Combining biotic surveys, GIS modeling, and ecological genetics to prioritize land purchases for the conservation of the federally threatened Red Hills salamander (*Phaeognathus hubrichti*).

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December 2010

Submitted to:
Alabama Department of Conservation and Natural Resources
State Wildlife Grants Program

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Introduction

The loss of biodiversity is of global concern, and amphibians are ranked as the most threatened group of vertebrates on the planet (Baillie et al. 2004). Thirty-two percent of the assessed amphibians are threatened with extinction, and 23% do not have sufficient data to assess extinction risk (GAA 2004). The southeastern United States is the world-center of salamander biodiversity. Habitat loss, introduced species, pollution, and climate change affect the population viability of species and threaten our natural heritage; in fact, Alabama is tied with California as the two mainland states with the highest number of recorded extinctions (Stein et al. 2000).

The Red Hills salamander (*Phaeognathus hubrichti*) is a rare, endemic fossorial plethodontid and the sole member of its genus. The species is only found in six counties in southern Alabama within the Red Hills physiographic province. The area of occupancy is approximately 500 km^2. Their restricted range makes them extremely vulnerable to the loss of genetic variability and extinction due to inbreeding and the susceptibility to demographic, environmental, and genetic stochasticity (Conner and Hartl 2004). *P. hubrichti* is especially subject to such threats because of its reliance on three narrowly distributed geologic formations in the Red Hills region of Alabama (Tallahatta, Nanafalia and Hatchetigbee) (Dodd 1991, Apodaca 2010). In fact, *P. hubrichti* was the first amphibian to obtain threatened status by the U.S. Fish and Wildlife Service (1976), due to the threats of habitat loss and over-collection (Dodd 1991). The quality and extent of remaining habitat is of concern because the amount and distribution of habitat affect reproductive rates and dispersal - two factors known to be low in *P. hubrichti* (Means 2003). Genetic issues loom especially large in this case; low gene flow between

populations would make individual populations especially vulnerable because genetic effective sizes can be up to three orders of magnitude smaller than the numbers of adults (Turner et al. 2002).

Remaining habitat is severely fragmented and threatened by a multitude of management practices. It is estimated that less than 40% of the supposed "area of occupancy" (123,553 acres; GAA 2004) supports populations of *P. hubrichti* (Bailey 1995; GAA 2004). The majority of remaining habitat is privately owned, predominately by timber companies. Prioritizing the remaining habitat within the Red Hills physiographic region for land purchases is critical because small, isolated populations are subject to stochastic events that could result in local extinctions. Currently there are seven Habitat Conservation Plans (HCPs) in effect for the Red Hills salamander. Although HCPs are a possible solution for protecting habitat outside of conservation reserves, HCPs are only effective for 10-30 years. In addition, if land is sold, the associated HCP is not necessarily transferred nor does the USFWS have a mechanism to evaluate the effectiveness of an HCP. Long-term conservation of critical habitat is essential for the continued persistence of rare species in the Red Hills physiographic region (10 P2, 1 P1, and 12 Watch List vertebrates). Therefore, prioritizing the remaining habitat within this region for land purchases will improve the probability that the Red Hills salamander and associated species do not succumb to stochastic events leading to local extinctions.

The opportunity to establish long-term habitat protection for *P. hubrichti* is an exciting prospect. Recently, land purchases made by a joint venture between the Alabama

Forever Wild Program, USFWS, and The Nature Conservancy have taken the crucial first steps in Red Hills conservation efforts. However, further recovery of *P. hubrichti* will require a combination of a strategic habitat-purchasing program and effective habitat management guidelines for private landowners. Each of these strategies will require a great deal of knowledge about the life history, ecology, genetic diversity, and distribution of *P. hubrichti*. To date, there is a lack of sufficient data in these areas. Here, we present a multi-faceted research project focused on gathering data vital to the recovery effort of *P. hubrichti*. We begin by using genetic data to investigate the current structure, effective size, and migration rates of *P. hubricthi* populations. This approach also allows us to explore the evolutionary history of the species, and therefore draw conclusions on whether current patterns are natural or have been caused by anthropogenic habitat modification. Additionally, genetic data can provide information on *P. hubrichti* life history characteristics, which would be difficult or impossible to obtain otherwise. Lastly, we use ecological modeling and spatially explicit data in order to help identify areas that may harbor unknown *P. hubrichti* populations. We conclude by suggesting a strategic plan to conserve *P. hubrichti* throughout its range, including habitat purchasing and management suggestions.

Literature Cited

Bailey MA (1995) Alabama's Threatened & Endangered Species: The Red Hills Salamander. Treasured Forests, Winter Issue 1995.

Baillie, J, Hilton-Taylor C, and Stuart S (2004) Red List 2004 Executive Summery International Union for the Conservation of Nature and Natural Resources, the World Conservation Union (IUCN). <//www.iucn.org> Downloaded on 2 January 2006

Conner, JK, Hartl DL (2004) A Primer of Ecological Genetics. Sinauer Associates Inc., Sunderland, MA.

Dodd Jr., CK (1991). The status of the Red Hills Salamander *Phaeognathus hubrichti*, Alabama, USA, 1976–1988. Biological Conservation 55:57–75.

IUCN, Conservation International, and NatureServe (2004) Global Amphibian Assessment. <www.globalamphibians.org>. Downloaded on 15 January 2006.

Means BD (2003) Notes on the Reproductive Biology of the Alabama Red Hills Salamander (*Phaeognathus hubrichti*) Contemporary Herpetology 2003 No. 3

Stein BA, Kutner LS, and Adams JS (2000) Precious Heritage: The status of biodiversity in the United States. Oxford University Press, Inc., New York, New York.

Turner TF, Wares JP, and Gold JR (2002) Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependent marine fish (*Sciaenops ocellatus*). Genetics 162:1329-1339.

US Fish and Wildlife Service (1976) Determination that the Red Hills Salamander is a threatened species. Federal Register 41:53032–53034.

I. Conservation genetics of the Red Hills salamander

Abstract

Estimating levels of gene flow and population connectivity are fundamental goals of conservation genetics, especially for imperiled species. Many factors can influence dispersal patterns and gene flow in natural landscapes. However, these patterns can be substantially distorted by the impacts of anthropogenic habitat modification. Studies of genetic divergence can provide insight into the impact of altered landscapes on gene flow; yet it is difficult to differentiate whether such patterns are due to historical or contemporary factors. In this study we investigate the effects of anthropogenic habitat degradation on spatial genetic patterns by accounting for historical patterns in the federally threatened Red Hills salamander (*Phaeognathus hubrichti*). By employing coalescent and non-equilibrium population genetic analyses, we were able to distinguish the effects of recent land use change from the naturally low migration rates characteristic of the species. Our results indicate that there are 5 well-supported populations (F_{ST} = 0.13009-0.1879) across the entire range of *P. hubrichti,* and that current migration rates between populations are low (m = 0.0025-0.0687). By accounting for history and species characteristics we demonstrate that this contemporary loss and fragmentation of habitat has had a negative impact on *P. hubrichti* in the form of reduced migration, bottlenecks, and high levels of inbreeding. We discuss the results of this study in terms of the direct impact to *P. hubrichti* and in the broader context of conservation genetics.

Introduction

The identification of intra-population genetic structure and diversity is fundamental to many fields within evolutionary biology and ecology. For conservation biology this information has become increasingly important in conservation planning for imperiled species (e.g. Geist & Kühn 2005; Pabijan et al. 2005; Dixon et al. 2006; Schwartz et al. 2007; Marshall et al. 2009; Matern et al. 2009; Straub & Doyle 2009). Maintaining genetic diversity, and therefore evolutionary potential, is fundamental to the long-term survival and recovery of at risk species (Avise 2004; Morgan et al. 2008). Theoretical models have demonstrated that the maintenance of genetic diversity and population viability is critically dependent on gene flow among local populations (Gilpin & Hanski 1991; Harrison 1991; Swindell & Bouzat 2005). This transfer of genes between populations influences a number of evolutionary processes, such as population persistence, release from inbreeding depression, and adaptive response (Frankham et al. 2002). Therefore, it has become a crucial goal of conservation genetics to not only identify the overall genetic structure, diversity and connectivity between populations, but also to understand the factors that shape such patterns.

Estimating migration and gene flow between populations is an increasingly important undertaking for conservation biologists, as habitat fragmentation continues to isolate populations and leave them more susceptible to the effects of genetic stochasticity and inbreeding depression (Frankham et al. 2002). In order to develop effective management and recovery plans it is essential to understand how and to what extent migration occurs in a modified landscape (Goldberg & Waits 2010). Accordingly, there has been a recent focus to identify landscape features that impede or facilitate gene flow

(e.g. Murphy et al. 2008; Zellmer & Knowles 2009; Goldberg & Waits 2010; Shirk et al. 2010). However, landscape features are not static through time, and a quickly changing landscape (as in the case of anthropogenic modification) can make it difficult to interpret current patterns (Zellmer & Knowles 2009).

Expanding our knowledge on the effects of anthropogenic habitat modification on gene flow requires a baseline understanding of migration in an unaltered setting. It can be difficult to interpret patterns of genetic variation because they may reflect the influence of landscape at several temporal scales (Zellmer & Knowles 2009; Anderson et al. 2010). Failing to take into account the importance of temporal variation in migration can obscure or even alter our interpretation of the influence that anthropogenic habitat alteration exerts on genetic differentiation in natural populations. Furthermore, these patterns can be complicated by natural history factors (e.g. high site fidelity, low dispersal ability, or specific habitat requirements). For example, in the case of naturally fragmented populations (or species with high site fidelity) it may be difficult to differentiate whether genetic structuring and gene flow patterns are due to their natural history, anthropogenic fragmentation, or a combination of both.

Fragmentation reduces gene flow in a wide variety of species (Epps et al. 2005; Proctor et al. 2005; Coulon et al. 2006; Cushman et al. 2006; Vandergast et al. 2007; Zellmer & Knowles 2009). However, without knowledge of a species natural dispersal patterns it is difficult to assess the impact of habitat fragmentation. In other words, before habitat fragmentation can be indicted as the cause of a decline in gene flow, we must first rule out natural attributes (low dispersal ability, site fidelity, etc). While relatively few studies have addressed this issue, new methods are emerging to deal with the temporal

scale of population genetic parameters across altered landscapes (Anderson et al. 2010). For instance, recent studies have accounted for the effects of landscape changes by first reconstructing historical landscapes and then using the residuals to quantify the effects of landscape change (e.g. Vandergast et al. 2007; Zellmer & Knowles 2009; Dyer et al. 2010). Furthermore, other recent studies have addressed the importance of temporal scale in population and landscape genetic studies (e.g. Fraser et al. 2007; Reed et al. 2009; Zellmer & Knowles 2009, Anderson et al. 2010, Knowles & Alvarado-Serrano 2010). Yet, comparatively few studies have explored the use of diverse analytical methods, which are available for molecular data, as a way of contrasting gene flow levels at distinct timescales. For example, two commonly used methods of estimating gene flow between populations (coalescent theory and non-equilibrium approaches) provide estimates of migration on altogether different time periods (Wilson & Rannala 2003). Combining these techniques can provide estimates of contemporary (non-equilibrium approaches) and historical (coalescent) gene flow.

The major goals of this study are to analyze the link between spatial genetic patterns with past and present landscape features and to gain insight into the basic population statistics of the federally threatened (IUCN: Endangered) Red Hills salamander (*Phaeognathus hubrichti*). Specifically we aim to: (I) define the population structure and population parameters across the range of *P. hubrichti*; (II) compare estimates of recent and historical patterns of migration; and (III) investigate the effect that anthropogenic habitat modification has had on recent patterns of migration and demographic events.

Methods

Population sampling:

Tissue samples were collected following U.S. Fish and Wildlife Threatened Species Permit TE136961-0. For each individual we collected a small tail-clip following standard protocols. Tissue was immediately preserved in 95% ethanol and transferred to a -80°C freezer at the University of Alabama Herpetology Collection. Sampling occurred across the entire known range of *P. hubrichti*, but was focused on sites that had been surveyed by Dodd (1991). In total we collected 105 individuals from 21 unique localities (Fig. 1). In view of the fact that *P. hubrichti* burrows are difficult to find and may be distributed patchily across even the best habitat, we considered a unique locality to consist of one USGS quadrangle. However, unique decimal degree coordinates were taken for each individual.

