Southern Flying Squirrel (*Glaucomys volans*) Den Site Selection and Genetic Relatedness of Summer Nesting Groups

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ABSTRACT

Southern flying squirrels (SFS) (*Glaucomys volans*) were trapped and radio collared from late May to August, 2011 at Pierce Cedar Creek Institute in south-central Michigan. Den site characteristics and genetic relatedness were investigated to gain further understanding of factors affecting habitat selection. SFS (n=15) were found to select for trees with larger dbh ($P<0.001$), lower autumn olive (*Elaeagnus umbellata*) presence ($P=0.012$), red oaks (*Quercus rubra*) ($\chi^2=34.25$, $0.025>P>0.010$), and snags ($\chi^2=27.20$, $P<0.05$). Seven SFS primers were amplified and sequenced to successfully identify all individuals and allow for calculation of pairwise relatedness. Two putative family groups (n=3, n=4), were identified within the sample, but overall relatedness was low ($r=0.036$, 95% CI: ± 0.068). SFS were not found to nest preferentially with relatives ($P=0.095$), which indicates social and mating factors may play a larger role in summer nest selection for this species. Our findings can be applied to better manage for SFS habitat, and contribute to a greater understanding of the influence of genetic relatedness on habitat use and communal nesting.

INTRODUCTION

The southern flying squirrel (*Glaucomys volans*, hereafter SFS) is a nocturnal, cavity-nesting resident of forested habitat in most of the eastern United States. Because flying squirrels spend their days in dens and are not hunted, less is known about their ecology than other squirrel species. Most previous work on SFS has been performed in the winter, and no studies examining den tree characteristics in south central Michigan have been found. Few studies have investigated the genetic relatedness of SFS nesting groups (Thorington *et al.* 2010, Thorington *et al.* 2011, Winterrowd *et al.* 2005), and we found no studies focusing on relatedness during the summer months.

It is known that SFS form aggregations during the winter for thermoregulatory benefits (Holloway and Malcolm 2007, Thorington *et al.* 2010). In the summer, there is no longer a thermoregulatory need for nesting together, yet occasionally squirrels are found in the same den. Few studies have been found on communal nesting in SFS during the warmer months, but one study by Layne and Raymond (1994) suggested aggregations in Florida may have an additional role in social organization in the species. Studies on SFS during winter have shown aggregations can be composed both of highly related individuals (Thorington *et al*. 2010) and of unrelated individuals (Winterrowd *et al*. 2005), so this topic warrants further research. It is unclear if only closely related SFS are nesting together, or if nest sharing is common across the population even in the summer months.

There are two peak breeding seasons for SFS, spring and summer, and it is common for females to produce litters in each season (Stapp and Mautz 1991). Squirrels breed from 11 February to 26 March and from 19 June to 8 July (Stapp and Mautz 1991). Litters produced in the spring are born between 22 March and 5 May, after a gestation of approximately 40 days, with average litter size of 2-3 pups (Stapp and Mautz 1991, Dolan and Carter 1977). Pups remain in the den where they were born until the weaning period begins, at approximately five weeks of age, and weaning is completed between seven and eight weeks of age (Linzey and Linzey 1979, Stapp and Mautz 1991). Thus, there is only a small period of time when a mother and pup could theoretically both be captured while still sharing the pup's natal nest, a guaranteed instance of a family group nesting together.

The appropriate habitat must be present to support a flying squirrel population. Favorable SFS habitat includes large, mature deciduous trees, snags, an open upper-understory, and sufficient cover to protect from aerial predators (Bendel and Gates 1987, Holloway and Malcolm 2007, Sawyer and Rose 1985, Taulman *et al.* 1998, Weigl 1978). Large, mature deciduous trees have a pattern of decay that is favorable to cavity formation and mast production, an important food resource to SFS (Bendel and Gates 1987, Holloway and Malcolm 2007). Snags are an important habitat characteristic because they provide plentiful natural cavities that can be used as nest sites and food storage areas since SFS are unable to excavate their own cavities (Bendel and Gates 1987, Holloway and Malcolm 2007, Taulman *et al*. 1998, Sawyer and Rose 1985). An open upper understory is important to allow for gliding from tree to tree (Bendel and Gates 1987) and there must be sufficient cover free of large clearings or edges, because SFS are slow to escape when on the ground, and open areas give no protection and few escape routes against aerial predators (Bendel and Gates 1987).

While the general habitat requirements of SFS are known, we found no research that looked specifically at the effects of invasive woody species on the species. Autumn olive (*Elaeagnus umbellata*) is an invasive shrub common to the Cedar Creek area. Due to its dense growth, it has the potential to invade the upper understory of the forest structure thus creating unsuitable SFS habitat. In contrast, the presence of autumn olive could benefit SFS by providing necessary cover in previously open areas, or provide an additional food resource in the spring and summer months.

Our main objectives for this research were to gain knowledge on SFS den site selection, and on the role of genetic relatedness in influencing SFS nest selection during the summer. We accomplished these goals by investigating SFS habitat features, nest tree characteristics, the presence of autumn olive around den trees, and the genetic relatedness of the SFS population. The results of this research are applicable in managing for habitat of the SFS.

STUDY SITE

Our study area was primarily located in the northwest parcel of the Pierce Cedar Creek Institute in south-central Barry County, Michigan. The plot was mixed deciduous forest

dominated by black cherry (*Prunus serotina*), red maple (*Acer rubrum*), American elm (*Ulmus americana*), red oak (*Quercus rubra*), sugar maple (*Acer saccharum*), white ash (*Fraxinus americana*), and green ash (*Fraxinus pennsylvanica*). Less dominant species included beech (*Fagus grandifolia*), pignut hickory (*Carya glabra*), black walnut (*Juglans nigra*), shagbark hickory (*Carya ovata*), and black ash (*Fraxinus nigra*). The majority of our study site was bordered by Pierce Cedar Creek Institutes's Red Trail and Brewster Lake (Figure 1).

