



From sky islands to river valleys: genetic structure in Appalachian woodland jumping mice (*Napaeozapus insignis*)

E. Claire Watersmith^{1,*}, Brian S. Arbogast¹, Alexandra G. DeLuca¹, Radmila Petric², Marketa Zimova³, Jenifer A. Mallinoff⁴, and Stephanie J. Kamel¹

¹Department of Biology and Marine Biology, University of NC Wilmington, 601 S College Road, Wilmington, NC 28403, United States

²Institute for the Environment, University of NC at Chapel Hill, 123 W Franklin Street, Chapel Hill, NC 27516, United States

³Department of Zoology and Physiology, University of WY, 1000 E. University Ave, Laramie, WY 82701, United States

⁴The Nature Conservancy, 5 Barbetta Drive, Asheville, NC 28806, United States

*Corresponding author: Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 S College Road, Wilmington, NC 28403, United States. Email: ewater10@su.edu

Abstract

At the southern edge of their range, woodland jumping mice (*Napaeozapus insignis*) are associated with the high-elevation spruce-fir “sky islands” of the Southern Appalachians, one of the most fragmented and endangered ecosystems in North America. Consequently, gene flow is expected to be limited among sky island populations, which could result in strong geographic patterns of genetic structuring. Here, we present the first assessment of population genetic structure in woodland jumping mice of the Southern Appalachian Mountains of Eastern North America. We identified and sampled male and female woodland jumping mice from seven distinct mountains/mountain ranges in the Southern Appalachians of North Carolina, the Allegheny Mountains in the Central Appalachians of West Virginia, and the White Mountains in the Northern Appalachians of New Hampshire. Using a ~350 base pair fragment of the mitochondrial DNA control region and 13 novel microsatellites, we estimated genetic diversity, relatedness among individuals, patterns of population genetic structure, and gene flow among populations. The geographic distribution of mtDNA haplotypes showed distinct haplogroups for the Northern Appalachian, Central Appalachian, and Southern Appalachian Woodland Jumping Mouse populations. Microsatellite analyses showed that within the Southern Appalachians, the northernmost and southernmost groups of spruce-fir sky island populations, separated geographically by the French Broad River, clustered separately. Surprisingly, however, there was minimal genetic differentiation among spruce-fir sky island populations on each side of the French Broad River; only the southernmost population was significantly differentiated from all others. Overall, females displayed greater genetic differentiation than males, suggesting differences in philopatry and dispersal among the sexes. Our study suggests that although Southern Appalachian sky islands serve as an important habitat for woodland jumping mice, it would be overly simplistic to conclude that the fate of this small mammal in the southern portion of its range is tied solely to that of this endangered ecosystem.

Keywords *Napaeozapus insignis*, population genetics, riverine barriers, small mammals, spruce-fir sky islands, Southern Appalachians

The current geographic distribution patterns of sky island ecosystems and their associated animal species are thought to be shaped by cycles of localized extinctions in lowland areas caused by past climate change (Brown 1971; Lomolino 1986; Wiens et al. 2019). The term sky island refers to ecosystems of unique community compositions restricted to mountaintops. The repeated isolation of populations on these mountaintops has led to the hypothesis that contemporary immigration between sky island populations is minimal or entirely absent, particularly for highly specialized taxa (Brown 1971; Lomolino 1986). However, this hypothesis has been challenged by studies suggesting that while some sky island specialists may indeed experience significant isolation, many sky island communities are largely composed of generalist woodland species potentially capable of dispersing between habitat patches. These generalists may experience fewer isolation effects and greater connectivity across fragmented landscapes (Lawlor 1998). This interplay between isolation and

connectivity highlights the complex dynamics shaping sky island communities, where the persistence of glacial relics contrasts with the broader ecological strategies of more generalist species. These dynamics underscore the importance of understanding the differential responses of species to isolation and environmental change within these unique refugial systems.

Geographic barriers such as lowland valleys characteristic of montane landscapes can significantly impede gene flow, promoting genetic drift and driving population differentiation. This genetic differentiation is shaped not only by contemporary population distributions but also by historical geographic patterns influenced by past geologic eras (Mastretta-Yanes et al. 2018). For instance, geographic patterns of genetic structure observed in certain Southern Appalachian salamander populations suggest that historical isolation, potentially due to competition or other ecological factors, has played a crucial role in shaping their genetic diversity (Crespi et al. 2003).

Associate Editor: Jesse Alston

Received: July 18, 2025. Accepted: April 3, 2026

© The Author(s) 2026. Published by Oxford University Press on behalf of the American Society of Mammalogists, www.mammalogy.org. All rights reserved. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

Additionally, genetic drift—strongest in small populations (Kimura 1983)—often leads to pronounced differentiation at the range margins of a species, where populations tend to be smaller and more isolated. This inverse relationship between genetic drift and population size has led to the hypothesis that edge-of-range populations exhibit the strongest genetic structure and reduced genetic diversity compared to core populations (Hampe and Petit 2005; Eckert et al. 2008).

The high-elevation mountain peaks of the Southern Appalachian Mountains form a classic sky island complex composed of small and disjunct patches of high-elevation Red Spruce (*Picea rubens*) and Fraser Fir (*Abies fraseri*) forests surrounded by expanses of mixed hardwood forests (Fig. 1). This spruce-fir ecosystem, covering only about 22,000 ha, is one of the most restricted and fragmented ecosystems in North America, making it exceptionally vulnerable to global change (Wear and Greis 2002). Additionally, the Fraser Fir is endemic to Southern Appalachian sky islands. Rising temperatures and shifting precipitation patterns are expected to further isolate these forest patches, reducing their already limited habitat range and increasing

the risk of localized extinctions. Compounding the effects of climate change, historical threats posed by logging, acid rain, and the introduction of the Balsam Woolly Adelgid (*Adelges piceae*), an invasive insect first detected in the region in 1957 (Speers 1958), have made this ecosystem less resilient to contemporary threats. The contemporary stressors, in conjunction with historic degradation, continue to threaten the spruce-fir ecosystem, including the animal communities that inhabit them.

The Southern Appalachian spruce-fir sky islands are considered Pleistocene refugia as they provide habitat for many animal and plant species that were more broadly distributed during glacial periods. These high-elevation habitats now host a diverse assemblage of taxa, including numerous glacial relics typically found at much higher latitudes, reflecting the role of sky islands in preserving biodiversity and acting as critical refugia for cold-adapted species through climatic fluctuations (Arbogast 1999; Walker et al. 2009; Mastretta-Yanes et al. 2018; He et al. 2019). However, with the threat of anthropogenic climate change, this refugial habitat may be lost entirely.

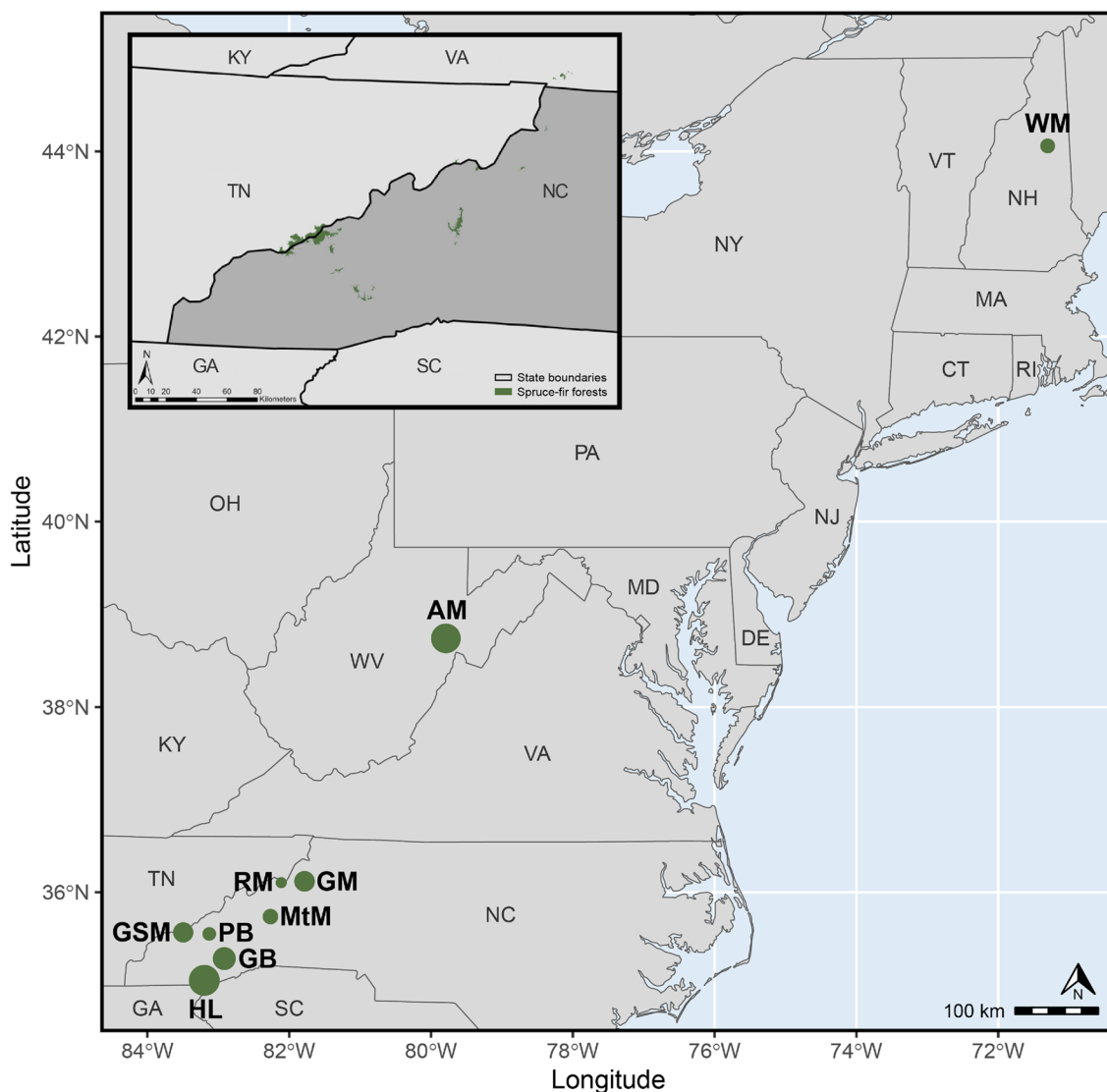


Fig. 1 Woodland Jumping Mouse (*Napaeozapus insignis*) sampling sites in the Southern Appalachians ($n = 7$), the Central Appalachians ($n = 1$), and the Northern Appalachians ($n = 1$). Inset: Distribution of the spruce-fir sky islands (green) of the Southern Appalachians in North Carolina, Tennessee, and Virginia. Abbreviations are described in the text.

