12-2018

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ABSTRACT Northern flying squirrel (*Glaucomys sabrinus*) and red squirrel (*Tamiasciurus hudsonicus*) populations are endemic to northern North America, including the Black Hills. The Black Hills populations are considered disjunct from other populations within their range. We examined insular populations to determine whether arboreal squirrels in the Black Hills each represent a unique population. We trapped and collected ear samples from northern flying and red squirrels in the Black Hills and in areas of Montana, Wyoming, Idaho, Utah, Minnesota, and Wisconsin to infer population phylogenies with special consideration of the Black Hills population. Microsatellite loci and two mtDNA sequences were used for phylogenetic data analyses, including neighbor-joining and maximum likelihood trees, percent divergence, and nucleotide diversity. For northern flying squirrels, mtDNA phylogenetic trees grouped individuals in the Black Hills population, suggesting extended isolation from other nearby mountain ranges. In both squirrels, phylogenetic trees inferred with nDNA provide similar topologies to the mtDNA of northern flying squirrels. Sequence divergence distances (range 0 to 1.0) for cytochrome-*b* among studied populations were relatively small (0.00 to 0.55 [northern flying squirrel] and 0.00 to 0.01 [red squirrel]), so divergence may be from an historical event. Nucleotide diversity (cytochrome-*b*) was higher than in some other ranges (0.07 [northern flying squirrel] and 0.08 [red squirrel]); however, heterozygosity was low in the Black Hills populations. These data suggest that northern flying squirrel and red squirrel populations in the Black Hills Mountains are not only geographically disjunct, but genetically unique from their conspecifics elsewhere.

KEY WORDS Black Hills, *Glaucomys sabrinus*, northern flying squirrel, red squirrel, *Tamiasciurus hudsonicus*.

Northern flying squirrels (*Glaucomys sabrinus*) and red squirrels (*Tamiasciurus hudsonicus*) have boreo-montane distributions that largely overlap in North America (Wilson and Ruff 1999). While red squirrels occur fairly continuously in the Appalachian Mountains in the east and the Rocky Mountains in the west, they are absent from the mountains of northern California and adjacent Washington, Oregon, and Nevada where northern flying squirrels occur. Northern flying squirrels are less continuously distributed than red squirrels in the Appalachian and Rocky Mountains with disjunct populations in both mountain chains. An insular population of northern flying squirrels occurs in the Black Hills of South Dakota and Wyoming and in the nearby Bear Lodge Mountains in northeastern Wyoming (Wells-Gosling and Heaney 1984), while red squirrels populations in these areas are probably also disjunct from populations in the Rocky Mountains to the west and forested areas to the east (Chapman and Feldhamer 1982, Kiesow et al. 2007).

With focus on the insular mountain ranges, the Black Hills Mountains are some of the oldest formations in North America. They were formed as a result of a series of uplifts starting in the Pre-Cambrian Period and ending in the Pleistocene (Froiland 1978). Much of the flora and fauna found in the Black Hills are relicts of past climate conditions dating to the late Pleistocene (~10,000 years ago) when ice sheets covered northern North America (Kiesow et al. 2012). The current boreo-montane character (*i.e.*, conifer-dominated woodlands) of the Black Hills formed late in the last glacial period (Turner 1974).

Northern flying and red squirrels have been isolated to the Black Hills Mountains since the late Pleistocene, and their preferred forested habitats are relatively continuous throughout the higher-elevation central-west crystalline core (≥ 2000 m) and limestone plateau (1000–2000 m; Kiesow at al. 2012). Of the two squirrel species in these regions, northern flying squirrels are more habitat specialized primarily using higher-elevation mesic areas with ponderosa pine (*Pinus ponderosa*) stands interspersed with quaking aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) (Krueger 2004), while red squirrels use primarily coniferous forests throughout their range (Kemp and Keith 1970, Rusch and Reeder 1978). We expected that boreo-montane habitat continuity would cause squirrels to be genetically similar throughout the Black Hills, but STRUCTURE and GENELAND assignment tests detected subtle population genetic structure for red squirrels but not for northern flying squirrels (Kiesow et al. 2012). Further, low heterozygosity within the Black Hills may indicate isolation of these squirrels to the region (Kiesow et al. 2012). Given the within population structuring in the Black Hills, it is likely additional genetic testing at a broader scale will elucidate a population clade specific to the Black Hills Mountains.

Historically, it was unknown how genetically divergent the northern flying squirrel population in the Black Hills was, causing the classification and reclassification of this population as a separate species or subspecies. King (1951) detected differences in morphometrics showing that northern flying squirrel specimens from the Black Hills had

larger skulls, longer bodies, and darker fur than specimens from other parts of their range. As a result, the Black Hills northern flying squirrel population was reclassified from *G. s. canescens* to *G. s. bangsi* (King 1951). Wells-Gosling and Heaney (1984) reported that, based on morphological data, the northern flying squirrel population in the Black Hills was disjunct and thus genetically isolated from other populations with the closest known population of *G. s. bangsi* located in western Wyoming. In addition, high cytochrome-*b* sequence variation was detected among northern flying squirrel populations (Arbogast 1999), indicating the presence of two clades, one of which corresponds to *G. s. bangsi*. More recently, Arbogast et al. (2017) proposed designating *G. sabrinus* populations in the Pacific Northwest as a new species, *G. oregonensis*, based on mtDNA control region sequences and eight microsatellite markers. Currently, northern flying squirrels in the Black Hills are classified as *G. s. bangsi*, which includes other regions of the northern Rocky Mountains (Hall 1981, Wells-Gosling and Heaney 1984).

