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Isolation by Distance Shapes Population Genetic Structure of a Rare Terrestrial Salamander, *Plethodon petraeus*, with an Extremely Small Range

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Abstract.—**Globally, amphibian species are experiencing declines at an alarming rate largely due to habitat loss, disease, and climate change. Species with limited distributions are at an elevated risk of a significant decline and extinction because of the inability to avoid and recover from these impacts. Long-term management plans are critical for conservation of species with small ranges; however, the knowledge required to develop effective plans is absent from the literature for many species. The distribution of the Pigeon Mountain Salamander, *Plethodon petraeus*, is restricted to roughly 17 km along the eastern flank of Pigeon Mountain in northwest Georgia, USA. Consequently, *P. petraeus* is highly vulnerable to the impacts associated with amphibian declines, a fact that placed the salamander on the list of rare and protected species in Georgia. The distribution of *P. petraeus* is highly correlated with patchily distributed rocky outcrops, which provide a tangible management habitat target. The development of an effective, long-term management plan requires an understanding of genetic population structure, gene flow, and habitat use patterns. We identified polymorphic cross-amplified microsatellites to determine how genetic diversity is structured across the distribution. Population genetic analyses revealed four distinct populations across the known range of *P. petraeus* and significant isolation-by-distance genetic structuring.**

Key Words.—amphibian; conservation; endemic; gene flow; management; microsatellites; Plethodontidae

Introduction

Size of geographic range consistently emerges as a key correlate of extinction risk across vertebrates (Murray and Hose 2005; Harris and Pimm 2008). Species with small ranges are inherently more susceptible to extinction level events because of their limited distributions. Leading causes of extinction level events, such as climate change, habitat loss, infectious diseases, and invasive species (Sodhi et al. 2008), are more likely to impact an entire species if it has a small distribution. Short-range endemic (SRE) species are among the most vulnerable to extinction (Harvey et al. 2011; Davis et al. 2015; da Silva and Tolley 2017). SRE species have disjunct and highly localized distributions with < 10,000 km^2 (Harvey 2002). Consequently, SRE species are often considered priorities for conservation management because of their inability to avoid and recover from anthropogenic and ecological stressors.

Amphibians have high rates of both endemism and extinction. Sixty-five percent of amphibians have ranges of < 50,000 km^2 (International Union for Conservation of Nature [IUCN] 2018) and roughly 60% of all amphibian species are declining globally. Among vertebrate taxa, amphibians have the highest proportion of species threatened with extinction (Stuart et al. 2004). The IUCN amphibian assessment in 2004 identified one in three species of amphibian as threatened with extinction (Baillie et al. 2004). The current global extinction rates for amphibians may be as much as 45,000 times greater than the background extinction rate (McCallum 2007).

Long-term management plans can be critical for the conservation of at-risk species with small ranges. Populations that reach a critically small population size have difficulty recovering from low levels of genetic diversity and inbreeding depression (Frankham et al. 2010). At that point, species or populations may require human intervention to manage genetic diversity; however, knowledge of population genetics required to develop effective plans is absent from the literature for many species. Obtaining estimates of genetic diversity of populations, gene flow, and structure is an important step in developing effective conservation plans or to establish recovery goals. Advances in the field of conservation genetics has allowed for data to be obtained relatively quickly and integrated into management plans (Eastman et al. 2009).

Integrating population genetics with amphibian conservation has been a successful approach for management of amphibian populations (Eastman et al. 2009; Spear and Storfer 2010; Apodaca et al. 2012). Although conservation genetics has brought new insights to the field of amphibian conservation, terrestrial lungless salamanders (Plethodontidae) remain relatively understudied in this field (Emel and Storfer 2012). Several taxa in the Plethodontidae are potentially imperiled (Milanovich et al. 2010) and are ecologically important vertebrate taxa in North American forests (Semlitsch et al. 2014).