Microsatellite analysis:

We used Qiagen DNeasy tissue kit and protocol (Qiagen Inc, Valencia, CA) to extract DNA from all tissue samples. We amplified 10 microsatellite markers using polymerase chain reaction (PCR). Each primer was developed specifically for use in *P. hubrichti* (Lance et al. 2009). Information on each primer as well as PCR conditions can be found in Lance et al. (2009). Each locus was amplified individually and labeled PCR products were run on an ABI 3730 Genetic Analyzer. Samples were genotyped using GeneMapper 3.7 software (Applied Biosystems, Inc.). Scoring and quality control of data were done using GeneMarker V. 1.7 (Softgenetics, LLC). Microchecker V2.2.3 (Van Oosterhout et al. 2004) was used to check for possible null alleles, linkage disequilibrium and scoring

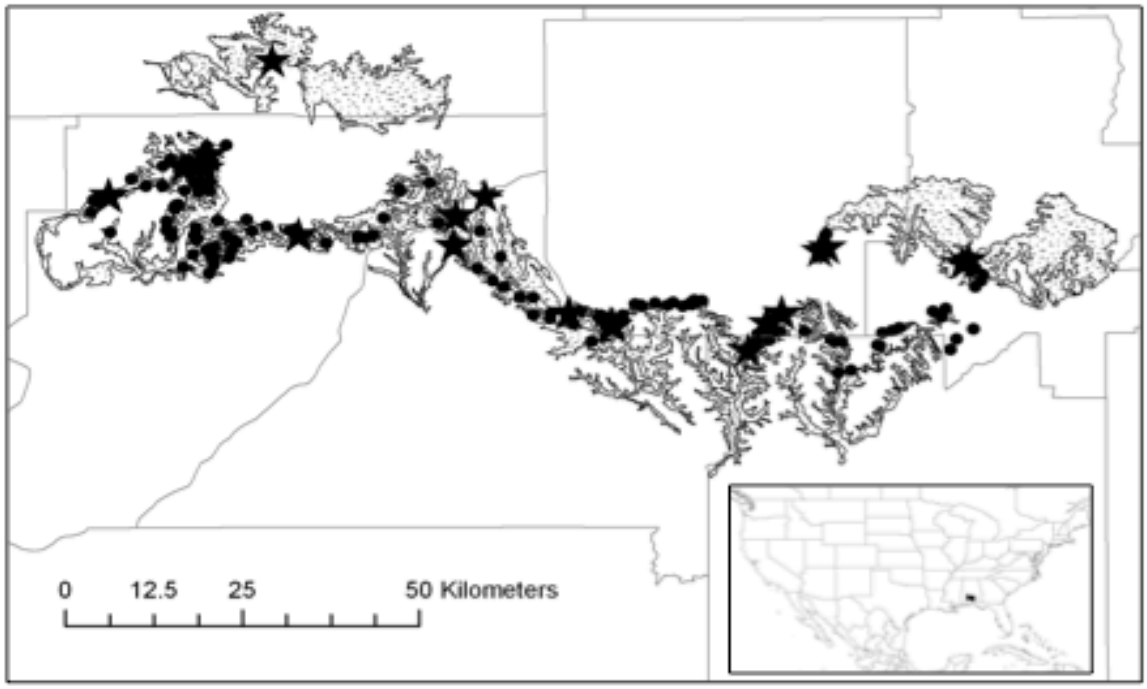

Fig. 1 Range map of *P. hubrichti* with an outline of geologic layers required by the species. Circles are known populations; stars represent tissue collection localities.

errors. Data quality and repeatability were tested by re-genotyping 10 individuals per locus. This test resulted in a repeatability success rate of 98%. Hardy-Wienberg equilibrium proportions for each population and locus were tested using an approach analogous to Fisher's exact test with a Markov chain of 1,000,000 iterations and 10,000 dememorization steps (Guo & Thompson 1992) in Arlequin version 3.11 (Excoffier et al. 2005).

Population genetic structuring and diversity:

We used two Bayesian methods to investigate the genetic structuring of populations. The first was implemented in TESS V. 2.3 (Chen et al. 2007). TESS uses hidden Markov random fields in order to model spatial dependence among individuals (Chen et al. 2007).

This approach has the advantage that it incorporates the *a priori* assumption that nearby individuals are more likely to have similar allele frequencies than individuals from distant localities. TESS was run for 100,000 simulations with a burn-in of 20,000. To estimate K we ran 100 replicates each for K values ranging from 3 to 9. For each K we averaged the 10 best DIC values and plotted them. Once we established the proper K value the 10 runs with the lowest DIC values for that K were exported to CLUMPP V. 1.1.2 (Jakobsson & Rosenberg 2007). CLUMPP uses three algorithms in order to properly match cluster membership over multiple runs. We repeated the above procedure using both the admixture and the no admixture models implemented in TESS V. 2.3 (Chen et al. 2007). Since the models did not vary significantly we used the results from the no admixture model as recommended by the authors. We also studied the spatial genetic patterns by using STRUCTURE 2.2 (Pritchard et al. 2000). Structure uses a Bayesian framework to assign individuals to populations based on their multilocus genotypes and is one of the most commonly used structuring programs. The ΔK method of Evanno et al. (2005) was used to assess the best value of K. For each run of STRUCTURE, the program was run for 1,000,000 MCMC cycles, with a burn-in of 100,000 and default settings. We also used Arlequin version 3.11 (Excoffier et al. 2005) in order to determine the ability of each Bayesian clustering method to assign the genotypes of individuals to the populations from which they came (Paetkau et al. 1995). Log-likelihoods were calculated using the allele frequencies from the observed data, and therefore providing the global individual likelihood. The global individual likelihood is the likelihood of that individual coming from the predetermined population for each locus of the individual's genotype (Cabe et al. 2006).

We investigated patterns of intra and inter population diversity among assigned populations using Arlequin V. 3.11. We performed a locus-by-locus AMOVA (Excoffier et al. 1992) using 10,000 permutations and computed pairwise and global values of F_{ST} using 1000 permutations for significance followed by a sequential Bonferroni correction. Observed heterozygosity (H_O), expected heterozygosity (H_E) (Nei 1987), allelic diversity, and fixation index (F_{IS}) for all populations were also calculated using Arlequin V. 3.11.

Estimates of recent and historical gene flow:

In order to estimate the historical patterns of gene flow we used the program MIGRATE V. 3.0.3 (Beerli & Felsenstein 1999,2001). MIGRATE uses coalescent theory and Markov chain Monte Carlo techniques to estimate historical pairwise migration rates ($M=m/\mu$, where m= migration rate and μ= mutation rate) and effective population sizes ($\Theta=4N_e\mu$, where N_e is effective population size). Distributions were estimated using the Bayesian implementation of Migrate based on the accuracy of the Bayesian approach under a wider variety of conditions (Beerli 2006). Following the recommendations of the author, we did an initial run on our data set using F_{ST} to find the start parameters, and the results of this run were used as start parameters for subsequent runs. Results from different runs were stable, indicating that the Markov chains had likely converged on the stationary distribution. For each run we used the continuous Brownian mutation model and ran 5,000,000 generations per long chain with a burn-in of 100,000. We also used BayesAss+ V.1.3 (Wilson and Rannala 2003) to estimate more recent migration rates. Although both programs use a Bayesian MCMC approach, BayesAss+ uses a genetic assignment method rather than a coalescent method to estimate gene flow. Genetic assignment methods tend to estimate more recent dispersal rates, as compared to

coalescent methods that are closer to long-term averages (Berry et al. 2004; Paetkau et al. 2004). We performed 5 runs (each with different seed values) of 5 million generations with a 2 million generation burn-in and sampled the chain every 2,000 generations. Estimates of M from MIGRATE were converted to proportion of migrants (m) for populations by using the formula $m = M\mu$, where $\mu = 5.4 \text{ X } 10^{-4}$ (Goldstein et al. 1995; Howes et al. 2009).

Recent and historical gene flow estimates (m) were compared using a Wilcoxon matched pairs test in STATISTICA V. 6.0. The Wilcoxon matched pairs test is a non-parametric method that assumes the samples are not independent of each other (Sokal & Rohlf 2003), making it an ideal fit for analyzing if there is a significant difference in migration estimates between two methods or time periods.

Recent demographic events:

To test for the signature of recent bottleneck events we used the program Bottleneck V1.2.02 (Cornuet & Luikart 1996; Piry et al. 1999). Bottleneck is based on the theory that in a population bottleneck the allelic diversity will decline more rapidly than the heterozygosity. That is to say, because of the loss of rare alleles the observed heterozygosity is larger than the heterozygosity expected based on the number of alleles present if the locus was at mutation-drift equilibrium. We ran the infinite alleles model (IAM) because it is most likely to fit the large number of interuted and compound microsatellites in our study (Cornuet and Luikart 1996). Because interrupted and compound microsatellites tend to fit the IAM much more closely they are among the most useful markers for detecting bottlenecks using this method (Cornuet and Luikart 1996). We also tested the robustness of the model by varying the parameters using the

two-phase model (TPM) with varying degrees of stepwise mutation model (SMM; 10 to 40% in steps of 10). All tests were run using 10,000 permutations. One-tailed Wilcoxon tests were used to determine the significance of heterozygosity excess or deficiency for each population.

Results

Genotypic data:

A total of 100 alleles were observed for the 10 loci sampled across all populations of *P. hubrichti.* The mean number of alleles per loci was 5.94 with a standard deviation of 0.641. We did not find the presence of linkage disequilibrium, scoring errors, or null alleles. However, some of the missing data (especially locus 62) could be due to null alleles causing a failure to identify the alleles as loci. There were deviations from HWE in some loci in some of the populations (no more than 2 for any single population), but since the deviations were not consistent across all populations we did not exclude them from the analyses.

Population genetic structuring and diversity:

TESS (Chen et al. 2007) and STRUCTURE (Pritchard et al. 2000) each identified 5 populations throughout the range of *P. hubrichti* (Fig. 2). Both programs identified the same populations whether or not admixture was used (for TESS), lending support that these populations are a biological reality. Furthermore, a likelihood assignment test (Paetkau et al. 1995) as performed in Arlequin (Excoffier et al. 2005) showed overwhelming support for each of the populations. Consistent with these results, the AMOVA (Table 1) indicated a high amount (15.58%) of variation between populations, as well as a high global fixation index (0.1558). Pairwise F_{ST} values were also high

