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Population Connectivity of the Eastern Collared Lizard Crotaphytus collaris in Arkansas

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ABSTRACT

Habitat reduction and fragmentation can isolate populations and decrease genetic diversity, making them susceptible to local extirpation. Additionally, geographic barriers can further impede dispersal among populations thus reducing gene flow. Field studies suggest these factors may be responsible for the decline in Eastern Collared Lizard (*Crotaphytus collaris*) populations in Arkansas. To address the impacts of habitat loss and fragmentation on the Eastern Collared Lizard (*C. collaris*) in Arkansas, I used DNA fragment analysis to examine genetic diversity, population structure and connectivity among *C. collaris* populations. I do so herein by employing microsatellite data from 138 adults across 11 loci to evaluate genetic diversity parameters and connectivity within and among populations in Arkansas. Results revealed that populations in geographic proximity are more genetically similar than populations more distant and isolated. Migration rates were higher within rather than between sites, ranging from 0.80 to 0.90, suggesting most populations are demographically independent and could comprise 'Management Units' (MUs). However, a Mantel test for isolation by distance (IBD) across all sites indicated a non-significant correlation between genetic and geographic distances. An Analysis of Molecular Variance (AMOVA) showed the majority of genetic variance exists within/among individuals (74%) and within populations (26%), which are moderately, but not significantly differentiated (F_{ST}=0.26). Results from assignment tests (Structure) and a Discriminant Analysis of Principal Components (DAPC) analyses suggest 5 or 8 distinct gene pools. High-population admixture characterized sites in Baxter and Stone counties, comprising the majority of samples (N=75). Overall, these data indicate populations are genetically isolated and susceptible to potential expiration. To mitigate loss of populations, local management, and conservation efforts such as habitat restoration and translocations will be beneficial if they

stabilize or increase population sizes, genetic diversity and promote gene flow in *C. collaris* in Arkansas.

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INTRODUCTION

Earth is currently facing an extinction crisis with a loss of biodiversity at rates not recorded since the Cretaceous-Tertiary extinctions (Ceballos et al. 2010). This has been dubbed the 'sixth mass extinction' (Frankham et al. 2010), but the current loss of species is unique in that it not only occurs at 1,000x the background extinction rate, but is also heavily driven by anthropogenic factors (Ceballos et al. 2010). Human activities that can lead to extinction, fragmentation, or destruction of habitat, include but are not limited to, deforestation, urbanization, agriculture, and fire suppression (Scanes 2018). Habitat fragmentation can be detrimental to the persistence of species, as it can lead to population isolation, and a reduction in population size, both of which may lead to a decrease in local genetic diversity that is essential for the evolutionary potential that allows species to adapt and persist in changing environments. Without genetic diversity, plants, and animals, are vulnerable to extinction (Templeton et al. 1990; Allendorf et al. 2013).

Habitat fragmentation can lead to the partitioning of habitat into "island" patches and can also reduce the total habitat area (Frankham et al. 2010). 'Island' patches are characterized by lack of connectivity, and populations that persist on such isolated patches are small, and susceptible to a loss of genetic diversity in the absence of gene flow (Willi et al. 2006). Small, isolated populations are also more susceptible to stochastic (random) processes, such as environmental changes, genetic drift, and changes in population sizes overtime (Pardini et al. 2017). Combined, these factors increase the risk of local extirpation. The effects that habitat fragmentation will have on gene flow between populations depends on a variety of factors such as population sizes within fragments, the number of population fragments, the distance and geographic distribution between fragments, migration rates among fragments, and the time since fragmentation (Frankham et al. 2010). Furthermore, the genetic impacts of fragmentation can

range from minimal to severe, depending on the amount of gene flow maintaining genetic diversity. However, when gene flow between fragments is sufficient, it may be considered a single large population. In summary, populations that are small and isolated are more susceptible to decreases in gene flow, genetic diversity, and even local expiration (Frankham et al. 2010; Montes-Carreto et al. 2020). Furthermore, genetic variation is an important measure of population structure, and in combination with other measures, may be used to identify population 'units' that need to be conserved (Allendorf et al. 2013). Therefore, investigating the extent of genetic diversity, population structure and the degree of connectivity among populations is essential for conservation management plans on both a local and species wide scale.

Eastern Collared Lizard (*Crotaphytus collaris*) populations in Arkansas are a prime example of how habitat loss and fragmentation can impact the persistence of a species within its habitat range. Historically the range of the Eastern Collared Lizard extended from Eastern Missouri to Arizona, as this species relies on dry rocky outcrops known as glades (McGuire et al. 2007; Grimsley 2012). However, Eastern Collared Lizard habitat was naturally reduced and fragmented due to climatic cooling which occurred 4,000 years ago and resulted in the growth of oak, hickory, and pine forests, reducing the number of open glade habitats (Smith 1957; Cole 1971; Brisson et al. 2003). Early European settlement resulted in years of widespread fire suppression, thus severely impacting glade habitats which are fire dependent. Both of these factors resulted in the increase of underbrush growth in glades. Over the past 30 years, dramatic declines in numbers of *C. collaris* in Arkansas have been recorded, with some populations locally extirpated (Brisson et al. 2003; Trauth 2011; Grimsley 2012). Furthermore, *C. collaris* populations in Arkansas are isolated from nearby populations in Missouri and Oklahoma and have also been identified as Evolutionarily Significant Units (ESU's) (Cole 2015). Consequently,

researchers and the Arkansas Game and Fish Commission have sought to study and protect these local at-risk populations. Additionally, several studies have focused on characterizing the physiological, ecological, and genetic properties of these populations (Hutchison 2003; Cole 2015; Elliot 2017; Brewster et al 2018; Brewster 2019). However, the extent to which habitat fragmentation and loss has impacted genetic diversity, gene flow and population structure of the Eastern Collared Lizard in Arkansas has yet to be determined.

Therefore, I seek to measure overall genetic diversity and connectivity among *C. collaris* populations in light of recent habitat loss and fragmentation. I will accomplish this by using microsatellite data and conservation genetic analyses to quantify genetic diversity, gene flow, population structure among Collared Lizard populations in Arkansas. Insights from this study will help to inform on-going and future conservation efforts aimed at generating connectivity among habitat patches and promote gene flow among Collared Lizard populations. The ultimate goal would be to establish connectivity among populations of *C. collaris*, so that the species may persist as a metapopulation in its naturally fragmented glade landscape.

LITERATURE CITED

Allendorf, FW, Aitken, SN and Luikart, G (2013) Conservation and the genetics of populations. John Wiley & Sons Hoboken.

Brewster, CL, Beaupre, SJ and Willson, JD (2018) Habitat loss and local extinction: linking population declines of eastern collared lizards (*Crotaphytus collaris*) to habitat degradation in Ozark glades. *Journal of Herpetology* 52:352–360.

Brewster, CL (2019) Eastern collared lizard (*Crotaphytus collaris*) population declines in Ozark landscapes: An assessment of environmental constraints. Dissertation, University of Arkansas.

Brisson, JA, Strasburg, JL and Templeton, AR (2003) Impact of fire management on the ecology of collared lizard (*Crotaphytus collaris*) populations living on the Ozark Plateau. *Animal Conservation* 6:247-254.

Ceballos, G, Andrés G, and Ehrlich, PR (2010) The sixth extinction crisis: Loss of animal populations and species. *Journal of Cosmology* 8:1821-1831.

Cole, KW (1971) Consideration of macro-Climatic and Macro-Biotic change in the Ozark highlands during post-glacial Times. *Journal of the Arkansas Academy of Science* 25:15-20.

Cole, JM (2015) Identifying evolutionarily significant units in eastern collared lizard (*Crotaphytus collaris*) from Arkansas. Thesis, University of Arkansas.

Elliott, HED (2017) Population structure of collared lizard (*Crotaphytus collaris*) in Arkansas and implications for conservation. Thesis, University of Arkansas.

Frankham, R, Ballou JD, and Briscoe, DA (2010) *Introduction to Conservation Genetics*. Ed. Karina H. McInnes. 2nd ed. Cambridge UP, New York.

Grimsley, AA (2012) A reexamination of the eastern collared lizard (*Crotaphytus collaris collaris*) in Arkansas. Dissertation, University of Arkansas.

Hutchison, DW (2003) Testing the central/peripheral model: analyses of microsatellite variability in the eastern collared lizard (*Crotaphytus collaris collaris*). *The American Midland Naturalist*, 149:148-162.

McGuire, JA, et al. (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. *Evolution* 61:2879-2897.

Montes-Carreto, LM, Guerrero JA, and Ortega, J (2020) Effects of habitat fragmentation on the genetic variability of the volcano rabbit (*Romerolagus diazi*). *Conservation Genetics in Mammals*. Springer, Cham. https://doi.org/10.1007/978-3-030-33334-8_9.

Pardini, R, Nichols, E and Püttker, T (2017) Biodiversity response to habitat loss and fragmentation. *Encyclopedia of the Anthropocene* 1:1-12.

Scanes, CG (2018) Human activity and habitat loss: destruction, fragmentation, and degradation. *Animals and Human Society*. Academic Press:451-482.

Smith, PW (1957) An analysis of post-Wisconsin biogeography of the Prairie Peninsula region based on distributional phenomena among terrestrial vertebrate populations. *Ecology* 38:205-218.

Templeton, AR, Shaw, K, Routman, E and Davis SK (1990) The genetic consequences of habitat Fragmentation. *Annals of the Missouri Botanical Garden* 77:13.

Trauth, SE (2011) Rapid reservoir inundation causes complete extirpation of the eastern collared lizard (Crotaphytus collaris) along the shoreline of Bull Shoals Lake in northern Arkansas. *Journal of the Arkansas Academy of Science* 65:133-137.

Willi, Y, Van Buskirk, J, and Hoffmann, AA (2006) Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics* 37:433-458.