The Pigeon Mountain Salamander (*Plethodon petraeus*) is a colorful, moderately large member of the *Plethodon glutinosus* complex of plethodontid salamanders (Marshall et al. 2004), and it is an SRE that could benefit from the integration of population genetics data into management planning. This species has an exceptionally small range (a liberal estimate is < 100 km^2) and, because of specific microhabitat requirements, a patchy distribution (Jensen et al. 2002). Additionally, species in the genus *Plethodon* and other terrestrial salamander species have low dispersal rates (Ousterhout and Liebgold 2010; Liebgold et al. 2011). Since its discovery in 1972 (Jensen et al. 2002), Pigeon Mountain Salamanders have been documented at fewer than 20 locations, all in caves, outcrops, or rocky areas extending along a 17 km southeastern ridge of Pigeon Mountain in Walker and Chattooga counties in northwest Georgia, USA (Wynn et al. 1988; Jensen et al. 2002). Because of the small number of occurrences within an extremely limited range, this species is state listed as Rare and protected by the state of Georgia (https://georgiabiodiversity.org). A petition for federal protection by the U.S. Fish and Wildlife Service (USFWS) in 2016 was dropped from the formal consideration due to lack of data on the species. It is listed as an At-risk Species by the IUCN because of its restricted range (IUCN 2018).

Although considered a rare species because of its limited distribution, *P. petraeus* is abundant in the few locations where it is found, and at some sites is more numerous than other species of sympatric salamanders (Jensen et al. 2002; Wynn et al. 1988). Also, the numbers of *P. petraeus* at the entrances and twilight zones of caves appear stable (Camp and Jensen 2007). The habitat occupied by *P. petraeus* is relatively undisturbed due to the steep topography of the area, and half of the range of the species is protected within Crockford-Pigeon Mountain Wildlife Management Area.

Individuals are generally found in and sometimes adjacent to rocky habitats, including cave entrances, rocky outcrops, and cliff faces (Wynn et al. 1988). Due to the generally low dispersal rate of terrestrial salamanders (Ousterhout and Liebgold 2010; Liebgold et al. 2011), *P. petraeus* possibly does not exist as a continuous, connected population throughout its range. Gene flow between habitats could be limited by distance between the patchily distributed rocky habitats or by competition for habitat with sympatric species. For example, when leaf litter dries, the Northern Slimy Salamander (*Plethodon glutinosus*) competes for refuge under rocks and logs where moisture levels remain high. Laboratory experiments have shown that the more aggressive *P. glutinosus* wins these territory disputes and evicts individual *P. petraeus* from cover (Marshall et al. 2004). Limited available cover for *P. petraeus* to use to avoid desiccation during movements across the forest floor could effectively limit dispersal (Marshall et al. 2004).

The limited distribution of this salamander increases the risk of extinction because it is more vulnerable to threats affecting a large percentage or the entirety of its range (Houlahan et al. 2000; Velo-Antón et al. 2013). A population genetics assessment of the Pigeon Mountain Salamander would provide wildlife managers with useful information regarding genetic diversity and population structure. Our study is an initial effort to analyze and describe the population genetics of this rare and protected amphibian. Our goals were to assess genetic diversity across the range, and to describe genetic population structure and identify management units. We generated genetic baseline data that can be used for furthering the conservation and management of *P. petraeus*. Due to seemingly large populations that have been relatively constant since the 1980s and protected, we expected that we would not find evidence of inbreeding depression. The levels of genetic diversity detected by this study can become a baseline to measure population genetic health in the future. Also, despite the small range of *P. petraeus*, we expected that more than one genetic population may be detected due to the patchy distribution and linear habitat of the species.

Materials and Methods

Field collections.—Pigeon Mountain (N34°39'41" W85°21'17") in Walker and Chattooga counties in northwest Georgia, USA (Fig. 1), is part of the Cumberland Plateau. This ecoregion is characterized by a flat upland plateau with steep, adjacent topographic relief and karst geology. Pigeon Mountain has numerous caves, and extensive sandstone and limestone outcroppings and bluffs. This habitat type, however, is not continuous throughout the small range, resulting in a fragmented distribution of the species (Jensen et al. 2002). A mesic deciduous forest composed primarily of oak (*Quercus* spp.) and hickory (*Carya* spp.) trees covers the majority of the landscape. The northern extension of the mountain is within the boundary of the 8,360 ha Crockford-Pigeon Mountain Wildlife Management Area (WMA). All