The Woodland Jumping Mouse, *Napaeozapus insignis* (Miller 1891), is a hibernating rodent widely distributed throughout the Appalachian Mountains of the eastern United States and southeastern Canada (Campbell et al. 2010). In the Southern Appalachian region, this species is recognized as a distinct subspecies, *N. i. roanensis* (Whitaker and Wrigley 1972). Historically, populations in the Southern Appalachians were thought to be confined primarily to high-elevation peaks and cool, moist coves (Whitaker and Wrigley 1972). However, more recent studies suggest that while the species is associated with spruce-fir forests, it can also be abundant in adjacent northern hardwood forests, which are found at mid-elevations in the region (DeGraaf et al. 1991). The Woodland Jumping Mouse typically occupies cool, moist, wooded habitats with abundant shrubby ground cover and decomposing woody debris, key features that support its ecological needs (Brower and Cade 1966; Brannon 2005). In these habitats, the Woodland Jumping Mouse also provides valuable ecosystem services such as long-distance dispersal of mycorrhizal fungi (Ovaska and Herman 1986). Although environmental conditions influence population density, the species can occasionally reach high abundances, with estimates of up to 59 individuals per hectare reported (Townsend 1935). Despite its prevalence and importance in these ecosystems, the population genetic structure of the Woodland Jumping Mouse within this portion of its range has never been studied.

The Southern Appalachian spruce-fir ecosystem is a biodiversity hotspot that harbors unique species and offers an invaluable opportunity to study the effects of geographic and climatic factors on genetic diversity and connectivity. Although other studies have examined aspects of the population genetic structure of small mammals in the Southern Appalachians (Sipe and Browne 2004; Arbogast et al. 2005; Browne and Ferree 2007), significant gaps remain in our understanding of these patterns. Previous research on other small mammal species such as the Southern Red-backed Vole (*Clethrionomys gapperi*), Smoky Shrew (*Sorex fumeus*), and Masked Shrew (*Sorex cinereus*), has revealed that the French Broad River may serve as a key geographic barrier influencing gene flow in these taxa (Sipe and Browne 2004; Browne and Ferree 2007), raising the possibility that the French Broad River could similarly impact the genetic structure of Woodland Jumping Mouse populations.

As a species nearing its southern range limit in the Appalachian Mountains, the Woodland Jumping Mouse may also display unique genetic patterns shaped by historical range contractions during the Pleistocene (Hewitt 1996). Interestingly, many species that were once widely distributed during the Pleistocene contracted into southern montane refugia during interglacial periods, potentially forming large and stable populations at the edge of their range (Hewitt 1996). As a result, these southern populations may exhibit higher genetic diversity than that found in conspecific northern populations. This disparity could arise because southern refugial populations retained relatively greater genetic diversity during bottleneck events associated with repeated cycles of range expansion and contraction (Hewitt 1996). Thus, both contemporary and historical processes must be considered when assessing the genetic structure and diversity of montane species. This pattern contrasts with the hypothesis that populations at range edges often experience reduced genetic diversity and heightened isolation, which can influence their long-term viability (Eckert et al. 2008).

Here, we employ mitochondrial DNA (mtDNA) and nuclear microsatellite markers to estimate genetic diversity indices and evaluate biogeographic patterns of population structure in woodland jumping

mice across 7 distinct mountains or mountain ranges in the Southern Appalachians in North Carolina. We also include populations from the Northern and Central Appalachians: White Mountains in New Hampshire and Allegheny Mountains in West Virginia, respectively, providing a comparative framework for understanding genetic patterns in the Southern Appalachians. We predict that: (i) sky island populations will be genetically differentiated from one another; (ii) the French Broad River will serve as a geographic barrier for Woodland Jumping Mouse populations in the Southern Appalachians; (iii) genetic diversity indices will be lower; and (iv) inbreeding and kinship coefficients will be higher in Southern Appalachian populations. We also ask if and how these metrics differ between males and females, potentially reflective of sex-biased dispersal. Overall, we aim to clarify how historical barriers, such as geological features like river valleys, and contemporary barriers, such as non-spruce-fir habitat between sky islands, shape genetic diversity and connectivity, which is crucial for assessing the broader implications of environmental and geographic constraints on montane biodiversity.

Methods

Sample collection and preparation

Data were collected on 7 distinct mountains and mountain ranges in North Carolina, which served as Southern Appalachian study areas. Six of these 7 mountains and mountain ranges contained spruce-fir sky islands (Fig. 1). Between May and August 2023 and 2024, 8 samples were collected from Roan Mountain (RM), 22 from Grandfather Mountain (GM), 8 from Mount Mitchell (MtM), 19 from the Great Smoky Mountains (GSM), 11 from the Plott Balsams (PB), 21 from the Great Balsam Mountains (GB), and 35 from Highlands (HL; Fig. 1; Supplementary Data SD1). Within each mountain or mountain range, we sampled between 1 and 4 sites; that were a minimum of 5 km and a maximum of 40 km apart. Sites were selected based on the presence of preferred Woodland Jumping Mouse habitat (i.e., water sources) and historical records of Woodland Jumping Mouse captures. At each site, we set transects, ~1,000 m in length, of Large Folding Heavy Duty (LFHD) Sherman traps (3" × 3.5" × 9"; H.B. Sherman Traps, Tallahassee, FL, United States) placed ~10 m apart. Traps were set and baited with a mixture of sunflower seeds, oats, and dried mealworms 1 to 2 h before sunset and checked the following morning at sunrise. We collected tissue samples from captured individuals and identified the sex of each individual. Sample collection consisted of Woodland Jumping Mouse ear tissue biopsies preserved in 90% to 95% ethanol. We captured and handled woodland jumping mice following applicable guidelines on the use of wild mammals in research from the American Society of Mammalogists (ASM; Sikes et al. 2016) and following protocols approved by the UNCW institutional animal care and use committee (IACUC). We obtained additional mammalian samples for genetic analysis from the West Virginia Division of Natural Resources, the North Carolina Museum of Natural Sciences, the University of North Carolina at Greensboro mammal collection, and the Museum of Southwestern Biology. Samples consisted of Woodland Jumping Mouse liver tissue preserved in ethanol: 12 samples from 1 locality in the White Mountains of NH; 33 from various localities in the Allegheny Mountains of WV; and 9 from various localities in the Great Balsam Mountains, Great Smoky Mountains, and Mount Mitchell in NC (Fig. 1; Supplementary Data SD1). The Northern Appalachian samples (NH) were collected in the 1980s and, as such, were excluded from microsatellite analysis so as not to introduce temporal differences into the spatial structure and genetic diversity analyses. They were, however,

included in the mtDNA analyses described below. We extracted genomic DNA from all samples using QIAGEN DNeasy Blood and Tissue Kits (Qiagen, Valencia, CA, United States) following the manufacturer's standard protocol for DNA isolation from tissue.

Mitochondrial DNA

We amplified and sequenced a ~350 base-pair (bp) segment of the mtDNA control region (D-loop) for all samples using primers CTRL-L (Bidlack and Cook 2001) and TDKD (Kocher et al. 1993). PCR conditions were as follows: an initial denaturation at 94 °C for 45 s followed by 34 cycles of a 30 s denaturation step at 94 °C, a 30 s annealing step at 52 °C, and a 45 s extension step at 72 °C, with a final 3 min extension at 72 °C. We sent unpurified PCR products to the NC State University Genomic Sciences Laboratory (Raleigh, NC, United States) for purification and Sanger sequencing.

We visually inspected mtDNA sequences for errors and multiple peaks, then trimmed and aligned them using SEQUENCHER v4.10.1 (Gene Codes Corporation, Ann Arbor, Missouri). To determine within-group mtDNA nucleotide diversity (π ; Nei 1987) and the average number of nucleotide differences between sequences (k ; Tajima 1983), we used DnaSP v6 (Rozas et al. 2017). Following these initial analyses, we separated individuals by sex and calculated diversity indices for male and female individuals separately. To create haplotype networks with a 95% parsimony connection limit (Templeton et al. 1992), we used TCS v1.23 (Clement et al. 2000). We used Geneious Prime 2024.0.7 (<https://www.geneious.com>) to infer haplotype trees using a GTR+Gamma substitution model, which is highly flexible and accounts for both variable substitution rates between nucleotides and rate variation across sites in a DNA sequence (Waddell and Steel 1997). The maximum likelihood (ML) approach was run with 1,000 ML replicates to assess nodal support. For the Bayesian approach, each MCMC run used a burn-in of 50,000 with a sampling frequency of 5,000 generations and a run length of 1,000,000 generations. *Napaeozapus abietorum* was used as an outgroup for these analyses.

Microsatellites

We genotyped individuals at 13 microsatellite loci using novel *Napaeozapus* primers developed by CD Genomics (Shirley, New York, United States; Supplementary Data SD2). PCR conditions were as follows: an initial denaturation at 95 °C for 15 min followed by 35 cycles of a 30 s denaturation step at 94 °C, a 30 s annealing step at 58 °C, and a 20 s extension step at 72 °C, with a final 10 min extension at 72 °C. We mixed 1 µl PCR product with 9 µl highly deionized formamide (HiDi) and 0.4 µl GeneScan-600 LIZ size standard (Applied Biosystems, Foster City, California, United States) for genotyping on an Applied Biosystems 3500 Genetic Analyzer at the UNCW Center for Marine Science Core Genetic Laboratory. We scored fragments using Applied Biosystems Microsatellite Analysis Software (ThermoFisher Scientific Inc.).