Conservation Genetics of the Imperiled Eastern Collared Lizard in Arkansas

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ABSTRACT

Habitat loss, fragmentation and isolation have reduced population sizes of many species world-wide and may also increase the risk of extinction. Conservation geneticist are particularly concerned with habitat loss and fragmentation as they can lead to a reduction of gene flow, genetic diversity, and population size and may ultimately increase the risk of expiration for small populations. Eastern Collard Lizard (*Crotaphytus collaris*) populations in Arkansas have suffered recent population declines as a result of a combination of natural and human mediated habitat loss and fragmentation. Conservation management strategies such as tree removal, prescribed burns, and translocations are already in effect. However there has yet to be a population genetic assessment of these populations. Therefore, in this study I employed microsatellite data derived from 138 adult *C. collaris* individuals, sampled from 14 sites, and genotyped across 11 loci to quantify genetic diversity, assess population structure, and evaluate connectivity among the remaining *C. Collaris* populations in Arkansas. Results revealed overall low genetic diversity, with genetic structure reflecting either five, or eight distinct gene pools, and a potential metapopulation among Baxter and Stone counties. The remaining populations appear to be isolated and small. Heterozygosity was found to be the highest among sites in Baxter and Stone counties (0.343-0.455). Gene flow appeared to be reduced between sites that were further apart from one another as indicated by low migration rates (<10%), suggesting these populations comprise 'Management Units'. Tests for isolation by distance (IBD) across all sites showed a non-significant correlation between genetic and geographic distance (P=0.260). Overall, my results indicate that isolated *C. collaris* populations may be at risk for further

isolation due to reduced gene flow. These findings can inform conservation efforts, so that translocations and habitat restoration can prioritize restoring gene flow among isolated populations.

INTRODUCTION

Habitat loss and fragmentation for many species has been steadily increasing as a result of anthropogenic activities and has been cited as one of the biggest threats to global biodiversity (Fahrig 2003). A severe loss of habitat reduction and increases in fragmentation may impact populations by reducing overall population size, inhibiting dispersal, and may ultimately lead to local expiration of a species. Expiration of local populations can impact long term species persistence as it can reduce genetic diversity, population structure and overall connectivity within a species range. Therefore, in order combat the further loss of species, conservation efforts should focus on preserving or creating habitat connectivity at the population level that will increase gene flow and genetic diversity.

Gene flow is essential between populations as aids in maintaining genetic diversity. A lack of, or reduction in gene flow, coupled with small populations sizes, has the potential to decrease genetic diversity within populations, potentially reducing its adaptive potential, which further threatens at risk populations and makes them susceptible to local extirpation (Slatkin 1987; Templeton et al. 2001; Montes-Carreto et al. 2020). Geneflow among populations is a function of an organism's dispersal ability and landscape-dependent factors (Elkin and Possingham 2008). Geographic barriers combined with patchy population distribution will impede dispersal and gene flow among populations. An example of the genetic consequences of habitat loss, fragmentation and isolation can be found in populations of the Eastern Collared Lizard (*Crotaphytus collaris*) within the Ozark region.

Biogeography and Conservation History of the Eastern Collared Lizard

The Eastern Collared Lizard (*C. collaris*) has occupied the Ozark region within Arkansas from about 8,000 years ago. They eventually became isolated from the Southwestern Eastern Collared

Lizard populations as a result of climatic cooling which occurred around 4,000 years ago when the prairie like Ozark plateau transitioned into dense oak and hickory forests (Smith, 1957; Cole, 1971; Brisson et al. 2003). As a result, the open glade habitat of *C. collaris* became naturally fragmented, inhibiting some dispersal by way of physical and geographic barriers (Templeton et al. 1990; Grimsley 2012).

It is also thought that this open woodland mosaic developed as a result of aboriginal fire regime and the Ozark Plateau became highly dependent upon fire (Nelson 1985; Nelson 1997). Furthermore, it is believed that *C. collaris* habitat fragmentation was exacerbated by European settlement and fire suppression, which increased the amount of woody vegetation, and forest understory, further creating barriers to dispersal, and isolating glade habitat, which the Eastern Collared Lizard depends on (Templeton et al. 1990; Nelson 1997; Brisson et al. 2003; Neuwald and Templeton 2013; Brewster 2019). The diminishing numbers of this charismatic species prompted multiple studies focusing on the physiological, ecological, and genetic characterization of *C. collaris*, as well as the conservation and restoration of their glade habitat (Templeton et al. 2001; Templeton 2011; Cole 2015; Elliot 2017; Elliot 2020; Brewster et al 2018; Brewster 2019). Studies on microsatellite variability and habitat loss and degradation of *C. collaris* populations support the idea that dispersal of individuals was hindered due to heavy forest and undergrowth surrounding populations, resulting in substantial population declines (Hutchison 2003; Brewster et al. 2018). Further, studies of *C. collaris* populations in Missouri revealed that a continued loss of habitat and glade fragmentation resulted in isolated *C. collaris* populations becoming more susceptible to genetic drift, and a reduction in gene flow which increased their vulnerability to local expiration (Hutchinson and Templeton 1999).

Collared Lizard populations in the Arkansas Ozark region are currently facing a similar fate to that of Collared Lizard populations in Missouri, where habitat loss and increased fragmentation due to environmental and anthropogenic factors may also have led to reduction of gene flow and a loss of genetic diversity. Declines of (*C. collaris*) populations in the Ozark glades have been linked to habitat degradation and previous studies suggest that without conservation intervention their populations will continue to deteriorate, leading to local extinction (Brewster et al. 2018, Grimsley 2012). Furthermore, a study on *C. collaris* populations in AR sought to identify evolutionary significant units (ESU's) by examining the evolutionary history of these populations through the analysis of mtDNA (Cole 2015). Although this study had a limited data set, researchers concluded that the Eastern Collared Lizard in Arkansas comprises a single ESU, thus identifying the importance of preserving *C. collaris* populations in Arkansas. Furthermore, it was concluded that the continued persistence of these populations' rests on increasing genetic diversity and restoring geneflow between *C. collaris* populations in Arkansas (Cole 2015). The conservation of *C. collaris* populations in Arkansas can be seen as extremely time sensitive. Evidence from previous studies on the distribution of these populations suggest that they have experienced past and recent local extinction throughout glade habitat patches in the Ozarks (Trauth, 1989; Hutchison 2003; Grimsley 2012). Additionally, local extinctions have already been documented in three *C. collaris* sites in Arkansas since the start of this study (Brewster et al. 2018).

Conservation efforts of habitat restoration and translocations may prove useful to conserving *C. collaris* populations in Arkansas. Populations of the Eastern Collared Lizard in the Ozark regions of Missouri were facing a similar fate to that of populations in Arkansas. Researchers found that in the 1980s, populations in the Missouri Ozarks were rapidly

disappearing due to habitat degradation and fragmentation, leaving populations isolated (Templeton et al. 2011). Conservation strategies to combat the loss of *C. collaris* in the Missouri Ozarks included prescribed burns and tree removal, which proved to have a positive effect on the dispersal of *C. collaris* and colonization of glade habitat (Templeton et al. 2001, 2011). This ultimately increased gene flow, which allowed for a stable metapopulation, and also increases in population size, thus aiding in the prevention of location extinction (Templeton et al. 2001, 2011). A metapopulation can be loosely defined as a set of subpopulations of the same species, which are isolated by unsuitable habitat, but, have some degree of gene flow between them (Levins 1970; Allendorf et al. 2013). Metapopulations are also defined by a balance of local extinction and recolonization events (Allendorf et al. 2013). Therefore, the current conservation management plan for the remaining Eastern Collared Lizard populations in Arkansas also includes controlled burns and tree removal to restore the glade habitat which this species depends on (Brisson et al. 2003; Neuwald and Templeton, 2013). The hope is that habitat management will allow populations of the Collared Lizard in Arkansas to become a stable metapopulation, with enough geneflow to be self-sustained.

Project Aims

The aim of this study is to assess the overall genetic structure and connectivity of the remaining *C. collaris* populations in Arkansas to inform on-going conservation management practices in the state. This will be accomplished through the analysis of microsatellite data. The use of microsatellites in conservation genetic studies assessing geneflow, fragmentation and population structure have been well documented and has led to a better understanding of endangered and/or threatened populations (Balloux and Lugon-Moulin 2002; Levine et al. 2016; Douglas et al. 2020; Hendricks et al. 2020; Montes-Carreto et al. 2020). I predict that the loss and

fragmentation of habitat and reduced migration between populations has resulted in low gene flow and low genetic diversity, with an increase in population structure. To test this, I used measures of population allelic diversity and values of *Fst* to evaluate overall population diversity, data quality, heterozygosity as well as looking for unique alleles and genetic differentiation between *C. collaris* sites. I also expected that *C. collaris* sites that are more distant and spatially segregated will have reduced genetic diversity and overall reduced geneflow, compared to sites that are closer in proximity to one another. I used an Analysis of Molecular Variance (AMOVA) to test for the presence of hierarchical population structure. I also used STRUCTURE and Discriminant Analysis of Principals Components (DAPC) to further identify population structure and identify the number of gene pools among *C. collaris* sites in Arkansas. I tested for recent gene flow by estimating migration rates and I also ran a Mantel test to evaluate Isolation-by-Distance (IBD). Results from my study can help inform management practices and conservation efforts regarding Eastern Collared Lizard populations in Arkansas.

METHODS

Study Organism: The Eastern Collared Lizard

The Eastern Collared Lizard (*C. collaris*) is a medium sized lizard, that is sexually dimorphic and known for their discernible color patterns (Brewster 2019). Its range extends from Eastern Missouri to Arizona, where it occupies dry rocky environments, as it is a saxicolous (rock dwelling) and heliothermic (sun-heated) species (McGuire et al. 2007; Grimsley 2012). The Ozark region of Arkansas is defined by forest habitat surrounding fragmented rocky outcrops known as glades, which provide ideal habitat for the Eastern Collared Lizard (Templeton et al. 2001; Brewster et al. 2018). However, historic, and current habitat fragmentation within the Ozark Plateau have plagued *C. collaris* populations in Arkansas, because they depend on glade

habitat for temperature regulation and foraging and mating (Brewster 2019). As such *C. collaris* populations have faced drastic declines and local extinctions over the past 30 years (Brisson et al. 2003 Trauth 2011; Grimsley 2012),

Sample Collection and Sites

This project is derived from *C. collaris* individuals sampled across 15 sites from 2011-2018. The collection of specimens was completed by Casey L. Brewster (2019). These sites encompassed all known populations of the Eastern Collared Lizard in Arkansas. GPS data for the site locations were also provided by Casey L. Brewster. A total of 446 toe samples were collected from adult, juveniles, and hatchlings and preserved in a solution of 95% ethanol. The handling and care of captured Collared Lizard specimens were approved and followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) Protocol #13036. The samples were derived from 15 sites which are distributed across eight counties in Arkansas (Baxter, Stone, Carroll, Conway, Izard, Logan, Marion, and Newton) (Figure 1). Initial STRUCTURE results were used as reasoning for the grouping of multiple sites within a county, with the exception of Optimus River Road (STCC) and Herd Creek Glade (STOP) in Stone county and Baxter 1 and Baxter 2 sites in Baxter county.