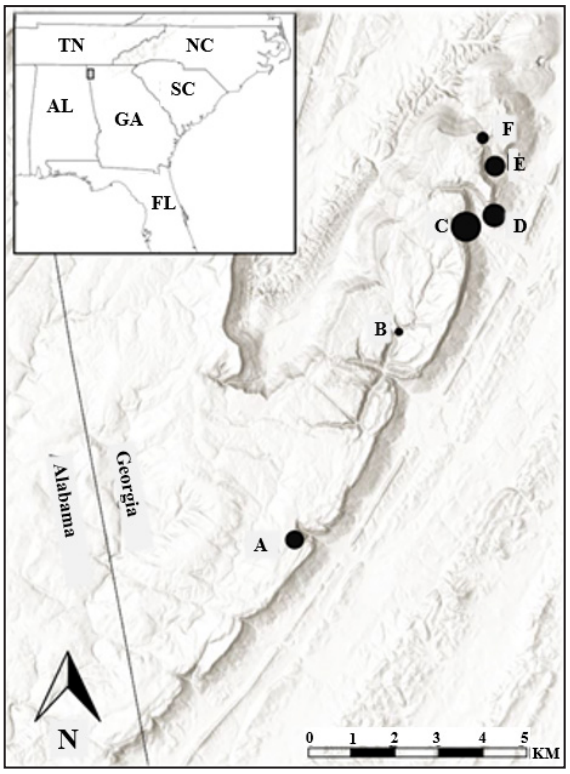

Figure 1. Sampling locations for Pigeon Mountain Salamanders (*Plethodon petraeus*) in northwestern Georgia, USA. Most sampling locations are within the Crockford-Pigeon Mountain Wildlife Management Area (WMA); exceptionally, Site A is outside the Crockford-Pigeon Mountain WMA. Size of symbols for sampled locations (solid circles) are proportional to sample size. Population reference letters are shown in black font. Sampling was conducted over most of the known range for the species.

known locations of *P. petraeus* exist in or to the southwest of the WMA. The WMA is used year-round by the public for recreation, including hunting, caving, rock climbing, hiking and mountain biking.

We sampled individual *P. petraeus* between Spring and Fall 2015. We selected sampling locations using Georgia Department of Natural Resources (GaDNR) species occurrence records. We selected six sampling locations based on accessibility. We conducted sampling on the Crockford-Pigeon Mountain Wildlife Management Area (sites B-F) and property protected through a conservation easement with The Nature Conservancy (TNC) of Georgia (site A; Fig. 1). The largest gap in our sampling scheme was between site A, the southern-most known location of *P. petraeus*, and site B. Records of *P. petraeus* exist between the locations but they occur on inaccessible private property. We visited the sites during the day and conducted visual searches of rock crevices. We captured juvenile and adult salamanders by hand, and we collected a 1.0 cm tail tip from each individual. Following tissue collection, we released the salamanders at their point of capture. We placed the tissue sample in a sterile 1.5 ml microcentrifuge tube and put the tubes on ice in the field. We transferred the samples to the laboratory for storage in a -20° C freezer prior to DNA extraction.

DNA extraction and genotyping.—Whole genomic DNA was extracted from tissue with the UltraClean Tissue & Cells DNA Isolation Kit (MoBio, Carlsbad, California, USA) at Kennesaw State University. We stored extracted DNA samples in 50 ul of elution buffer at -20° C. We used polymerase chain reaction (PCR) to cross-amplify a panel of Western Slimy Salamander (*Plethodon albagula*) microsatellite markers for use in *P. petraeus* (Spatola et al. 2013). Eight polymorphic loci were identified, and we used PCR to amplify DNA from each individual (Table 1). Primers were 5-prime end-labeled with a fluorescent dye (6-FAM, NED or HEX; Applied Biosystems). Reactions consisted of 0.5 uM of each primer, Taq DNA polymerase, PCR Master Mix, and 1 ul DNA in a 15 ul reaction. PCR conditions followed *Taq* DNA polymerase guidelines and included an initial activation step of 15 min at 95° C, followed by 25 cycles through three steps: denature (30 sec at 94° C), annealing (90 sec at 60 or 62° C), and extension (90 sec at 72° C; Table 1). We performed all PCR reactions on S1000 thermal cyclers (Bio-Rad, Hercules, California, USA). PCR product sizes were estimated on an Applied Biosystems 3130xl DNA analyzer (Applied Biosystems, Foster City, California, USA) using Liz 600 size standard at the Savannah River Ecology Laboratory. Results were scored using GENEMARKER (v. 1.97; Softgenetics, State College, Pennsylvania, USA). We excluded individuals with poor-quality genotype data (> 25% genotype data missing) from downstream analyses. We binned alleles with the program TANDEM (Matschiner and Salzburger 2009). We tested for the presence of full siblings within our data set using COLONY (Jones and Wang 2010). When we detected full siblings, we randomly retained one individual from each family and removed the others from our data set.