METHODS

Sampling Area

We established five trapping transects and one additional trapping station in the study area (Figure 1). Within each transect, we positioned each trapping station approximately 25 m apart, each containing two live traps (7.6 cm x 7.6 cm x 25.4 cm, H.B. Sherman Traps, Tallahassee, FL). We left a minimum of 50 m between parallel transects. Our trapping station not within a transect contained six live traps in a cluster around a tree known to contain flying squirrels. We had no more than 118 traps set out at any time.

We attached traps to tree trunks at chest height in a horizontal orientation using 17 gauge steel wire. We measured trees with traps for diameter at breast height (dbh), identified trees to species, and classified the tree's decay level. We assessed decay using a scale from 1 to 5, with 1 being a snag (completely dead) and 5 being completely healthy. This scale was adapted from a method described by Holloway and Malcolm (2007). Their scale involved three levels of decay including healthy (intact canopy), declining ($\geq$50% canopy showing dieback), and dead (snag). Upon observing our trap and den trees we found the need to have a more detailed scale. With the 1 to 5 scale we were capable of having three levels of decline with level five indicating 0% dieback, level 4 indicating 1-25% canopy dieback, level 3 indicating 25-50% canopy dieback, level 2 indicating 50-75% canopy dieback, and level 1 indicating $\geq$75% dieback.

Small Mammal Sampling

We trapped from May 23rd through July 31st, 2011. From May 23rd through June 4th, traps were set just before dusk and checked approximately four hours later (approximately 2000 and 2400, respectively), a method to discourage disturbance by raccoons. After four nights of

trapping this way with little success and virtually no disturbance, we began checking traps at sunrise (approximate 0630). We baited traps with a mixture of peanut butter and rolled oats formed into a ball. We recorded the location for all small mammal captures (i.e. transect, station, and trap number) and all non-target species were immediately released.

Southern Flying Squirrel Processing

We processed all southern flying squirrel captures in Pierce Cedar Creek Institute's Research Laboratory. To ensure safety of the animal and researcher during processing we anesthetized all captures using Isofluorane. To facilitate this, we constructed an anesthetization chamber using a large plastic jar with cotton balls secured to the underside of the lid with chicken wire. We wet the cotton balls on the lid with a small amount of Isofluorane, then placed the squirrel in the jar and replaced the lid. We kept squirrels in the anesthetization chamber only long enough to calm them for safe handling. Because each squirrel reacted to anesthetization slightly differently depending on weight and stress level, some individuals required more Isofluorane initially or more frequent re-anesthetization throughout processing.

All squirrels were first fitted with a standard metal ear tag with a unique number (Monel 1005-1, National Band & Tag Company, Newport KY) as a form of individual identification in case of radio collar loss or removal. After ear tagging, we equipped each squirrel with a radio collar (Holohil Systems Ltd., Ontario, Canada, Model BD-2C, 2.1g ~2.75 g with attachment hardware). Radio collars were attached using a zip tie encased in a section of plastic tubing (~25 mm) to protect the back of the squirrel's neck from irritation. We did not collar squirrels weighing less than 55 g to avoid having the collar exceed 5% of the animal's body weight. Following radio collar fitting, we took a small ear punch to serve as a tissue sample for genetic testing. Ear punches were stored in Eppendorf tubes in a -20^{o}C freezer in the research laboratory until time of DNA extraction. After completion of all processing, we returned squirrels to their capture site and released them.

All capturing and handling of animals during the course of this study followed the guidelines established by the American Society of Mammalogists (Sikes *et al*. 2007), and was approved by the Grand Valley State University Animal Care and Use Committee (Project No. 10-02-A)

Den-Site Location

We located radio collared SFS using a Very High Frequency (VHF) receiver (Advanced Telemetry Systems, Isanti, MN, Model R410) and three-element Yagi antenna. Telemetry began after the capture of our first flying squirrel in late May and ran through the first week of August (~10 weeks). We located squirrels during the day to assess den site characteristics.

Upon locating a den we recorded the den tree species, size, location, decay level, and autumn olive presence. We measured dbh in centimeters and recorded den site location in Universal Transverse Mercator (UTM) using a handheld GPS. We measured autumn olive presence by pacing out a 10 m x 10 m quadrat around each den tree and counting the number of autumn olive stems within that quadrat, a method we adopted from Flory and Clay (2006). We recorded decay level using the method detailed for trap-tree measurements. After measurements were completed, we flagged and numbered each den tree for individual identification.

Forest Composition Measurements

In order to allow for comparison between the available types of trees and the trees squirrels selected for den sites, we collected a random sample of 100 trees throughout the study site. We paced to our first random tree starting from a known den tree to be sure we were in suitable SFS habitat. We used a random number generator to choose a compass bearing and a distance between 10 and 100 m, then paced to that location and picked the nearest tree larger than 10.2 cm (4.0 in) in dbh. We reasoned that a squirrel could not find a suitable nest in a tree any smaller than this. For sampling of subsequent trees, we did not return to the den tree, rather we paced from the last tree sampled.

If the direction and distance given led us out of suitable habitat (i.e. prairie, a road, body of water, or other area void of trees) we returned to the last tree measured and picked a new random bearing and distance. To ensure that we did not resample any trees, and that we covered our entire study area, we measured 25 trees a day and started at a den tree located in a different section of our study area each day over a four-day period. We performed the same measurements on all random trees as we did on all den trees.

DNA Extraction & Amplification

We performed DNA extraction of the tissue from each squirrel in a research lab at our home institution, Grand Valley State University (Allendale, MI) using a QIAGEN DNeasy Blood and Tissue Kit (QIAGEN Sciences, MD), and we followed the guidelines of the bench protocol for animal tissues (July, 2006). To ensure our DNA extraction was successful, we ran a portion of the products on a 1% polyacrylamide gel, stained using Ethidium Bromide (EtBr), and photographed the gel using Carestream Molecular Imaging Software (Standard Edition, V. 5.0.7.22, Carestream Health INC).