Similarly, red squirrel subspecific designation is perplexing. Red squirrel populations within the Rocky Mountain region are proximate to each other, which suggest that subspecies designations would include clusters of nearby ranges. However, the Black Hills population is apparently disjunct (Jones et al. 1985); therefore, South Dakota populations are designated as a separate subspecies, *Tamiasciurus hudsonicus dakotensis*, and considered endemic to this area (Hall 1959, Turner 1974, Steele 1998). This classification was based solely on darker hair color and longer, heavier body size and does not take into account any molecular (DNA-based) data (Turner 1974). Mitochondrial DNA research shows three lineages of New World red squirrels: 1) a western lineage, 2) a southwestern lineage, and 3) a widespread lineage comprising all other populations of *T. hudsonicus* (Arbogast et al. 2001); *T. h.* is found in the widespread lineage. Hope et al. (2016) used published nuclear DNA sequences, mtDNA cytochrome-*b* sequences, and niche variables from red squirrels sampled across North America to suggest revisions to red squirrel taxonomy. This more recent analysis suggests that these lineages constitute separate species: the western lineage (*T. douglasi*), the southwestern lineage (*T. mearnsi*), and the widespread lineage (*T. hudsonicus*) (Hope et al. 2016). Black Hills red squirrels are included in *T. hudsonicus* in this taxonomic revision (Hope et. al 2016).

Our comparative study of the phylogenetics of the Black Hills northern flying squirrel and red squirrel populations was motivated by three factors. First, the insularity of the Black Hills suggests that these populations have the potential to be phylogenetically distinct and therefore may show unique genetic and ecological properties. Second, there are few studies examining insular, isolated mountain ranges, and our research will fill the gap in sampling represented by some of these ranges. Finally, by comparing phylogenetic patterns for a species of conservation concern (northern flying squirrel) with an ecologically similar one that is not of concern within the Black Hills (red squirrel), we aimed to inform management of both species. Our prediction was that the northern flying squirrel would show greater phylogenetic differences than the more abundant red squirrel in the Black Hills.

STUDY AREA

The Black Hills are unique geological formations with a continental climate and coniferous and prairie vegetation (Peterson 1974), disjunct from other boreal and montane biomes. They are one of the oldest geologic formations in North America and were formed as a result of a series of uplifts starting in the Pre-Cambrian Period and ending in the Pleistocene (Froiland 1974).

During the last glacial phase of the Pleistocene, glacial ice sheets covered much of northern North America. The Black Hills and nearby areas, including the plains between the Black Hills and Bighorn Mountains, were not glaciated during the last Pleistocene glacial episode, but were adjacent to glaciers to the east (Froiland 1978). Peterson (1974) suggested that a montane biota was present in the region during the last glacial maximum, whereas boreal elements may not have appeared in the Black Hills and other periglacial areas until late in the Pleistocene (~10,000 years ago). The boreo-montane elements of the Black Hills that support both squirrel species are disjunct from contiguous boreal forests; the nearest boreal forest is 700 km to the north (Turner 1974), and the nearest mountain range and probable populations of these squirrels are 241 km northwest of the Black Hills in the Bighorn Mountains (Froiland 1978). Due to this isolation, the northern flying and red squirrel populations in the Black Hills are likely distinct (or rather unique) from other arboreal squirrel populations.

METHODS

Field Sampling

Squirrels were live-trapped with Havahart® and Tomahawk® traps baited with peanut butter, bacon grease, and oatmeal placed in trees every 60–80 meters. Ear samples were collected throughout the Black Hills/Bear Lodge Mountains from 2005–2007 and areas of the northern Rocky Mountains (west) and western Midwest (east) in 2006 (Fig. 1). The University of South Dakota (USD) and South Dakota State University (SDSU) Institutional Animal Care and Use Committees evaluated and approved all handling and care techniques associated with this project (Permit Number 04-A021). We used a subset of the collected northern flying squirrel and red squirrel samples for mtDNA

Figure 1. Sample sites in the Black Hills/Bear Lodge, west (Montana, Idaho, Wyoming, and Utah), and east (Minnesota and Wisconsin) regions, with two-letter or three-letter identification codes for each, depending on the sample source and associated identification (see Table 1).

analyses due to sequence quality and length. In addition to these samples, we included mtDNA sequences available via National Center for Biotechnology Information (NCBI) nucleotide database in our mtDNA sequence analyses. For the microsatellite data analyses, we used all our collected samples due to success in microsatellite genotyping techniques. Table 1 provides information on sample numbers and sites as well as sample subsets used for data analyses. Accession numbers are available upon request.

DNA Extraction and Amplification

We extracted DNA from ear clippings collected throughout the areas specified in Table 1. We used reagents and methods provided in the QIAGEN (Valencia, CA) DNeasy blood and tissue extraction kit to conduct the DNA extraction.