We screened loci for the presence of linkage disequilibrium using the log-likelihood ratio statistic for each pair of loci in the population using Genepop v4.2 (Raymond and Rousset 1995). We also used Genepop v4.2 (Raymond and Rousset 1995) to screen loci for deviations from Hardy-Weinberg equilibrium (HWE) at each locus and in each population using Fisher's Exact Test. We used the default Markov chain parameters for both tests, and we applied a Bonferroni correction to *P* values for the number of comparisons to assess statistical significance (Rice 1989). We used Micro-Checker v2.20 (Van Ossterhout et al. 2004) to test for the presence of null alleles in the microsatellite data.

Table 1. Description of eight cross-amplified polymorphic loci for Pigeon Mountain Salamanders (*Plethodon petraeus*) from Georgia, USA. Locus name, primer sequences (Spatola et al. 2013), florescent dye, and annealing temperature (T_a) are listed for each locus. Also indicated are the number of individuals genotyped (N), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosities for each locus.

Locus	Primer sequence, 5' to 3'	Labelling dye	T_a (°C)	Size Range (bp)	N	N_a	H_o	H_e	F_{is}
PG_3XI	F: AGCGGTGGATAGTCGTACAC	6-FAM	60	138–150	97	4	0.693	0.473	-0.457
	R: ATAGCACATAGGCAGATCAGTC								
PG_43M	F: AGTCATTGTCAGCTTGCGC	HEX	60	103-131	99	8	0.761	0.680	-0.110
	R: GGGAGCTTGCATCAGGAAAG								
PG_POG	F: ACCTGTATTTCACGCTGCAC	NED	60	208-256	96	7	0.570	0.529	-0.010
	R: CTGCACCTCTCACCCTACTG								
PLAL_084	F: ACTCCACAAACTCACTACCTG	6-FAM	60	326-354	98	4	0.768	0.552	-0.446
	R: TGTGGACCCTATTCTTGGCC								
PLAL_127	F: ATGTCCGAGCTATGAAACCC	HEX	60	97-122	89	7	0.360	0.473	0.085
	R: GCACTCGCCTTGACCATTAC								
PLAL_402	F: AGTGGTGAGGGAGATGGATG	NED	62	156-308	97	29	0.659	0.837	0.081
	R: TGGACTGTTGCTTTCTTGTGC								
PLAL_542	F: ATGCCTTAGGACCGCAGTAG	6-FAM	62	164-280	89	22	0.833	0.786	0.031
	R: TGGGTTTCCTGGCATACTCC								
PLAL_701	F: CATGCGTACAGGATTAGGTCAG	HEX	60	201-241	99	9	0.268	0.247	-0.093
	R: CAGTCTGCCTCTTTGTAAGGC								
Overall						11.25	0.614	0.572	-0.115

Genetic diversity and population structure.—We used GENODIVE v2.0 (Meirmans 2006) to calculate observed and expected heterozygosity, the fixation index (F_{ST}), and inbreeding coefficients (F_{IS}). We calculated the overall correlation of pairwise F_{ST} and distance separating sampling sites to detect the presence or absence of isolation by distance (IBD). We used a Mantel test (1,000 permutations) in GENODIVE v2.0 (Mantel 1967; Sokal et al. 1986; Slatkin 1993; Meirmans 2006) to determine the significance of matrix correlations between pairwise F_{ST} and distance separating the sites. We assessed population genetic diversity and evaluated the overall genetic differentiation between sampling locations using an Analysis of Molecular Variance (AMOVA) framework. We assessed genetic variation across sampling locations, within sampling locations and within individuals with an AMOVA in GENODIVE 2.0 with 10,000 permutations to assess significance (Meirmans and Tienderen 2004).

We estimated population genetic structure using two Bayesian methods. First, we used the Bayesian assignment methods implemented in the software programs STRUCTURE v2.3.4 (Pritchard et al. 2000) and GENELAND 4.0.0 (Guillot et al. 2005) to detect population genetic clustering. In STRUCTURE, we ran 10 independent simulations at each value of K between 2–6 (exploratory analyses supported K > 1). Each run consisted of 100,000 burn-in steps followed by 1,000,000 Markov Chain Monte Carlo (MCMC) iterations using an admixture model with correlated allele frequencies and no location prior. This approach infers genetic assemblages by estimating the probability of the observed genetic data given K number of genetic clusters. The appropriate K value was selected using PopHelper 2.2.7 (Earl and VonHoldt 2011; Francis 2017) to determine the most likely number of populations using the DeltaK criterion (Evanno et al. 2005). This analysis was repeated hierarchically for genetic clusters in which multiple sampling locations were grouped in a single cluster, K = 1, to address limitations of STRUCTURE when populations are defined by strong IBD (Meirmans 2012). We displayed the population membership probability of each individual (to each cluster) to provide a visual representation of genetic structure using PopHelper 2.2.7 (Francis 2017).