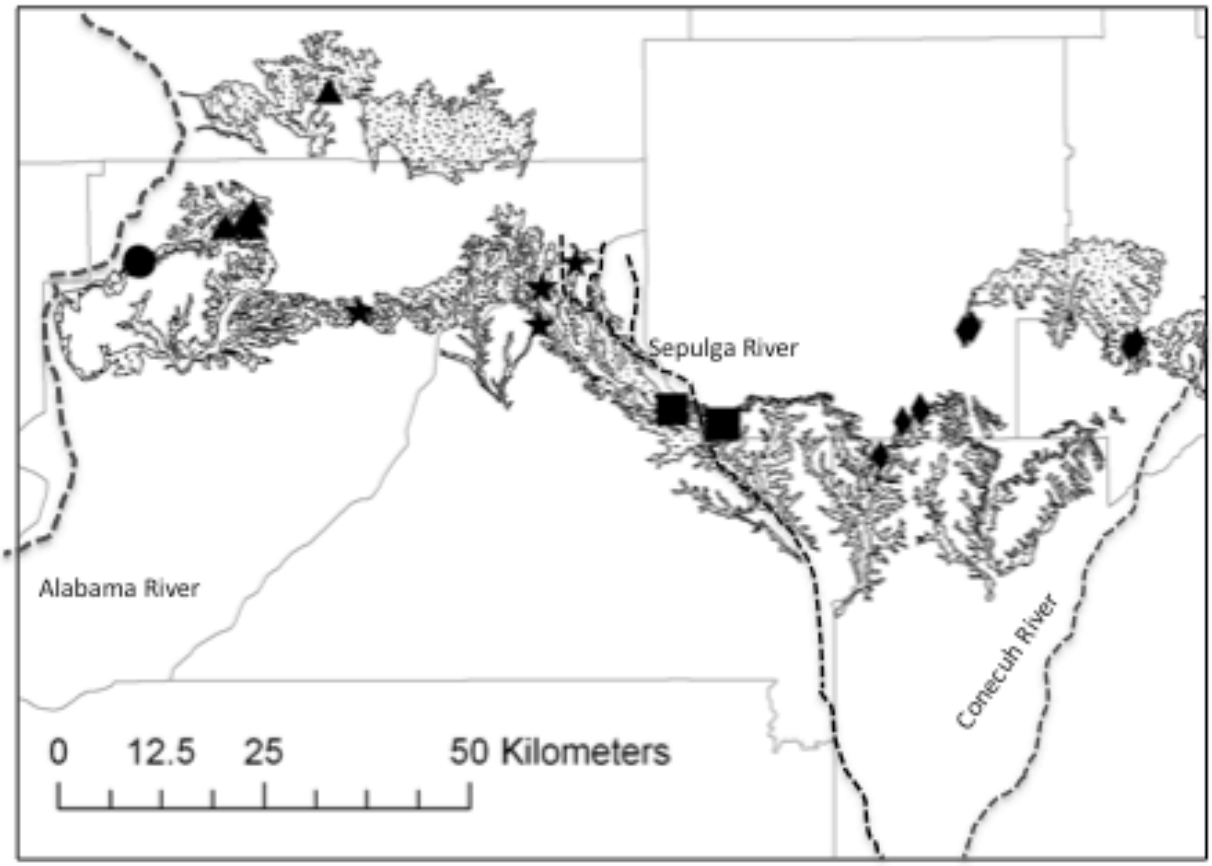

Fig. 2 Populations of *P. hubrichti* as determined by Bayesian clustering techniques. Populations are numbered from left to right and represented by unique symbols (1, circles; 2 triangles; 3, stars; 4, squares; 5, diamonds).

Table 1. Results of the Analysis of Molecular Variance (AMOVA). *P*-values were determined using 10 000 permutations.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	*P*-value
Among Populations	4	105.717	0.6084 Va	15.58	< 0.00001
Within Populations	203	669.149	3.2963 Vb	84.42	< 0.00001
Total	207	774.865	3.9047		
Fixation Index (F_{ST})	0.15582				

(Table 2), with each population pair having a value above 0.13. All F_{ST} values were highly significant after Bonferroni correction. We found large differences in F_{IS} values between populations (Table 3), although each population did exhibit a heterozygosity deficiency.

Table 2. Pairwise F_{ST} values for *P. hubrichti* populations. * indicates statistically significant to the < 0.05 level. Significance determined using 10 000 permutations and a sequential Bonferroni correction.

Population	1	2	3	4	5
1	0	*	*	*	*
2	0.1423	0	*	*	*
3	0.1766	0.1313	0	*	*
4	0.1351	0.1372	0.1399	0	*
5	0.1675	0.1879	0.1846	0.1301	0

Table 3. Summation of allelic information for each of the five populations of *P. hubrichti*. H_O, heterozygosity observed; n, number of individuals per population; H_E, heterozygosity expected; F_{IS}, fixation index.

Population	n	H_O(mean)	H_E(mean)	F_{IS}
1	15	0.5797	0.7342	0.2104
2	17	0.5602	0.6498	0.1378
3	34	0.5669	0.6728	0.1574
4	19	0.5996	0.6723	0.1081
5	19	0.6105	0.6958	0.1226

Estimates of recent and historical gene flow:

Recent estimates of gene flow (using BayesAss+) were highly variable between populations (Table 4). Estimates ranged from a mean of 0.002 to a mean of 0.06. We found no asymmetric gene flow (non-overlapping 95% confidence intervals) for any of the population pairs. For one population (Pop. 1) the analysis indicated that the results

were close to those being generated by uninformative data (Wilson & Rannala 2003), so we urge the use of caution when considering BayesAss+ estimates for that population. However, apart from one seemingly high estimate of migration from population 2 into population 1, the remaining estimates concerning that population are not outside the realm of normalcy nor are they biologically unfeasible. The estimates for the 3 runs of BayesAss+ did not vary statistically (t-tests; $P < 0.05$), indicating that the method produced consistent results pertaining to this data set.

Historical estimates of gene flow using MIGRATE (Beerli 2006) had less variation than recent estimates (Table 4) but still showed a fair amount of variation between population pairs. One population pair did exhibit a pattern of historically asymmetric migration; population 3 showed a much higher rate of migration into population 2 than vice versa. Overall, the migration rates from MIGRATE fall in line with what one would normally expect (i.e. neighboring populations seem to have the highest rate of migration), and none of the populations appear to be genetic sinks. Estimates of Θ across all populations were nearly identical (Table 5), with all values between 0.09 and 0.1. The Wilcoxon test indicated that the BayesAss+ estimates for recent m (migration rate) were significantly lower ($P < .001$) than historical rates from MIGRATE, with most of the values being a magnitude lower.

Table 4. Rates of migration (m) as inferred from a coalescent method (MIGRATE), and from a Bayesian assignment (BayesAss+). All rates are represented as *m*.

Populations	MIGRATE	95% Confidence Interval	BayesAss+	95% Confidence Interval
2 -> 1	0.0283	0.0108-0.0378	0.0687	0.000004-0.2963
3 -> 1	0.0310	0.0108-0.0459	0.0124	0.000003-0.0698
4 -> 1	0.0283	0.0081-0.0405	0.0122	0.000003-0.0641
5 -> 1	0.0121	0-0.0189	0.0108	0.000004-0.0632
1 -> 2	0.0148	0-0.0243	0.0063	0.000001-0.0377
3 -> 2	0.0526	0.0297-0.0756	0.0075	0.000001-0.0450
4 -> 2	0.0256	0.0054-0.0350	0.0061	0.000001-0.0358
5 -> 2	0.0256	0.0081-0.0351	0.0059	0.000001-0.0376
1 -> 3	0.0121	0-0.0189	0.0029	0.000004-0.0150
2 -> 3	0.0094	0-0.0162	0.0059	0.000051-0.0231
4 -> 3	0.0175	0-0.0243	0.0028	0.000002-0.0149
5 -> 3	0.0121	0-0.0189	0.0025	0.000001-0.0147
1 -> 4	0.0256	0.0054-0.0351	0.0053	0.000001-0.0298
2 -> 4	0.0202	0.0027-0.0270	0.0062	0.000001-0.0339
3 -> 4	0.0175	0-0.0270	0.0098	0.000003-0.0475
5 -> 4	0.0337	0.0162-0.0432	0.0054	0.000001-0.0305
1 -> 5	0.0148	0-0.0243	0.0056	0.000001-0.0324
2 -> 5	0.0175	0-0.0270	0.0054	0.000002-0.0293
3 -> 5	0.0202	0-0.0297	0.0134	0.000105-0.0489
4 -> 5	0.0202	0.0027-0.0297	0.0061	0.000003-0.0350

Table 5. Estimates of Θ and N_e for all populations of *P. hubrichti*. N_e was estimated from Θ using a well-accepted vertebrate mutation rate of 5.4×10^{-4} (Goldstein et al. 1995; Howes et al. 2009).

Population	Θ ($4N_e\mu$)	95% Confidence Interval	N_e
1	0.0972	0.0885 - 0.1	45.0231
2	0.0977	0.0905 - 0.1	45.2546
3	0.0987	0.0950 - 0.1	45.7175
4	0.0982	0.0930 - 0.1	45.4861
5	0.0982	0.0925 - 0.1	45.4861

Recent demographic events:

We found evidence for recent genetic bottlenecks in up to 3 of the 5 populations (Table 6). The IAM model indicates that 3 populations have experienced a bottleneck in the recent past (Table 6). The sensitivity analysis indicates that two of the populations lose support for a recent bottleneck as more of the SMM model is introduced into the algorithm (Table 6). However, because the IAM model is more appropriate given our data (Cornuet and Luikart 1996) we feel that there is strong support for at least two of these populations (1&4). Population 3 loses support quickly as SMM is added into the model and therefore is considered the least likely of the 3 to have experienced a recent bottleneck.

Table 6. Results of bottleneck analysis for all populations of *P. hubrichti*. All values are presented as *P*-values. Bold values indicate statistically significant.

Population	Model	% of SSM	Prob of H def	Prob of H excess
1	IAM	0	0.9877	**0.0161**
2	IAM	0	0.5771	0.4609
3	IAM	0	0.9877	**0.0161**
4	IAM	0	0.9975	**0.0034**
5	IAM	0	0.9033	0.1162
1	TPM	10	0.9580	**0.0527**
2	TPM	10	0.2783	0.7539
3	TPM	10	0.8837	0.1377
4	TPM	10	0.9965	**0.0048**
5	TPM	10	0.7841	0.2460
1	TPM	20	0.9580	**0.0527**
2	TPM	20	0.2783	0.7539
3	TPM	20	0.8388	0.1875
4	TPM	20	0.9965	**0.0048**
5	TPM	20	0.7841	0.2460
1	TPM	30	0.9472	0.0654
2	TPM	30	0.2783	0.7539
3	TPM	30	0.7841	0.2460
4	TPM	30	0.9951	**0.0068**
5	TPM	30	0.7539	0.2783
1	TPM	40	0.9472	0.0654
2	TPM	40	0.2158	0.8125
3	TPM	40	0.8623	0.1611
4	TPM	40	0.9877	**0.0161**
5	TPM	40	0.7216	0.3125

Discussion

We found significantly different estimates of migration rates when comparing historical and recent gene flow in the Red Hills salamander. Our hypothesis that levels of gene flow have been reduced in recent times was strongly supported by several lines of data (Table 4; Table 6). Furthermore, we found that reduced levels of gene flow have had a considerable impact on recent demographic parameters, including genetic bottlenecks in many populations (Table 6).

Population genetic structuring and diversity:

Results from our study demonstrate that the federally threatened Red Hills salamander exhibits a strong pattern of genetic structuring across its entire geographic range. Bayesian clustering identified five distinct and well supported populations (Fig. 2). These groupings were highly supported by an abundance of evidence, including high F_{ST} values between populations (Table 2). It is not surprising that a species with the characteristics of *P. hubrichti* would exhibit such a high degree of spatial structuring across their range. However, based on our previous knowledge of the species distribution and genetic history (Shwaner & Mount 1970; McKnight et al. 1991) the geographic nature of the population structuring was unexpected. Mitochondrial data have suggested that there were only two main lineages within the species (McKnight et al. 1991), thus our results are novel in that they identify five strongly supported populations. Previous surveys have shown that *P. hubrichti* is closely tied to three geologic formations, all of which are a "claystone" that help retain moisture and sustain burrow shape (Shwaner & Mount 1970; French & Mount 1978; Dodd 1991, Apodaca 2010). Nonetheless, our results demonstrate that the absence of these geologic formations does not necessarily translate to an absence

of gene flow (Fig. 2; Table 2; Table 4). Additionally, previous research utilizing mtDNA has suggested that large rivers may impede gene flow (McKnight et al. 1991), but our results show little support for this hypothesis (Fig.2; Table 2; Table 4). In fact, populations seem to be maintained by the continuous presence of suitable slope (Apodaca 2010), which often accompanies river drainages.

Estimates of recent and historical gene flow:

Estimates of historical rates of migration were fairly low (Table 4), but are similar to values found for other low vagility amphibians (e.g. Wang 2009). However, estimates of recent migration rates were significantly lower than historical rates (Wilcoxon matched pairs Test; $P < 0.001$). By comparing these rates (Table 4) we can infer that migration rates in the recent past have declined a great deal. There is little doubt that this decline can be attributed to anthropogenic habitat destruction and modification. In fact, other analyses corroborate strongly with this conclusion. For example, a previous fragmentation analysis indicated that *P. hubrichti* has lost between 69.5 and 86.1% of their original habitat (Apodaca 2010). It is unlikely that losing this amount of habitat would not affect a species' migration rates, especially in a naturally fragmented species with low dispersal ability.