We ran Micro-Checker v2.2.3 to check for null alleles, large allele drop-out, and stuttering (Van Oosterhout et al. 2004). To determine basic diversity indices, we calculated the number of alleles, allelic richness rarified to the smallest population sample size ($n = 8$) and observed and expected heterozygosity (H_o and H_e) using the packages "PopGenReport" and "hierfstat" in R v4.4.0 (Adamack and Gruber 2014; Gruber and Adamack 2015; Goudet and Jombart 2022; R Core Team 2024). We determined inbreeding coefficients (F_{IS}) and tested for significance with 1,000 permutations of the data using GenoDive (Meirmans and Van Tienderen 2004). To determine family structure and contemporary patterns of association, we calculated kinship

coefficients (k ; Loiselle et al. 1995) using GenoDive (Meirmans and Van Tienderen 2004). We binned individuals according to the following levels of kinship: "nearly identical," $0.57 > k > 0.375$; "full siblings," $0.375 > k > 0.1875$; "half siblings," $0.1875 > k > 0.09375$; and "quarter siblings," $0.09375 > k > 0.047$, following the methods used by Iacchei et al. (2013). Following these initial analyses, we separated individuals by sex and calculated diversity indices, inbreeding, and kinship coefficients for male and female individuals separately. We then tested for significant differences of these variables between males and females using t -tests with unequal variance.

To infer spatial patterns of genetic structure across sampled sites, we calculated all pairwise values of population differentiation using Weir and Cockerham's F_{ST} (Weir and Cockerham 1984) and tested for significance with 10,000 permutations of the data using GenoDive (Meirmans and Van Tienderen 2004). To test whether patterns of differentiation within Woodland Jumping Mouse populations fit an isolation by distance model, we performed multiple regression analysis with 10,000 permutations using package "ecodist" in R (Goslee and Urban 2007; R Core Team 2024). The geographic distance (in km) between locations was determined as the shortest straight-line path connecting those points. For this analysis, we removed the Allegheny Mountains population from consideration as it was the only population that we sampled that was >200 km in distance from the other populations used in this study. We used the same methods to test for F_{ST} and isolation by distance in male and female woodland jumping mice separately.

We used STRUCTURE v2.3.4 to detect genetic structure and assign individuals to genetic clusters (Pritchard et al. 2000). We assessed the number of genetic clusters (K) for values of K ranging from 1 to 9 using an admixture model with allelic frequencies correlated among populations and population information assigned. We ran 10 Bayesian Markov chain Monte Carlo (MCMC) searches of 350,000 steps with a 50,000-step burn-in. STRUCTURE results were collated and ΔK was computed using the Evanno method (Evanno et al. 2005) implemented in STRUCTURE SELECTOR (Li and Liu 2018) to find the value of K that best fit the observed distribution of genotypes. Given that ΔK frequently identifies $K = 2$ as the top level of hierarchical structure, even when more subpopulations are present, we also conducted hierarchical STRUCTURE runs by first identifying major clusters, then analyzing each cluster separately (Janes et al. 2017). Results were visualized using the package "mapmixture" in R (Jenkins 2024; R Core Team 2024).

To further partition genetic variance within and among populations, we performed an AMOVA under the assumptions of the infinite allele model using GenoDive with 1,000 permutations of the data to test for statistical significance (Meirmans and Van Tienderen 2004). Populations were grouped in part based on the STRUCTURE results (see below) such that variance was assessed among and within major regions (those sampled south of the French Broad River, north of the French Broad River, and in the Central Appalachians), among individual populations within regions, and among and within Woodland Jumping Mouse individuals within populations. Finally, to visualize genetic clusters, we created discriminant analysis of principal components (DAPC) plots using the function DAPC from the package "adegenet" in R (Jombart 2008; Jombart and Ahmed 2011; R Core Team 2024). We also created separate DAPC plots for male and female woodland jumping mice following the same methods (Jombart 2008; Jombart and Ahmed 2011; R Core Team 2024).

To test for population bottlenecks, we used BOTTLENECK v1.2.02 (Piry et al. 1999). We used both the mode-shift test, which can detect recent bottlenecks (within the past 25 to 50 generations) and the

Wilcoxon's sign-rank test, which can detect older bottlenecks (within the past 250 generations; Cornuet and Luikart 1996). For the Wilcoxon's sign-rank test, we chose to use the 2-phase model (TPM; Di Rienzo et al. 1994) and set the parameters to 95% single-step mutations and 5% multiple-step mutations and set the variance among multiple steps to 12% as suggested by Piry et al. (1999). To estimate θ —defined as $4N_e\mu$ where N_e is the effective population size and μ is the mutation rate—and immigration rates among populations, we used LAMARC v2.1.10 (Kuhner et al. 2006). We estimated θ and immigration rates using 10 initial chains with 500 genealogies sampled and 2 final chains with 10,000 genealogies sampled.

Results

Mitochondrial DNA

We successfully obtained ~350 bp mtDNA control region sequences from most of the collected Woodland Jumping Mouse tissues (total $n = 176$ out of 179; Table 1). Analysis of the mtDNA control region sequence data revealed a total of 14 polymorphic sites and 30 different haplotypes across the 9 populations. The number of haplotypes per population was lowest at Roan Mountain ($n = 1$) in the Southern Appalachians and highest in the Allegheny Mountains in the Central Appalachians ($n = 12$; Table 1). Moreover, both mtDNA nucleotide diversity (π) and the average number of nucleotide differences between sequences (k) were lowest at Grandfather Mountain in the Southern Appalachians (0.001 and 0.355, respectively) and highest in the Allegheny Mountains in the Central Appalachians (0.005 and 1.890, respectively; Table 1). We were unable to determine π and k for Roan Mountain because all individuals shared the same haplotype. We found no significant differences in mtDNA diversity indices between males and females (Supplementary Data SD3).

The network shows all 30 Woodland Jumping Mouse haplotypes joined together, with the 2 main clusters of Southern Appalachian haplotypes being separated by 1 nucleotide difference (Fig. 2). Within the Southern Appalachians, we identified 12 unique haplotypes and 2 shared haplotypes, 1 with the White Mountains population (Northern Appalachians) and the other with the Allegheny Mountains population (Central Appalachians). Of the 12 unique haplotypes, a clear pattern of spatial arrangement emerged, with only 2 haplotypes shared

between populations separated by the French Broad (Fig. 3). We also identified 5 unique haplotypes in the White Mountains and 11 unique haplotypes in the Allegheny Mountains. We recovered a single best ML tree and a consensus Bayesian tree using Geneious. There was no internal clade structure, with 1 exception that lacked support (Supplementary Data SD4).

Microsatellites

We obtained microsatellite genotypes from 158 out of 179 individuals (Table 2). Overall, 98.42% of those Woodland Jumping Mouse loci were successfully amplified and scored (4,043 of 4,108 alleles). All loci were polymorphic, ranging from 7 alleles (NI-3684441) to 26 alleles (NI-3521084; Supplementary Data SD5). A global test for the presence of null alleles and stuttering revealed that several loci, including NI-3521084, NI-1509778, NI-155143, NI-3886996, and NI-241994, showed potential evidence of null alleles (i.e., homozygote excess), though the frequency was low, this pattern was not detected in all populations. We ran analyses with and without these loci, and the results were qualitatively similar. As such, we report results using the full dataset.

The mean number of alleles varied across populations and was lowest at Roan Mountain in the Southern Appalachians ($\bar{x} = 5.77$) and highest in the Allegheny Mountains in the Central Appalachians ($\bar{x} = 9.69$), though this might have been driven by differences in sample size ($n = 8$ and 33, respectively). Comparisons of rarefied allelic richness were relatively similar across populations, with the lowest ($\bar{x} = 5.12$) and highest ($\bar{x} = 6.70$) values at the Plott Balsams and Highlands-Cashiers Plateau in the Southern Appalachians, respectively (Table 2). Observed heterozygosity (H_o) values were lower than expected heterozygosity (H_e) values in all cases, but both diversity indices were relatively similar across all sampled populations (Table 2). We detected significant F_{IS} estimates in all populations (Table 2). Estimates of k were relatively consistent across populations, with the lowest kinship estimate ($k = 0.016$) recorded at Roan Mountain and the highest kinship estimate ($k = 0.041$) recorded in the Allegheny Mountains, values both within the unrelated category (Table 2). Diversity indices and inbreeding coefficients were not significantly different between males and females, but estimates of mean kinship were significantly higher in females ($k = 0.048$; quarter-siblings) than in males ($k = 0.004$; unrelated) (t -test = -3.035 , $df = 11$, P -value = 0.011; Supplementary Data SD6).

Pairwise comparisons of genetic differentiation were highest between the Allegheny Mountains and Mount Mitchell and lowest between Roan Mountain and Mount Mitchell (Table 3; Supplementary Data SD7). Within sampled Southern Appalachian populations, Highlands was significantly differentiated from all populations (mean pairwise $F_{ST} = 0.023$). Minimal genetic differentiation was detected among populations southwest ($n = 0$ significant comparisons) and northeast ($n = 1$ significant comparison) of the French Broad River, though significant pairwise differentiation was detected in all cross-river comparisons (mean $F_{ST} = 0.028$; Table 3). Genetic distance was weakly correlated with geographic distance ($r^2 = 0.031$, P -value = 0.045; Supplementary Data SD8). Pairwise F_{ST} values were significantly higher between females than males (t -test = -6.399 , $df = 28$, P -value = $6.3E-7$; Fig. 4), though weakly significant isolation by distance was observed in males ($r^2 = 0.064$, P -value = 0.042) but not in females ($r^2 = 0.025$, P -value = 0.285; Supplementary Data SD9).

The STRUCTURE analysis returned 3 clusters ($\Delta K = 3$; Supplementary Data SD10), with Cluster A containing the Highlands, the Great Balsams, the Plott Balsams, and the Great Smoky Mountains; Cluster

Table 1 Summary statistics from mtDNA control region sequences for Woodland Jumping Mouse (*Napaeozapus insignis*) populations sampled across 9 mountain sites from the Southern, Central, and Northern Appalachians, including sample size (n), number of haplotypes (N_h), number of polymorphic sites (P_s), nucleotide diversity (π), and the average number of nucleotide differences between sequences (k).