We analyzed ear clippings from five to ten individuals (of both squirrel species) from each sample site using two different mitochondrial DNA (mtDNA) regions (i.e., cytochrome-*b* and mtDNA control region). These regions were amplifiable in these squirrel species. All amplifications were conducted with 15 uL reactions containing 1.0 uL of genomic DNA, 0.5 uL of 10 uM forward primer, 0.5 uL of 10uM reverse primer, and 7.5 uL of 2X PCR Master Mix (Promega). The mtDNA cytochrome-*b* region (forward and reverse primers) consisted of L14724 (5-CGAAGCTTGATATGAAAAACCATCGTTG-3) and H15149 (5-AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3) (Arbogast 1999). Amplification occurred under the following conditions: 93º C for 3 min followed by 3 cycles of 93º C for 3 min, 41º C for 15 sec increasing to 72º C over 3 min, and 72º C for 2 min then followed by 29 cycles of 93º C for 45 sec, 56º C for 1 min 30 sec, and 72º C for 2 min 30 sec followed by 72º C for 10 min. We amplified the mtDNA control region (forward and reverse primers) using primers H16359 (5-GGAAGGGGATAGTCATTTGG-3; Barratt et al. 1999) and RScont6 (5-CCTTCAACTCCCAAAGCTGA-3; Hale et al. 2001), which targeted a 543 bp stretch of the mtDNA control region. Amplification occurred under the following conditions: 94º C for 4 min, then 30 cycles of 94º C for 30 s, 50ºC for 30 s, 72º C for 1 min 30 sec, with a final extension of 72º C for 10 min (Hale et al. 2001).

Polymorphic microsatellite loci were also used to study both squirrel species. Ten loci were analyzed for the northern flying squirrels and included GS2, GS4, GS13 (Zittlau et al. 2000), FLS1 and FLS6 (Winterrowd et al. 2005), and GLSA12, GLSA22, GLSA48, GLSA52, and GLSA65 (Kiesow et al. 2011). Sixteen loci used for the red squirrels included Thu03, Thu08, Thu14, Thu21, Thu 23, Thu25, Thu31, Thu32, Thu33, Thu37, Thu38, Thu41, Thu42, Thu50, Thu55, and Thu59 (Gunn et al. 2005). Using conditions specified by each respective publication, we performed 15 uL reactions containing 50 ng genomic DNA.

Amplified mtDNA regions were purified with polyethylene glycol (PEG) precipitation, where mtDNA was precipitated with 20% PEG 800/2.5M NaCl, washed with 80% ethanol, and resuspended in sterile water. Purified mtDNA products were sequenced using the T3 primer and the DYEnamic ET Terminator Cycle Sequencing Kit (General Electric) in 1/8th volume reactions. Sequences were electrophoresed on an Avant 3100 Genetic Analyzer. Forward and reverse sequences (contigs) were generated for each locus in each individual. Then, for each locus these contigs were edited and assembled into a consensus sequence using DNAStar© Lasergene and edited with BioEdit© (Hall 1999).

Microsatellite samples were multiplexed with five loci per sample and electrophoresed on an ABI PRISM sequencer, where Liz-500 was used as a size-standard (Applied Biosystems). Each forward primer was fluorescently-labeled with VIC (green), PET (red), or 6-FAM (blue) dye. Sizing of alleles for each locus was conducted using GeneMapper software (Applied Biosystems) and then transformed into a data matrix of all individuals and all loci, per squirrel species.

Data Analyses

Sequences were aligned in MEGA7 (Kumar et al. 2016) using the Clustal-W algorithm with the BLOSUM weight matrix and gap opening costs of 10 and gap extension costs of 0.1 in pairwise and multiple alignments. The aligned lengths of the mtDNA regions varied per squirrel species, 166 bp for cytochrome-*b* and 286 bp for control region for northern flying squirrels and 299 bp for cytochrome-*b* and

Table 1. Regions, identification codes, and number of samples used for northern flying squirrels (NFS) and red squirrels (RS) mtDNA analyses. The ID Code referenced here refers to the site in which the samples were collected that are represented in the phylogenies. For example, PFxx represents Payette National Forest in Idaho, while the number (represented by xx) is the actual sample number. Because of its sample location, samples from PFxx are from the "west" region and indicated as "West" on the phylogenies. A total of one northern flying squirrel sample and four red squirrel samples were used for mtDNA analyses from sample source Payette National Forest (or PFxx).

Region (ID Color)	Sample Source (ID Code)	State	Samples (NFS, RS)
West	Payette National Forest (PFxx)	ID	1, 4
("West")	Kootetnai National Forest (KFxx)	MT	5, 5
	Western Montana (FSWxx)	MT	6, 3
	Shoshone National Forest (SFxx)	WY	4, 2
	Bighorn Mountains (BFxx)	WY	0, 7
	Laramie Mountains (LMxx)	WY	0, 6
	Wasatch - Cache National Forest (AFxx)	UT	5, 4
East	Western Minnesota (RSExx/FSExx)	MN	4, 2
('East")	Paul Bunyon State Forest (PBxx)	MN	0, 7
	Cloquet Valley State Forest (CVxx)	MN	0, 6
	Cheuamegon Nicolet National Forest (CNxx)	WI	2, 0
	Northern Highland State Forest (NHxx)	WI	3, 6
	Central Wisconsin (ORxx)	WI	3, 16
Black Hills/	Black Hills National Forest (xxBH)	SD	25, 34
Bear Lodge	Bear Lodge National Forest (BLxx)	WY	6, 6

192 bp for control region for red squirrels. An outgroup, a sequence from the Siberian flying squirrel (*Pteromys volans*; GenBank Accession number AB164478.1), was used for comparison of cytochrome-*b* and control regions in all northern flying squirrel trees, and an outgroup, a sequence from the Douglas squirrel (*T. douglasii*; GenBank Accession number KU977145.1), was used for comparison of cytochrome-*b* control region in all red squirrel trees. Outgroups were selected based on previous studies (Arbogast et al. 2001, Hope et. al 2016) and genetic similarities between species that allow for comparisons within these squirrel species.