The effects of fragmentation can also be seen through an examination of recent demographic events, which reveal that multiple populations have undergone a recent bottleneck (Table 6). This number is most likely a low end estimate since the two populations that were not identified as having undergone a recent bottleneck (pops. 2& 5; Table 6) break one of the key assumptions of bottleneck analyses, the absence of sub-population structure (Busch et al. 2007; Marshall et al. 2009). Estimates of very small

effective population sizes (N_e) for all populations (Table 5) support the evidence for recent bottlenecks. However, low effective population sizes could also result from other factors such as a strong reproductive skew or uneven sex ratios (Beebee 2005), both of which are unknown in *P. hubrichti*. Effective population sizes in this range are rare in natural populations and the other amphibians with similar N_e are either threatened species or found in populations that have experienced heavy fragmentation (e.g. Rowe & Beebee 2004; Funk et al. 1999; Wang 2009). However, it should be noted that some amphibian species that do not appear to be facing an imminent threat also exhibit similar N_e values (e.g. Funk et al. 1999; Jehle et al. 2001).

Conservation implications:

Our study has several important implications for the recovery and management of *P. hubrichti*. Protection of continuous slope habitat is seemingly the most important element in any recovery plan for this species. We provide evidence for the negative effects of anthropogenic habitat fragmentation along this slope by showing a dramatic decline in recent migration rates and the presence of bottlenecks. Clearly, the long-term survival of this species is dependent on restoring intervening habitat. The restoration of intervening habitat would help restore historical levels of gene flow and alleviate the pressures of inbreeding depression. Although it has been estimated that as much as 86.1% of the current habitat has been lost or altered (Apodaca 2010), there is no way to estimate how much of the habitat has been negatively effected by forestry practices such as clear cutting to the edge of the hardwood canopy, or chemical preparation of surrounding areas. Therefore, we urge the adoption of the habitat guidelines laid out by Dodd (1991).

The future status of *P. hubrichti* is highly dependent on the establishment of protected areas. During the nearly 35 years that *P. hubrichti* has been listed as a USFWS threatened species the only protection afforded them has been in the form of habitat conservation plans (HCPs), generally with large timber companies. Although HCPs are a viable tool for the conservation of a species, they do not always incorporate sufficient scientific data to ensure species persistence (Harding et al. 2001). In fact, most current *P. hubrichti* HCPs allow for selective harvest on any habitat with slope less than 28 degrees. This type of habitat may not contain the highest number of individuals, but it is likely vital to gene flow between populations.

Literature Cited

Apodaca JJ (2010) Prioritizing regions for the conservation of amphibians with special emphasis on the Red Hills salamander (*Phaeognathus hubrichti*) Dissertation, The University of Alabama.

Austin JD, Lougheed SC, Boag PT (2004) Controlling for the effects of history and nonequilibrium conditions in gene flow estimates in northern bullfrog (*Rana catesbeiana*) populations. Genetics 168, 1491-1506.

Avise JC (2004) Molecular markers, natural history and evolution (Second Edition) Sinauer, Sunderland, MA.

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics 22, 341-345.

Beerli P, Felsenstein J (1999) Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152, 763-773.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences, USA 98, 4563-4568.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? Molecular Ecology 13, 551-561.

Bouzat JL, Lewin HA, Paige KN (1998) The ghost of genetic diversity past: Historical DNA analysis of the greater prairie chicken. American Naturalist 152, 1-6.

Busch JD, Waser PM, DeWoody JA (2007) Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). Molecular Ecology 16, 2450-2462.

Cabe PR, Page RB, Hanlon TJ, et al. (2006) Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat. Heredity 98, 53-60.

Chen C, Durand E, Forbes F, Francois O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Molecular Ecology Notes 7, 747-756.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001-2014.

Coulon A, Guillot G, Cosson JF, et al. (2006) Genetic structure is influenced by landscape features: empirical evidence from a roe deer population. Molecular Ecology 15, 1669-1679.

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation 128, 231-240.

Dixon JD, Oli MK, Wooten MC, et al. (2006) Effectiveness of a regional corridor in connecting two Florida black bear populations. Conserv Biol 20, 155-162.

Dodd CK (1991) The Status of the Red Hills Salamander *Phaeognathus-Hubrichti*, Alabama, USA, 1976-1988. Biological Conservation 55, 57-75.

Dyer RJ, Nason JD, Garrick RC (2010) Landscape modelling of gene flow: improved power using conditional genetic distance derived from the topology of population networks. Molecular Ecology 19, 3746-3759.

Epps CW, Palsboll PJ, Wehausen JD, et al. (2005) Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. Ecology Letters 8, 1029-1038.

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

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1, 47-50.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes - application to human mitochondrial-DNA restriction data. Genetics 131, 479-491.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics Cambridge University Press, Cambridge.

Fraser DJ, Hansen MM, Ostergaard S, et al. (2007) Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. Molecular Ecology 16, 3866-3889.

French TW, Mount RH (1978) Current status of the Red Hills salamander *Phaeognathus hubrichti* Highton, and factors affecting its distribution. Journal of the Alabama Academy of Science 49, 172-179.

Funk WC, Tallmon DA, Allendorf FW (1999) Small effective population size in the long-toed salamander. Molecular Ecology 8, 1633-1640.

Geist J, Kuehn R (2005) Genetic diversity and differentiation of central European freshwater pearl mussel (*Margaritifera margaritifera L.*) populations: implications for conservation and management. Molecular Ecology 14, 425-439.

Gilpin ME, Hanski IA (1991) Metapopulation dynamics: empirical and theoretical investigations. Academic Press, London.

Goldberg CS, Waits LP (2010) Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. Molecular Ecology 19, 3650-3663.

Goldstein DB, Linares AR, Cavallisforza LL, Feldman MW (1995) Genetic absolute dating based on microsatellites and the origin of modern humans. Proceedings of the National Academy of Sciences of the United States of America 92, 6723-6727.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48, 361-372.

Harding EK, Crone EE, Elderd BD, et al. (2001) The scientific foundations of habitat conservation plans: a quantitative assessment. Conservation Biology 15, 488-500.

Harrison RG (1991) Molecular-changes at speciation. Annual Review of Ecology and Systematics 22, 281-308.

Howes BJ, Brown JW, Gibbs HL, et al. (2009) Directional gene flow patterns in disjunct populations of the black ratsnake (*Pantheropis obsoletus*) and the Blanding's turtle (*Emydoidea blandingii*). Conservation Genetics 10, 407-417.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23, 1801-1806.

Jehle R, Arntzen W, Burke T, Krupa AP, Hodl W (2001) The annual number of breeding adults and the effective population size of syntopic newts (*Triturus cristatus, T-marmoratus*). Molecular Ecology 10, 839-850.

Jordan RJ, Mount RH (1975) The status of the Red Hills salamander, *Phaeognathus hubrichti* Highton. Journal of Herpetology, 211-215.

Knowles LL, Alvarado-Serrano DF (2010) Exploring the population genetic consequences of the colonization process with spatio-temporally explicit models: insights from coupled ecological, demographic and genetic models in montane grasshoppers. Molecular Ecology 19, 3727-3745.

Lance SL, Hagen C, Glenn TC, Apodaca JJ, Rissler LJ (2009) Development and characterization of twelve polymorphic microsatellite loci in the threatened Red Hills salamander, *Phaeognathus hubrichti*. Conservation Genetics 10, 1919-1921.

Lee-Yaw JA, Davidson A, Mcrae BH, Green DM (2009) Do landscape processes predict phylogeographic patterns in the wood frog? Molecular Ecology 18, 1863-1874.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution 18, 189-197.

Marshall JCJ, Kingsbury BA, Minchella DJ (2009) Microsatellite variation, population structure, and bottlenecks in the threatened copperbelly water snake. Conservation Genetics 10, 465-476.

Matern A, Desender K, Drees C, et al. (2009) Genetic diversity and population structure of the endangered insect species *Carabus variolosus* in its western distribution range: Implications for conservation. Conservation Genetics 10, 391-405.

Mcknight ML, Dodd CK, Spolsky CM (1991) Protein and mitochondrial-DNA variation in the salamander *Phaeognathus Hubrichti*. Herpetologica 47, 440-447.

Morgan MJ, Hunter D, Pietsch R, Osborne W, Keogh JS (2008) Assessment of genetic diversity in the critically endangered Australian corroboree frogs, *Pseudophryne corroboree* and *Pseudophryne pengilleyi*, identifies four evolutionarily significant units for conservation. Molecular Ecology 17, 3448-3463.

Murphy MA, Evans JS, Cushman SA, Storfer A, (2008) Representing genetic variation as continuous surfaces: an approach for identifying spatial dependency in landscape genetic studies. . Ecography 31, 685-697.

Nei M (1987) Molecular Evolutionary Genetics Columbia University Press New York, USA.

Pabijan M, Babik W, Rafinski J (2005) Conservation units in north-eastern populations of the Alpine newt (*Triturus alpestris*). Conservation Genetics 6, 307-312.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population-structure in Canadian polar bears. Molecular Ecology 4, 347-354.

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90, 502-503.

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

Proctor MF, McLellan BN, Strobeck C, Barclay RMR (2005) Genetic analysis reveals demographic fragmentation of grizzly bears yielding vulnerably small populations. Proceedings of the Royal Society B-Biological Sciences 272, 2409-2416.

Reed DH, Teoh V-H, Stratton GE, Hataway RA (2009) Levels of gene flow among populations of wolf spider in a recently fragmented habitat: current versus historical rates. Conservation Genetics In Press.

Rowe G, Beebee TJC (2004) Reconciling genetic and demographic estimators of effective population size in the anuran amphibian *Bufo calamita*. Conservation Genetics 5, 287-298.

Rowe G, Beebee TJC, Burke T (1998) Phylogeography of the natterjack toad *Bufo calamita* in Britain: genetic differentiation of native and translocated populations. Molecular Ecology 7, 751-760.

Schwaner TD, Mount RH (1970) Notes on the distribution, habits, and ecology of the salamander *Phaeognathus hubrichti* Highton. Copeia 1970, 571-573.

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22, 25-33.

Shirk AJ, Wallin DO, Cushman SA, Rice CG, Warheit KI (2010) Inferring landscape effects on gene flow: a new model selection framework. Molecular Ecology 19, 3603-3619.

Sokal RR, Rohlf FJ (2003) Biometry 3rd edn. W.H. Freeman and Company New York, USA.

Storfer A, Murphy MA, Evans JS, et al. (2007) Putting the "landscape" in landscape genetics. Heredity 98, 128-142.

Straub SC, Doyle JJ (2009) Conservation genetics of *Amorpha georgiana* (Fabaceae), an endangered legume of the Southeastern United States. Molecular Ecology 18, 4349-4365.

Swindell WR, Bouzat JL (2005) Modeling the adaptive potential of isolated populations: Experimental simulations using Drosophila. Evolution 59, 2159-2169.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4, 535-538.

Vandergast AG, Bohonak AJ, Weissman DB, Fisher RN (2007) Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera : Stenopelmatidae : Stenopelmatus). Molecular Ecology 16, 977-992.

Wang IJ (2009) Fine-scale population structure in a desert amphibian: landscape genetics of the black toad (*Bufo exsul*). Molecular Ecology 18, 3847-3856.

Wang IJ, Summers K (2010) Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. Molecular Ecology 19, 447-458.

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

Zellmer AJ, Knowles LL (2009) Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. Molecular Ecology 18, 3593-3602.

II. Evidence of sex-biased dispersal in the Red Hills salamander

Abstract

Sex-biased dispersal has received a great deal of attention in ecology and evolutionary biology. Yet our knowledge on this subject is mostly based on data gathered from birds and mammals. Despite the increased focus on dispersal patterns of imperiled species, little attention has been paid to how sex-biased dispersal can affect rates of gene flow or migration patterns. Understanding these characteristics in threatened and endangered species is critically important to long-term recovery efforts. In this study we investigate whether the federally threatened Red Hills salamander (*Phaeognathus hubrichti*) exhibits a sex bias in dispersal using 10 microsatellite loci. We tested whether there was a significant difference in F_{IS} values between sexes and we calculated the assignment index (*AIc*) along with the variance in the assignment index (*vAIc*) for each sex. We found that there is a significant pattern of male biased dispersal for this species. Along with other life history data, this finding suggests that *P. hubrichti* likely operates under a breeding system similar to female defense polygyny. Consequently, the breeding system of the Red Hills salamander coupled with anthropogenic habitat alteration could help explain the high levels of inbreeding exhibited by this species.