Population	n	N_h	P_s	π	k
Highlands-Cashiers plateau	34	5	4	0.004	1.392
Great Balsam Mtns	23	5	4	0.004	1.277
Plott Balsams	11	3	3	0.004	1.527
Great Smoky Mtns	20	5	4	0.004	1.505
Mt. Mitchell	13	4	3	0.003	0.897
Grandfather Mtn	22	5	4	0.001	0.355
Roan Mtn	8	1	0	–	–
Allegheny Mtns	33	12	6	0.005	1.890
White Mtns	12	6	4	0.003	0.955

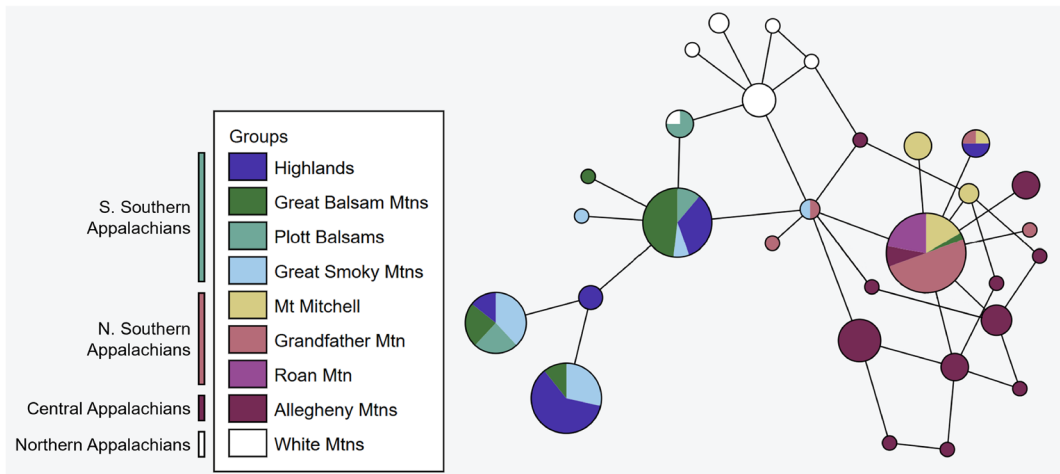


Fig. 2 Network of 30 Woodland Jumping Mouse (*Napaeozapus insignis*) haplotypes in the Southern, Central, and Northern Appalachians, with colors indicating sampled populations, and connecting lines representing single nucleotide differences between haplotypes. Circles are proportional to their sample size.

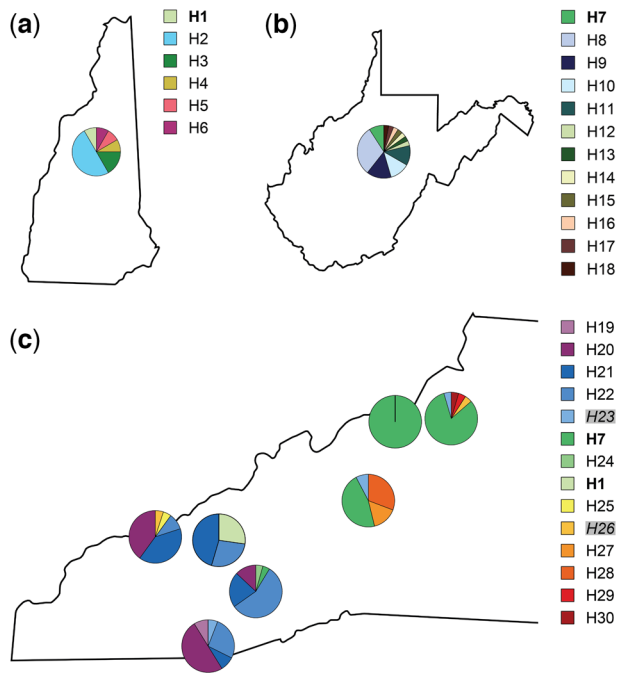


Fig. 3 Geographic distribution of Woodland Jumping Mouse (*Napaeozapus insignis*) mtDNA haplotypes across all sampled populations in a) the Southern Appalachians, b) the Central Appalachians, and c) the Northern Appalachians; each color represents a unique haplotype. Bolded haplotypes are shared among regions and shaded haplotypes are shared across the French Broad River.

B containing Mount Mitchell, Grandfather Mountain, Roan Mountain; and Cluster C containing the Allegheny Mountains. Clusters A and B were separated by the French Broad River. Subsequent hierarchical analyses on each cluster revealed further population structure within Cluster A: all individuals at Highlands were assigned to subcluster A1; the remaining 3 populations contained individuals from both sub-clusters A1 and A2 (Fig. 5). The DAPC plot showed similar patterns of spatial structure across the 3 regions (Fig. 6). When only males were included, spatial structure appeared more pronounced than when only females were analyzed (Supplementary Data SD11).

Similarly, the AMOVA analysis revealed significant spatial structuring among regions southwest and northeast of the French Broad

Table 2 Summary statistics from microsatellite genotypes for Woodland Jumping Mouse (*Napaeozapus insignis*) populations sampled across 8 mountain sites from the Southern and Central Appalachians including sample size (n), allelic richness averaged across loci (A), rarefied allelic richness (AR), inbreeding coefficients (F_{IS} bolded where significant at $P < 0.5$), observed heterozygosity (H_o), expected heterozygosity (H_e), and mean kinship coefficients (k).

Population	n	A	AR	F_{IS}	H_o	H_e	k
Highlands-Cashiers plateau	27	9.85	6.70	0.19	0.66	0.82	0.019
Great Balsam Mtns	23	8.69	6.05	0.23	0.61	0.79	0.016
Plott Balsams	10	5.85	5.12	0.17	0.63	0.76	0.024
Great Smoky Mtns	20	8.62	6.39	0.19	0.67	0.82	0.017
Mt. Mitchell	13	7.00	5.57	0.18	0.63	0.77	0.031
Grandfather Mtn	22	8.00	6.00	0.14	0.67	0.78	0.019
Roan Mtn	8	5.77	5.20	0.13	0.67	0.77	0.016
Allegheny Mtns	33	9.69	6.22	0.21	0.62	0.77	0.041

Table 3 Matrix of pairwise genetic distance (F_{ST}) with distance (km) above the dashed lines, bolded values are significant at $P < 0.05$.

	HL	GB	PB	GSM	MtM	GM	RM	AM
HL	-	37.933	55.403	57.086	113.087	173.746	152.335	511.333
GB	0.014	-	32.906	35.617	75.509	136.024	114.423	473.612
PB	0.030	0.012	-	2.763	80.742	136.654	110.661	463.961
GSM	0.012	0.007	0.009	-	82.266	137.610	111.341	463.804
MtM	0.034	0.030	0.039	0.029	-	60.714	43.344	401.526
GM	0.018	0.025	0.025	0.031	0.014	-	29.161	342.533
RM	0.029	0.005	0.036	0.030	-0.010	0.009	-	359.578
AM	0.043	0.046	0.041	0.042	0.047	0.038	0.030	-

River in the Southern Appalachians, and in the Central Appalachians (variance explained = 2.3%, P -value = 0.009; Table 4). Less pronounced spatial structure was also apparent among sky island populations within regions (variance explained = 1.2%, P -value = 0.001), though values overall were low and the majority of the variation was explained by differences within and among individuals in a population (Table 4).

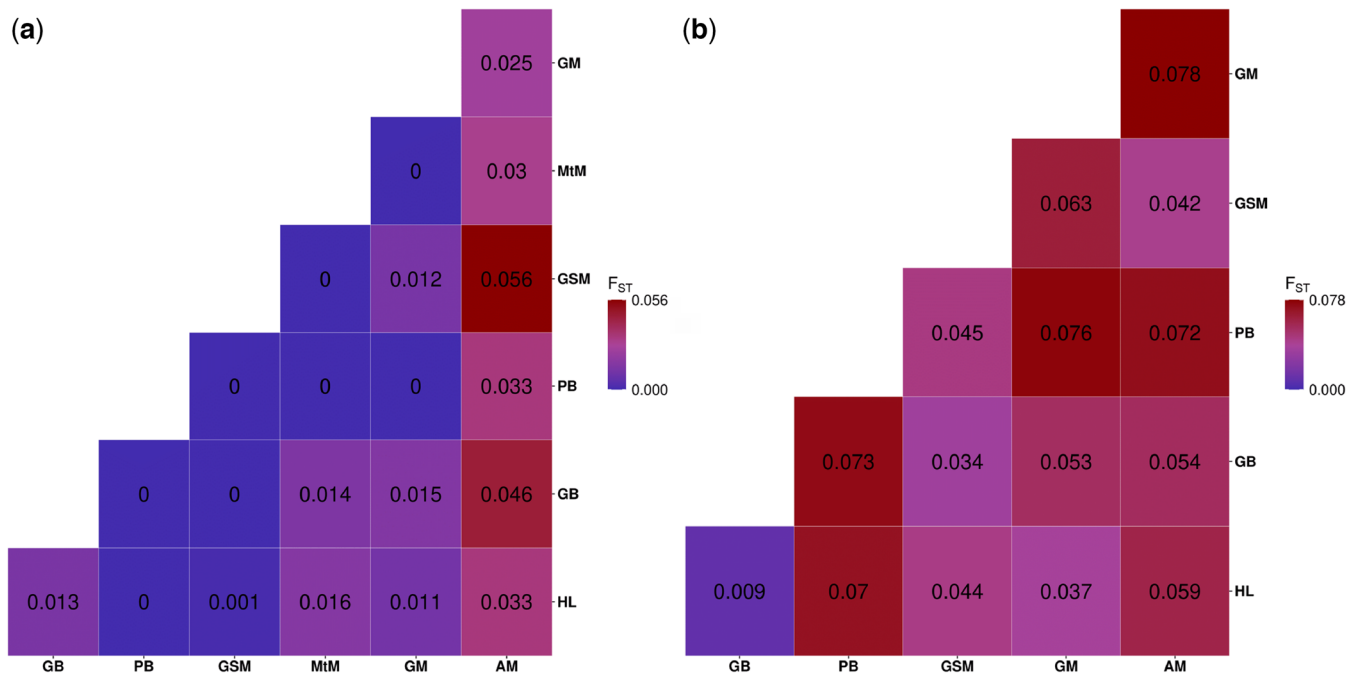


Fig. 4 Pairwise genetic distance (F_{ST}) using microsatellite data between populations of a) male and b) female woodland jumping mice (*Napaeozapus insignis*). Red indicates higher estimates of F_{ST} and thus, genetic differentiation; blue indicates the converse.