Phylogenetic analyses were inferred with neighbor joining (NJ) and maximum likelihood (ML) methods in MEGA7 (Kumar et al. 2016). We conducted NJ analyses with a Maximum Composite Likelihood model with 1,000 bootstrap replications, a common practice with NJ analysis (Kumar et al. 2016). We conducted ML analyses with 100 bootstrap replicates using the Tamura 3-parameter model (Kumar et al. 2016), which was determined to be the most appropriate nucleotide substitution model for our data set based on the Bayesian (BIC) and corrected Akaike Information Criterion (AICc) in the ML model selection feature of MEGA7 (Kumar et al. 2016). We inferred trees using each region (mtDNA cytochrome-*b* and control) separately, and with the regions combined. We used multiple tree-building methods with different data sets to assess phylogenetic results.

In addition to trees, we estimated sequence divergence distances (and percent sequence divergence) and nucleotide diversity (i.e., the degree of polymorphism) for each region. Estimates of sequence divergence for all possible pairwise estimates of taxa were calculated under the Tamura 3-parameter (as determined via BIC and AIC*c* analyses) and estimates of nucleotide diversity were calculated using MEGA7.

We obtained matrix values for microsatellite data using MSA 4.05 (Dieringer and Schlštterer 2003). We incorporated microsatellite distance matrices (i.e., Nei's distances) into PHYLIP (i.e., NEIGHBOR; Felsenstein 1993) to infer phylogenetic relationships and create an unrooted neighbor-joining tree with no outgroup.

RESULTS

We collected 237 northern flying squirrel (NFS) ear clippings (i.e., 151 Black Hills, 53 west, and 33 east) and 208 red squirrel (RS) ear clippings (i.e., 96 Black Hills, 57 west, 55 east), all of which were used from microsatellite analyses, as microsatellite analyses requires more samples per site with more amplified loci to conduct proper data analyses. For mtDNA analyses, we used a total of 64 northern flying squirrel and 118 red squirrel samples for mtDNA analyses, in addition to an existing 43 northern flying squirrel and 83 red squirrel mtDNA cytochrome-*b* sequences available via Genbank. (Note: no mtDNA control region sequences were available for these squirrels.) Because phylogenetic analyses requires fewer samples per site for analyses, we used sequences with the same length and quality (hence the reason a subset of collected samples were used for analyses). General locations and regional designations for the analyzed samples are provided in Table 1.

Based on the neighbor-joining and maximum likelihood trees developed from the cytochrome-*b* regions (Fig. 2), individuals from the Black Hills (xxBH) and Bear Lodge (BLxx) Mountains were identified in a separate clade/group (indicated in red on the figure) in northern flying squirrels but not in red squirrels. Sequence divergence distances ranged from 0.00 to 0.55 (0.00%–0.85% sequence divergence, NFS) and 0.00 to 0.01 (0.00%–1.58% sequence divergence, RS), and nucleotide diversity was 0.07 (NFS) and 0.10 (RS).

Based on the neighbor-joining and maximum likelihood trees developed from the control region (Fig. 3), individuals from the Black Hills (xxBH) and Bear Lodge (BLxx) Mountains are identified in a separate clade/group (indicated in red on the figure) in northern flying squirrels but not in red squirrels. Sequence divergence distances range from 0.00 to 0.61 (overall 0.00%–1.43% sequence divergence, NFS) and 0.00 to 0.03 (overall 0.00%–1.04% sequence divergence, RS) and nucleotide diversity was 0.10 (NFS) and 0.15 (RS).

When mtDNA regions were combined for phylogenetic analyses, little information was revealed regarding relationships between these squirrels. Thus, data reported herein reflect cytochrome-*b* and control regions for each squirrel.

Analyses of northern flying squirrel and red squirrel populations from all sample localities indicated that each grouped together based on similarities in microsatellite frequencies (Fig. 4). In both squirrels, populations from the Black Hills and Bear Lodge Mountains (samples indicated by xxBH and BLxx, and indicated in red) are most similar. The Black Hills (xxBH) and Bear Lodge Mountains (BLxx) are most similar to the west region (indicated in dark blue on the figure), such as Utah [AFxx; northern flying squirrel] and Wyoming [SFxx, BFxx, LMxx; northern flying squirrel, red squirrel] populations). In addition, red squirrel populations showed an allele presence on locus Thu08 estimated around 308 bp in the Black Hills population rather than 180 bp, which indicates an insertion or mutation in this region of the nuclear DNA and could suggest differentiation of the Black Hills red squirrel population. In both squirrels, populations from the east region (indicated in light blue on the figure) in northern Wisconsin (from sample sources CNxx, NHxx, and ORxx) are more similar to the west region (indicated in dark blue).