Introduction

The study of population connectivity in fragmented landscapes is an important topic in conservation genetics (e.g. Shaffer et al. 2000; Funk et al. 2005; Cabe et al. 2006; Wang 2009; Zellmer and Knowles 2009). One of the fundamental determinants of genetic structure across populations is the level and geographic extent of individual migration between populations (Clobert et al. 2001). Dispersal ability is thus a key life-history trait affecting population structure and the long-term evolutionary history of a species (Clobert et al. 2001; Palo et al. 2004). Gene flow between populations can be especially important for small populations, subject to genetic drift and the perils of inbreeding depression (Frankham 2002). Therefore, understanding the dispersal characteristics of threatened and endangered species is critically important to long-term recovery. These data can help identify appropriate management units, determine the severity of threat to any single population, help identify corridors, and determine which populations (or subpopulations) should be protected as distinct units (Shaffer et al. 2000).

Sex-biased dispersal has received a great deal of attention in evolutionary ecology; yet understanding differences in dispersal patterns between the sexes remains a major topic in evolution and ecology (Palo et al. 2004). The majority of our knowledge on sex-biased dispersal comes from studies of mammals and birds. Numerous studies have demonstrated that mammals tend to have male biased dispersal (Reviewed in Handley and Perrin 2007), and birds tend to have female biased dispersal (Shorey 2002). The difference in bird and mammal sex-biased dispersal is thought to be reflective of the unique mating systems of the groups (Greenwood 1980). In the thirty years that have passed since Greenwood's landmark paper we have learned that although the relationship

between sex-biased dispersal and mating system is complex, his basic hypothesis tends to hold (Handley and Perrin 2007). However, information on the extent and direction of sex-biased dispersal in other groups is quite limited (for a brief review see Austin et al. 2003).

Amphibians present a particularly interesting and pressing study system for sex-biased dispersal. Knowledge on dispersal patterns of amphibians is particularly urgent due to the rate at which amphibians are declining (Stuart et al. 2004). Genetic research on amphibian sex-biased dispersal has been limited to a handful of studies, focused almost exclusively on anurans (Austin et al. 2003; Lampert et al. 2003; Monsen and Blouin 2003; Palo et al. 2004; Vietes et al. 2006; Knopp and Merilä 2009; but see: Cabe et al. 2006). These studies have failed to find a consistent pattern of either sex dispersing more frequently. This is not surprising since amphibians have a wider array of reproductive strategies than any other vertebrate class (Halliday and Adler 1986). One would expect that most pond breeding amphibians would exhibit female biased dispersal, since their mating systems are similar to resource-defense polygyny (a common breeding system in birds) and that terrestrial direct developers would be more akin to female-defense polygyny (a common mammal breeding system) with a male dispersal skew. This life-history complexity make amphibians a system that invites much more research on sex-biased dispersal.

In this study we aim to test whether there is a sex-bias in the dispersal patterns of the federally threatened (IUCN: Endangered) Red Hills salamander (*Phaeognathus hubrichti*). It is imperative to the long-term survival of the species that we gather and integrate as much data into recovery plans as possible. Gaining information on the

dispersal habits of the species will enlighten our view on population connectivity and allow us to make informed predictions on their mating patterns, both of which we know very little about.

Methods

We collected 105 individuals from 21 unique localities across the entire range of *P. hubrichti* following the protocols in U.S. Fish and Wildlife Threatened Species Permit TE136961-0. However, we could only definitively sex 92 individuals, thus we included 45 female and 47 male samples in all analyses. All samples were collected from 2007-2009 and no single location was surveyed more than once, therefore eliminating the chance of a recapture. Tissue was immediately preserved in 95% ethanol and transferred to a -80°C freezer at the University of Alabama Herpetology Collection.

To extract DNA from the tissue we used Qiagen DNeasy tissue kit and protocol (Qiagen Inc, Valencia, CA). We amplified 10 microsatellite markers using polymerase chain reaction (PCR). Each primer was developed specifically for use in *P. hubrichti* (Lance et al. 2009). Information on each primer as well as PCR conditions can be found in Lance et al. (2009). Each locus was amplified individually, and labeled PCR products were run on an ABI 3730 Genetic Analyzer. Samples were genotyped using GeneMapper 3.7 software (Applied Biosystems, Inc.). Scoring and quality control of data were done using GeneMarker V. 1.7 (Softgenetics, LLC). In order to assure repeatability, we re-genotyped 10 individuals per locus. This test resulted in a repeatability success rate of 98%. We used the populations of Apodaca (2010) for all analyses. All other analyses were run using FSTAT v. 2.9.3 (Goudet 2001).

To test for a bias in sex dispersal we compared the F_{IS} values for both males and females. Additionally we calculated the assignment values for individuals (*AIc*) and the variance in *AIc (vAIc).* Statistical significance for each test was assessed using the randomization method of Goudet, Perrin, and Waser (2002), with 10 000 randomizations. In this method, each individual is randomly assigned as either a male or female, while respecting the empirical sex ratio. Random samples are then generated under the null hypothesis of no bias in dispersal between the sexes. The mean *AIc* determines the probability that a particular genotype should appear in its sampled population (Goudet et al. 2002). Mean *AIc* values are distributed around a mean of zero, with positive values indicating that an individual is likely philopatric, and negative values indicating that an individual is more likely than average to be a recent immigrant. Because the sex that disperses more frequently is likely to have more immigrants and resident individuals in a population, the variance of the mean *AIc (vAIc*) is more likely to be higher (Goudet et al. 2002).

Results and discussion

Our results revealed a significant male bias in the dispersal of *P. hubrichti* (Table 1). This result was strongly supported by the mean assignment (*mAIc*) and the variance assignment (*vAIc*) tests (Table 1), but not by a difference in F_{IS} values between sexes. However, because populations of *P. hubrichti* have experienced recent bottlenecks and a large reduction in migration rates due to anthropogenic habitat modification (Apodaca 2010). we found a large range-wide F_{IS} (0.1330). The high range-wide F_{IS} value indicates that there is a large amount of inbreeding throughout the range of *P. hubrichti*.

Consequently, all individuals, regardless of sex, exhibit a high inbreeding coefficient, thus rendering a comparison of F_{IS} values ineffective.

Table 1. Inbreeding coefficient (F_{IS}), mean assignment (*mAIc*), and variance assignment (*vAIc*) values for males and females of *P. hubrichti*. Significance was determined using 10 000 randomizations and the randomization method of Goudet et al. (2002).

	N	F_{IS}	*mAIc*	*vAIc*
Males	47	0.1319	-0.39799	17.90466
Females	45	0.1437	0.41568	6.91068
P		0.479	0.021	0.016

Due to the secretive nature of *P. hubrichti* we know very little about their mating system. Yet we can make some inferences based on familial relationships and the data we present here. We suggest that *P. hubrichti* most likely operates under a breeding system similar to female defense polygyny (as is common in mammals). In this mating system females will cluster, typically around a resource, and males will compete against each other for the right to mate with individuals within the cluster (Alcock 2001). In this case females are most likely not clumping together as a social system, such as we would see in mammals, but rather clumping around the resource of possible burrow localities. Ideal soil conditions for burrow sites are limited, even in good habitat, creating a resource for females to cluster around, which is what we find in natural populations (Dodd 1991; Apodaca 2010). Additionally, it has been documented that males are much more heavily scarred than females (Bakkegard and Guyer 2004), suggesting they enter into intense male-male combat. Often, when plethodontid males defend a specific territory (such as in resource defense polygyny), conflicts are resolved via methods such as pheromonal markers and threat displays before the escalation leads to injury (Jaeger 1984). Thus, if

male Red Hills Salamanders were involved in a resource defense polygyny type system we would not expect to see such heavy scarring on males.

From a conservation perspective our findings may help to explain high levels of inbreeding. Dodd (1991) found that in undisturbed habitat *P. hubrichti* burrows are concentrated on the upper two-thirds of the inhabited slope. However, when ridge top habitat is either selectively or clear-cut there is a statistically significant change in the locations where burrows are found, with a tendency for burrows to shift towards the middle of the slope (Dodd 1991). This shift effectively concentrates burrows and may allow a single male to control a larger harem. This pattern combined with lower dispersal rates (Apodaca 2010) could have a detrimental effect on the long-term outlook for the species.

Our finding of male biased dispersal in a plethodontid salamander is unique. In fact, the only other study to use molecular markers to investigate such questions in a plethodontid (Cabe et al. 2006) found no evidence of a distinction between the sexes. In general there have been inconsistent results of sex-biased dispersal concerning amphibians in the seven previous studies that have investigated this issue (see Knopp and Merilä 2009). With such a low sample size across such a varied set of taxa it is impossible, at this stage, to draw broad conclusions concerning the evolution of sex-biased dispersal across amphibians. However, this is an area of study that begs to be explored more thoroughly in the future.

Literature Cited

Alcock J (2001) Animal Behavior (Seventh Edition) SinauerAssociates, INC, Sunderland.

Apodaca JJ (2010) Prioritizing regions for the conservation of amphibians with special emphasis on the Red Hills salamander (Phaeognathus hubrichti) Dissertation, The University of Alabama

Austin JD, Davila JA, Lougheed SC, Boag PT (2003) Genetic evidence for female-biased dispersal in the bullfrog, Rana catesbeiana (Ranidae). Molecular Ecology 12, 3165-3172.

Bakkegard KA (2002) Activity patterns of Red Hills salamanders (Phaeognathus hubrichti) at their burrow entrances. Copeia, 851-856.

Bakkegard KA, Guyer C (2004) Sexual size dimorphism in the Red Hills Salamander, Phaeognathus hubrichti (Caudata : Plethodontidae : Desmognathinae). Journal of Herpetology 38, 8-15.

Cabe PR, Page RB, Hanlon TJ, et al. (2006) Fine-scale population differentiation and gene flow in a terrestrial salamander (Plethodon cinereus) living in continuous habitat. Heredity 98, 53-60.

Clobert J, Danchin E, Dhondt A, Nichols J (2001) Dispersal Oxford University Press, Oxford

Dodd CK (1991) The Status of the Red Hills Salamander Phaeognathus-Hubrichti, Alabama, USA, 1976-1988. Biological Conservation 55, 57-75.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to conservation genetics Cambridge University Press, Cambridge.

Funk WC, Greene AE, Corn PS, Allendorf FW (2005) High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. Biol Lett 1, 13-16.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). Available from http://www.unil.ch/izea/software/fstat.html.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Molecular Ecology 11, 1103-1114.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28, 1140-1162.

Halliday T, Adler K (1986) The encyclopedia of reptiles and amphibians Andromeda Oxford ltd, New York.

Handley LJL, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. Molecular Ecology 16, 1559-1578.

Jaeger RG (1984) Agonistic behavior of the Red-backed salamander. Copeia 1984, 309-314.

Knopp T, Merila J (2009) Microsatellite variation and population structure of the moor frog (Rana arvalis) in Scandinavia. Molecular Ecology 18, 2996-3005.

Lampert KP, Rand AS, Mueller UG, Ryan MJ (2003) Fine-scale genetic pattern and evidence for sex-biased dispersal in the tungara frog, Physalaemus pustulosus. Molecular Ecology 12, 3325-3334.

Lance SL, Hagen C, Glenn TC, Apodaca JJ, Rissler LJ (2009) Development and characterization of twelve polymorphic microsatellite loci in the threatened Red Hills salamander, Phaeognathus hubrichti. Conservation Genetics 10, 1919-1921.

Means DB (2003) Notes on the reproductive biology of the Alabama Red Hills salamander (Phaeognathus hubrichti). Contemporary Herpetology 2003, 1-5.

Monsen KJ, Blouin MS (2003) Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. Molecular Ecology 12, 3275-3286.