DNA Extraction and Isolation

Genomic DNA from toe clips was isolated from the 446 samples using Qiagen DNeasy Extraction Kits and followed the standard protocols. The extracted and isolated DNA was then quantified via a Qubit fluorometer, which was aliquoted to standardized concentrations. Samples were then used for polymerase chain reactions (PCR) to amplify across 11 microsatellite (Msat) loci for *C. collaris* (Hutchinson et al. 2004). Msat fragments were run through a capillary sequencer, to separate them electrophoretically. Finally, all alleles were manual scored using the (GeneMapper v6) software.

This work was performed by Brenna Levine and Avery Elliott (Elliott 2017). This data was used to evaluate the genetic diversity and connectivity for the 14 locations that C. collaris samples were taken from (Figure 1).

Of the 446 samples collected, only 400 were deemed useable for initial analyses based on the quality of the microsatellite data. From this data, it was determined that only the adult samples (N=138) from each site should be used in the final analyses in order to prevent misinterpretation of the data, which may occur if comparing individuals to their direct offspring and siblings. Additionally, having multiple closely related members among samples violates certain model assumptions. One site (CNPJ) was also removed from further data analyses, because it only contained 1 individual. The number of adults from each site can be found in Tables 1 and 2 as well as in Figure 2. Microsatellites are short tandem repeats (STR) that are found at a high frequency in most organisms and because of their high mutation rates, they reveal allelic diversity within individuals and populations, necessary for genetic studies of short time scales (Schlotterer 2000; Selkoe and Toonen 2006). Further, the microsatellite data collected allowed me to examine allelic differentiation within the population, evidence of gene flow and population structure which is essential in tackling conservation biology questions (Balloux and Lugon-Moulin 2002).

Data Analyses

I used GenAIEx v6.5 to calculate mean heterozygosity, pairwise *Fst*, the number of different alleles, number of private alleles, percent polymorphism and the expected heterozygosity under Hardy-Weinberg equilibrium (Peakall and Smouse 2012). The mean number of alleles found at each of the 11 microsatellite loci was used to calculate genetic diversity and allelic patterns across populations. The genetic diversity of each site was evaluated by estimating the expected heterozygosity and calculating percent polymorphism. Pairwise *Fst* values were calculated to

show the genetic variance differences among loci and among sample sizes. Significant values of genetic differentiation between sites were also highlighted.

Analysis of Molecular Variance (AMOVA) was used to test for the presence of hierarchical population structure (Meirmans 2012). The AMOVA was also used to test the percentage of genetic differentiation among populations, and whether it was caused by differences between populations, individual samples of a populations, or within individuals (Meirmans 2006). F-statistics were calculated to determine the significance of the AMOVA results, as F-statistics are applicable to dimorphic populations where there are two alleles at the locus (Nei 1977). Additionally, *Fis* was used as a potential indicator of inbreeding within a population (Conner and Hartl 2004).

STRUCTURE v2.3.3 was used to infer population structure across all *C. collaris* individuals at all 14 sites to estimate the number of unique populations (Pritchard et al. 2000a). The STRUCTURE program can infer population structure from multilocus data, by assigning individuals to a population based on shared alleles and then finding groupings of individuals to estimate genetic clusters, within Hardy-Weinberg equilibrium (Pritchard et al. 2000a). In STRUCTURE, I selected the Admixture model, which allows individuals to possess blended ancestry during the analyses. This allows for individuals to be calculated as if they may have received some fraction of their genome from multiple populations (K) (Pritchard et al. 2000a). Initial tests were run with a burn-in of 150,000, an MCMC of 500,000 and tested for multiple populations (K) from 1-12. Final analyses parameters (i.e., burn-in and MCMC) were chosen based off of alpha values, divergence distance between populations, and likelihood values from the summary statistics (Pritchard et al. 2000a). A final burn-in of 500,000 iterations was chosen and used in analyses, followed by 10^6 Markov chain Monte Carlo iterations, with three iterations

of each K and values of K, or populations, ranging from 2-12 were evaluated using this model. A maximum number of 12 populations (K) was chosen based on the geographic location of the sites. The most likely K was determined using the methods described in (Pritchard et al. 2000a), as well as the Dela K method proposed by (Evanno et al. 2005). They showed that the number of groups or populations was detected best using delta K (Evanno et al. 2005). Additionally, Cluster Markov Packager Across K (CLUMPAK) was used to process STRUCTURE results and generate figures (Kopelman et al. 2015).

To further identify populations and genetic clusters of *C. collaris* in Arkansas I used a Discriminant Analysis of Principal Components (DAPC) through the Adegenet package in R 2.13.1 (Jombart 2008; R Development Core Team 2011). DAPC was chosen in addition to STRUCTURE analyses because it is able to identify possible subpopulations through estimating allele combination within each individual. DAPC will also try to estimate the largest between-group variance, while also minimizing variation within the clusters (Jombart et al. 2010; Jombart and Collins 2015). Finally, DAPC also estimates genetic cluster membership probabilities for each individual (Jombart and Collins 2015). Prior to running the DAPC, a cross-validation test was run to determine the number of PC's retained during analyses. The number of clusters (K=8) for DAPC analyses were initially determined using the lowest Bayesian Information Criterion (BIC) (138) (Jombart and Collins 2015). In addition, I also visually examined the DAPC to look for clusters.

To further detect levels of geneflow and population connectivity among sites of *C. collaris*, I estimated recent migration rates between sites, using the program BA3-SNPS Version 1.1 (BA3-SNPS) which is created from the BayesAss version 3.0.4, algorithm to automate MCMC parameter tuning (Wilson and Rannala 2003; Mussmann et al. 2019). This program

estimates the rate of migration among populations through a Bayesian method that utilizes individual genotype data, as well as using Markov chain Monte Carlo (MCMC) analyses in order to estimate posterior probabilities (Wilson and Rannala 2003).

I used a Mantel test to look for isolation by distance (IBD), by testing for a statistical relationship between a geographic distance matrices and a genetic matrix (Mantel 1967). The P-value from a Mantel test represents the relative number of times the shuffled (randomized) regression coefficient is equal to or larger than the observed coefficient and was used to determine the significance of the statistical test (Séré et al. 2017). I conducted the Mantel test using GenAIEx and created a correlation coefficient for two data matrices (genetic and geographic), ranging from –1 to +1, and also tested for significance via random permutation (GenAIEx Manual Peakall, R. and Smouse P.E. (2012). The null hypothesis for the Mantel test used, is that there is no relationship between the geographic and genetic matrices (Rxy=0). The alternative hypothesis is that there is a relationship between the genetic and geographic distances (Rxy>0). I ran a total of 99 permutations for 14 samples (sites). The genetic matrix was calculated using pairwise *Fst* values and the geographic distance matrix was created using decimal Latitude and Longitude data for all 14 *C. collaris* sites.

RESULTS

Allelic Patterns Across Populations

When examining allelic patterns for all 138 individuals across 11 loci, I found that the number of alleles ranged from 1.55 in MRRU to 3.18 in STCE. Heterozygosity among sites ranged from 0.27 to 0.45 (Table 3). The highest values of heterozygosity were found in sites BACG (0.45), STCE (0.42), and STHC (0.43), all of which are located in Baxter county.

Pairwise *F*ST values were used as an indicator of gene flow between *C. collaris* sites, where larger values indicate greater genetic distance between groups of the Eastern Collared Lizard. Results from the pairwise *F*ST estimates (Tables 4a & 4b) revealed that gene flow is most reduced between sites which are geographically further from one another. Sites in Marion county (MRRU & MRFR) had the greatest differentiation from sites in Logan county with values of (0.36-0.38). (STOP) and sites in Carroll county are also significantly differentiated, with reduced gene flow from sites in Logan county with *F*ST values of (0.36) and (0.31-0.36) respectively (Table 4b.).

Analysis of Molecular Variance (AMOVA)

Results from the AMOVA, revealed that that the majority of allelic variance exists within individuals (66%), rather than among populations (26%) for 138 individuals across 14 sites (Figure 3) (Table 5). Additionally, *C. collaris* across all sites were found to be moderately, but not significantly differentiated from one another (*F*ST =0.264) (Table 5).

Assignment Test (STRUCTURE)

The Delta K plot indicates that there are likely 5 or 8 distinct gene pools (Figure 4). Bar plots displaying estimates of Q which plots individuals based on their portion of ancestry from each gene pool were also used to identify distinct gene pools (Figure 5). When examining the K=8 Q plot, eight distinct gene pools can be seen, separated by color, and individuals are sorted into either: Baxter, Carroll, Logan, Marion, Newton, Izard and into Optimus River Road (STCC) and Herd Creek Glade (STOP) in Stone county. Optimus River Rd. and Herd Creek Glade sties were found to be their own distinct genetic cluster. Additionally, the Calico Rock Pittman site in Izard county was found to be somewhat genetically different from those in Stone and Baxter counties.

Additionally, results reveled distinct genetic clusters in Carroll, Logan, and Marion and Newton counties.