Following DNA extraction, we performed polymerase chain reaction (PCR) in 20 μL reactions with final concentrations as follows: 10X Colorless Go Taq reaction buffer with 1.5mM $MgCl_2$, 0.25 mM dNTP mix, 0.1 μM forward and reverse primers, 0.1 μM M13 Primer labeled with FAM, VIC, NED, or PET, 0.35 units Go Taq DNA polymerase, and 10% diluted genomic DNA. We amplified seven SFS primers (SFS-02, 03, 04, 05, 07, 14, 15), and two northern flying squirrel (*Glaucomys sabrinus*) primers (GS-08, 10) (Fokidis *et al.* 2003) and then visualized amplified products using a 2% gel and 6x GelRed loading dye as opposed to EtBr.

Genetic Analysis

We ran amplified products on the genetic sequencer (3130xl Genetic Analyzer, Applied Biosystems, Inc.) located at the Annis Water Resource Institute (Muskegon, MI). Following sequencing, we scored alleles using PeakScanner (ABI, Inc.), which allowed us to assign genotypes to individuals for each locus. We used the program Relatedness 5.0.8 (Goodnight Software) to calculate the average coefficient of relatedness (R) among individuals in the population, and also among individuals found nesting in the same tree. We then used Kinship 1.1 (Goodnight Software) to sort samples into putative family groups. This is done by examining the pairwise genetic relatedness between individuals, and calculating the likelihood that two individuals fit into a hypothesized relationship. For our SFS sample we used Kinship to calculate the likelihood that individuals were unrelated or full siblings (R=0.5), unrelated or parent/offspring (R=1), and unrelated or aunt/niece (R=0.25). Genepop on the Web (version 4.0.10) was used to calculate expected heterozygosity and to determine if our loci were in Hardy-Weinberg equilibrium.

Data Analysis

We performed statistical tests using SPSS 17.1 and Program R with an alpha value of 0.05 representing significance. We calculated the mean number of consecutive days all squirrels remained in a den tree and then used a Student's t-test to determine if this number differed significantly between males and females. To compare characteristics of the general forest composition to the SFS den trees, we used t-tests to compare the size (dbh) and autumn olive prevalence between den and random trees and we used Chi-square tests to look for random or nonrandom selection for decay level and den tree species. We also used a Chi-square test to determine if squirrels were captured more frequently on any particular tree species. We ran three ANOVA tests with Tukey's and LSD Post-Hoc tests to compare tree size to species, tree decay level to species, and tree size to decay level. We used a Mantel test to compare matrices for pairwise relatedness and time spent nesting together to determine if squirrels displayed nonrandom selection for nest mates based on level of relatedness.

RESULTS

Southern Flying Squirrel Captures

We captured a total of 15 squirrels (7 males & 8 females) over 2,675 trap nights resulting in a trapping success of 1.27%. Of the 15 individuals captured, 14 (7 males & 7 females) were radio collared (Table 1). We did not collar the 15th squirrel because it did not meet the weight requirement for the size of the collars. We had a maximum of 12 squirrels collared at one time. We collected a total of 266 telemetry locations with an average of 19.07 ($\pm$3.68 *SE*) locations per squirrel (range 1-48). Over the duration of the study we lost seven squirrels either due to depredation or collar loss. Three of the seven squirrels were most likely depredated as we found squirrel parts with the collar, or we recovered the whole squirrel carcass with evidence of injury. The remaining four squirrel losses could have been from collars being slipped off or from depredation, but we found no conclusive evidence for either fate.

We captured two non-target small mammal species throughout the study. White-footed mice (*Peromyscus leucopus*) made up the majority of our non-target small mammal captures

(694 captures, 33.34% trapping success), but we also captured a small number (2 captures, 0.11% trapping success) of eastern chipmunks (*Tamias striatus*).

Den Tree Characteristics

We tracked squirrels to a total of 56 den trees (Figure 2, Table 2), with an average of 5.57 ($\pm$1.06 *SE*) den trees per squirrel (range 1-13). Squirrels spent an average of 2.19 ($\pm$2.80 *SE*) days in each den, and no significant difference was found between males and females (t-test, t=-0.239, df=98 *P*=0.811). The longest recorded use of a single den tree for a squirrel was 20 consecutive days, however the squirrel likely left each night to forage.

Den trees had significantly fewer autumn olive stems surrounding their base than random trees (t-test, t=2.554, df=154, *P*=0.012, Figure 3a). We found a mean of 0.14 ($\pm$0.14 *SE*, range 0-8) autumn olive stems for den trees compared to a mean of 4.16 ($\pm$1.17 *SE,* range 0-66) for the random sample. We also found den trees to be significantly larger in size (dbh) than random trees (t-test, t=-3.639, df=162, *P*<0.001, Figure 3b). Den trees had a mean dbh of 45.2 cm ($\pm$3.64 *SE*, range 11.7-179.3 cm) compared to the mean we found for random trees of 31.2 cm ($\pm$2.02 *SE*, range 10.7-109.5 cm).

Among den trees, we found that live trees had an average dbh of 47.7 cm ($\pm$29.36 *SE*, range 11.7-179.3 cm) whereas snags had an average dbh of 32.2 cm ($\pm$19.31 *SE*, range 15.2-83.3 cm). We found nonrandom selection for decay, den tree species, and trap tree species using Chi-square analysis. Dens were more common in snags and less common in trees with level 3 decay (χ^2=27.20, *P*<0.001). In terms of tree species, there were significantly more dens in red oaks and significantly fewer dens in black cherry and green ash (χ^2=34.249, 0.025>*P*>0.010, Figure 4). Squirrels selected for red oak and white ash trap trees and avoided traps set on black walnut and red maple (χ^2=31.43, 0.05>*P*>0.001).