Palo JU, Lesbarreres D, Schmeller DS, Primmer CR, Merila J (2004) Microsatellite marker data suggest sex-biased dispersal in the common frog Rana temporaria. Molecular Ecology 13, 2865-2869.

Perrin N, Mazalov V (2000) Local competition, inbreeding, and the evolution of sex-biased dispersal. American Naturalist 155, 116-127.

Schwaner TD, Mount RH (1970) Notes on the distribution, habits, and ecology of the salamander Phaeognathus hubrichti Highton. Copeia 1970, 571-573.

Shaffer G, Fellers GM, Magee A, Voss R (2000) The genetics of amphibian declines: population substructure and molecular differentiation in the Yosemite Toad, Bufo canorus (Anura, Bufonidae) based on single-strand conformation polymorphism analysis (SSCP) and mitochondrial DNA sequence data. Molecular Ecology 9, 245-257.

Shorey L (2002) Mating success on white-bearded manakin (Manacus manacus) leks: male characteristics and relatedness. Behavioral Ecology and Sociobiology 52, 451-457.

Stuart SN, Chanson JS, Cox NA, et al. (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306, 1783-1786.

Vieites DR, Chiari Y, Vences M, et al. (2006) Mitochondrial evidence for distinct phylogeographic units in the endangered Malagasy poison frog Mantella bernhardi. Molecular Ecology 15, 1617-1625.

Wang IJ (2009) Fine-scale population structure in a desert amphibian: landscape genetics of the black toad (Bufo exsul). Molecular Ecology 18, 3847-3856.

Zellmer AJ, Knowles LL (2009) Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. Molecular Ecology 18, 3593-3602.

Recommendations for the recovery of the Red Hills salamander

Abstract

Despite being federally listed for well over three decades *Phaeognathus hubrichti* continues to decline. Past conservation efforts have focused on the use of habitat conservation plans with large landowners. However, this method is focused on mitigating further habitat losses rather than promoting the recovery of the species. The goal of this section is to devise effective measures that will lead to the stabilization and eventual recovery of the Red Hills salamander. We base all of our recommendations on data pertaining to the species' habitat requirements, climatic suitability, available habitat, habitat fragmentation, gene flow patterns, genetic structure and genetic variability. We recommend the acquisition and proper management of twenty-one new conservation areas for the species. Additionally, we urge that large landowners with populations of *P. hubrichti* enter into safe harbor agreements, which we have provided guidelines for.

Introduction

The year of 2010 marks the 50-year anniversary of the discovery of the Red Hills salamander (*Phaeognathus hubrichti)* by Leslie Hubricht. Since the description of *P. hubrichti* (Highton, 1961) there has been a great deal of research on this species. Yet, there still remains a large gap in basic knowledge about the salamander due to its secretive and fossorial nature. This lack of data has hampered conservation efforts for *P. hubrichti* despite being federally listed as a threatened species since 1976. The original federal listing came in response to the concerns of local herpetologists based on the impact that forestry practices were having on the few known populations at the time (Schwaner, 1970: Jordan and Mount, 1975; Mount 1975). Since the listing it has become abundantly clear that timber practices have a strong negative effect on the persistence of *P. hubrichti* (Dodd, 1991; Godwin, 2008). Unfortunately, nearly the entirety of the range of *P. hubrichti* is owned by large timber conglomerates or managed for timber production (Bailey and Means, 2004).

The federal listing of *P. hubrichti* provided some degree of protection for the salamander through the employment of habitat conservation plans (HCPs) with large landholders. However, HCPs do not offer a solid long-term option for the conservation of a species, as oftentimes they are based on limited data (Harding et al., 2001). Additionally, HCPs do not transfer with landownership when a piece of property changes possession (Bonnie, 1999; Harding et al., 2001; Godwin, 2008). Thereby exposing imperiled species to the threats of landscape alteration and fragmentation immediately following a land transaction.

The HCPs for *P. hubricthi* are particularly inadequate as the majority of them focus solely on preserving small patches of high quality habitat, while ignoring how this habitat is impacted by forestry practices on surrounding habitat or the connectivity between patches (Table 1). These highly fragmented patches are extremely vulnerable to the loss of genetic variability and extinction due to inbreeding and the susceptibility to demographic, environmental, and genetic stochasticity (Conner and Hartl, 2004; Frankham et al., 2007; Apodaca 2010). Currently it is believed that there are eight HCPs in place with large timber companies (J. Smithem pers. comm.). These HCPs represent roughly 37% of the entire range of *P. hubrichti*. However, there have been a large number of timber transactions within the last 5 years in the Red Hills, possibly nullifying the benefits of these HCPs (E. Soehren pers. comm.).

Table 1. An example of the typical habitat management guidelines for a habitat conservation plan designed to minimize and mitigate habitat losses for the Red Hills salamander (*Phaeognathus hubrichti)*.

HABITAT CLASSIFICATION
Potentially occupied salamander habitat can be classified into three categories, Class A (optimal), Class B (moderately suitable), and Class C (marginal).
CLASS A (or Optimal) habitats are bluffs and ravines with 27-degree angle slope or greater, or other extensive areas of steep slopes that are underlain by the Tallahatta formation, and are dominated by deciduous trees. No timber harvest will be permitted in optimal habitats.
CLASS B (or Moderately Suitable) habitats are areas of 18 to 27-degree slope within either the Tallahatta or Hatchetigbee formations, and with naturally occurring mixed hardwood/pine and pine/hardwood forest types. Siltstone outcroppings may or may not be evident. These habitats may receive increased levels of selective cutting (followed by natural regeneration of tree species characteristic of Red Hills salamander habitat), provided total hardwood canopy cover is not reduced to less than 65 percent.
CLASS C (or Marginally Suitable) to unsuitable habitats (<18-degree slope) within the Tallahatta or Hatchetigbee formations occur immediately adjacent to optimal or moderately suitable habitats with naturally occurring mixed hardwood/pine or pine hardwood forest types; siltstone may or may not be evident. Options on these areas will include normal silvicultural practices, such as clearcutting, select tree harvest, chemical and mechanical site preparation, planting, and prescribed burning. Clearcut areas may be planted with pine or hardwood seedlings. Site preparation methods vary depending on the site but usually include a combination of herbicides and fire. Although rotation lengths may change in the future due to economic and/or biological considerations, plantations are currently managed on a pulpwood/sawtimber rotation averaging 20-35 years.

For *P. hubrichti,* current management via HCPs will only lead to increasingly fragmented populations with a greater degree of edge effects in remaining habitat. Edge effects can be detrimental to amphibians as edges are typically warmer, have a lower relative humidity, are more susceptible to frequent and longer frosts, have increased soil temperature, decreased soil moisture, and decreased amounts of leaf litter (Klein 1989; Parker 1989). A survey twelve years after the initial listing of *P. hubrichti* found that edge effects had a major impact on the distribution of *P. hubrichti* burrows on inhabited slopes and that there had been a large number of heavily impacted sites despite HCPs (Dodd, 1991). This trend has continued as numerous sites surveyed for this study were highly degraded due to forestry practices. Undoubtedly, we must seek out more effective conservation measures if we wish to ensure the long-term persistence of *P. hubrichti.*

The acquisition of protected areas for conservation purposes is one of the most effective methods for assuring the persistence of a species (Soulé, 1991; Balmford et al., 1996; Redford and Richter, 1999; Rodrigues and Gaston, 2002). However, identifying proper land tracts for attainment is not a straightforward undertaking. There are several factors that should be considered when pursuing tracts of land for the conservation of a species, including but not limited to: habitat feasibility, climatic suitability, population connectivity, population viability, population size, acquisition cost, genetic distinctiveness, and genetic diversity. For *P. hubrichti* the attainment of such data is complicated by the fact that they spend the majority of their time underground within

their burrow (Bakkegard, 2002). Therefore, it is necessary to depend on indirect methods, such as genetic techniques and GIS approaches, to estimate these data.

Here we aim to apply several sources of data in order to make both broad and specific recommendations for the purchase and management of protected areas for *P. hubrichti* and for the improvement of privately managed lands. We base these recommendations on habitat requirements, climatic suitability, available habitat, habitat fragmentation, gene flow patterns, genetic structure and genetic variability. We hope that this data can be applied to a large-scale habitat purchasing guideline for the recovery of *P. hubrichti*. Additionally, we use a GIS habitat modeling approach in order to identify areas that may harbor unknown populations of *P. hubrichti.* Identifying unknown populations is fundamental to the conservation of threatened and endangered species. We test the validity of this model by surveying possible localities within Wilcox County.

Methods

Species data

Phaeognathus hubrichti is a monotypic species that diverged from its nearest living relative over 35 million years ago (Highton, 1961, Vieites et al. 2007, Kozak et al. 2009). They are restricted to a narrow geologic band that runs across Alabama known as the Red Hills. Within the Red Hills they are thought to be restricted to the Tallahatta and Hatchetigbee formations (Dodd, 1991), though a more recent discovery (Bailey and Miller, 2006) indicates they may also be found in the Nanafalia. These formations are Eocene in age and consist of claystone, siltstone and sandstone (Scott, 1972). It is likely that these geologic layers provide a substrate that allows *P. hubrichti* burrows to be easily

created and persist for months (Jordan, 1975; Gunzburger and Guyer, 1998). *Phaeognathus hubrichti* has several habitat requirements in addition to their geologic restrictions.

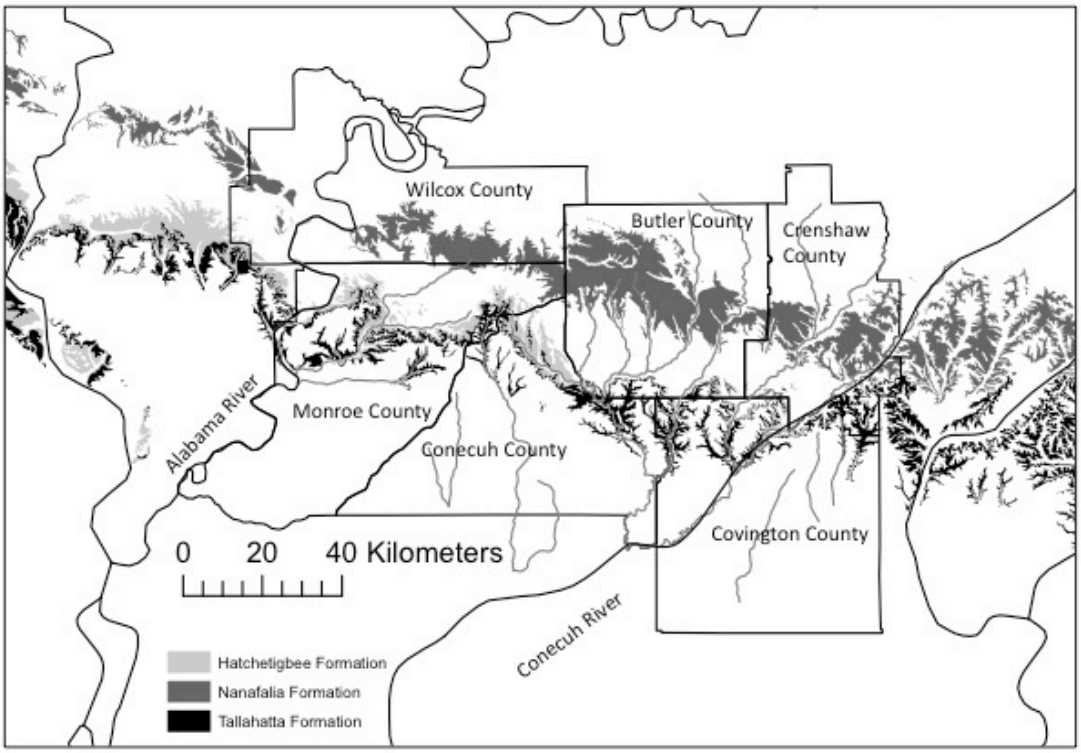

Fig. 1. Outline of the three geologic layers known to contain populations of the Red Hills salamander (*Phaeognathus hubrichti*).