Discriminant Analysis of Principal Components (DAPC)

Results from the Discriminant Analysis of Principal Components (DAPC) can be seen in Figure 6. During analyses, I chose to retain 20 PC's based on results from the cross-validation test, as seen in Figure 7. Furthermore, *C. collaris* individuals were initially clustered into eight genetic clusters based on BIC scores (Figure 8) and STRUCTURE results. However, visual examination of the DAPC and groupings of individuals by genetic similarity revealed three genetic clusters as seen in Figure 6. Results also revealed a larger genetic cluster which comprises City Bluff, Cataract Creek, Logan, Carroll, and Izard sites. As well as a cluster which contains Optimus Rover Road and Marion sites, and finally a cluster containing only individuals from Newton county.

Bayesian Analyses

Results from Bayesian analyses using the BA3-SNPS program, which looks at rates of migration as evidence of gene flow, revealed that overall, migration rates were higher within, rather than between sites (Table 6). Values closer to 1 indicate little to no migration. Additionally, when migration is <=10%, then populations are considered 'Management Units' (MU's). Therefore, *C. collaris* sites that are considered MU's are Logan county (LOSL & LOSU), Optimus River Road (STCC), Marion county (MRFR & MRRU), and Newton county (NWPR). Significant migration rates between populations of *C. collaris* can be seen in relation to their location within Arkansas and shows the geographic distance between populations and sites in Figure 9. BayesAss results also revealed moderate values of inbreeding coefficients in Cataract Creek (BACG) and Table Rock (BATE) in Baxter county and also in (IZCP) in Izard county (Figure 10).

Mantel Test

The results from the Mantel test, which tests for isolation by distance (IBD), showed that across all sites there was a very weak, but non-significant, correlation between the genetic and geographic distance (Rxy=0.105, P=0.260) (Figure 11).

DISCUSSION

The purpose of this study was to determine the levels population connectivity and genetic diversity among Eastern Collared Lizard populations in Arkansas. The results and data from this study will be useful in the continued management of *C. collaris* sites in Arkansas by identifying the impact of habitat reduction and fragmentation on population structure and gene flow between *C. collaris* populations. Furthermore, this study may also aid in identifying populations and sites for future Collared Lizard translocations within Arkansas.

Genetic Diversity

Results for allelic diversity revealed low to moderate levels of heterozygosity among *C. collaris* populations. The highest values for heterozygosity which were found in Baxter and Stone counties may be due the fact that there were more samples from these sites, and they are closer to one another. Therefore, the potential for migration and gene flow is higher. Furthermore, populations and sites which were more isolated tended to have lower levels of heterozygosity, such as in Carroll county. Overall, heterozygosity was generally lower than expected. This was likely influenced by small sample sizes. The gathering of more samples would allow for a more accurate representation of genetic variability within populations.

Population Connectivity & Structure

Genetic structure within and among *C. collaris* populations was detected at a low levels and revealed there are likely several distinct genetic clusters. In particular, results from

STRUCTURE and Delta K analyses indicated that either five or eight genetic clusters exist for all *C. collaris* sites in Arkansas. The population structure seen in *C. collaris* populations in Arkansas, may be result of prolonged isolation due to a loss of habitat, thus leading to a reduction in dispersal and gene flow between populations. For example, populations which were geographically distant appeared to be their own distinct genetic cluster. Bayesian analyses for migration rates revealed low levels of gene flow between populations which were more geographically distant, such as in Newton, Logan, Carroll. These results may be due to decreased gene flow and genetic drift due to isolation. However, tests for IBD indicate that there is not a strong correlation between genetic distance and geographic distance across all *C. collaris* sites in Arkansas. This is likely a result of having small sample sizes and testing for IBD across all sites, and not just between sites which are geographically distant.

Interestingly, higher levels of genetic similarity and gene flow were seen in the neighboring sites within Baxter and Stone counties. Individuals within these sites tended to be more similar and may comprise a potential metapopulation, though this was not explicitly tested for. Surprisingly, Herd Creek Glade (STCC), which is also located in Stone county and close to sites in Baxter 2, had low levels of gene flow and appeared to be genetically distinct from other nearby sites in Baxter and Stone county. It is unclear why individuals in Herd Creek Glade are more genetically distinct than the remaining Stone and Baxter counties. Perhaps there are unknown physical barriers of dispersal. Optimus River Road (STOP) which is also in Stone county, but is much more geographically isolated, was found to have reduced gene flow and was genetically distinct. Because of the distance it is unlikely that *C. collaris* individuals could naturally disperse to the other populations in Stone and Baxter county. For this reason, sites in

Baxter, Stone, and Izard counties which are closer to one another may be the best candidates for establishing a metapopulation structure.

Results from this study may be indicative of similar patterns of Collared Lizard populations in Missouri. These studies found that habitat loss and fragmentation disrupted gene flow and decreased genetic diversity. However, sites within these studies were closer to one another, contained more samples and were studied over a longer period of time (Templeton 2001; Neuwald & Templeton 2013). Additionally, studies on the impact of habitat loss and fragmentation on *C. collaris* populations in Missouri found that continued dispersal and gene flow were necessary to maintain populations amongst the naturally fragmented glade habitat (Brisson 2003), and that translocations and prescribed burns aided the transition of isolated populations of *C. collaris* to a metapopulation structure (Templeton et al. 2011).

Results from this study may be indicative of reduced gene flow and genetic diversity as a result of habitat loss and fragmentation, but small and uneven sample sizes likely led to an underrepresentation of the genetic status of certain *C. collaris* populations in Arkansas. For example, an uneven number of samples were available from each population, in part due to an effort to capture every individual and some sites having small population sizes. The low number of adult lizards that I was able to use for the genetic analyses impacted my ability to make definitive interpretation of genetic diversity, population structure and connectivity. Therefore, any future genetic analyses or monitoring of these populations should include additional sampling.

The continued genetic monitoring of *C. collaris* populations in Arkansas would pair well with on-going conservation efforts to restore Eastern Collared Lizard glade habitat and the initiation of translocations within in Arkansas. This conservation project is funded by The

Arkansas Game and Fish Commission, as well as the National Parks Service, and the US Forest Service, and is primarily focused on restoring *C. collaris* glade habitat by removing cedar from 200 acres of current glade habitat in addition to using prescribed burns (Web 2021). The goal is to improve glade-habitat quality as well as connectivity between glades. This direct conservation plan also includes the translocation and reintroduction of the Eastern Collared Lizard into restored glade habitat, with the hope of potentially creating new Eastern Collared Lizard populations. Translocations of the Eastern Collared Lizard and the creation of new populations is important in helping to re-establish a metapopulation structure among *C. collaris* populations.

CONCLUSION

Impact of Habitat Loss and Fragmentation on the Eastern Collared Lizard

Results from this study indicate that fragmentation and habitat loss may be having an impact on the genetic diversity within small, isolated populations. STRUCTURE, BayesAss and pairwise *Fst* results indicated that gene flow is decreased between sites which are geographically distant and leads to a reduction in overall population structure. Low values of genetic diversity within populations, coupled with low levels of gene flow due to fragmentation can have serious impacts on the future of *C. collaris* populations in Arkansas. The continued loss of genetic diversity, and gene flow could make it difficult for *C. collaris* populations in Arkansas, to persist in a metapopulation structure and may ultimately increase their risk of local expiration. The loss of local populations is important because it contributes to the larger problem of biodiversity loss on both a regional and global scale.

Future directions

Since the management of *C. collaris* populations are ongoing, the continued use of conservation genetic methods and research will further aid in conserving these populations in Arkansas, by

allowing researchers and managers to track gene flow and genetic diversity. For example, genetic samples from Eastern Collared Lizard populations can be taken pre- and post-translocation to monitor genetic diversity of these populations. Additionally, genetic monitoring of these populations will allow us to further understand how gene flow and population structure may change in response to the direct conservation management strategies of brush removal, prescribed burns, and translocations. This could then be used as a measure of how successful these management strategies are for fragmented populations.

LITERATURE CITED

Allendorf, FW, Aitken, SN, and Luikart, G (2013) Conservation and the genetics of populations. John Wiley & Sons Hoboken.

Balloux, F and Lugon-Moulin, N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology* 11:155-165.

Brewster, CL, Beaupre SJ, and Willson, JD (2018) Habitat loss and local extinction: linking population declines of eastern collared lizards (*Crotaphytus collaris*) to habitat degradation in Ozark glades. *Journal of Herpetology* 52:352-360.

Brewster, CL (2019) Eastern collared lizard (*Crotaphytus collaris*) population declines in Ozark landscapes: an assessment of environmental constraints. Dissertation, University of Arkansas.

Brisson, JA, Strasburg, JL and Templeton, AR (2003) Impact of fire management on the ecology of collared lizard (*Crotaphytus collaris*) populations living on the Ozark plateau. *Animal Conservation* 6:247-254.

Cole, KW (1971) Consideration of macro-climatic and macro-biotic change in the Ozark highlands during post-glacial times. *Journal of the Arkansas Academy of Science* 25:15-20.

Cole, JM (2015) Identifying evolutionarily significant units in eastern collared lizard (*Crotaphytus collaris*) from Arkansas. Thesis, University of Arkansas.

Conner, JK, and Hartl, DL (2004) *A primer of ecological genetics*. Sinauer Associates Incorporated.

Douglas, MR, Anthonysamy, WJB, Mussmann, SM, Davis, MA, Louis, W, Douglas, ME (2020) Multi-targeted management of upland game birds at the agroecosystem interface in midwestern North America. *PloS one* 15: e0230735.

Elliott, A (2020) Conservation genetics of collared lizard (*Crotaphytus collaris*) Populations in Arkansas. Thesis, University of Arkansas.

Elliott, ED (2017) Population structure of collared lizard (*Crotaphytus collaris*) in Arkansas and Implications for Conservation. Thesis, University of Arkansas.

Elkin, CM, and Possingham, H (2008) The role of landscape-dependent disturbance and dispersal in metapopulation persistence. *The American Naturalist* 172:563-575.

Evanno, G, Regnaut, S, and Goudet, J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology* 14:2611-2620.

Fahrig, L (2003) Effects of habitat fragmentation on biodiversity. *Annual review of ecology, evolution, and systematics* 34:487-515.