We used GENELAND as a second method to look for convergence among different techniques characterizing genetic structure. GENELAND is a Bayesian model that uses both genotypic data and spatial coordinates to identify genetic clusters based on Hardy-Weinberg equilibrium. Studies indicate that GENELAND performs better than other Bayesian clustering methods in terms of correctly assigning individuals to groups (François and Durand 2010). In our GENELAND analysis, we first performed a series of runs to infer the number of genetic

clusters in the dataset. First, we performed 10 runs of 100,000 MCMC iterations with a thinning interval of 100 and a maximum number of populations of 10. We used correlated allele frequencies and geographic coordinates to parameterize all runs. We inferred K as the modal number of genetic groups estimated among the best of the 100,000 iterations for the 10 runs. Then we performed 100 independent runs with K fixed to the number of populations previously inferred. We ranked the 100 runs according to their mean logarithm of posterior density and used the results of assignments from the highest ranking to visualize the data. In addition, runs of the model using the same parameters but longer iterations, 250,000 and 500,000, confirmed that longer runs would not affect the results.

Results

Population genetic analysis.—We sampled 114 *P. petraeus* at six locations, and all individuals were genotyped for eight cross-amplified microsatellite loci (Table 1). Genotyping failed for 10 individuals. Failure was defined as individuals missing > 25% genotype data. Following removal of four individuals after the detection of siblings, 100 individuals were included in the data set. Among the six sampling locations, the mean sample size was 16.7 individuals, with a minimum of five and a maximum of 29 (Table 2).

The eight loci surveyed had 4–29 alleles (mean = 11.25 ± 3.24 standard deviation) across all samples (Table 1). There was a risk that the microsatellite loci would have low diversity because they were initially developed for *P. albagula* (Spatola et al 2013) and cross-amplified for our study instead of developing species-specific primers. Nevertheless, allelic diversity parameters were suitable for use in this population genetic study. Allelic diversity per locus measures are comparable to the allelic diversity of the loci in the species for which the primers were developed (Spatola et al. 2013). There was no evidence of linkage dis-equilibrium between the eight loci surveyed. There was also no evidence of scoring errors, larger allele dropout, or null alleles detected.

Table 2. Genetic diversity of six sampling locations of Pigeon Mountain Salamanders (*Plethodon petraeus*) in northwestern Georgia, USA. Number of individuals amplified (N), number of alleles (N_a), observed (H_o) and expected (H_e) heterozygosities, and inbreeding coefficient (F_{is}).

Location	N	N_a	H_o	H_e	F_{is}
A	10	3.125	0.545	0.43	-0.269
B	18	5.500	0.655	0.569	-0.151
C	22	5.250	0.591	0.615	0.038
D	29	8.125	0.679	0.645	-0.053
E	5	3.000	0.543	0.562	0.034
F	16	5.375	0.607	0.581	-0.045
Overall	100				-0.122

All loci and populations were within HWE expectations. Observed heterozygosity at each sample location ranged from 0.543 to 0.679 (mean = 0.607; Table 2). When compared to other more widely distributed species within the same genus, the overall heterozygosity was similar (Table 3). Global genetic differentiation measures provided evidence for genetic structure among populations. A hierarchical AMOVA showed significant genetic structure with 12.7% variation explained among populations ($P < 0.001$). Pairwise F_{ST} revealed significant differentiations among populations; all 15 pairwise comparisons were significantly different from zero (F_{st}, Bonferroni correction applied; Table 4).

Isolation by distance.—Significant isolation by distance indicates that gene flow was more prominent among neighboring populations. Isolation by Euclidean (geographic) distance results indicated a strong correlation with genetic distance for *P. petraeus* ($r^2 = 0.804$. $P = 0.005$; Fig. 2).

Population structure.—Despite significant genetic differentiation between all pairwise F_{st} values (Table 4), sampling locations were assigned to only two clusters in the initial Structure analyses (K = 2); however, Δ K support for K = 4 was similar to K = 2 (Fig. 3, Appendix able). For both K = 2 and K = 4, we found support for a shift from one group (blue, the dominate groups for sites A and B) to a second group (red, dominant for sites C, D, E and F; Fig. 3). This pattern followed a north to south trend, with individuals from centrally located sites assigned to both clusters (Fig. 4).