DISCUSSION

All samples in our study were taken from areas found in Arbogast's (1999, 2017) eastern clade. Pairwise sequence divergence distances ranged from 0 – 0.61 (0.0%) in our samples, within the range noted by Arbogast (1999). Our two mtDNA regions present different trees based on neighbor-joining and maximum likelihood analyses. The tree based on cytochrome-*b* sequences shows Black Hills *G. sabrinus* samples nested within those from our western sites in Idaho, Montana, Wyoming, and Utah, forming a monophyletic clade basal to the west. In contrast, our control region tree places a monophyletic Black Hills clade separate from western and eastern samples with stronger bootstrap support than cytochrome-*b* sequences. Additionally, our neighbor-joining tree based on nuclear microsatellites suggests that disjunct Black Hills *G. sabrinus* are somewhat isolated from other sampled locations. We see a west to east to Black Hills trend in sequence divergence distances within both *G. sabrinus* datasets.

Arbogast (1999) collected northern flying squirrel samples from West Virginia, North Carolina, Utah, Michigan, Alberta, British Columbia, Washington, Oregon, California, Louisiana, and Tennessee, but not from the Black Hills. Arbogast (1999) resolved two clades for northern flying squirrels based on 315 bp of mtDNA cytochrome-*b* sequences: a western clade (i.e., Pacific Northwest) and an eastern clade (Cascade Mountains, CA; northern Rocky Mountains and much of boreal Canada). Interestingly, the western and eastern clades were split by samples of *G. volans* (southern flying squirrel) that formed a monophyletic clade within the *G. sabrinus* sequences leading to the conclusion that *G. sabrinus* is paraphyletic (Arbogast 1999). Pairwise sequence divergence across *G. sabrinus* distribution ranged from 0–7.2% and was 0–2.6% within each clade ("eastern" and "western") of northern flying squirrels (Arbogast 1999).

As with northern flying squirrels, our red squirrel sample locations fell within the eastern clade detected by Arbogast et al. (2001, see also Hope et. al 2016), and we are able to expand on that study with the inclusion of Black Hills *T. hudsonicus* mtDNA sequences. Pairwise sequence divergence distances among our sampled red squirrels ranged from 0–0.03 (0.00%), within the range reported by Arbogast et al. (2001). We found little evidence of phylogeographic structure in our red squirrel samples. Neighbor-joining and