Grimsley, AA (2012) A reexamination of the eastern collared lizard (*Crotaphytus collaris collaris*) in Arkansas. Dissertation, University of Arkansas.

Hendricks, S, et al. (2020) Patterns of genetic partitioning and gene flow in the endangered San Bernardino kangaroo rat (*Dipodomys merriami parvus*) and implications for conservation management. *Conservation Genetics* 21:819-833.

Hutchison, DW (2003) Testing the central/peripheral model: analyses of microsatellite variability in the eastern collared lizard (*Crotaphytus collaris collaris*). *The American Midland Naturalist* 149:148-162.

Hutchison, DW, Strasburg, JL, Brisson, JA, and Cummings, S (2004) Isolation and characterization of 11 polymorphic microsatellite loci in collared lizards (*Crotaphytus collaris*). *Molecular Ecology Notes* 4:54-556.

Hutchison, DW, and Templeton, AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* 53:1898-1914.

Jombart, T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24:1403-1405.

Jombart, T, Devillard, S and Balloux, F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* 11:1-15.

Jombart, T, and Collins, C (2015) A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0. 0. *London: Imperial College London, MRC Centre for Outbreak Analysis and Modelling*.

Kopelman, NM, Mayzel, J, Jakobsson, M, Rosenberg, NA, & Mayrose, I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources* 15:1179-1191.

Levine, BA, et al. (2016) Population genetics of the copperhead at its most northeastern distribution. *Copeia* 104:448-457.

Levins, R (1970) Extinction. Some mathematical questions in biology. *American Mathematical Society* 77-107.

Mantel, N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* 27:209-220.

McGuire, JA, et al. (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. *Evolution: International Journal of Organic Evolution* 61:2879-2897.

Meirmans, PG (2006) Using the amova framework to estimate a standardized genetic differentiation measure. *Evolution* 60:2399-2402.

Meirmans, PG (2012) AMOVA-based clustering of population genetic data. *Journal of Heredity* 103:744-750.

Montes-Carreto, LM, Guerrero, JA and Ortega, J (2020) Effects of habitat fragmentation on the genetic variability of the volcano rabbit (*Romerolagus diazi). Conservation Genetics in Mammals*. Springer, Cham 197-215.

Mussmann, SM, Douglas, MR, Chafin, TK, Douglas, ME (2019) BA3-SNPs: Contemporary migration reconfigured in BayesAss for next-generation sequence data. *Methods in Ecology and Evolution* 10:1808-1813.

Nei, M (1977) F-statistics and analysis of gene diversity in subdivided populations. *Annals of human genetics* 41:225-233.

Nelson, PW (1985) The terrestrial natural communities of Missouri. Missouri natural areas Committee, Jefferson City. 197 p.

Nelson, JC (1997) Presettlement vegetation patterns along the 5th Principal Meridian, Missouri Territory, 1815. *American Midland Naturalist*:79-94.

Neuwald, JL, and Templeton, AR (2013) Genetic restoration in the eastern collared lizard under prescribed woodland burning. *Molecular ecology* 22:3666-3679.

Peakall, R and Smouse, PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28:2537-2539.

Pritchard, JK, Stephens, M, and Donnelly, P (2000a) Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.

R Development Core Team, *R: A Language and Environment for Statistical Computing*, 2011 Vienna, Austria, R Foundation for Statistical Computing.

Schlötterer, C (2000) Evolutionary dynamics of microsatellite DNA. *Chromosoma* 109:365-371.

Selkoe, KA, and Toonen, RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology letters* 9:615-629.

Sere, M, Thevenon, S, Belem, AMG, & De Meeûs, T (2017) Comparison of different genetic distances to test isolation by distance between populations. *Heredity 119*:55-63.

Slatkin, M (1987) Gene flow and the geographic structure of natural populations. *Science* 236:787-792.

Smith, PW (1957) An analysis of post-Wisconsin biogeography of the Prairie Peninsula region based on distributional phenomena among terrestrial vertebrate populations. *Ecology* 38:205-218.

Templeton, AR, Shaw, K, Routman, E, and Davis, SK (1990) The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden* 77:13-27.

Templeton, AR, Robertson, RJ, Brisson, J, and Strasburg, J (2001) Disrupting evolutionary processes: the effect of habitat fragmentation on collared lizards in the Missouri Ozarks. *Proceedings of the National Academy of Sciences* 98:5426–5432.

Templeton, AR, Brazeal, H, and Neuwald, JL (2011) The transition from isolated patches to a metapopulation in the eastern collared lizard in response to prescribed fires. *Ecology* 92:1736-1747.

Trauth, SE (1989) Distributional survey of eastern collared lizard, *Crotaphytus collaris collaris* (Squamata: Iguanidae), Within the Arkansas River Valley of Arkansas. *Journal of the Arkansas Academy of Science* 43:101-104.

Trauth, SE (2011) Rapid reservoir inundation causes complete extirpation of the eastern collared lizard (*Crotaphytus collaris*) along the shoreline of Bull Shoals Lake in northern Arkansas. *Journal of the Arkansas Academy of Science* 65:133-137.

Wilson, GA, & Rannala, B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* 163:1177-1191.

(2021) "Cooperative Fish and Wildlife Research Units Program: Arkansas Education, Research and Technical Assistance for Managing Our Natural Resources." *Arkansas Project: Glade Restoration and Conservation Management of Eastern Collared Lizards in Northern Arkansas*. Web. 19 Mar. 2021.

TABLES

Table 1. Collared Lizard samples (N=400) sorted by site (15) and county (8) into either Adult, Yearling, Hatchling or Unknown and showing the total samples (N) for each site. Samples are listed by age class. The individual from CNPJ was dropped from analyses due to insufficient samples. Baxter and Stone counties contain the most samples.

County	Sites	Site(s)	Adult	Yearling	Hatchling	Unknown	N
Baxter	City Bluff Calico Rock Central & East	**STCE STHE**	30	47	15	12	104
	Cataract Creek Glade & Table Rock East	**BACG BATE**	6	3		2	11
Carroll	Lake Leatherwood Central	**CRLC**	9	14		6	29
	Lake Leatherwood South	**CRLS**	7			3	10
Conway	Petit Jean	**CNPJ**				1	1
Logan	Schwartz Quarry Lower	**LOSL**	9	10	6		25
	Schwartz Quarry Upper	**LOSU**	8	6	28		42
Marion	Flippin Rock Quarry, Rush BRNP	**MRFR MRRU**	18	13			31
Newton	Pruitt BRNP	**NWPR**	12	18	1	3	34
Izard	Calico Rock Pittman	**IZCP**	6	2			8
Stone	Optimus River Road	**STCC**	21	52		13	86
	Herd Creek Glade / Forest	**STOP**	12	7			19
Total:		15	138	172	50	40	400

Table 2. Adult *Crotaphytus collaris* samples (N=138) listed by their respective county, with the exception Optimus River Road and Herd Creek Glade in Stone county for a total of (N=8).

County	Sites	Site Code(s)	Adults	Site #
Baxter	City Bluff Calico Rock Central & East	**STCE, STHE**	30	1
	Cataract Creek Glade & Table Rock East	**BACG, BATE**	6	
Carroll	Lake Leatherwood Central	**CRLC**	9	2
	Lake Leatherwood South	**CRLS**	7	
Logan	Schwartz Quarry Lower	**LOSL**	9	3
	Schwartz Quarry Upper	**LOSU**	8	
Marion	Flippin Rock Quarry, Rush BRNP	**MRFR, MRRU**	18	4
Newton	Pruitt BRNP	**NWPR**	12	5
Izard	Calico Rock Pittman	**IZCP**	6	6
Stone	Optimus River Road	**STCC**	21	7
	Herd Creek Glade / Forest	**STOP**	12	8
Total: 7		**14**	**138**	**8**

Table 3. Standard molecular diversity measures for 138 adult *Crotaphytus collaris* samples from all 14 sites. Genetic diversity parameters are based on genotypes across 11 microsatellite loci. Significant values are highlighted in bold.

Mean Allelic Patterns Across Populations														
Population	**BACG**	**BATE**	**CRLC**	**CRLS**	**IZCP**	**LOSL**	**LOSU**	**MRFR**	**MRRU**	**NWPR**	**STCC**	**STCE**	**STHC**	**STOP**
Na	2.45	2.18	1.73	2.00	2.55	2.09	2.18	2.45	1.55	2.18	2.45	**3.18**	2.82	2.36
Ne	2.06	1.59	1.54	1.63	1.94	1.65	1.78	1.82	1.44	1.81	1.68	2.00	2.04	1.95
I	0.74	0.56	0.40	0.48	0.67	0.53	0.53	0.60	0.34	0.58	0.59	0.76	0.74	0.64
No. Private Alleles	0.09	0.09	0.00	0.09	**0.27**	0.09	0.09	0.09	0.00	0.09	0.09	0.00	0.00	0.00
He	**0.45**	0.34	0.27	0.30	0.39	0.34	0.31	0.36	0.24	0.37	0.36	**0.42**	**0.43**	0.39
uHe	0.55	0.41	0.29	0.32	0.43	0.36	0.34	0.37	0.32	0.39	0.37	0.43	0.46	0.41

Table 4.a. Pairwise *Fst* values were calculated using Msat data from 138 *Crotaphytus collaris* individuals for 14 sites in Arkansas. Larger *Fst* values are highlighted in Table 4.b.