We detected no evidence of relatively old bottlenecks (ca. 250 generations) in the sampled Woodland Jumping Mouse populations, and weak evidence of recent bottlenecks (ca. 25 to 50 generations) in 2 populations. Roan Mountain showed a signature of heterozygote excess, although this signature was not significant (Supplementary Data SD12). The mode-shift tests revealed shifted distributions at Roan Mountain and the Plott Balsams, though sample sizes for both were small ($n = 8$ and 11 , respectively) and results should be interpreted with caution (Supplementary Data SD12). Effective population size estimates were generally high; lowest in Roan Mountain and the highest in the Allegheny Mountains, with θ ranging from 1.095 to 10.431 (Supplementary Data SD13). Overall, immigration rates between populations were high (Supplementary Data SD13).

Discussion

In this study, our data suggest that the population genetic structure of Southern Appalachian woodland jumping mice is primarily driven by historical riverine barriers rather than by the more recent, contemporary barriers posed by the distribution of the non-spruce-fir forests that separate the disjunct, high-elevation spruce-fir sky islands in the region. Although spruce-fir sky islands clearly serve as an important habitat for woodland jumping mice in the Southern Appalachians, contrary to our initial predictions, our results indicate that the present-day fragmented nature of this habitat is not reflected in the population genetic structure of woodland jumping mice in the Southern Appalachians. Several factors could have contributed to this surprising result, including: (i) the ability of woodland jumping mice to inhabit not just spruce-fir forests, but also adjacent high-mid-elevation hardwood forest habitats in the Southern Appalachians; (ii) the relatively recent (i.e., post-Pleistocene) fragmentation of a previously much more continuous spruce-fir forest ecosystem in the region; and (iii) an extremely large effective population size of woodland jumping mice in the Southern Appalachians. However, Southern Appalachian populations of this species form separate genetic clusters northeast

and southwest of the French Broad River, consistent with our prediction that the French Broad River acts as an important geographic and genetic barrier for woodland jumping mice. Additionally, we documented that the Highlands-Cashiers Plateau population is significantly differentiated from all other populations we sampled.

Whereas previous studies of other spruce-fir-associated small mammals, such as smoky shrews, southern red-backed voles, and Carolina northern flying squirrels (*Glaucomys sabrinus coloratus*), have reported significant population structure across spruce-fir sky islands (Sipe and Browne 2004; Browne and Ferree 2007; Schumacher 2012), we found no evidence of similar patterns in woodland jumping mice. Rather, and contrary to our prediction, we found limited inter-island genetic differentiation among the sky island populations of woodland jumping mice, which may reflect immense Woodland Jumping Mouse effective population sizes. Although estimating effective population size in non-isolated populations can be challenging (Ryman et al. 2019), it is worth noting that our estimates of θ were very high (ranging from 1.095 to 10.431; Supplementary Data SD7). Because genetic drift is weak in large populations (Kimura 1983), low levels of drift may contribute to the lack of pronounced genetic structure in Southern Appalachian woodland jumping mice, which appear to have extremely large contemporary population sizes.

Conversely, while woodland jumping mice are a key component of the high-elevation spruce-fir forests of the Southern Appalachians, they are not restricted to this habitat. For example, there are records (albeit rare) of woodland jumping mice occurring at elevations well below the spruce-fir/hardwood ecotone in the Southern Appalachians (including 1 outlier record in South Carolina at 251 m in elevation; Williams and Webster 2022). Thus, while extensive ongoing connectivity between geographically distant Southern Appalachian montane island populations seems unlikely, occasional instances of immigration along riparian corridors (especially during cool weather; Hall 1946; Lawlor 1998) may promote higher than expected levels of gene flow between populations of woodland jumping mice in the Southern Appalachians. This possibility is supported by a study of a similar

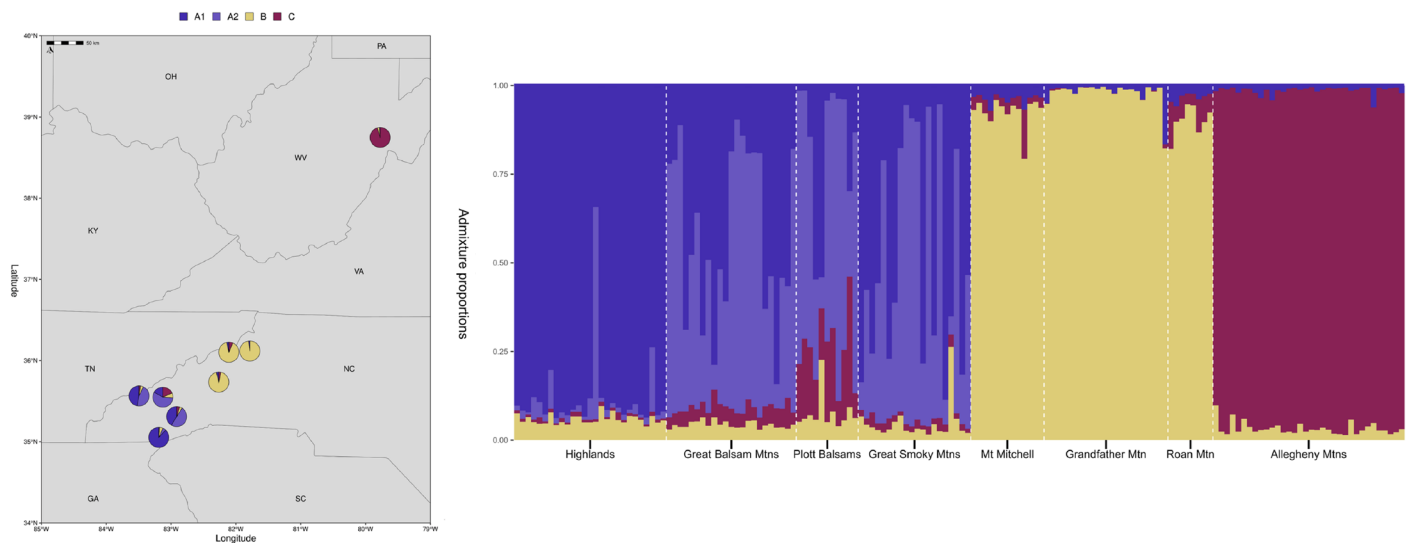


Fig. 5 Geographic distribution of STRUCTURE population clusters ($n=4$) and admixture proportions per Woodland Jumping Mouse (*Napaeozapus insignis*) population in the Southern and Central Appalachians. Each color represents a unique genetic subcluster.

species, the Pacific Jumping Mouse (*Zapus trinotatus*), that revealed riparian habitats facilitate dispersal of the species, even across topographic barriers (Vignieri 2005). Riparian habitats may therefore similarly facilitate dispersal of woodland jumping mice where there are small, mid- to high-elevation mountain streams, which could contribute to the lack of significant genetic differentiation between sky islands. Therefore, the theory that sky island small mammals do not migrate outside of their respective sky island habitat, posed by Brown (1971), may not apply to many of the small mammals associated with the spruce-fir sky islands of the Southern Appalachians. Finally, woodland jumping mice almost certainly had extensive geographic connectivity in the relatively recent past (i.e., during cooler periods of the Pleistocene). As such, factors such as ancestral polymorphism may be influencing contemporary patterns of genetic structure. This possibility may be especially likely in our study given the large estimated effective population size for Southern Appalachian Woodland Jumping Mouse populations today and because populations were likely much larger during cooler periods of the Pleistocene when spruce-fir forests were much lower and more extensive in the study region.

We found strong evidence that the French Broad River functions as a geographic barrier for Woodland Jumping Mouse populations. This result was supported by the existence of 2 main genetic clusters within the Southern Appalachians that occur southwest and northeast of the French Broad River, in addition to a third cluster representing the Allegheny mountains (Fig. 5). Hierarchical structure analysis identified additional subclusters (A1 and A2) that occur southwest of the French Broad River, indicative of differentiation with respect to Highlands. Similarly, the geographic distribution of mtDNA haplotypes shows that only 2 out of the 12 unique haplotypes that we identified in the Southern Appalachians are shared between populations of woodland jumping mice found on the southwest and northeast sides of the French Broad River. Moreover, the DAPC plot and pairwise estimates of F_{ST} based on microsatellite data show similar geographic patterns of genetic structure to those based on the distribution of mtDNA haplotypes and the microsatellite genetic cluster analysis (Fig. 6, Table 3). Thus, our combined genetic data support the idea that the French Broad River is likely a geographic barrier for woodland

jumping mice (and presumably many other species of small mammals; Sipe and Browne 2004; Browne and Ferree 2007), across which there is little contemporary gene flow.

Additionally, pairwise values of F_{ST} indicate that the Highlands-Cashiers Plateau population is significantly differentiated from all other sampled populations (Table 3); individuals from this location were primarily assigned to subcluster A1 (Fig. 5), whereas other populations sampled from southwest of the French Broad River were more admixed. This observed pattern may also be driven by the position of multiple low-lying river valleys that separate the Highlands-Cashiers Plateau from other populations located south of the main stem of the French Broad River, including the Tuckasegee, the Chattooga, and the Cullasaja.

This pattern is consistent with several previous studies that have found that riverine barriers appear to play a primary role in shaping geographic patterns of genetic variation in both the Southern Appalachians (Sipe and Browne 2004; Browne and Ferree 2007) as well as in many other places, such as the Eastern Arc Mountains in Tanzania (Stanley and Esselstyn 2010). Alternatively, the genetic differentiation of woodland jumping mice from the Highlands-Cashiers Plateau may be explained by the fact that other areas that we sampled southwest of the French Broad River (the Great Smoky Mountains, the Plott Balsams, and the Great Balsams) are located on adjacent ridges and separated by relatively small gaps of non-spruce-fir forest habitat. Furthermore, the elevations in these gaps do not drop below those commonly inhabited by woodland jumping mice (Linzey 1995). In contrast, the Highlands-Cashiers Plateau is more distant from these ridges, and no patches of high-elevation spruce-fir occur between it and the Great Balsam Mountains, the nearest area sampled. Therefore, differences in elevation and corresponding intervening habitat between these sites may have also played a role in shaping the geographic patterns of genetic structure observed.