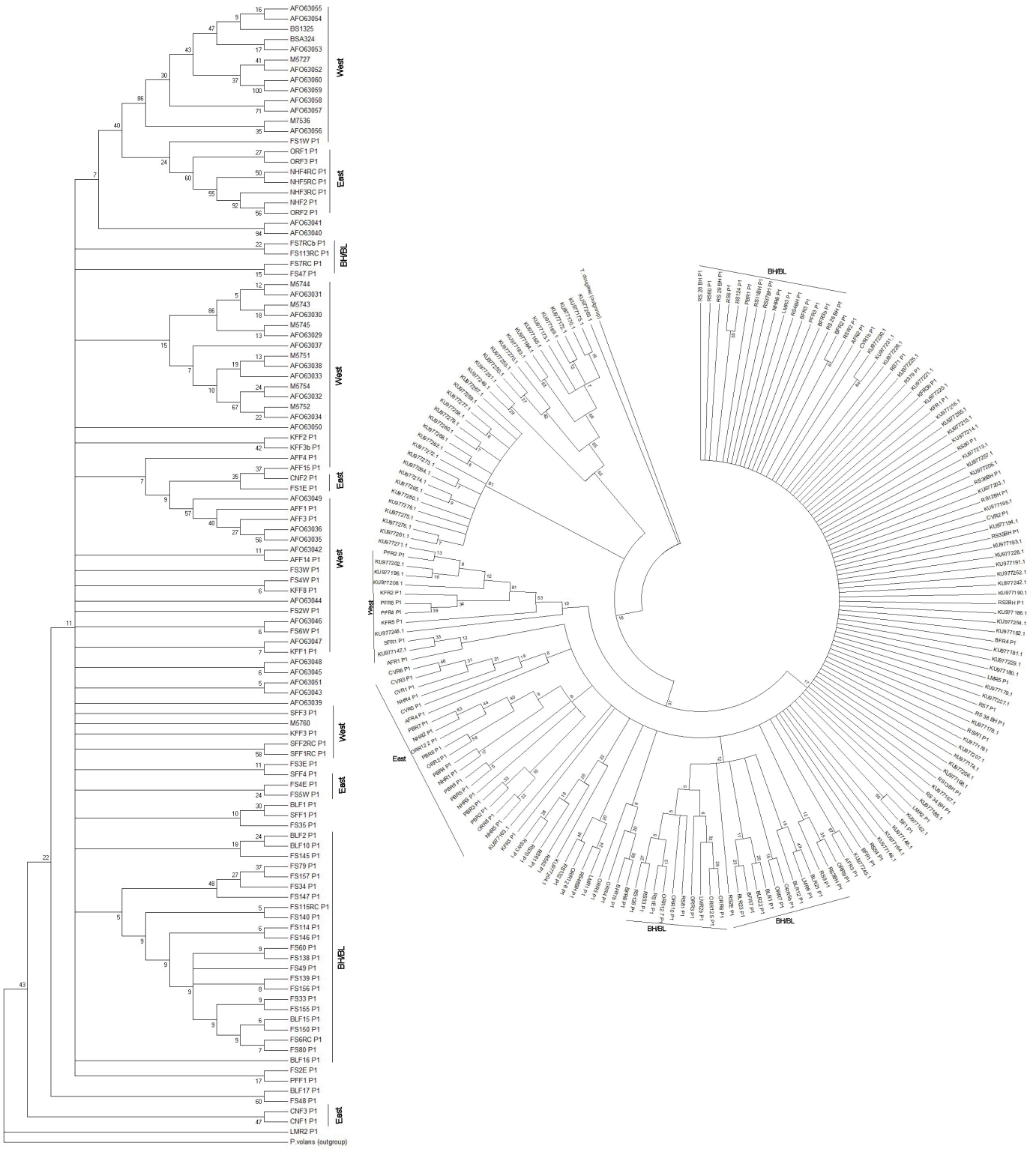

Figure 2. Phylogenetic relationship of A) northern flying squirrel (rectangular tree with *P. volans* outgroup) and B) red squirrel (circular tree with *T. douglasii* outgroup) using the cytochrome-*b* region (coded as P1 behind sample source identifier) based on maximum likelihood analysis. (Note: neighbor-joining analyses showed similar results.) Samples labeled with RSxx, BLxx, or xxBH (xx represents individual sample number) are from the Black Hills or Bear Lodge Mountains (indicated as BH/BL), while the rest are samples from the west (indicated West) and east (indicated as West) regions. Numbers on branches indicate ML bootstrap support values. Low bootstrap support values are included for the higher-level relationships

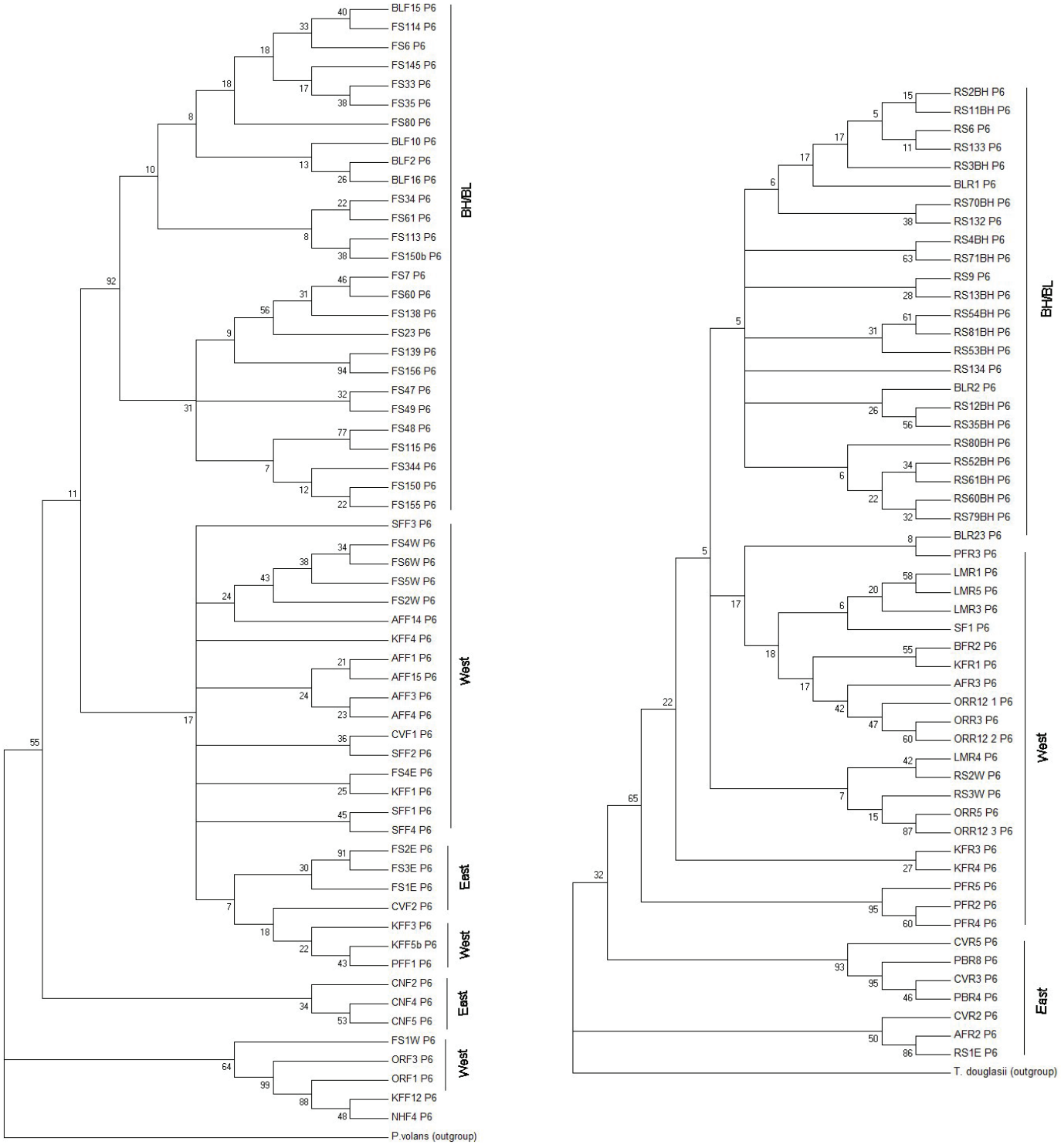

Figure 3. Phylogenetic relationship of A) northern flying squirrel (rectangular tree with *P. volans* outgroup) and B) red squirrel (rectangular tree with *T. douglasii* outgroup) using the control region (coded as P6 behind sample source identifier) based on maximum likelihood analysis. (Note: neighbor-joining analyses showed similar results.) Samples labeled with RSxx, BLxx, or xxBH (xx represents individual sample number) are from the Black Hills or Bear Lodge Mountains (indicated as BH/BL), while the rest are samples from west (indicated as West) and east (indicated as East) regions. Numbers on branches indicate ML bootstrap support values. Low bootstrap support values are included for the higher-level relationships.