	BACG	BATE	CRLC	CRLS	IZCP	LOSL	LOSU	MRFR	MRRU	NWPR	STCC	STCE	STHC	STOP
BACG	0.00													
BATE	0.18	0.00												
CRLC	0.14	0.32	0.00											
CRLS	0.13	0.31	0.02	0.00										
IZCP	0.14	0.14	0.25	0.26	0.00									
LOSL	0.23	0.26	0.31	0.32	0.26	0.00								
LOSU	0.23	0.27	0.35	0.36	0.25	0.04	0.00							
MRFR	0.13	0.20	0.20	0.20	0.14	0.26	0.26	0.00						
MRRU	0.16	0.23	0.25	0.25	0.19	0.36	0.38	0.17	0.00					
NWPR	0.15	0.18	0.20	0.19	0.17	0.27	0.25	0.10	0.18	0.00				
STCC	0.11	0.15	0.22	0.20	0.11	0.29	0.27	0.14	0.13	0.12	0.00			
STCE	0.09	0.12	0.22	0.21	0.08	0.26	0.25	0.11	0.16	0.12	0.04	0.00		
STHC	0.10	0.13	0.21	0.20	0.09	0.22	0.21	0.11	0.15	0.10	0.06	0.02	0.00	
STOP	0.19	0.24	0.33	0.30	0.16	0.35	0.36	0.21	0.24	0.24	0.12	0.12	0.12	0.00

Table 4.b. Significant pairwise Fst values listed by site and county. Values were calculated from (N=138) across 14 sites. The most significant values- indicating the greatest genetic differentiation between *Crotaphytus collaris* populations are in bold.

Greatest Genetic Differentiation		
Carroll	**0.31-0.36**	Logan
Carroll	**0.31-0.32**	Baxter-1
Logan	0.23-0.27	Baxter-1
Logan	**0.36-0.38**	Marion
Marion	0.25	Carroll
Newton	0.25-0.27	Logan
City Bluff Calico R.C	0.25-0.26	Logan
Optimus River Rd.	0.27-0.29	Logan
Herd Creek Glade	**0.33-0.33**	Carroll
Herd Creek Glade	**0.35-0.36**	Logan
Herd Creek Glade	0.24	Baxter-1

Table 5. Summary table for F-statistics calculated from Analysis of Molecular Variance (AMOVA) results, using (N=138) *Crotaphytus collaris* individuals for 14 sites. *Fst* = 0.26, *Fis* = 0.11, *Fit* = 0.34. Corresponding percentages of molecular variance can be found in Figure 4.

F-Statistics	Value	P(rand >= data)
Fst	0.26	0.00
Fis	0.11	0.00
Fit	0.34	0.00

Table 6. Values indicating migration rates were calculated from Bayesian Analyses using Msat data from 138 *Crotaphytus collaris* individuals, grouped into 9 areas. Values range from 0 to 1, where 1 indicates no migration. Values in bold represent sites where migration is low. Further, migration <=10%, in populations comprise 'Management Units' (MUs).

Migration Rates	
Baxter-1	**0.80**
Baxter-2	0.69
Carroll	**0.88**
Izard	0.69
Logan	**0.90**
Marion	**0.89**
Newton	**0.87**
STCC	**0.90**
STOP	**0.86**

FIGURES

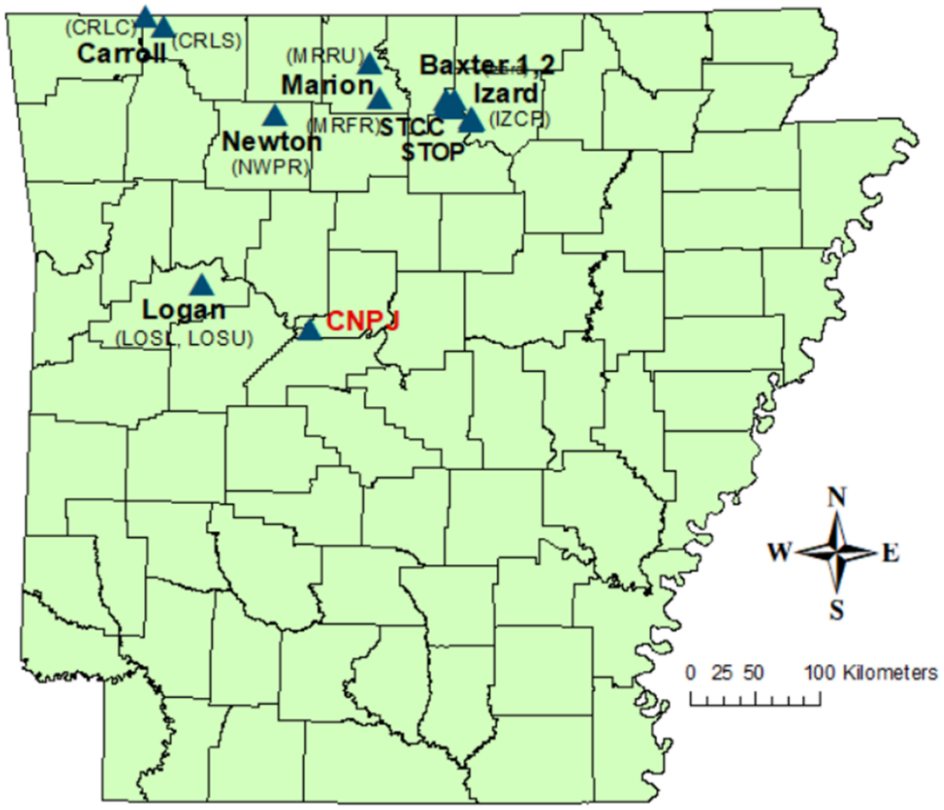

Figure 1. Geographic distribution of 14 sites in Northwest Arkansas where Eastern Collared Lizard (*Crotaphytus collaris*) was sampled from 2011-2018. CNPJ is in red because it was removed from all analyses due to low sample size. Sample sizes for each site are listed in Table 1.

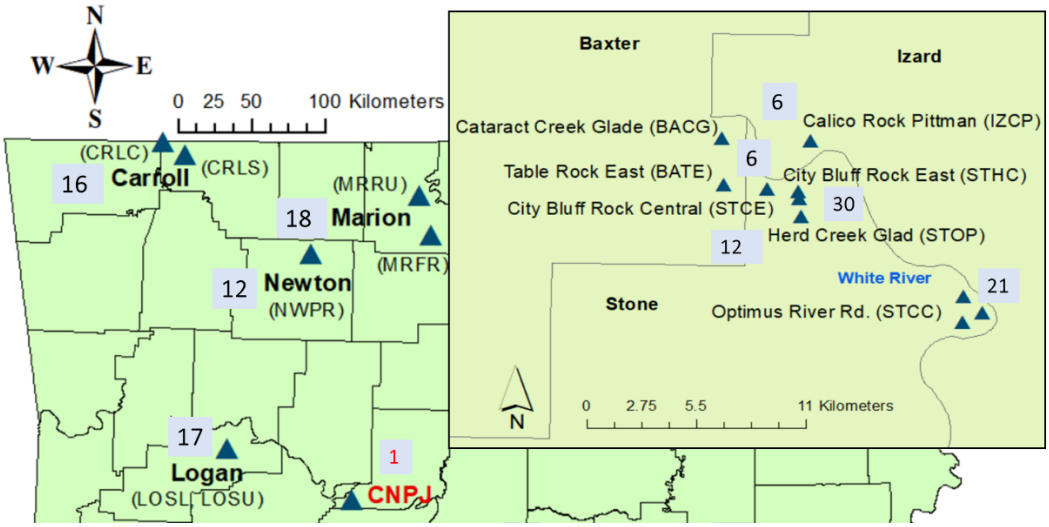

Figure 2. Sample sites with corresponding number of adult *Crotaphytus collaris* samples that were used from each site and county in Arkansas. An inset of Baxter, Stone, and Izard county shows closeness of sites in these counties, as well as the potential geographic barrier of the White River.

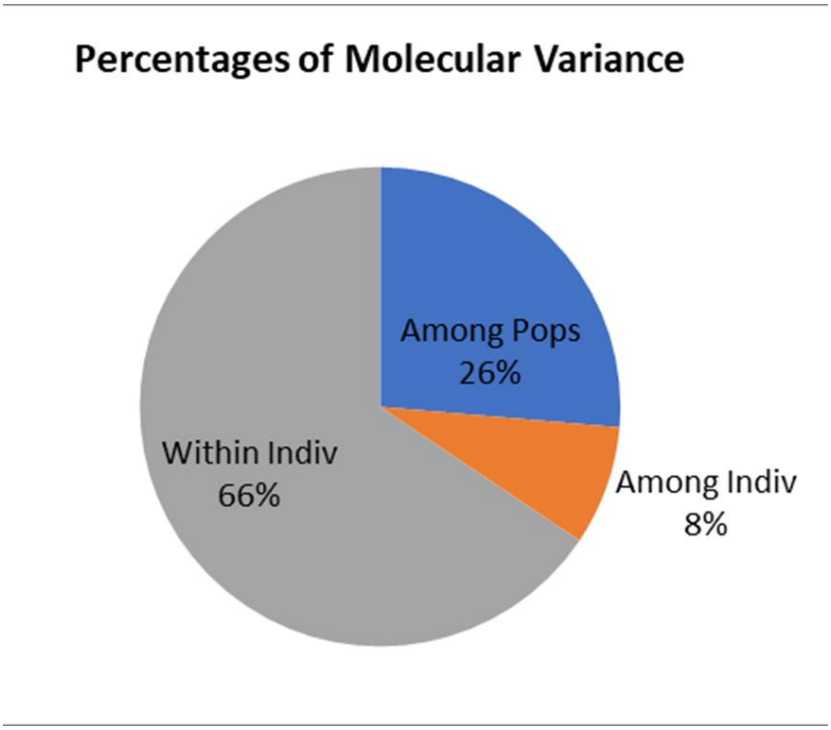

Figure 3. Results from the Analysis of Molecular Variance (AMOVA) are shown in the pie chart. AMOVA was calculated using microsatellite data from 138 *Crotaphytus collaris* individuals across 14 sites.