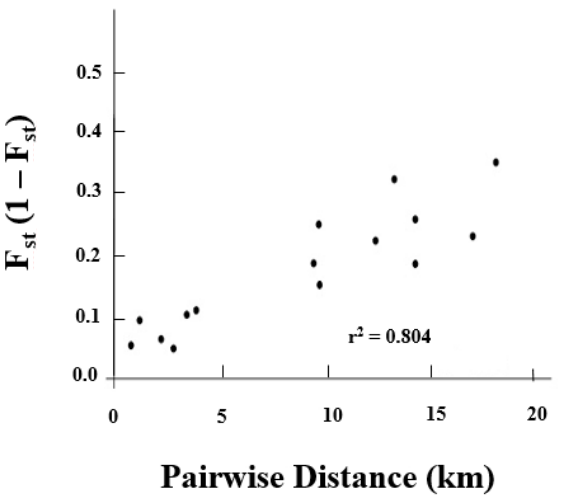

Figure 2. Isolation by distance. Results of a Mantel test for correlations between measures of distance and genetic distance. Significant value with 10,000 permutations.

Table 3. Comparison of observed (H_o) and expected (H_e) heterozygosities reported in this study to other species in the genus *Plethodon* in the eastern USA. Mean H_o and H_e were claculated from the data provided in referenced manuscripts when they were not included.

Species	Common Name	H_o	H_e	Reference
P. petraeus	Pigeon Mountain Salamander	0.610	0.570	This Study
P. albagula	Western Slimy Salamander	0.470	0.495	Spatola et al. 2013
P. shermani	Red-legged Salamander	0.676	0.781	Spatola et al. 2013
P. albagula	Western Slimy Salamander	0.490	0.500	Peterman et al. 2014
P. websteri	Webster's Salamander	0.410	0.430	Feist et al. 2017
P. websteri	Webster's Salamander	0.380	0.280	Feist et al. 2019
P. cinereus	Red-backed Salamander	0.620	0.620	Cabe et al. 2007
P. cinereus	Red-backed Salamander	0.530	0.610	Noël and Lapointe 2010
P. cinereus	Red-backed Salamander	0.391	0.516	Cameron et al. 2017
P. cinereus	Red-backed Salamander	0.320	0.320	Waldron et al. 2019
P. cinereus	Red-backed Salamander	0.383	0.400	Wilk et al. 2020

In the hierarchical analysis of the K = 2 scenario, we identified four genetic groups within the north genetic cluster (Fig. 4, Appendix Table). Site division for hierarchical analysis included sites A and B, which had little probability of assignment to the second group (red), and sites C, D, E and F, which had a few individuals with a modest probability of assignment to the first group (blue). At the secondary level, sites A and B were grouped together with strongest support for K = 1 after visual inspection of structure within this group. We found support for a genetic cluster at sampling site C and another cluster that included D, E and F (K = 2, yellow and green). The Structure bar graph, however, does not support strong differentiation between the two clusters at this level. A south to north trend of individuals assigned to two clusters at a gradually changing rate indicates an isolation by distance genetic pattern. At the third level, we found support for K = 3 genetic clusters, with sites D, E and F all being distinct. After the hierarchical analysis, K = 5 genetic clusters were detected; sampling locations A and B grouped together and the northern sites, C, D, E and F all identified as individual clusters (Appendix Table).

The GENELAND analysis reached a consensus with the number of genetic clusters identified in the hierarchical Structure analysis. In the initial 10 runs performed to estimate K, the modal number of genetic groups estimated along the MCMC was 5. Therefore, subsequent runs were performed with K set to 5. The 100 runs of 100,000 iterations performed for the assignment step also converged on five genetic clusters. All runs assigned individuals to one of the five populations. When we ranked the 100 runs by mean posterior probability densities, their qualities only showed a slight decrease from 100 through the runs. The general clustering pattern was similar to Structure (Fig. 4). The analysis supported a north to south pattern of population differentiation.

