus* habitat varies from northern hardwoods to hemlock forest. *Betula alleghaniensis* and *Fagus grandifolia*, both components of northern hardwood forest (North Carolina Wildlife Resources Commission), are dominant species at Big Junction and Hooper Bald. Whigg Branch differs from Big Junction and Hooper Bald in its considerable *Tsuga canadensis* component. *Glaucomys sabrinus coloratus* habitat

surrounding the Cherohala Skyway is unique in that the conifer component is hemlock rather than spruce or fir. Results from the vegetation surveys suggest that as the hemlocks die due to the devastating effects of the hemlock wooly adelgid, *G. s. coloratus* might not be affected and the possibility of increased food resources exists (fungal bloom) with the increased decaying matter. When assessing the effect of *T. canadensis* loss on *G. s. coloratus*, however, one must also consider the genetic implications of expansion by *G. volans* into this area due to climate change and conifer loss. On the one hand, competition for resources (den sites, food types) between the two species may occur due to their similar resource requirements resulting in the decline of *G. s. coloratus* (Weigl, 1978 and 1999). Contrarily, Bowman et al. (2005) saw limited competition and in fact, documented hybridization between the two species in Pennsylvania and Ontario.

Hooper Bald had the least amount of canopy cover when compared to Big Junction and Whigg Branch. Hooper Bald also had the highest litter depth compared to the other sites. *Known activity* plots tended to have a more closed canopy and shallower litter depth than *random* plots. These results suggest *G. s. coloratus* prefer relatively dense canopy with low litter depth. Presumably, dense canopies can provide some level of protection from both predators and harsh weather elements while also providing optimal conditions (cool and wet) for fungal growth. The squirrels may prefer a shallow leaf litter depth for ease of foraging ability. Moisture content of litter, however, is also likely reduced with decreasing depth. Since fungal growth is dependent upon moist conditions, the seemingly preferred shallow litter depth may actually be in response to some other factor contributing to low litter depth in the microhabitat.

Understory species, including herbs, varied among all three sites. Dominant species at Big Junction and Hooper Bald included various grasses, *Drypoteris intermedia* (intermediate woodfern), *Eurybia divaricata* (white wood aster), and *Viburnum sp*. Dominant species at Whigg Branch were *Rhododendron sp*. and *Eurybia divaricata*. These results are indicators of *G. s. coloratus* habitat and must be viewed in conjunction with other factors such as structural composition of the forest, amount of herbaceous cover, conditions promoting fungal growth, and interactions between *G. s. coloratus* and *G. volans* (Weigl, 1999). Open midstories that facilitate gliding and sufficient herbaceous ground cover may be more important than understory/herbaceous species composition (Weigl et al., 1999; Smith, 2007; Hackett and Pagels, 2003).

Den site availability seems to be much more important to the smaller *G. volans* than to the hardier *G. s. coloratus*. *G. volans* tends to use tree cavities most often, probably for increased protection from predators and extreme weather elements they are ill prepared for (Bendel and Gates, 1987). *G. s. coloratus* will use cavities as well as dreys (outside nests in the branches of trees) in both hardwoods and conifers. This is one reason why *G. volans* is almost always associated with hardwood forests while *G. s. coloratus* is found utilizing both northern hardwood and conifer forests. In a food habits analysis of *G. volans* performed by Harlow and Doyle (1990), acorns were identified as the most prevalent food item. Other food items identified included pine seeds, insect matter, hickory seeds, and most notably when regarding *G. s. coloratus*, American holly fruit, tree moss, fungal spores, and blueberry fruit. These last four items were found in areas surrounding the Cherohala Skyway during my vegetation sampling. Fortunately for *G. s. coloratus*, these species do not occur in large numbers in this area.

Conclusions and Future Work:

At the largest scale, suitable *Glaucomys sabrinus coloratus* habitat along the Cherohala Skyway is fragmented. At the smallest scale, forest habitat surrounding Whigg Branch, Big Junction, and Hooper Bald is relatively uniform and unfragmented. Small potential habitat patches scattered throughout larger areas of potential habitat could provide stepping stones for movement and increase gene flow. Large-scale connectivity could be enhanced by future forest management techniques including the introduction of spruce trees along wide roadside areas along the Cherohala Skyway.

G. s. coloratus habitat ranges from northern hardwood forest with little conifer component at Hooper Bald and Big Junction to eastern hemlock dominated forest at Whigg Branch, suggesting they may not be directly affected by loss of *T. canadensis*. Effects of the encroachment by *G. volans* into a pure northern hardwood stand, however, must still be considered.

Glaucomys sabrinus coloratus forage on common fungi that might include fungi associated with *T. canadensis* and evidence suggests the squirrels might forage underground during the winter. I recommend soil and hypogeal fungi sampling be performed at both *G. s. coloratus* activity areas and random areas, particularly in areas deemed unsuitable for squirrel persistence. To my knowledge, the only diet analyses performed in this area have been visually/microscopically. It would be interesting to determine the hypogeal fungal species available for consumption using molecular techniques.

The landscape analyses performed here have revealed an area to the north of Whigg Branch, Big Junction, and Hooper Bald (Stratton Bald) of suitable *G. s. coloratus*

habitat that, to my knowledge, has not been surveyed. Trapping efforts should commence at this site to determine if another population does indeed exist in this area. Radio telemetry should also be performed to determine movement patterns of the squirrels and whether they cross into the unsuitable habitat delineated by the landscape analyses.

Assuming there is another population of *G. s. coloratus* residing at Stratton Bald, genetic testing could determine the relatedness of that population to the population residing at Whigg Branch, Big Junction, and Hooper Bald. Additionally, with the recent findings of hybridization between *G. volans* and *G. sabrinus* by Garroway et al. (2010), it would be interesting to determine the relatedness of any *Glaucomys volans* found in the Cherohala Skyway area to the *G. s. coloratus* population that resides there.

In 2008, the North Carolina Wildlife Resources Commission, along with Duke Energy, installed three sets of crossing structures along the Cherohala Skyway. Cameras mounted at the tops of these structures have documented their use by *G. s. coloratus*. These structures have effectively created a corridor connecting habitat on the north side of the Cherohala Skyway to that on the southern side. Genomic DNA analysis of *G. s. coloratus* immediately following crossing structure installation versus that several years from now would be interesting to explore. The crossing structures in effect, serve as another "stepping stone" between larger, more permanent *G. s. coloratus* habitat. The potential exists for very rapid population gene flow surrounding the Cherohala Skyway.

Glaucomys sabrinus coloratus is an endangered species that plays an important role in its forest community by its contribution to the perpetuation of the surrounding forest. The northern flying squirrel of the Pacific Northwest is thought to indicate overall

ecosystem productivity due to their diet of nuts, fruits, and fungi, their existence as a prey item, and their ability to inhabit a variety of forest habitats (Wilson, 2003). When continuing research of *G. s. coloratus*, we must look at this community as a whole, as a sum of its many parts as opposed to its individual components.

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