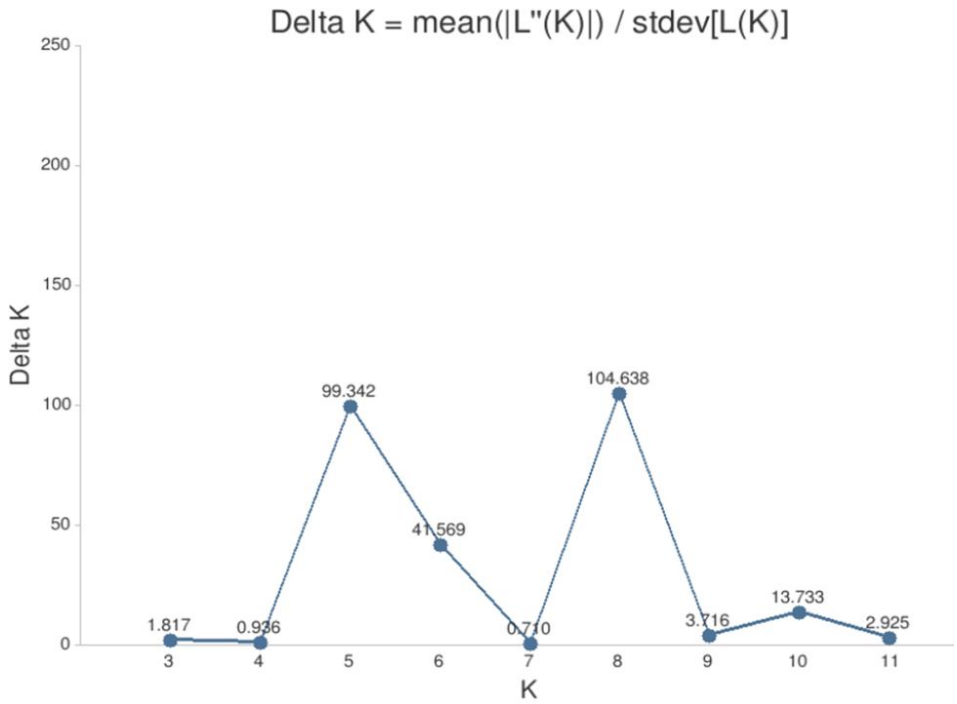

Figure 4. Delta K plot was generated from structure data for 138 *Crotaphytus collaris* individuals, grouped into 9 areas. K is representative of the number of potential genetic clusters. Using the program Clumpak. Ln(Pr(X|K) values were used in Clumpak to identify the k, for which Pr(K=k) is the highest, which is an indicator of the most likely genetic clusters found.

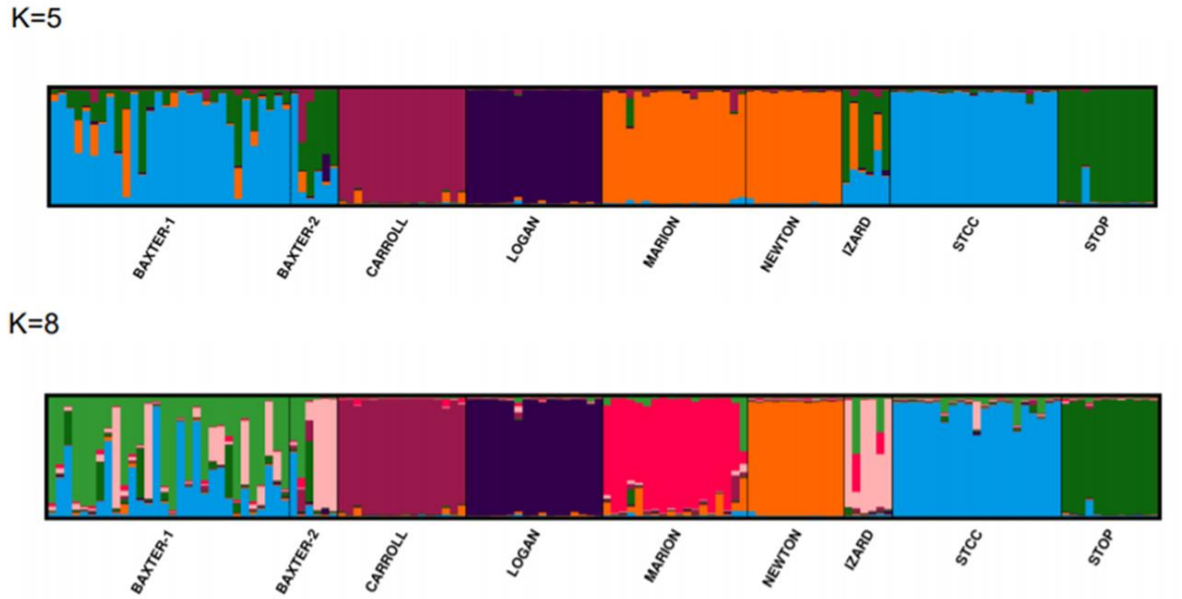

Figure 5. Q bar plots from STRUCTURE output, made using CLUMPAK. 138 *Crotaphytus collaris* individuals from 9 geographic areas, are represented by a single vertical line (bar) and gene pools represented by color (K = number of gene pools). Results were generated using a burn-in of 500,000 and a MCMC of 10^6. The proportion of color reflects ancestry of individual in particular gene pool. Both K=5 and K=8 were identified in the Delta plot as being the most likely number of genetic clusters or populations.

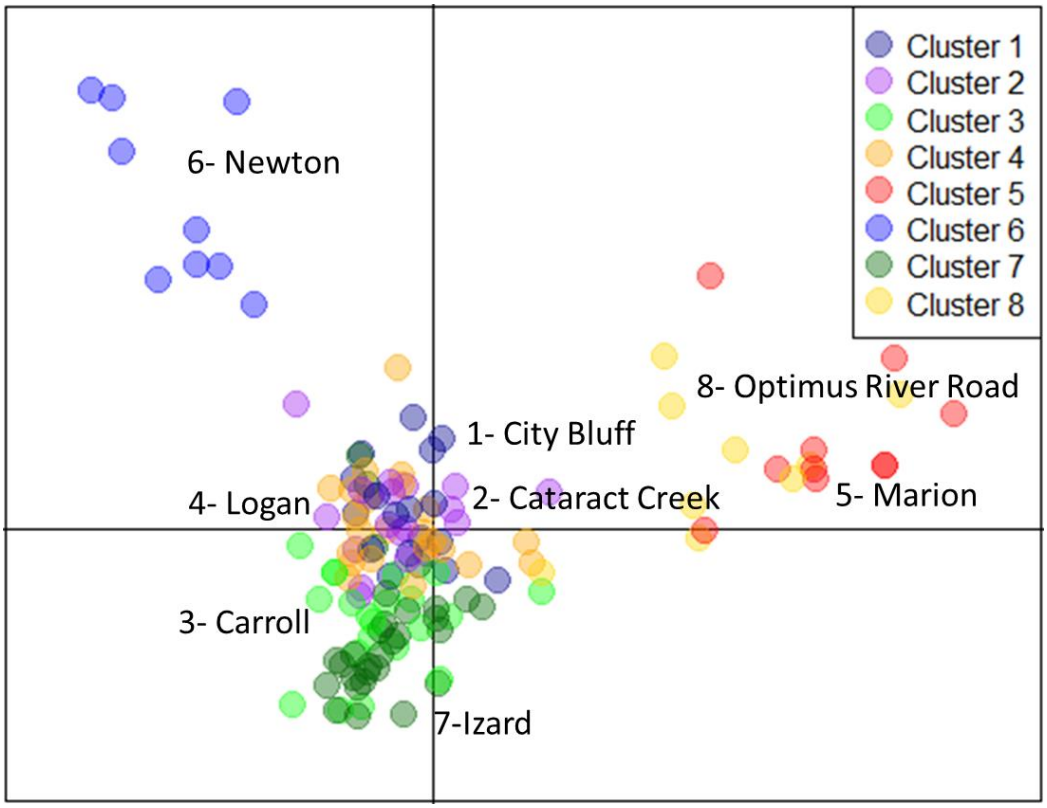

Figure 6. Discriminant analysis of principal coordinate (DAPC) results for 138 *Crotaphytus collaris* individuals, from 9 geographic areas, across 11 loci. K=40 groups were used, and 20 PC's were retained, with five discriminant functions and 8 clusters were chosen based on Delta K and Structure results. Individuals are represented as dots and are assigned to clusters, which are colored coded.

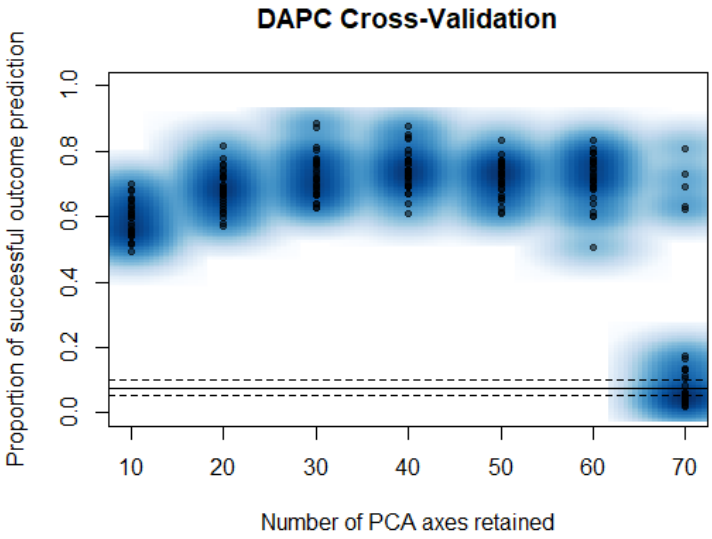

Figure 7. DAPC Cross-Validation test for microsatellite data for 138 *Crotaphytus collaris* individuals, which were grouped into 9 geographic areas. The x-axis represents the number of PCA axes retained, and the y-axis is the proportion of successful outcome prediction and ranges from 0-1. The DAPC Cross-Validation test shows the successful outcome prediction based off of the number of PCA axes which are retained. Therefore, 20 PC's were retained during analyses.