In order to discern if squirrels were nesting in red oaks because they happened to be the largest in size, the most decayed or for some alternative reason, we looked for correlations between tree species, size and decay. To do this we ran three ANOVAs—one comparing tree species and size, one comparing tree species and decay level, and one comparing tree size and decay level (Table 2). Our results showed red oaks were only significantly larger in size than two other tree species—American elm and black cherry ––and were not significantly different from

any other species based on decay level (Table 2). When comparing tree size to decay level including all trees sampled, no significant results were found. However, when we ran the ANOVA taking only den tree data into account we found multiple significant results. We found snags were significantly smaller in size than trees decayed at both level 3 (P=0.026) and level 4 (P=0.041). We also found that trees decayed at levels 2, 3 and 4 were all larger in size than healthy level 5 trees (P= 0.045, 0.009, 0.013 respectively).

DNA Extraction, Amplification & Analysis

We successfully extracted DNA from all of the tissue samples collected from the 15 captured SFS. We then ran PCR with all nine of the primers, seven of which amplified and genotyped successfully and were used in further genetic analysis (SFS-02, 03, 04, 07, 14, 15, GS-10). The remaining two primers (SFS-05 and GS-08) did not produce scorable, reliable peaks and were excluded from further analysis.

We identified 64 total alleles across the seven loci, with16 alleles at our most polyallelic locus (SFS-02), and 4 alleles at our least polyallelic locus (SFS-03, Relatedness 5.0.8, Goodnight Software, Table 3). With the exception of one locus (SFS-04, P=0.026), all of our loci were in Hardy-Weinberg equilibrium (χ_2=19.36, df=14, Prob.=0.152, Genepop on the Web, version 4.0.10, accessed October 12, 2011, Table 3).

Relatedness Analysis

We found the relatedness of the population to be low with an average degree of relatedness of 0.036 (95% CI: $\pm$0.068, Relatedness 5.0.8). The average degree of relatedness within nesting groups was very low (0.0144, 95% CI: $\pm$0.0466, Relatedness 5.0.8), suggesting squirrels were not nesting with close relatives. Further analysis comparing the number of days squirrels nested together to their pairwise genetic relatedness revealed a similar result, supporting that SFS nesting groups are composed primarily of unrelated individuals (P=0.095, Mantel test in Program R, 999 permutations).

Kinship 1.1 (Goodnight Software) analyses for proposed relationships using 10,000 simulated pairs resulted in two putative family groups (one group of 4, one group of 3) that were more likely related than unrelated (Table 4). For these groups the log of the ratio of the

likelihood values indicates the hypothesized relationship is more likely to be true than there being no relationship at the alpha level of 0.05 (Table 4).

DISCUSSION

Den Site Selection

In our study, SFS selected for larger trees and snags, similar to previous studies (Bendel & Gates 1987, Holloway & Malcolm 2007). Bendel and Gates (1987) had a similar average den tree dbh (42.4 cm $\pm$3.2 *SE* compared to 45.2 cm $\pm$3.6 *SE* in this study), and also found snag dbh to be smaller than live tree dbh. SFS may be selecting for snags despite having a smaller dbh because they tend to have favorable decay patterns for the creation of natural cavities (Bendel & Gates 1987, Taulman *et al*. 1998). Bendel & Gates (1987) found that larger trees provide for longer glides and a larger surface area for landing, which are two characteristics that make larger trees highly suitable for den tree use.

Red oaks were selected for regardless of size or decay. Thomas & Weigl (1998) as well as Weigl (1978) found that SFS prefer to eat acorns and store hickory nuts, therefore, it could be more favorable to den in red oaks in the summer when mast is a main food source than in the winter when the mast of hickories is favored. It would be interesting to study the same population through both summer and winter to see if there is seasonal variation in trees selected for denning. If den tree selection is based on mast production in the summer, then the selection against green ash and black cherry trees in our study could be related to unfavorable mast production as neither species was significantly smaller than any other species in the study.

Because we were unable to see individual dens we were forced to assume that squirrels were in the same den when multiple squirrels were located in the same tree on the same day. In the future, the use of nest boxes or Swedish climbing ladders could eliminate this assumption in many cases. Both Layne & Raymond (1994) and Reynolds *et al*. (2009) had year-round success with nest-boxes, however the use of nest-boxes might create unnaturally large aggregations that would not be viable in natural cavities.

Trap Tree Selection

We found that squirrels selected for traps on red oaks and white ash, but avoided traps on black walnuts and red maple. SFS are likely to frequent red oaks more than other tree species in our study area due to the favorable mast production and selection as SFS den trees. For future studies we would suggest focusing trapping efforts on red oaks and white ash as well as raising the trap height to approximately 6 m given that Risch & Brady (1996) increased SFS trapping success by placing traps between 4.5 and 8.5 m high.

Impact of Autumn Olive

We found that den trees were surrounded by significantly fewer autumn olive stems than non-den trees, indicating SFS are selecting against autumn olive presence. This result could be due to ecological factors, but it also could have been due to edge effect, a recent burn in the area, or methodological bias.

There was a potential edge effect seen in our study because previous work has found many invasive plants including autumn olive grow thickest along forest edge (Flory & Clay 2006). Our SFS may appear to avoid autumn olive, but actually avoid edge in order to remain in the forest interior to minimize contact with predators, an effect found in other small mammal species (Bendel & Gates 1987). Autumn olive density at PCCI was thickest on the forest edge due to both this edge effect and to a recent burn, which may have further confounded our results.

After choosing our study site we later discovered the forest understory had been burned on March 29th, 2010 specifically for autumn olive removal. This potentially had a large impact on our finding, as den trees may have had high autumn olive presence prior to the burn, but not after.

During our study, we discovered that our method for measuring autumn olive density was not sufficient for what we were trying to quantify. Often we found many small, young autumn olive stems, or a mature autumn olive shrub that occupied a large understory area but had only a few stems. Due to our method of counting stems for density, results showed many small stems had an inaccurately large impact, when really the mature shrub with fewer stems occupied more understory. We adapted the method from Flory and Clay (2006), who were measuring the density of invasive plants in relation to roads, so the method worked well for them, but was

insufficient for our quantification. Any future studies investigating autumn olive impact on SFS should use a different methodology that better measures the understory area shrubs occupy.