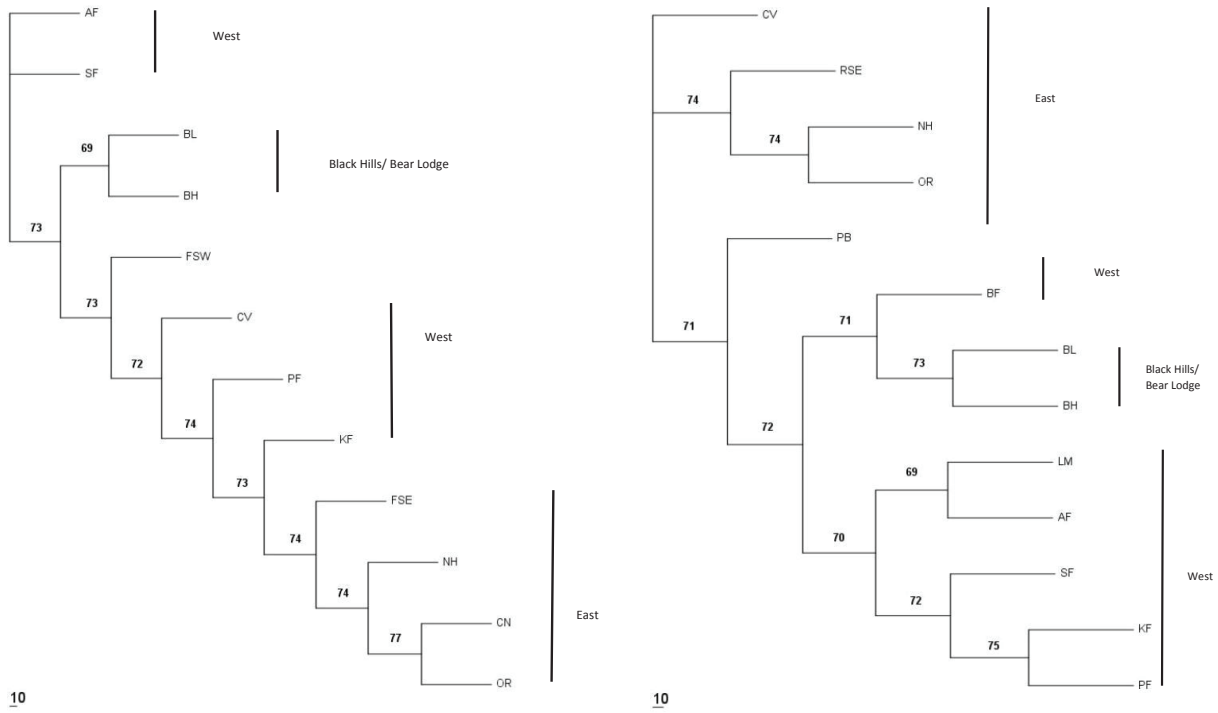

Figure 4. Relationship of northern flying (rectangular tree with no outgroup; west region is top clade) and red squirrel (rectangular tree with no outgroup; east region is top clade) populations from different forests based on microsatellite data (with Nei's distances) via neighbor-joining method of analysis, where East represents the east region, Black Hills/Bear Lodge represents the Black Hills/ Bear Lodge, and West represents the west region (as identified in Table 1).

maximum likelihood trees based on both cytochrome-*b* and control region mtDNA regions show Black Hills sequences interspersed with those of red squirrels sampled both from the east and west of the Black Hills. A neighbor-joining tree based on nuclear microsatellites suggests some potential differentiation of Black Hills red squirrels with moderate bootstrap support. Overall, unlike the results for northern flying squirrels, our data do not suggest strong differentiation of Black Hills red squirrel populations from nearby locations. Interestingly, Klicka et al. (2011) found a phylogeographic pattern for the non-migratory forest-obligate Hairy Woodpecker (*Picoides villosus*) similar to that of the red squirrel using mtDNA sequences. As with our results, Hairy Woodpeckers sampled from the Black Hills were placed in a "north and east" clade corresponding to the eastern clade of Arbogast et al. (2001) and distinct from a "south and west" woodpecker clade that corresponds to the western red squirrel clade of Arbogast et al (2001). Even though there is little evidence of phylogenetic structure in red squirrels, we see a west to east to Black Hills trend in sequence divergence distances within both *T. hudsonicus* datasets, similar to that seen in *G. sabrinus*. Arbogast et al. (2001) sampled *Tamiasciurus* spp. across their North American range with most samples originating in the United States and Canadian Rocky Mountains and the Cascade Mountains in California. Arbogast et al. (2001) analyzed eight isozyme loci and 402 bp sequences of the mtDNA cytochrome-*b* locus. Allozyme data for Black Hills T. hudsonicus were included in the study, but no mtDNA sequences from the Black Hills were reported in Arbogast et al. (2001). The main conclusions were that molecular data do not suggest that *T. hudsonicus* and *Tamiasciuris douglasii* are separate species and that *G. sabrinus* and North American *Tamiasciuris* spp. exhibit similar phylogeographic patterns (Arbogast et al. 2001): an eastern and a western clade, with sequences divergences from 0 to 2.4%, possibly a result of historical events. However, recent data by Hope et al. (2016) suggest that there is novel regional genetic diversity across the *Tamiasciurus* spp. distribution in western North America with contact zones of hybridization.