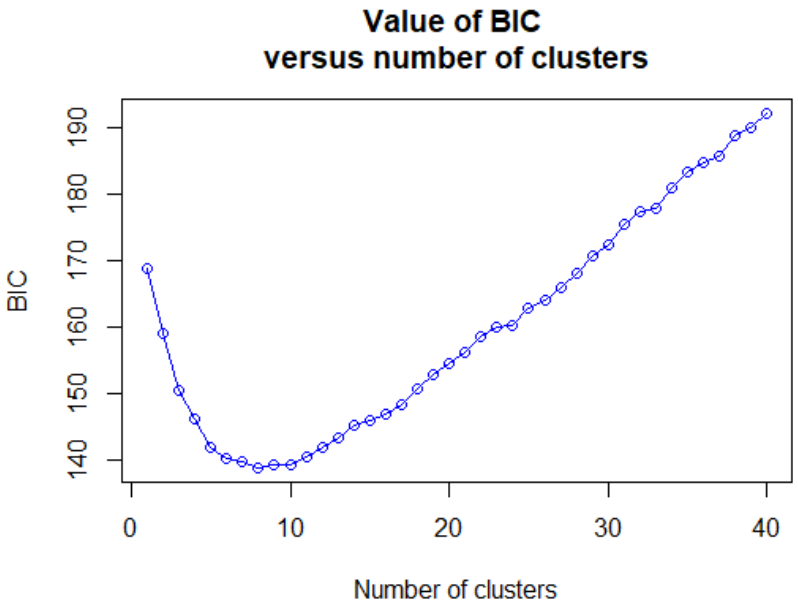

Figure 8. BIC values based on the number of selected clusters in DAPC analyses. 8 genetic clusters were chosen because it was associated with the lowest BIC value (138).

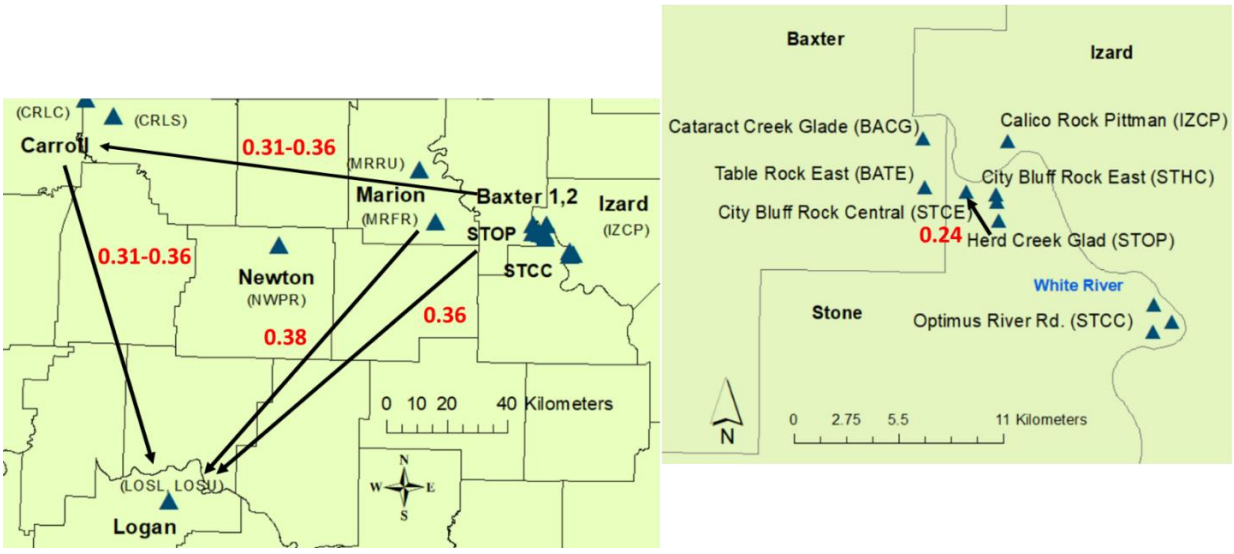

Figure 9. Significant migration rates between populations of *Crotaphytus collaris*. Values are based off of pairwise *Fst* values calculated from Msat data for 138 individuals across 14 sites. These *Fst* values can also be found in Table 4.b. All data for site locations were provided by Casey L. Brewster.

Inbreeding Coefficients	
Baxter 1	0.33
Baxter 2	**0.51**
Carroll	0.23
Izard	**0.48**
Logan	0.35
Marion	0.21
Newton	0.07
STCC	0.13
STOP	0.19

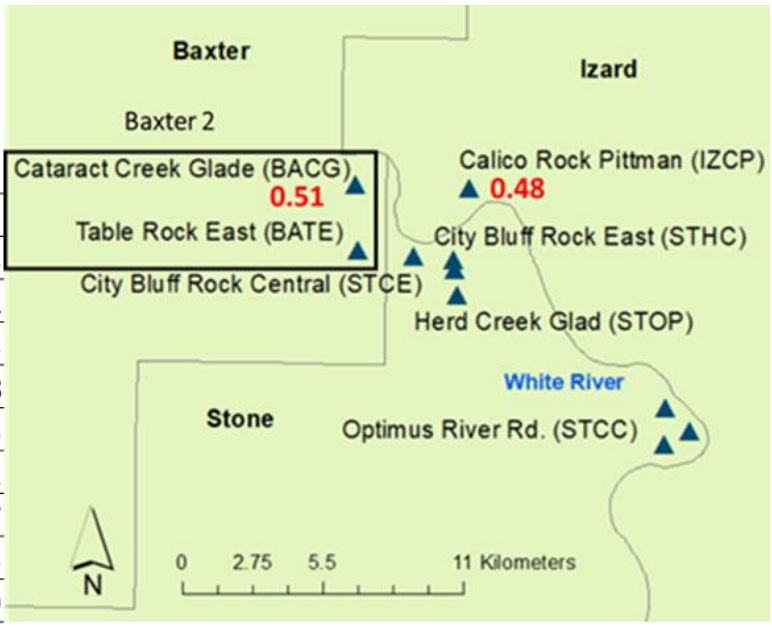

Figure 10. Values for moderate inbreeding coefficients were detected in *Crotaphytus collaris* samples within Baxter 2 (0.51) which comprises Cataract Creek (BACG) and Table Rock (BATE). A inbreed coefficient of 0.48 was in (IZCP) in Izard county. Inbreeding coefficients were calculated using Msat data for (N=138) individuals for 9 areas. Sites were grouped by county, and Baxter county was split into two separate areas.

Rxy	P(rxy-rand >= rxy-data)
0.105	0.260

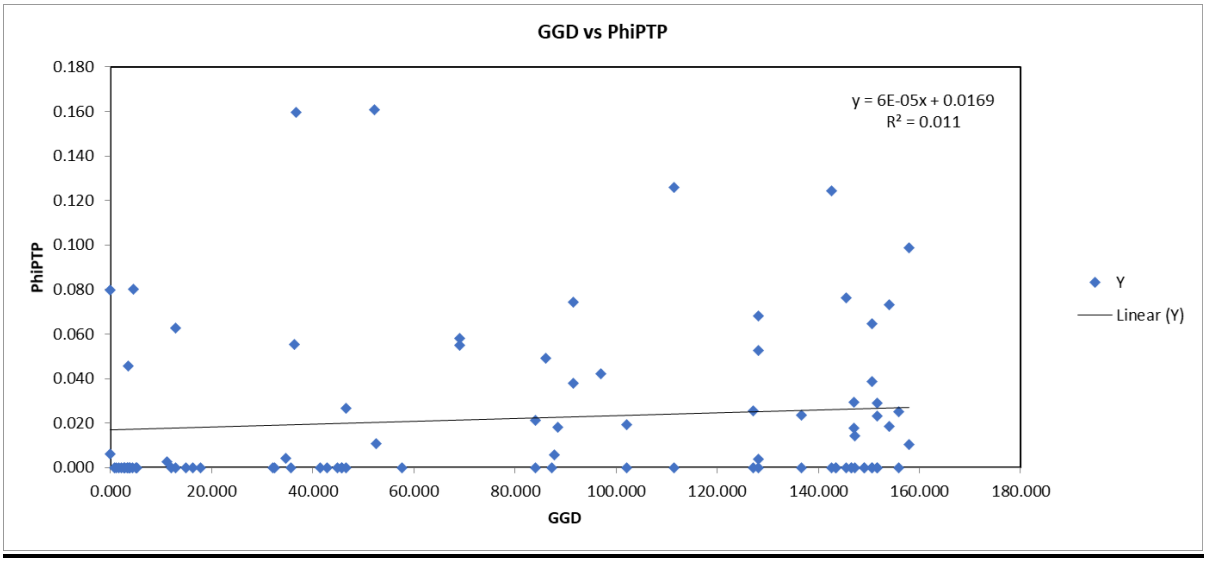

Figure 11. Mantel test was performed using GenAIEx to test Isolation by Distance (IBD) by looking for a correlation between the genetic matrix (PhiPTP) for 138 *Crotaphytus collaris* individuals and geographic distances (GGD) for 14 sites, and 99 permutations. Results revealed no significant correlation between genetic and geographic distances (Rxy=0.105, P=0.260).

CONCLUSION

In light of the sixth mass extinction, the conservation of species in response to natural and human meditated habitat loss and fragmentation is of serious concern to conservation biologists and land managers alike. Anthropogenic activities, such as fire suppression and habitat destruction, can have severe impacts on the conservation of locally adapted species by diminishing their habitat, therefore leading to habitat loss and fragmentation. Habitat fragmentation can be detrimental to populations because it disrupts dispersal, which can lead to habitat patches and isolated populations. Furthermore, habitat loss and fragmentation play a crucial role in diminishing gene flow and genetic variation among individuals and populations, which are essential to maintaining populations and ultimately species distributions.

Eastern Collared Lizard populations in Arkansas provide an example of how loss and fragmentation of habitat can lead to isolated populations, a reduction in gene flow and lack of genetic diversity. Overall, I detected moderate population structure among *C. collaris* sites in Arkansas. Results also indicated five or eight distinct gene pools, with evidence for potential metapopulation structure in two counties. This study also revealed that there are several small semi-isolated populations of the Eastern Collared Lizard in Arkansas, with reduced gene flow and migration between these populations. I also detected lower genetic diversity in isolated populations. More specifically, this work illustrates the need for conservation efforts to focus on managing critical habitat for species so as to revive the potential for gene flow between nearby populations. This is especially true if population persistence is maintained by a metapopulation structure. Future research could focus on estimating the success of translocations and habitat restoration in facilitating gene flow and aiding in re-colonization of empty habitat space.