Genetic Relatedness

Our SFS sample showed very low relatedness, which could be an indicator that the population is large and we did not get a sufficient sample, or that young disperse far from natal dens thus keeping genetic diversity high (Selonen & Hanski 2009). Despite having putative family groups present, we found groups of squirrels nesting together to be composed primarily of unrelated adults ($r=0.014 \pm 0.047$), which was a similar finding by Winterrowd *et al.* (2005) ($r=0.029 \pm 0.052$), however Thorington *et al*. (2010) found squirrels often nest with relatives. SFS at PCCI may select against nesting with relatives due to seasonal differences in social behavior. Previous studies have shown that during the winter, squirrels nest communally for thermoregulatory benefits and indirect fitness gains may exist for squirrels nesting with relatives (Thorington *et al*. 2010). In contrast, during the summer squirrels may select against nesting with relatives in order to mate with non-relatives, thus increasing genetic diversity. Previous studies have also shown female SFS tend to become aggressive and territorial during the breeding season, which may further impact nesting patterns (Madden 1974). Future studies could focus on monitoring a single SFS population year round to detect changes in nesting patterns.

Management Implications

In order to manage for SFS in the future, managers should retain snags and larger trees. Red oaks should be conserved in particular, no matter the size. Autumn olive should be controlled through fire, herbicide, or physical removal.

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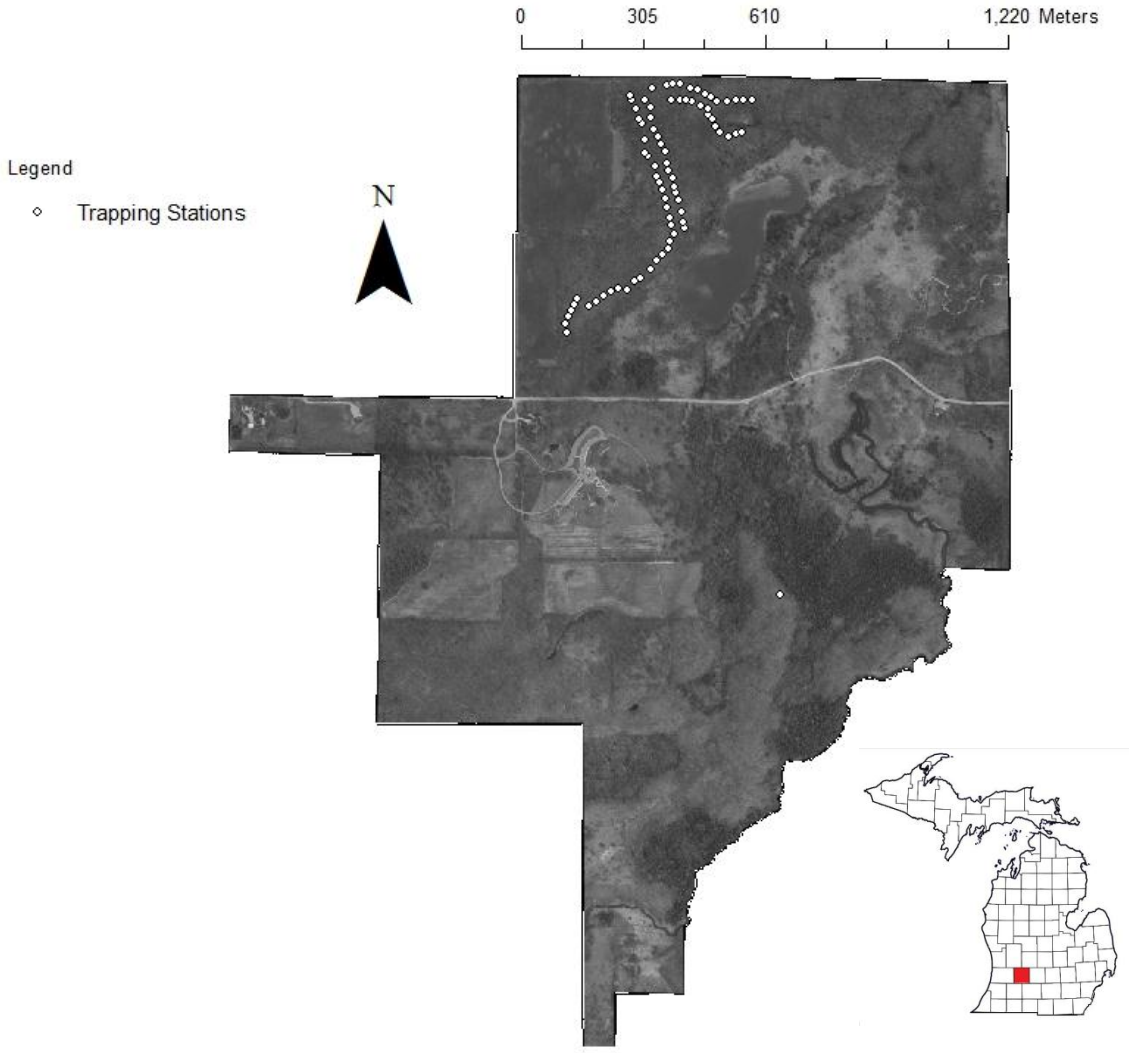

Figure 1. Aerial photo of study area with southern flying squirrel (*Glaucomys volans*) trapping stations marked. Pierce Cedar Creek Institute, Barry Co., Michigan