Although we predicted that genetic diversity indices would be lower in the Southern Appalachians than in the Central or Northern Appalachians, support for this prediction was mixed. While allelic richness varied widely across sites, rarefied allelic richness—wherein estimates of allelic richness are based on a sample size rarefied to that of the population with the smallest sample size—was relatively

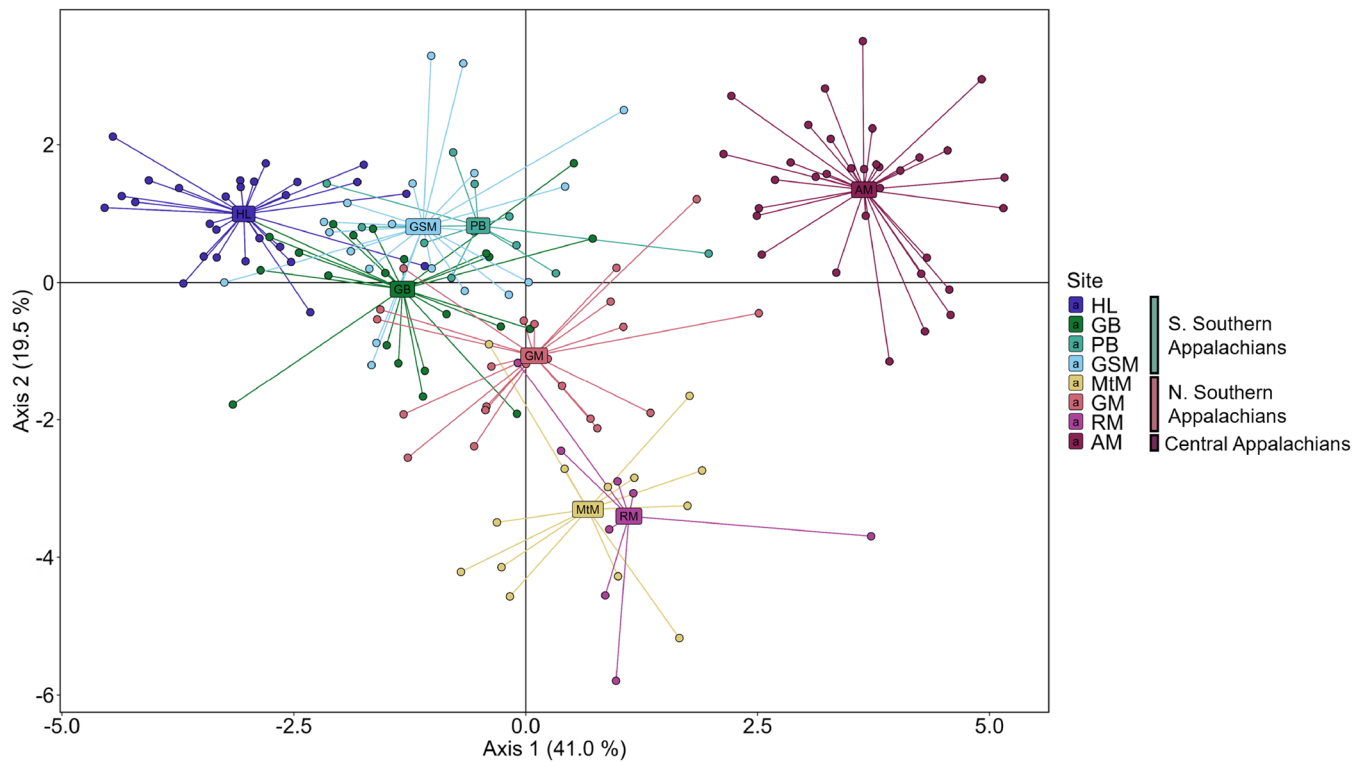


Fig. 6 Discriminant analysis of principal components (DAPC) plot using microsatellite data from the eight Woodland Jumping Mouse (*Napaeozapus insignis*) populations in the Southern and Central Appalachians.

Table 4 Hierarchical analysis of molecular variance (AMOVA) as a weighted average over all loci among regions (north and south of the French Broad, and Central Appalachians), among populations within regions, and within and among individuals within populations.

Source of variation	% of variation	P value
Among population groups	2.3	0.009
Among populations within population groups	1.2	0.001
Among individuals within populations	17.5	0.001
Within individuals	79.0	–

consistent across populations (Table 2). Other diversity indices calculated with our microsatellite data were also fairly consistent and generally high (Table 2). This pattern differs from that observed by Arbogast et al. (2005), where Carolina northern flying squirrels were found to have lower indices of genetic diversity than other populations of northern flying squirrels. It is worth noting, though, that since 2005, *G. sabrinus* has been split into 2 species, *G. sabrinus* and *G. oregonensis* (Arbogast et al. 2017), so the inclusion of *G. oregonensis* individuals in the aforementioned 2005 study likely greatly inflated the diversity estimates for populations outside the Southern Appalachians (Arbogast et al. 2005). However, mtDNA diversity indices varied more widely (Table 1). For example, the number of haplotypes observed at each locality was highest in the Central Appalachians, where we identified 12 haplotypes, whereas it ranged from 1 to 5 per population in the Southern Appalachians (Table 1). Additionally, nucleotide diversity and the average number of nucleotide differences between sequences were lowest in Grandfather Mountain and Roan Mountain. Interestingly, these 2 sites have the lowest spruce-fir coverage of any

spruce-fir sky island sampled, which could factor into their lower levels of genetic diversity.

Despite the relatively high genetic diversity and very large estimated effective size of these populations, we detected significant inbreeding coefficients in all Southern Appalachian populations and in the Allegheny Mountains population. This result could be due to the Wahlund effect, which is a reduction in heterozygosity in a population caused by its subdivision into smaller, reproductively isolated groups (Dharmarajan et al. 2013). When individuals from these subpopulations are pooled together, the overall population shows a higher frequency of homozygotes and a lower frequency of heterozygotes than would be expected if the entire population were mating randomly. In this study, sampling tended to occur across several localized patches, resulting in the identification of both positive inbreeding coefficients and large estimates of θ (which can be used as a proxy for effective population size). The Wahlund effect might reconcile these patterns, especially given that behaviors thought to result in inbreeding avoidance have been observed in other species of jumping mice (genus *Zapus*; Vignieri 2007). High levels of promiscuity have also been observed in other species of jumping mice and small mammals more generally, raising the likelihood of limited inbreeding and low kinship among breeding individuals (Vignieri 2007). However, it remains an open question whether similar avoidance behaviors and mating patterns are present in woodland jumping mice.

Finally, we asked whether patterns of relatedness and population connectivity might differ among the sexes, potentially reflecting sex-biased dispersal. We found that within-population estimates of kinship were significantly higher in females than in males and that measures of genetic differentiation between populations were significantly higher in females than in males (Fig. 6). Indeed within-population kinship estimates in females tended to reflect

degrees of relatedness attributed to “quarter-siblings,” whereas male kinship estimates tended to suggest that individuals were unrelated. This pattern may indicate that female woodland jumping mice are more philopatric than males as high levels of kinship are often associated with strong philopatry (Arbogast et al. 2022), suggesting they may not disperse as far from their natal area as do their male counterparts. This pattern complements mark-recapture and genetic studies that uncovered evidence of male-biased dispersal in Pacific jumping mice (Vignieri 2007). Indeed, studies of movements and home ranges in woodland jumping mice have found that males travel further over time and have larger home range sizes (Ovaska and Herman 1988). In contrast to previous work, however, we did not detect the same pattern of isolation by distance, which found that females were significantly isolated by distance while males were not (Vignieri 2007). This difference leads us to believe that while dispersal may be male-biased, it does not occur over far enough distances to drive patterns of population genetic structure. Indeed, Ovaska and Herman (1988) found that the distance between the 2 farthest captures of male woodland jumping mice between June and August was 447 m.

Although our results reveal subtle patterns of population genetic structure in the woodland jumping mice of the Southern Appalachians, much is still unknown about subpopulations within this portion of their range and familial structure in the species as a whole. Due to our sampling protocol, we likely missed genetic patterns at the subpopulation or familial level, as high F_{IS} estimates likely indicate cryptic substructure rather than true inbreeding. Therefore, future studies of woodland jumping mice with modifications to sample collection (i.e., multiple sampling sites within each sky island with sufficient sample sizes for statistical tests) may provide additional insights into the patterns driving this result, and more samples are needed from the Plott Balsams and Roan Mountain to confirm the recent bottlenecks that we inferred in these populations with the mode shift test (Supplementary Data SD5). Additionally, we recommend more sampling in the Highlands-Cashiers Plateau area, where the single site sampled was genetically differentiated from other sites sampled south of the French Broad River. Additional sampling there may help to further clarify patterns of genetic structure near the southern edge-of-range of the Woodland Jumping Mouse. Moreover, the scope of our study was limited primarily to the southern extent of the geographic range of this species; similar studies of conspecific populations would provide additional context for interpreting Woodland Jumping Mouse population genetic structure in the Southern Appalachians. Finally, while some studies have addressed similar questions about other Southern Appalachian spruce-fir sky island-associated small mammals (Sipe and Browne 2004; Arbogast et al. 2005; Browne and Ferree 2007), much is still unknown about the genetic health and resilience of most species of small mammals associated with the spruce-fir ecosystem of the Southern Appalachians.