Phaeognathus hubrichti are generally found on mature mesic slopes in the ravines of the Red Hills (Jordan, 1975). They are most common on steep (> 30°) north-facing slope, with a full canopy of mature hardwood trees (Dodd, 1991). However, individual burrows are not uncommon in small isolated patches of microclimate, such as isolated ledges or at the base of large trees (Dodd, 1991; Pers. Obs.). A full hardwood canopy allows a greater degree of moisture retention and a vast array of invertebrate prey (Dodd,

1991). These hardwood ravines were historically surrounded by mature longleaf (*Pinus palustris)*(or possibly a longleaf shortleaf (*Pinus echinata)* mixture) pine forests (Harper, 1920; Mohr, 1901). Though, longleaf may not have been completely dominant across the entire range (Mohr, 1901). The clear cutting of these ridge tops followed by their replacement with pine plantations has had detrimental effects on *P. hubrichti*.

Timber harvest on the ridge tops increases the edge effects on the mesic slopes. This type of disturbance causes *P. hubrichti* to no longer use burrows on the top third of the slope, thereby effectively shrinking the amount of available habitat (Dodd, 1991). Furthermore, it is likely that shifting the ridge tops from a mature open canopy system (as in longleaf systems) to a densely packed canopy of growing pines drastically alters the hydrology of the system (Godwin, 2008). This can occur through two mechanisms. The first mechanism being that a dense canopy causes rainfall interception, and thus a loss of water through direct evaporation (Godwin, 2008). The second mechanism is that vigorously growing plantation trees will remove a great deal more water from the soil than a mature forest (Vertessy, 2001). This problem is exacerbated by the fact that a natural longleaf system would consist of widely spaced trees and an intervening grassland habitat. Godwin (2008) hypothesized that this reduction of water on the ridge tops leads to a reduction in moisture levels in the underlying Tallahatta, Hatchetigbee, and Nanafalia formations, and may lead to physiological stress, impaired reproduction, unsuccessful egg development, increased mortality, and a decrease in prey availability. In addition to a restricted habitat, *P. hubrichti* also has a highly specialized life history. They have evolved many adaptations to life within a burrow, including: elongated body,

numerous vertebrae, solidly constructed skull, small nostrils, modified eyelids, small limbs, absence of lateral line organs, and direct development within the burrow (Highton, 1961; Jordan, 1975; Means, 2003). A specialized life history is one of the strongest predictors of extinction risk (Hunter and Gibbs, 2007).

In 2006 Bailey and Miller discovered a new population of *P. hubrichti* in Wilcox County, Alabama, which was not known to harbor any populations. Up until this discovery, it was believed that *P. hubrichti* only inhabited the Tallahatta and Hatchetigbee formations within the Red Hills (Dodd, 1991). Significantly, the newly discovered Wilcox County population was found to be in an entirely different geologic formation, the Nanafalia. This discovery immediately raised the question of how many additional, and currently unprotected, populations are found outside of the perceived range.

Habitat modeling

In order to identify suitable areas for *P. hubrichti* we divided the range of *P. hubrichti* and the surrounding area into grid cells of 0.005°. The total size of the model was 215 km x 170 km, allowing for the inclusion of the known six counties that contain *P. hubrichti* and a 20 km buffer. Each cell was then assigned a value of suitability based on the addition of three variables: 1) slope, 2) proper habitat type, and 3) proper geologic layer. We then eliminated cells that were outside of the climatic envelope created using environmental niche modeling.

Slope was calculated using the national elevational dataset (NED) available from the USGS. NED is available at 1/9 arc second (about 3 meters) resolution. Slope was calculated using the ARCGIS v9.3 spatial analyst extension. This slope calculation finds the maximum rate of change between each cell and its neighbors. Therefore, the maximum change in elevation between neighboring cells receives the highest value, and the lower the slope value the flatter the terrain. Since *P. hubrichti* are most commonly found on the steep slopes, this technique allows us to identify suitable tracts of slope. For the habitat suitability analysis, each cell was grouped into one of six groups using the Jenks optimization method (Jenks, 1967). This method is also known as the goodness of variance fit, and is analogous to a one-way analysis of variance. Essentially the Jenks method seeks to maximize variance between natural breaks in the data by minimizing the squared deviations of the class means. Each category was then assigned a value, with the lowest slope values receiving a low number and vice versa.

We used the AL-GAP dataset (Kleiner et al. 2007) to identify habitat suitable for *P. hubrichti*. By using recently collected *P. hubrichti* points and life history knowledge of the species we determined that category 51 (East Gulf Coastal Plain Southern Mesic Slope Forest) was the only category that properly represented *P. hubrichti* habitat. This category was assigned a value of 1 and all others a 0. Though it is likely that there are small patches of habitat not identified by this method they are probably too small or infrequent to harbor a viable population. For the geologic layers we used the Alabama geologic map made available by the USGS. From this data set we selected the three geologic layers required by *P. hubrichti* (Tallahatta, Hatchetigbee, and Nanafalia).

We used ecological niche modeling (ENM) in order to identify areas that are climatically similar to known *P. hubrichti* populations. We chose to use Maxent (Phillips et al. 2006) because it has been shown to be one of the most reliable ENM methods (Elith et al., 2006; Pearson et al., 2007). In general, Maxent uses a machine learning approach (maximum entropy) to predict the probability of occurrence of a species given an equal effort of sampling across the locality data. Maximum entropy modeling is part of a family of statistical approaches (machine learning) that typically outperforms traditional statistical approaches (e.g. generalized linear models) in complex ecological situations (Olden et al., 2008). We used a least point threshold (LPT) in order to determine the Maxent value that would serve as a cutoff point for what we would consider as suitable climatic conditions. All though there are several threshold methods available for niche modeling (see Pearson et al. 2007), the LPT is one of the most conservative and has the biological reality that the model is identifying area at least suitable as areas where the species has been reported. All cells with values above the threshold were deemed as climatically suitable, and all of those below were categorized as unsuitable.

To evaluate the accuracy of our distributional model we tested for a statistically significant difference between the values of the cells that contain known *P. hubrichti* localities and an equal number of randomly selected cells using an unpaired two-tailed t-test. We used all known locality data from the Alabama Natural Heritage Program (ALNHP), which included 130 unique localities. We then generated two sets of randomly selected cells, one set was randomly selected from the six counties known to contain *P. hubrichti* and another set that was selected just from the area within the six known

counties that contained the proper geologic layers. This process allowed us to evaluate if our modeling technique identified *P. hubrichti* habitat significantly better than random.

We then used the model as a guide to identify areas within Wilcox County that may contain populations of *P. hubrichti*. In total we surveyed 16 localities predicted to be in high quality habitat by the model (categories 4&5), four localities predicted to be in suitable habitat (categories 3&4), and four localities where the model did not predict presence (Categories 1&2) but where topography was suitable for *P. hubrichti*. Surveys were conducted by extensively searching slope habitat for the characteristic burrows of *P. hubrichti*. Following Dodd (1991), only sites that had unmistakable burrows or where salamanders were observed were considered to have active populations of *P. hubrichti*.

Genetic data

We incorporated the genetic data of Apodaca (2010) into our recommendations for habitat purchasing. These data indicate that there are 5 major populations throughout the range of *P. hubrichti* (Fig. 2). Additionally, these populations have very little gene flow between each other and all display a high amount of inbreeding. Therefore, any conservation efforts for the species should consider each of these populations as essentially an independent management unit.

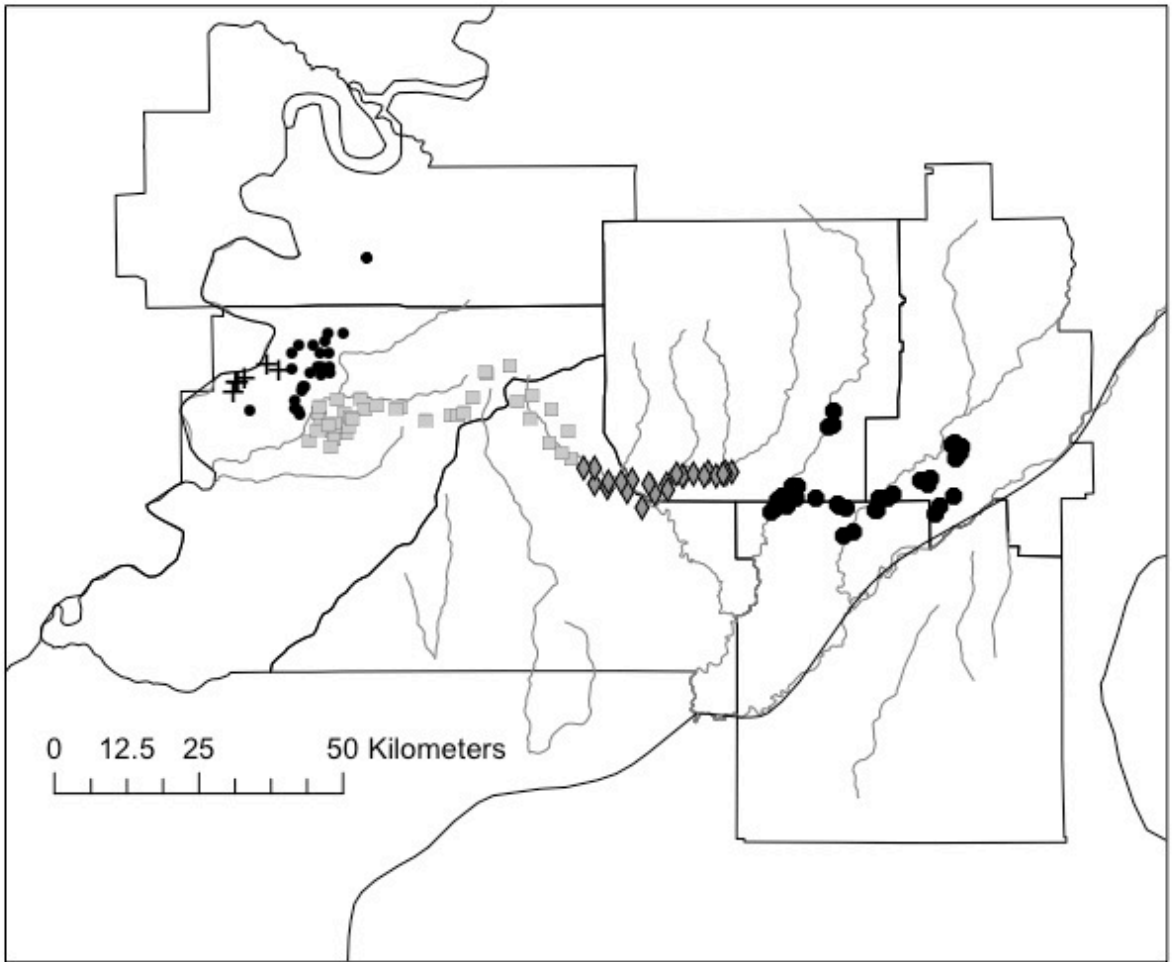

Fig. 2. Known localities of the Red Hills salamander grouped into their respective populations as determined by genetic data. Populations are from left to right: population 1- black crosses, Population 2- black circles, Population 3- grey squares, Population 4- dark-grey diamonds, Population 5- black octagons.

Results

Our model included a total of 173,056 cells (Fig. 3). The highest two categories, representing the most suitable *P. hubricthti* habitat, contained 196 (0.11%) and 745 (0.43%) cells, respectively. The next two highest categories, which still represent high quality habitat, contained 1925 (1.11%) and 3690 (2.13%) cells. The next to last category, which contains habitat that is still viable but not ideal, contained 10856 cells (6.27%). The lowest category, representing unsuitable habitat, contained the vast majority

of cells (155,644, 89.9%). The ENM indicated that all of the areas within our habitat model were climatically similar and therefore we do not feel that climate limits the distribution of *P. hubrichti*.