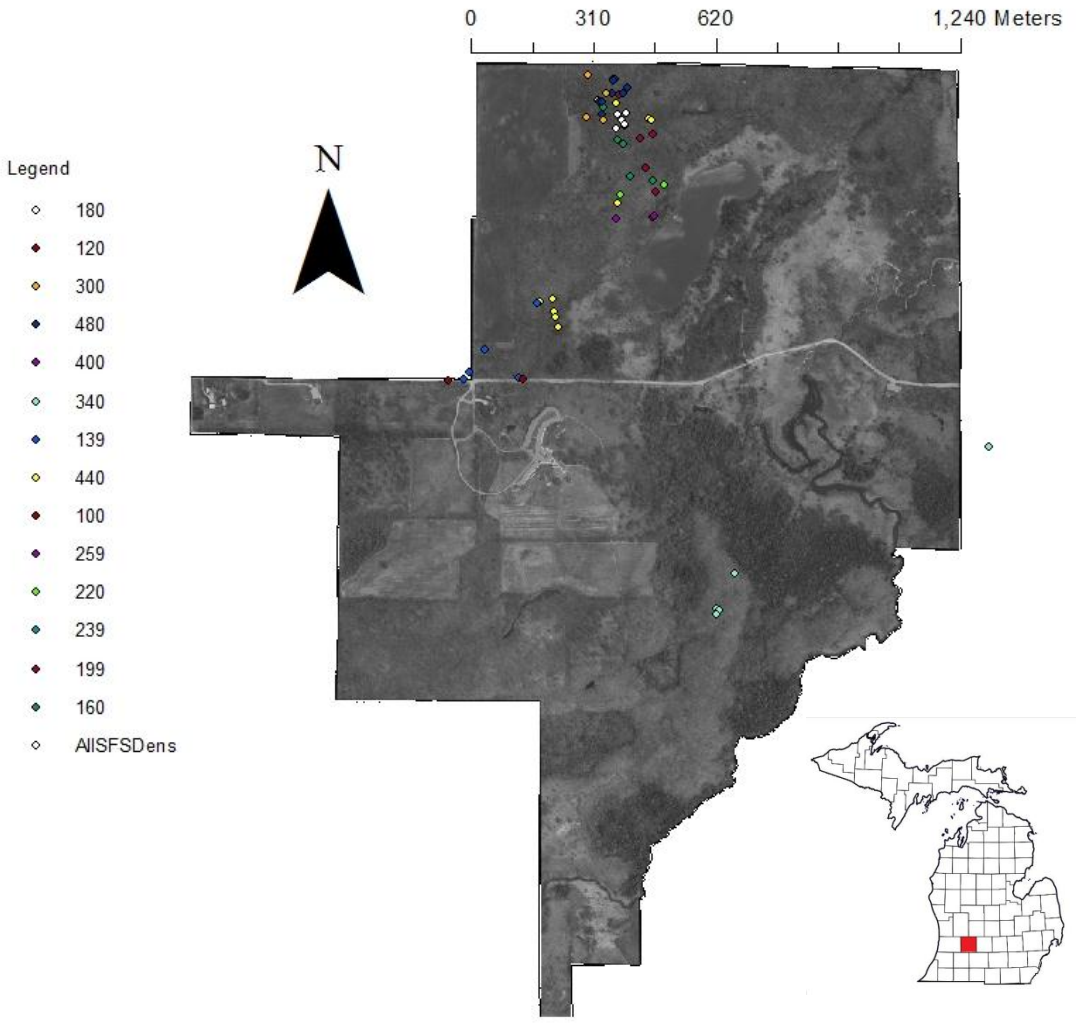

Figure 2. Locations of 14 radio collared southern flying squirrels (*Glaucomys volans*) at Pierce Cedar Creek Institute, Barry Co. Michigan.