Both the highly fragmented nature of the spruce-fir sky islands of the Southern Appalachians and the many threats facing this ecosystem have generated concern about the future of spruce-fir associated organisms. Moreover, projections of range reductions for many Appalachian mammals due to climate change threaten to increasingly fragment and isolate these populations (Zhu et al. 2022), which may result in future threats to their genetic health. Establishing temporal baselines on genetic diversity and structure for these populations will assist long-term monitoring efforts (Pimm et al. 2006); however, the woodland jumping mice of the Southern Appalachians presently do not warrant conservation concern. Indeed, our findings suggest gene flow among Southern Appalachian Woodland Jumping Mouse

populations in the recent past. We conclude that although spruce-fir sky islands are an important habitat for woodland jumping mice in the Southern Appalachians, their present-day fragmented distribution has not resulted in similarly fragmented patterns of contemporary population genetic structure in the species. From a conservation perspective, our study suggests that while woodland jumping mice are an important ecological component of Southern Appalachian spruce-fir sky islands (Ovaska and Herman 1986), the near- and long-term fate of the species is certainly not solely dependent on that of contemporary spruce-fir sky islands. This conclusion has important implications given the predicted changes in elevational distributions and overall reduction in coverage of spruce-fir forests in the Southern Appalachians associated with climate change.

Acknowledgments

We thank the North Carolina Wildlife Resources Commission (NCWRC) and the American Society of Mammalogists for providing funding for this project. We thank the WVDNR, North Carolina Museum of Natural Sciences, University of North Carolina at Greensboro Mammal Collection, and Museum of Southwestern Biology at the University of New Mexico for collecting, providing, or loaning us samples for use in this study. Special thanks to the staff of these museums who assisted us and the collectors of samples loaned to us, including Lisa Gatens, Michael Cove, Joseph Cook, Ryan Stephens, Mariko Yamasaki, Bryan McLean, Olivia Chapman, Amanda Reyes, and Leo Ivey. Additional thanks to Joseph Cook as well as Zachary Long, who provided helpful comments on earlier versions of this manuscript. Finally, thank you to Melissa Smith, who assisted in editing 2 of the figures. S.J.K. serves at the National Science Foundation—any opinions, findings, conclusions, or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Author contributions

Emma Claire Watersmith (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Visualization, Writing—original draft, Writing—review & editing), Brian S. Arbogast (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing), Alexandra G. DeLuca (Investigation, Methodology, Resources, Writing—review & editing), Radmila Petric (Investigation, Methodology, Resources, Writing—review & editing), Marketa Zimova (Investigation, Resources, Writing—review & editing), Jenifer A. Mallinoff (Investigation, Resources, Writing—review & editing), and Stephanie Kamel (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing)

Supplementary data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1. Woodland Jumping Mouse (*Napaeozapus insignis*) sample information, including sample ID, mtDNA, and microsatellite success, collection year and locality, and tissue catalog ID.

Supplementary Data SD2. Woodland Jumping Mouse (*Napaeozapus insignis*) microsatellite locus ID, size range, and primer sequences.

Supplementary Data SD3. Summary statistics from both mtDNA control region sequences for male and female woodland jumping mice (*Napaeozapus insignis*) from all sampled Woodland Jumping Mouse populations, including sample size (n), the number of haplotypes (N_h), number of polymorphic sites (P_s), nucleotide diversity (π), and the average number of nucleotide differences between sequences (k).

Supplementary Data SD4. Maximum likelihood haplotype tree depicting the phylogenetic relationships of all 30 identified Woodland Jumping Mouse (*Napaeozapus insignis*) haplotypes with bootstrap support values shown along the node branches. A *N. abietorum* sequence was included as an outgroup in which the tree is rooted. This tree is representative of the relationships observed from the Bayesian haplotype tree as well.

Supplementary Data SD5. Summary statistics from microsatellite genotypes at 13 microsatellite loci, including the effective number of alleles (A_E), inbreeding coefficients (F_{IS}), observed heterozygosity (H_O), and expected heterozygosity (H_E).

Supplementary Data SD6. Summary statistics from both microsatellite genotypes for male and female woodland jumping mice (*Napaeozapus insignis*) from all sampled Woodland Jumping Mouse populations including sample size (n), allelic richness averaged across loci (A), rarefied allelic richness (AR), inbreeding coefficients (F_{IS}), observed heterozygosity (H_O), expected heterozygosity (H_E), and mean kinship coefficients (k).

Supplementary Data SD7. Matrix of pairwise genetic distance (F_{ST}) calculated from microsatellite data. Red indicates higher estimates of F_{ST} and thus genetic differentiation; blue indicates lower estimates F_{ST} and genetic differentiation.

Supplementary Data SD8. Relationship between geographic distance (km) and genetic distance (F_{ST}) between North Carolina localities of sampled Woodland Jumping Mouse (*Napaeozapus insignis*) populations. The r -squared value and slope of the fitted line are included in the lower right corner of the plot.

Supplementary Data SD9. Relationship between geographic distance (km) and genetic distance (F_{ST}) between North Carolina localities for: A) Woodland Jumping Mouse (*Napaeozapus insignis*) males; and B) woodland jumping mouse females. The r -squared values and slope of the fitted lines are included in the lower right corner of each plot.

Supplementary Data SD10. Delta K analysis (top) and Q -Plot (bottom) from STRUCTURE analysis. A peak is evident at $K = 3$, suggesting 3 subclusters, which correspond to southwest of the French Broad (populations 1 to 4), northwest of the French Broad (populations 5 to 7), and the Allegheny mountains.

Supplementary Data SD11. Discriminant analysis of principal components (DAPC) plot of the 8 microsatellite sampling sites. Each color represents a different sampling site. A) shows the DAPC plot for male woodland jumping mice (*Napaeozapus insignis*); and B) shows the DAPC plot for female woodland jumping mice.

Supplementary Data SD12. Bottleneck detection under the TPM model and allele frequency distribution, bolded values are significant at $P < 0.05$.

Supplementary Data SD13. Immigration rates from a source population to a receiving population; θ , a proxy for the effective population size of each source population, is listed in parentheses following the name of the source population.

Funding

Funding for this project was provided by the North Carolina Wildlife Resources Commission (NCWRC; grant number: 584180) and the American Society of Mammalogists (ASM).

Conflict of interest

None declared.

Data availability

All sequences generated for this study have been uploaded to GenBank (accession numbers PX515011 to PX515188).

References

- Adamack AT, Gruber B. 2014. PopGenReport: simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution* 5(4):384–387. <https://doi.org/10.1111/2041-210X.12158>
- Arbogast BS. 1999. Mitochondrial DNA phylogeography of the new world flying squirrels (*Glaucomys*): implications for Pleistocene biogeography. *Journal of Mammalogy* 80(1):142–155. <https://doi.org/10.2307/1383215>
- Arbogast BS, Browne RA, Weigl PD, Kenagy GJ. 2005. Conservation genetics of endangered flying squirrels (*Glaucomys*) from the Appalachian Mountains of Eastern North America. *Animal Conservation* 8(2):123–133. <https://doi.org/10.1017/S1367943004001830>
- Arbogast BS, Kamel SJ, Akers NT, Hall JG. 2022. Kinship and breeding site philopatry drive fine-scale genetic structure in fragmented populations of the Gopher Frog (*Rana capito*) in North Carolina. *Journal of Herpetology* 56(2):249–257. <https://doi.org/10.1670/20-140>
- Arbogast BS, Schumacher KI, Kerhoulas NJ, Bidlack AL, Cook JA, Kenagy GJ. 2017. Genetic data reveal a cryptic species of New World flying squirrel: *Glaucomys oregonensis*. *Journal of Mammalogy* 98(4):1027–1041. <https://doi.org/10.1093/jmammal/gyx055>
- Bidlack AL, Cook JA. 2001. Reduced genetic variation in insular northern flying squirrels (*Glaucomys sabrinus*) along the North Pacific Coast. *Animal Conservation Forum* 4(4):283–290. <https://doi.org/10.1017/S1367943001008885>
- Brannon MP. 2005. Distribution and microhabitat of the woodland jumping mouse, *Napaeozapus insignis*, and the white-footed mouse, *Peromyscus leucopus*, in the Southern Appalachians. *Southeastern Naturalist* 4(3):479–486. [https://doi.org/10.1656/1528-7092\(2005\)004\[0479:DAMOTW\]2.0.CO;2](https://doi.org/10.1656/1528-7092(2005)004[0479:DAMOTW]2.0.CO;2)
- Brower JE, Cade TJ. 1966. Ecology and physiology of *Napaeozapus insignis* (Miller) and other woodland mice. *Ecology* 47(1):46–63. <https://doi.org/10.2307/1935743>
- Browne RA, Ferree PM. 2007. Genetic structure of Southern Appalachian “Sky Island” populations of the southern red-backed vole (*Myodes gapperi*). *Journal of Mammalogy* 88(3):759–768. <https://doi.org/10.1644/06-MAMM-A-049R1.1>
- Brown JH. 1971. Mammals on Mountaintops: Nonequilibrium Insular Biogeography. *The American Naturalist* 105(945):467–478.
- Campbell JW, Mengak MT, Castleberry SB, Mejia JD. 2010. Distribution and status of uncommon mammals in the Southern Appalachian Mountains. *Southeastern Naturalist* 9(2):275–302. <https://doi.org/10.1656/058.009.0206>
- Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10):1657–1659. <https://doi.org/10.1046/j.1365-294x.2000.01020.x>
- Cornuet JM, Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144(4):2001–2014. <https://doi.org/10.1093/genetics/144.4.2001>
- Crespi EJ, Rissler LJ, Browne RA. 2003. Testing Pleistocene refugia theory: phylogeographical analysis of *Desmognathus wrighti*, a high-elevation salamander in the Southern Appalachians. *Molecular Ecology* 12(4):969–984. <https://doi.org/10.1046/j.1365-294X.2003.01797.x>
- DeGraaf RM, Snyder DP, Hill BJ. 1991. Small mammal habitat associations in poletimber and sawtimber stands of four forest cover types. *Forest Ecology and Management* 46(3-4):227–242. [https://doi.org/10.1016/0378-1127\(91\)90234-M](https://doi.org/10.1016/0378-1127(91)90234-M)
- Dharmarajan G, Beatty WS, Rhodes OE Jr. 2013. Heterozygote deficiencies caused by a Wahlund effect: dispelling unfounded expectations. *The Journal of Wildlife Management* 77(2):226–234.
- Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB. 1994. Mutational processes of simple sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences* 91(8):3166–3170. <https://doi.org/10.1073/pnas.91.8.3166>
- Eckert CG, Samis KE, Loughheed SC. 2008. Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond.