Our results suggest that the currently disjunct Black Hills northern flying squirrel population may have diverged genetically during the late Pleistocene within the unglaciated Black Hills. Although our genetic data provide limited evidence of endemism, based on short sequence reads, future studies with more comprehensive genetic coverage may help to resolve phylogeographic relationships among northern flying squirrel populations that have diversified rapidly in response to post-Pleistocene climate changes. A study of Russian flying squirrels (*Pteromys volans*) shows similar patterns of distinctiveness, in which glacial refugia occupied by Russian flying squirrels are associated with

forest dynamics in the Pleistocene (Oshida et al. 2005). Similarly, remnants of the boreal forest remain in the Black Hills. Phylogenetic analyses have also shown that Black Hills populations of two woodpeckers (Black-backed [*Picoides arcticus*, Pierson et al. 2010] and Three-toed [*P. dorsalis*, Ervin 2011]) are phylogenetically distinct from conspecifics found in more continuous boreo-montane habitat. Therefore, species like the northern flying squirrel inhabiting the Black Hills during the late Plesitocene were likely isolated by habitat. Further, the northern flying squirrel is somewhat of a habitat specialist with limited ability to disperse long distances across nonforested areas. Even within the Black Hills, genetic structure data indicate subtle population structuring is likely a result of a backbone of granite outcrops serving as a natural migration barrier for squirrels (Kiesow et al. 2012). The current disjunction of Black Hills northern flying squirrels and monophyly based on mtDNA sequences and microsatellite frequencies suggest that the Black Hills northern flying squirrel population may be divergent from populations found nearby. In contrast, the relatively continuous distribution of Black Hills red squirrels with main-stem Rocky Mountain populations and its lack of cohesiveness in the phylogeographic analyses presented here indicate that Black Hills red squirrels are not particularly distinct from red squirrels found to the east and west.

Data provided herein suggest that northern flying squirrels may form a population in the Black Hills isolated from nearby populations. The presence of (weak) genetic structure among our sample locations potentially suggests that squirrel movements may be inhibited by topography or unsuitable habitat within the Black Hills (Kiesow et al. 2012). Ultimately, this could limit population admixture to nearby mountain ranges in the insular United States, resulting in unique, isolated populations of these squirrels in the Black Hills.

The extent to which geographic isolation during the Pleistocene resulted in separate "populations" for management purposes is an important question, albeit notoriously difficult to answer. While Waples and Gaggiotti (2006), Palsbøll et al. (2006), and Funk et al. (2012) acknowledge that there is no consensus regarding a quantitative definition of "population," populations as defined under different paradigms can be meaningful to managers at different spatial scales. The geographic disjunction of arboreal squirrel habitat with its resultant insularization in the Black Hills in conjunction with a signal of phylogenetic uniqueness (for northern flying squirrels, in particular) justify the recognition of these two squirrel populations as worthy of focused conservation efforts in the Black Hills. As noted in Kiesow et al. (2012), these efforts have begun for northern flying squirrels in the Black Hills (and South Dakota in general), as they are classified as imperiled by the South Dakota Natural Heritage Program (South Dakota Department of Game, Fish and Parks 2006) and are a designated "Species of Local Concern" by the USDA Forest Service (USDA Forest Service 2005). Though the red squirrel is not imperiled in South Dakota, it and the northern flying squirrel face a variety of threats to their Black Hills habitat (Kiesow et al. 2012).

Because these arboreal squirrels are dependent on connectivity of suitable habitat from a micro- and macroscale perspective, recognizing that these arboreal squirrels are unique to the Black Hills and/or upgrading their conservation status in concert with improved habitat management measures (see Kiesow et al. 2012) is imperative to maintain the genetic integrity of the two isolated populations. Isolated populations are vulnerable to decline in forested lands managed for timber harvest (Koprowski 2005). Forest management practices (i.e., anthropogenic disturbances), along with natural disturbances (e.g., weather, fire, insect and pathogen infestations, root rot, and neighboring tree fall; Lundquist 1995) can help establish forest heterogeneity, which can affect the ecology of the forest ecosystem. Natural disturbances, in particular, create snags and different-aged ponderosa pine stands, which provide den-sites and food resources for squirrels.

MANAGEMENT IMPLICATIONS

Because forest management practices seemingly have the largest effect on species dependent on forest heterogeneity, it is particularly important that forest managers consider both plant and animal species in forest management decisions. According to Shepperd and Battaglia (2002), forest managers should consider species needs as well as natural disturbances to maintain old-growth heterogeneity and minimize impacts to species dependent on such forests. By doing so, species reliant on heterogenic forests, like the northern flying squirrel and red squirrel, can thrive into the future.

ACKNOWLEDGMENTS

We thank the South Dakota Game, Fish and Parks for funding this research as part of a State Wildlife Grant (Grant 2414), and thank the University of South Dakota and South Dakota State University for additional assistance through student aid and staff support.

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Submitted: 19 July 2017. Accepted: 28 November 2018. Associate Editor: Colter Chitwood