Discussion

Characterizing the genetic diversity and population structure not only contributes to the fundamental knowledge of SREs but is also a vital part of forming a modern conservation plan for *P. petraeus* (Allendorf et al. 2012). The present study provides strong evidence for multiple genetically distinct subpopulations across the range of *P. petraeus*. These data strongly support IBD as an explanation for this pattern despite the relative short distance considered (i.e., < 20 km). Without considering additional aspects, such as life-history

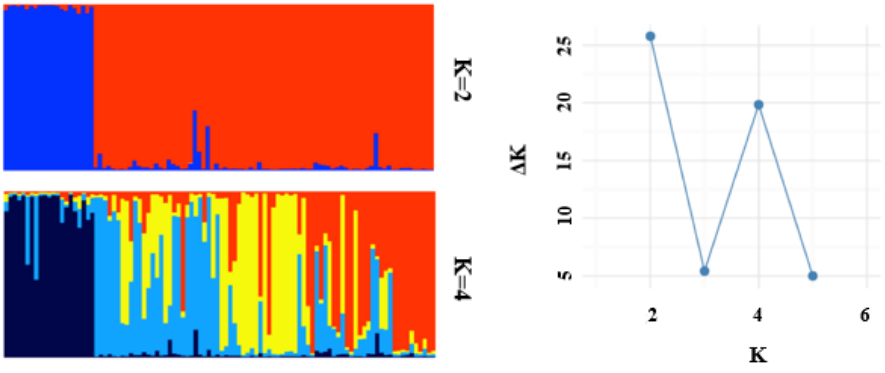

Figure 3. Delta K analysis (Evanno et al. 2005) supported the presence of two genetic clusters; K = 2 and K = 4 (see Appendix Table).

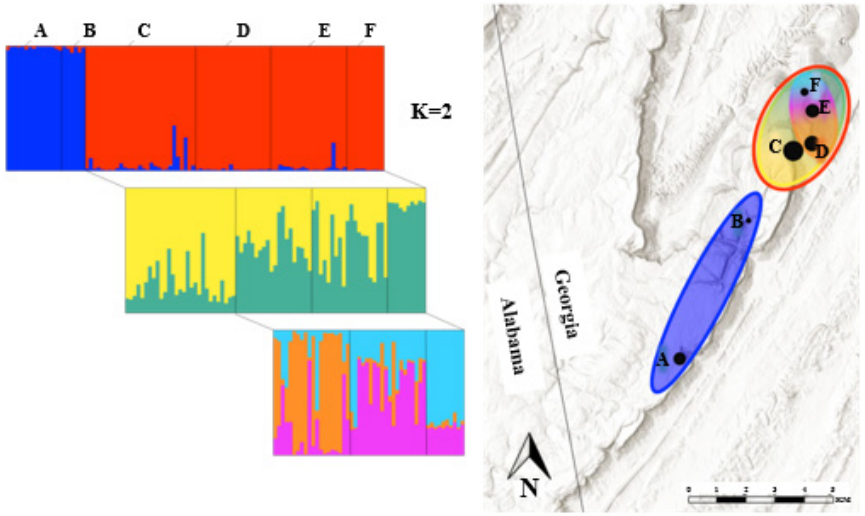

Figure 4. Structure results for six sampling locations of Pigeon Mountain Salamanders (*Plethodon petraeus*) in northwestern Georgia, USA. Letters correspond to sampling locations. Each vertical bar represents one individual. Colors indicate the most likely genetic cluster assignments. Black vertical bars denote individuals from the same sampling locations. Each cluster was hierarchically analyzed for nested structure; nested structure results are shown directly below the original cluster. Hierarchical analyses were repeated until terminal clusters (K = 1) were reached. Nested, colored outlines on the map correspond to population clusters.

traits and landscape, it may be easy to assume that a species with such a small range is a single genetic population.

Despite the small range of this endemic species, genetic diversity within sampling locations and across the entire range is relatively high. The overall heterozygosity is similar to other more widely distributed species within the same genus. There was no detection of inbreeding depression at any of the sampling locations. Interestingly, one of the sampling locations (F) showed evidence of outbreeding depression. The positive F_{is} value indicates there may have been an influx of individuals to this site. Overall, the small range of the Pigeon Mountain Salamander does not seem to be limiting the abundance of salamanders (*P. petraeus* individuals often outnumber all other species of salamanders combined at observed sampling locations; Marshall et al. 2004; Camp and Jensen 2007; pers. obs.). This high local abundance seems to be large enough to protect the species from inbreeding.

Table 4. Matrix of sampling locations F_{ST} (lower left) and *P* values (upper right).