- Molecular Ecology 17(5):1170–1188. <https://doi.org/10.1111/j.1365-294X.2007.03659.x>
- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14(8):2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* 22(7):1–19. <https://doi.org/10.18637/jss.v022.i07>
- Goudet J, Jombart T. 2022. hierfstat: estimation and tests of hierarchical F-statistics. R package version 0.5-11
- Gruber B, Adamack AT. 2015. landgenreport: a new r function to simplify landscape genetic analysis using resistance surface layers. *Mol Ecol Resour* 15(5):1172–8. <https://doi.org/10.1111/1755-0998.12381>
- Hall ER. 1946. *Mammals of Nevada*. Berkeley, CA, USA: The University of California Press.
- Hampe A, Petit RJ. 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* 8(5):461–467. <https://doi.org/10.1111/j.1461-0248.2005.00739.x>
- He K, Gutierrez EE, Heming NM, Koepfli KP, Wan T, He SW, Jin W, Liu SY, Jiang XL. 2019. Cryptic phylogeographic history sheds light on the generation of species diversity in sky- island mountains. *Journal of Biogeography* 46(10):2232–2247. <https://doi.org/10.1111/jbi.13664>
- Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* 58(3):247–276. <https://doi.org/10.1111/j.1095-8312.1996.tb01434.x>
- Iacchei M, Ben-Horin T, Selkoe KA, Bird CE, García-Rodríguez FJ, Toonen RJ. 2013. Combined analyses of kinship and F_{ST} suggest potential drivers of chaotic genetic patchiness in high gene-flow populations. *Molecular Ecology* 22(13):3476–3494. <https://doi.org/10.1111/mec.12341>
- Janes JK, Miller JM, Dupuis JR, Malenfant RM, Gorrell JC, Cullingham CI, Andrew RL. 2017. The $K = 2$ conundrum. *Molecular Ecology* 26(14):3594–3602. <https://doi.org/10.1111/mec.14170>
- Jenkins TL. 2024. mapmixture: an R package and web app for spatial visualisation of admixture and population structure. *Molecular Ecology Resources* 24(4):e13943. <https://doi.org/10.1111/1755-0998.13943>
- Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24(11):1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>
- Jombart T, Ahmed I. 2011. adegenet 1.3-1: new tools for the analysis of genome- wide SNP data. *Bioinformatics* 27(21):3070–3071. <https://doi.org/10.1093/bioinformatics/btr521>
- Kimura M. 1983. *The neutral theory of molecular evolution*. Cambridge, UK: Cambridge University Press.
- Kocher TD, Conroy JA, McKaye KR, Stauffer JR. 1993. Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. *Molecular Phylogenetics and Evolution* 2(2):158–165. <https://doi.org/10.1006/mpev.1993.1016>
- Kuhner MK. 2006. LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics* 22(6):768–770. <https://doi.org/10.1093/bioinformatics/btk051>
- Lawlor TE. 1998. Biogeography of Great Basin mammals: paradigm lost? *Journal of Mammalogy* 79(4):1111–1130. <https://doi.org/10.2307/1383003>
- Linzey DW. 1995. *Mammals of the Great Smoky Mountains National Park—1995 update*. *Journal of the Elisha Mitchell Scientific Society* 111(1):1–81.
- Li Y-L, Liu J-X. 2018. A web-based software to select and visualize the optimal number of clusters using multiple methods. *Molecular Ecology Resources* 18(1):176–177. <https://doi.org/10.1111/1755-0998.12719>
- Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* 82(11):1420–1425. <https://doi.org/10.1002/j.1537-2197.1995.tb12679.x>
- Lomolino MV. 1986. Mammalian community structure on islands: the importance of immigration, extinction and interactive effects. *Biological Journal of the Linnean Society* 28(1-2):1–21. <https://doi.org/10.1111/j.1095-8312.1986.tb01746.x>
- Mastretta-Yanes A, Xue AT, Moreno-Letelier A, Jorgensen TH, Alvarez N, Piñero D, Emerson BC. 2018. Long-term in situ persistence of biodiversity in tropical sky islands revealed by landscape genomics. *Molecular Ecology* 27(2):432–448. <https://doi.org/10.1111/mec.14461>
- Meirmans PG, Van Tienderen PH. 2004. genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes* 4(4):792–794. <https://doi.org/10.1111/j.1471-8286.2004.00770.x>
- Miller Jr GS. 1891. Description of a new jumping mouse from Nova Scotia and New Brunswick. *The American Naturalist* 25:742–743.
- Nei M. 1987. *Molecular evolutionary genetics*. New York, NY, USA: Columbia University Press. <https://doi.org/10.7312/nei-92038>
- Ovaska K, Herman TB. 1986. Fungal consumption by six species of small mammals in Nova Scotia. *Journal of Mammalogy* 67(1):208–211. <https://doi.org/10.2307/1381029>
- Ovaska K, Herman TB. 1988. Life history characteristics and movements of the woodland jumping mouse, *Napaeozapus insignis*, in Nova Scotia. *Canadian Journal of Zoology* 66(8):1752–1762. <https://doi.org/10.1139/z88-253>
- Pimm SL, Dollar L, Bass OL Jr. 2006. Genetic rescue of the Florida panther. *Animal Conservation* 9(2):115–122. <https://doi.org/10.1111/j.1469-1795.2005.00010.x>
- Piry S, Luikart G, Cornuet JM. 1999. BOTTLENECK: a program for detecting recent effective population size reductions from allele data frequencies. *Journal of Heredity* 90(4):502–503. <https://doi.org/10.1093/jhered/90.4.502>
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155(2):945–959. <https://doi.org/10.1093/genetics/155.2.945>
- R Core Team. 2024. R: a language and environment for statistical computing. Version 4.4.0. Vienna (Austria): R Foundation for Statistical Computing. www.R-project.org.
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* 34(12):3299–3302. <https://doi.org/10.1093/molbev/msx248>
- Ryman N, Laikre L, Hössjer O. 2019. Do estimates of contemporary effective population size tell us what we want to know? *Molecular Ecology* 28(8):1904–1918. <https://doi.org/10.1111/mec.15027>
- Schumacher KL. 2012. *Conservation genetics of the northern flying squirrel (Glaucomys sabrinus) in the Central and Southern Appalachians* [master's thesis]. [Wilmington (NC, USA)]: University of North Carolina Wilmington.
- Sequencher® version 4.10.1 DNA sequence analysis software. Ann Arbor (MI USA): Gene Codes Corporation. <http://www.genecodes.com>.
- Sikes RS, Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97(3):663–688. <https://doi.org/10.1093/jmammal/gyw078>
- Sipe TW, Browne RA. 2004. Phylogeography of masked (*Sorex cinereus*) and smoky shrews (*Sorex fumeus*) in the Southern Appalachians. *Journal of Mammalogy* 85(5):875–885. <https://doi.org/10.1644/214>
- Speers CF. 1958. The balsam woolly aphid in the southeast. *Journal of Forestry* 56(7):515–516.
- Stanley WT, Esselstyn JA. 2010. Biogeography and diversity among montane populations of mouse shrew (Soricidae: *Myosorex*) in Tanzania. *Biological Journal of the Linnean Society* 100(3):669–680. <https://doi.org/10.1111/j.1095-8312.2010.01448.x>

- Tajima F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105(2):437–460. <https://doi.org/10.1093/genetics/105.2.437>
- Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132(2):619–633. <https://doi.org/10.1093/genetics/132.2.619>
- Townsend MT. 1935. Studies on some of the small mammals of central New York. *Roosevelt Wildlife Annals* 4:1–120.
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4(3):535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
- Vignieri SN. 2005. Streams over mountains: influence of riparian connectivity on gene flow in the Pacific jumping mouse (*Zapus trinotatus*). *Molecular Ecology* 14(7):1925–1937. <https://doi.org/10.1111/j.1365-294X.2005.02568.x>
- Vignieri SN. 2007. Cryptic behaviours, inverse genetic landscapes, and spatial avoidance of inbreeding in the Pacific jumping mouse. *Molecular Ecology* 16(4):853–866. <https://doi.org/10.1111/j.1365-294X.2006.03182.x>
- Waddell PJ, Steel MA. 1997. General time-reversible distances with unequal rates across sites: mixing Γ and inverse Gaussian distributions with invariant sites. *Molecular Phylogenetics and Evolution* 8(3):398–414. <https://doi.org/10.1006/mpev.1997.0452>
- Walker MJ, Stockman AK, Marek PE, Bond JE. 2009. Pleistocene glacial refugia across the Appalachian Mountains and coastal plain in the millipede genus *Narceus*: evidence from population genetic, phylogeographic, and paleoclimatic data. *BMC Evolutionary Biology* 9(1):25–11. <https://doi.org/10.1186/1471-2148-9-25>
- Wear DN, Greis JG. 2002. Southern Forest resource assessment—summary report. Asheville (NC, USA): US Department of Agriculture, Forest Service, Southern Research Station.
- Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38(6):1358–1370. <https://doi.org/10.2307/240864>
- Whitaker JO, Wrigley RE. 1972. *Napaeozapus insignis*. *Mammalian Species* 14:1–6.
- Wiens JJ, Camacho A, Goldberg A, Jezkova T, Kaplan ME, Lambert SM, Miller EC, Streicher JW, Walls RL. 2019. Climate change, extinction, and sky island biogeography in a montane lizard. *Molecular Ecology* 28(10):2610–2624. <https://doi.org/10.1111/mec.15073>
- Williams JE, Webster WMD. 2022. Noteworthy record of the woodland jumping mouse (*Napaeozapus insignis*) in western South Carolina. *Journal of North Carolina Academy of Science* 135(3–4):37–39. <https://doi.org/10.7572/JNCAS-D-18-00004.1>
- Zhu G, Papeş M, Armsworth PR, Giam X. 2022. Climate change vulnerability of terrestrial vertebrates in a major refuge and dispersal corridor in North America. *Diversity and Distributions* 28(6):1227–1241. <https://doi.org/10.1111/ddi.13528>