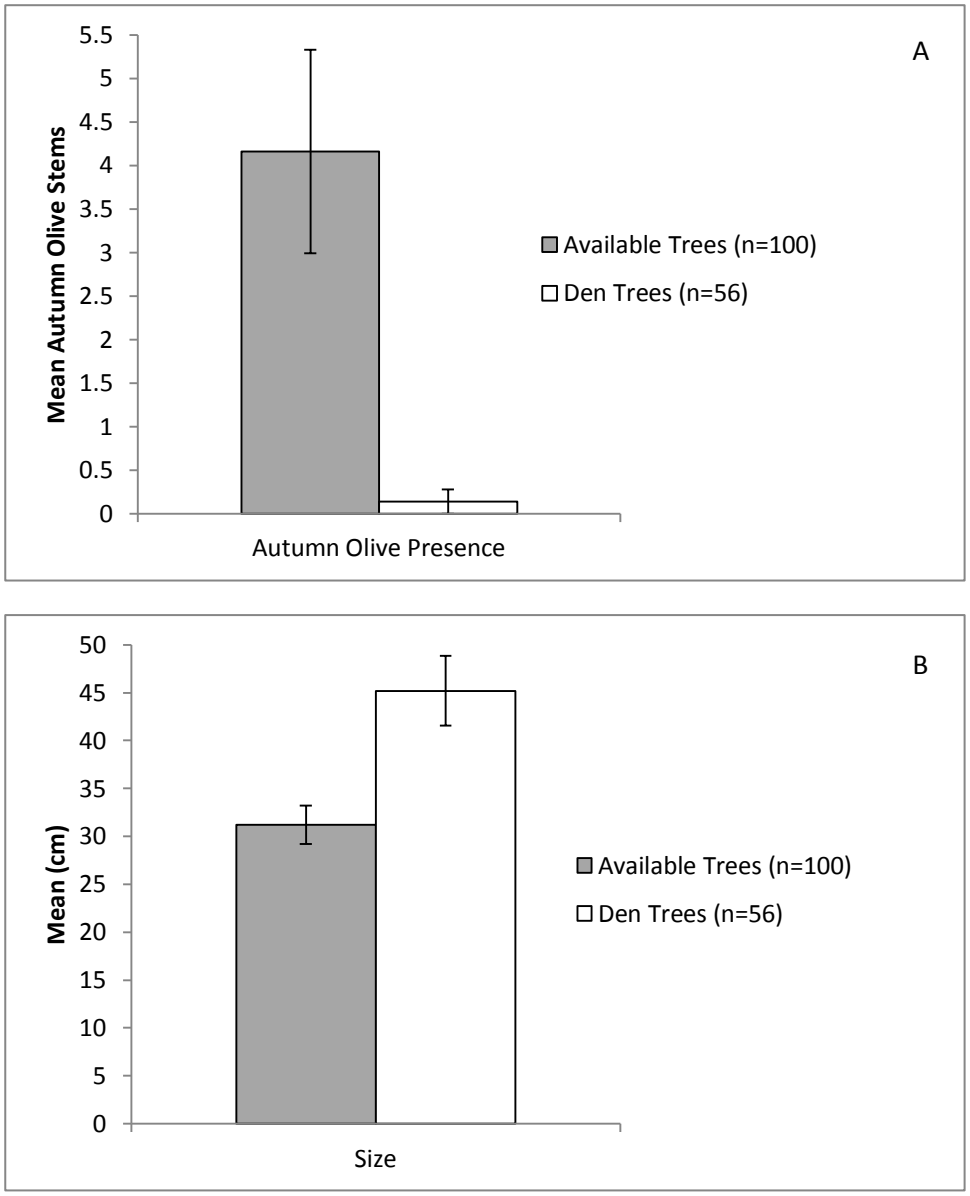

Figure 3. Comparison of mean Autumn Olive (*Elaeagnus umbellata*) presence (A) and mean size (B) for available trees and den trees selected by southern flying squirrels (*Glaucomys volans*) at Pierce Cedar Creek Institute, Barry Co., MI. Summer, 2011. Den trees selected by squirrels were found to have significantly fewer autumn olive stems in a 10 m^2 area surrounding the tree when compared to a random tree sample representing available trees (A, t-test, t=2.55, df=154, *P*=0.012). Den trees were also found to be significantly larger in size (mean dbh) than available trees (B, t-test, t=3.64, df=162, *P*<0.001)

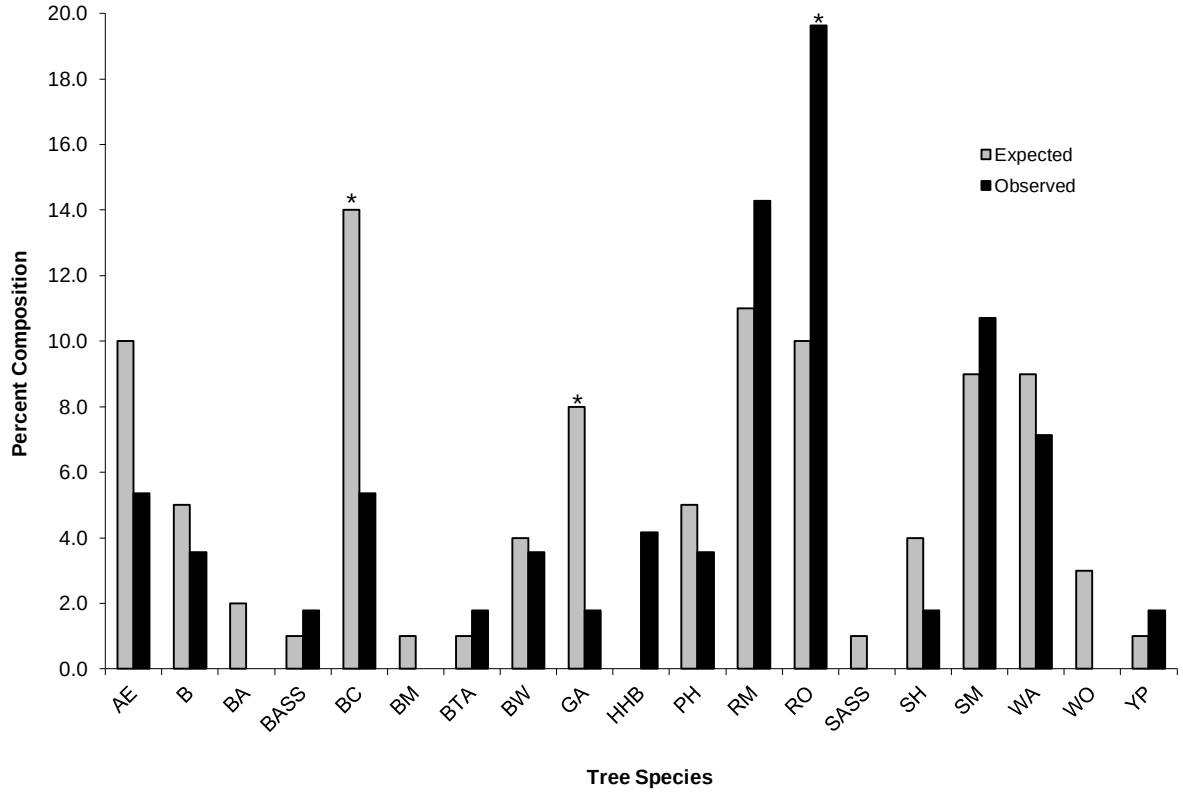

Figure 4. Den site selection based on tree species by southern flying squirrels (*Glaucomys volans*) at Pierce Cedar Creek Institute, Barry, Co., MI. Summer, 2011. Chi-square analysis of tree species selected for denning by SFS (observed) versus tree species available (expected) from a random sample. Results show non-random selection for tree species (χ^2=34.25, 0.025>*P*>0.010) with SFS selecting to den in red oaks (*Quercus rubra*), and selecting against black cherry (*Prunus serotina*) and green ash (*Fraxinus pennsylvanica*) (starred bars, based on a difference of $\geq$5%). Tree species idenfication is as follows: AE=American elm, B=beech, BA=black ash, BASS=basswood, BC=black cherry, BM=black maple, BTA=big toothed aspen, BW=black walnut, GA=green ash, HHB=hophornbeam, PH=pignut hickory, RM=red maple, RO=red oak, SASS=sassafrass, SH=shagbark hickory, SM=sugar maple, WA=white ash, WO=white oak, YP=yellow poplar. Reference Table 2 for scientific names.

Table 1. Characteristics of captured southern flying squirrels (*Glaucomys volans*) at Pierce Cedar Creek Institute, Barry Co., Michigan, Summer, 2011.