	A	B	C	D	E	F
A	—	0.001	0.001	0.001	0.001	0.001
B	0.093	—	0.001	0.001	0.001	0.001
C	0.102	0.063	—	0.001	0.001	0.001
D	0.109	0.048	0.053	—	0.001	0.001
E	0.318	0.22	0.246	0.184	—	0.002
F	0.345	0.227	0.254	0.183	0.146	—

The spatial and genetic structure of *P. petraeus* seems to be strongly influenced by the landscape and habitat availability. The distribution of *P. petraeus* along the narrow southeast-facing slope of Pigeon Mountain and the patchy occurrence across this landscape of the species can be best described by the stepping-stone model of IBD (Wright 1943). In this simple model, subpopulations are most likely to exchange migrants with adjacent subpopulations. The high genetic differentiation (F_{st}) between sampling locations indicates limited gene flow occurs between these adjacent locations. The genetic differentiation between sampling locations of *P. petraeus* is more similar to the genetic differentiation between locations of *Plethodon* fragmented by urbanization than those measured in connected habitat in the study conducted by (Cabe et al. 2007). Several studies have quantified the genetic diversity of salamanders in the genus *Plethodon*; the results of our study indicate stronger genetic differentiation (upwards of 10× larger Fst values) than other studies that have quantified the genetic diversity of terrestrial woodland salamanders sampled across similar distances (Feist et al. 2017; Cabe et al. 2007; Bayer et al. 2012; Peterman et al. 2014).

Other factors, such as competition with other terrestrial salamanders, could be limiting the dispersal ability of *P. petraeus* (Marshall et al. 2004) and contributing to strong genetic differentiation. The previous research on increased aggression in another terrestrial salamander on Pigeon Mountain was driven by the hypothesis that the Northern Slimy Salamander may limit the dispersal of Pigeon Mountain Salamanders. The results could also be indicating that dispersal may be more limited for this species than other species in the genus *Plethodon*.

Conservation implications.—Conservation genetics can be an integral component of assessing the status of threatened and endangered species with small ranges. The conservation status of *P. petraeus* is based on its highly restricted range. The protection provided to the species includes listing as a rare species by the State of Georgia, which prohibits direct take, and the preservation of roughly half of its known range within a WMA. Our findings, however, show that genetically distinct populations fall outside the protection offered by the WMA where indirect take, such as habitat conversion, is not regulated.

These data emphasize that strong isolation by distance shapes the spatial genetic structure of *P. petraeus* across its small range. Fortunately, from a conservation standpoint, the species in not suffering from decreased genetic diversity often associated with inbreeding and small population size. These results highlight a potential difficulty in managing *P. petraeus*. Individuals sampled from the southernmost extent of the species range are genetically distinct from those 10 km to the north and therefore, should be considered for management separately. Efforts to educate private landowners about the biodiversity on their properties and benefits of conservation should be pursued along with perpetual land protection (e.g., establishment of conservation easements and fee-simple acquisition, when possible).

This study also provides a genetic baseline for long-term management. For example, future population genetic studies could be conducted to monitor changes in population genetic parameters. The importance of incorporating population genetic data in the management of species at risk can be influential in their persistence. Also, population genetic data could be used to select individuals from locations across the species range to promote a genetically diverse captive amphibian breeding program, if deemed necessary to maintain or recover the species. Genetic diversity should be considered in future conservation plans.

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Appendix Table. Delta K calculations and log likelihoods for STRUCTURE output.

All individuals: Figure 3

#

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-2414.81	0.31	NA	NA	NA
2	**10**	**-2230.85**	**8.41**	**183.96**	**216.86**	**25.786**
3	10	-2263.75	17.71	-32.9	96.07	5.425
4	10	-2200.58	6.1	63.17	121.08	19.849
5	10	-2258.49	17.69	-57.91	88.36	4.995
6	10	-2404.76	36.14	-146.27	NA	NA

Following K = 2 and K = 3 (D, E, F analysis) for all individuals (results visualized in Figure 4).

Sampling locations A and B:

#

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	**10**	**-391.03**	**1.29**	**NA**	**NA**	**NA**
2	10	-391.95	2.83	-0.92	0.8	0.283
3	10	-393.67	2.88	-1.72	NA	NA

Sampling locations C, D, E and F:

#

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-1779.64	1.37	NA	NA	NA
2	**10**	**-1793.72**	**15.51**	**-14.08**	**54.61**	**3.521**
3	10	-1753.19	29.74	40.53	21.55	0.725
4	10	-1734.21	8.49	18.98	NA	NA

Sampling locations D, E and F:

#

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-1000.15	0.16	NA	NA	NA
2	10	-1097.88	129.13	-97.73	239.4	1.854
3	**10**	**-956.21**	**2.43**	**141.67**	**164.44**	**67.671**
4	10	-978.98	3.18	-22.77	NA	NA