Ear Tag	Radio Collar Freq.	Sex	Avg. Mass (g)	Dates collared (# of days)	Den Trees (#)	Recaps (#)	Avg. Days/Den
862/603	160/280	M	61.0	5/24-8/6 (74)	12	3	1.96
866	199/212	F	63.4	6/7-7/7, 7/20-8/6 (47)	13	8	1.35
870	100	M	56.8	6/10-7/8 (28)	1	0	--
868	220	M	64.5	6/10-6/26 (16)	4	3	1.33
869	239	M	82.0	6/10-7/8 (28)	5	0	3.14
867	259	F	78.0	6/10-6/26 (16)	2	0	1.00
872	139	F	72.6	6/13-7/8 (25)	5	3	1.89
871	440	M	63.0	6/13-7/17 (34)	11	0	1.53
873	340	M	70.7	6/14-8/6 (55)	5	0	3.33
874	399	F	67.2	6/15-6/21 (6)	2	0	1.00
601	480	F	66.8	6/17-8/6 (52)	8	2	2.55
602	300	F	72.2	6/18-8/6 (51)	5	0	9.00
604	120	F	74.0	7/21-7/30 (9)	1	0	--
606	180	M	66.8	7/25-8/6 (14)	4	0	1.25
605*	--	F	44.7	--	--	0	--

*too small to radio collar

Table 2. Characteristics of all den trees used by 15 captured southern flying squirrels (*Glaucomys volans*) at Pierce Cedar Creek Institute, Barry Co. MI. Data is organized based on tree species from most frequently used species to least frequently observed species.

Tree Species	Quantity used as Den Trees	Mean Size (avg. dbh cm ±*SE*)	Mean Decay (avg. decay class ±*SE*)	Mean A.O.[+] (avg. stems ±*SE*)	Total Days used as a Den Tree
Red Oak (*Quercus rubra)*	11	56.7 ±20.0	3.2 ±1.3	0	109
Red Maple (*Acer rubrum)*	8	37.4 ±13.5	4.1 ±1.1	0	19
Sugar Maple (*Acer saccharum)*	6	50.3 ±33.6	4.3 ±0.5	1.3 ±3.3	19
Unidentifiable*	6	27.7 ±7.8	1	0	35
Hophornbeam (*Ostrya virginiana)*	4	15.5 ±2.7	1.4 ±2.0	0	13
White Ash (*Fraxinus Americana)*	4	46.9 ±12.2	3.3 ±1.0	0	12
American Elm *(Ulmus americana)*	3	34.7 ±13.2	3.0 ±1.7	0	6
Black Cherry *(Prunus serotina)*	3	33.3 ±16.8	2.3 ±1.5	0	6
Beech *(Fagus grandifolia)*	2	26.7 ±2.9	5.0 ±0.0	0	2
Black Walnut *(Juglans nigra)*	2	77.4 ±42.9	2.5 ±0.7	0	2
Pignut Hickory *(Carya glabra)*	2	32.8 ±3.3	3.0 ±2.8	0	14
Basswood (*Tilia americana)*	1	55.9	4	0	1
Big Toothed Aspen (*Populus grandidentata)*	1	30.2	3	0	1
Green Ash *(Fraxinus pennsylvanica)*	1	179.3	2	0	1
Shagbark Hickory *(Carya ovate)*	1	47.2	3	0	2
Yellow Poplar (*Liriodendron tulipifera)*	1	72.6	5	0	1

[+]A.O. = Autumn Olive presence (i.e. average number of stems observed in a 10 m^2 area surrounding den trees
*Trees were too dead to accurately identify

Table 3. Characteristics of the seven successfully amplified loci used to identify individual southern flying squirrel (*Glaucomys volans*) captures at Pierce Cedar Creek Institute, Barry Co., MI. All values estimated using Genepop on the Web (version 4.0.10: Hardy-Weinberg test—Option 1.3, and Basic Data—Option 5.1, accessed October 12, 2011). Locus sequences (forward and reverse) and repeat motifs available in original primer note (Fokidis *et al*. 2003). Starred *P* value indicates significance.

					Hardy-Weinberg Equilibrium Estimates			
							Fis Estimates	
Locus	Allele Size Range (bp)	Number of Alleles	Observed Heterozygosity (H_o)	Expected Heterozygosity (H_E)	*P*	*SE*	W&C	R&H
SFS-02	193-273	16	0.93	0.95	0.281	0.034	0.020	0.004
SFS-03	237-249	4	0.60	0.64	0.496	0.008	0.060	0.009
SFS-04	148-188	13	0.73	0.89	0.026*	0.009	0.183	0.132
SFS-07	253-267	7	0.67	0.72	0.375	0.017	0.082	0.020
SFS-14	153-171	6	0.67	0.61	0.782	0.013	-0.094	-0.056
SFS-15	119-131	7	0.93	0.79	0.893	0.007	-0.184	-0.106
GS-10	217-237	11	0.67	0.89	0.067	0.014	0.261	0.191

Totals: 7 Loci, 64 Alleles Overall (Fisher's Method) χ^2=19.36, df=14, Prob.=0.152

Table 4. Resulting putative family groups from genetic pairwise relationship analysis using Kinship 1.1 (Goodnight Software). Hypothesized relationships included: parent/offspring or unrelated (R=1, PO vs. UN), full siblings or unrelated (R=0.5, FS vs. UN), and aunt/niece or unrelated (R=0.25, AUNT vs. UN). The log of the ratio of the likelihood values given under each hypothesized relationship indicate the value needed for significance based on 10,000 simulated pairs. Stars indicate significance at the *P*=0.05 (*), 0.01 (**), and 0.001 (***) levels. Resulting putative groups are given in a summary at the bottom of the table. Squirrels are referenced using ear tag number and further individual details can be found in Table 1.

		log(Ratio)		
		PO vs. UN	FS vs. UN	AUNT vs. UN
ID #1 (Ear Tag)	ID #2 (Ear Tag)	≥0.879	≥0.1584	≥0.4246
874	869	1.543**	0.617*	0.545*
874	866		0.284*	0.468*
869	606	1.305**	0.801*	0.432*
866	869		3.499***	0.951**
605	867	1.450**	0.534*	0.531*
605	873		0.272*	

Resulting Putative Family Groups:

(1) 874, 869, 866, and 606
(2) 605, 867, and 873