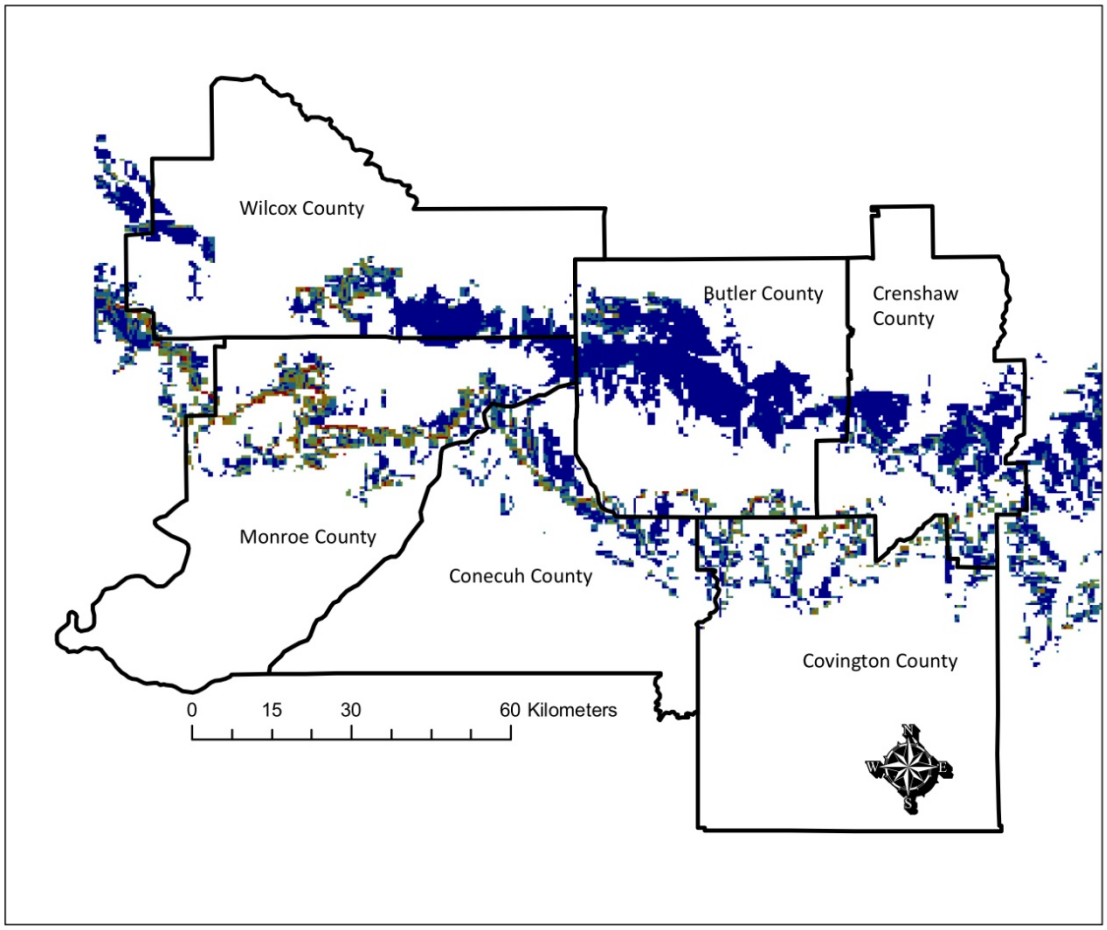

Fig. 3. Distribution model for the Red Hills salamander (*Phaeognathus hubrichti*). Warmer colors (reds, oranges, etc) represent a greater possibility of suitable habitat.

Statistical analysis indicated that our model did significantly better than random when identifying suitable habitat for *P. hubrichti*. Known localities of *P. hubrichti* were found in cells had significantly higher model values than locations drawn from random from the six counties that contain *P. hubrichti* ($P < 0.0001$). Additionally, known localities of *P. hubrichti* were found in cells that also had significantly higher model values than

locations drawn from random from within the three geologic layers known to contain *P. hubrichti (P* = 0.0026).

The survey results (Fig. 4) revealed several previously undiscovered populations within Wilcox County, Alabama. In total we discovered 14 previously unknown populations. Our model appears to be effective at identifying areas that may harbor unknown populations or *P. hubrichti*. However, more surveys would need to be completed in order to test this statistically. In total we found new populations in 13 out of 16 surveyed localities for the highest categories (4&5), 1 out of 4 in categories 2 &3, and no new populations in categories 0 or 1. The results of this survey indicate that current range maps for *P. hubrichti* do not accurately reflect the extent of occurrence for the species; thus, we present an updated range map (fig. 5).

We have identified 14 sites that we feel have a good possibility of containing unknown populations of *P. hubrichti* (Fig 6). We have based these predictions on the combination of our habitat model and our ENM. ENMs have been successfully employed for the discovery of unknown populations or closely related species in other settings (Raxworthy et al., 2003; Pearson et. al., 2007). We placed each predicted sites in one of three categories based on how likely we felt they were to contain unknown populations (Table 2). The categories were based on the following features: 1.) Amount of available habitat, 2.) Proximity to known populations, and 3.) whether the area is separated from other populations by a major barrier (i.e. Alabama or Conecuh Rivers, etc.).

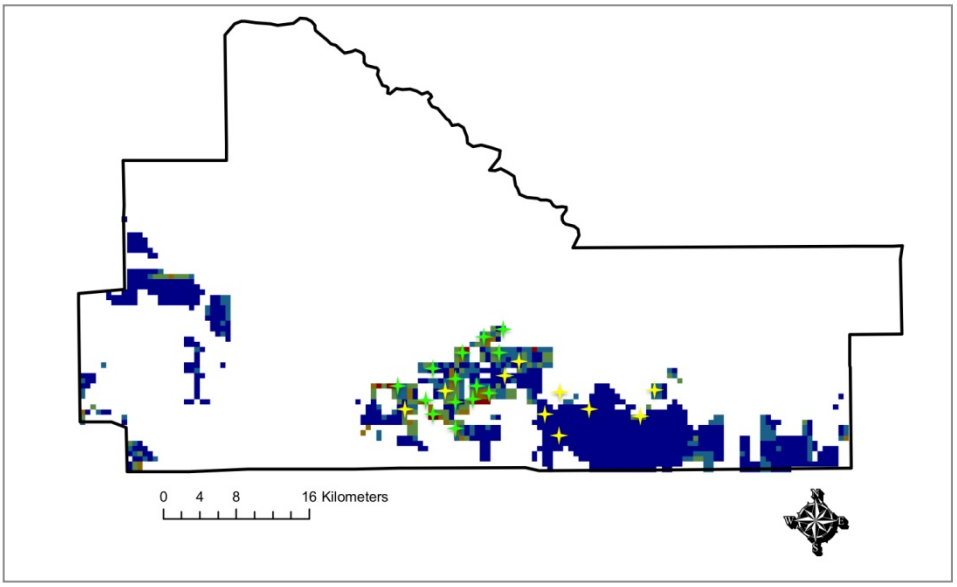

Fig. 4. Survey localities within Wilcox County, Alabama. Green symbols indicate a population of the Red Hills salamander (*Phaeognathus hubrichti*) was present; yellow indicates we were not able to detect individuals or burrows.

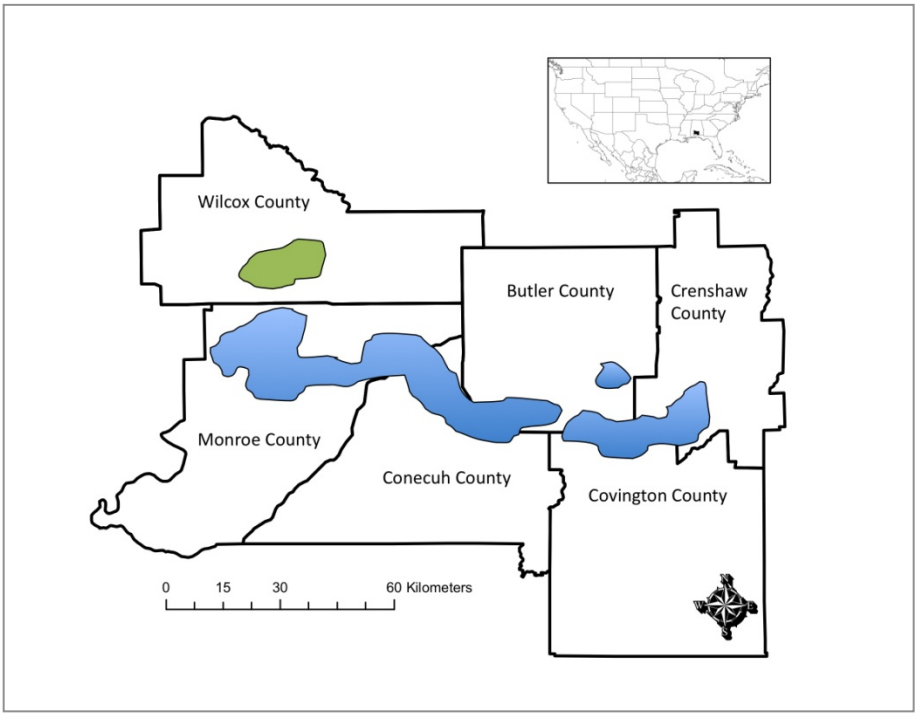

Figure 5. Species distribution map for the Red Hills salamander (*Phaeognathus hubrichti*). The blue polygons indicate the extent of the range known prior to this study. The green polygon represents the extent of the populations discovered in this study.

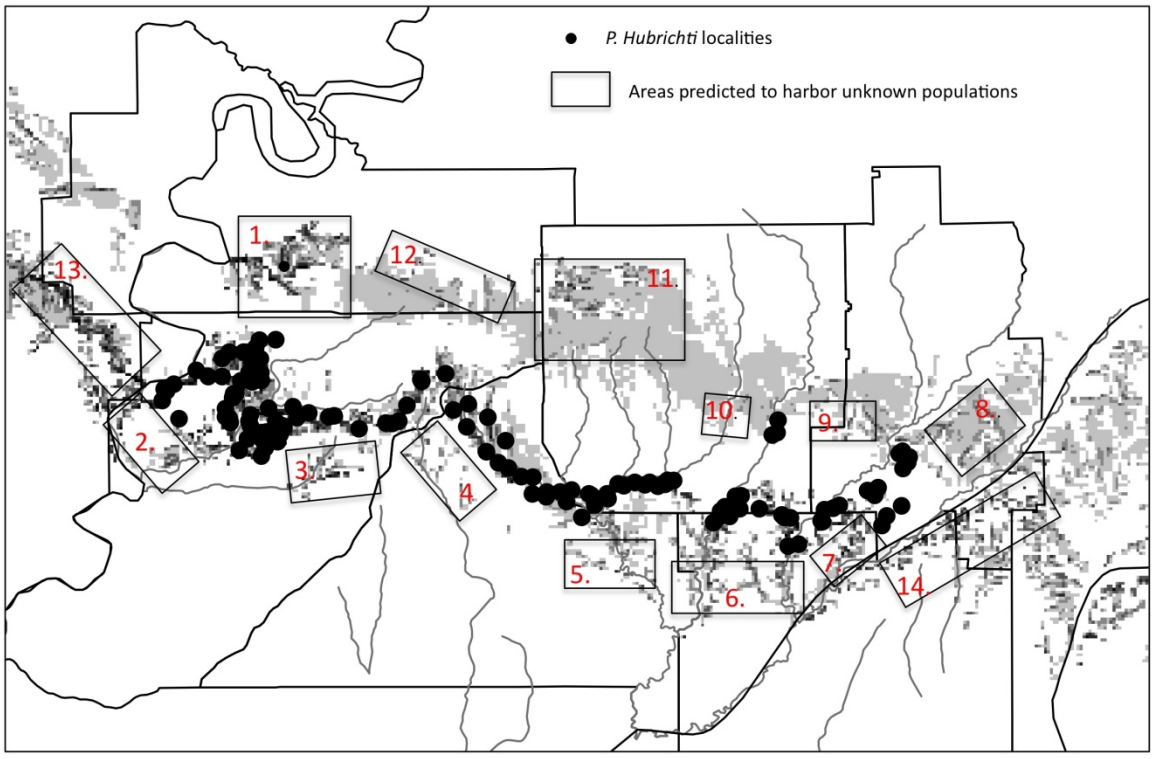

Fig. 6. Areas suggested for surveys to determine the presence of unknown Red Hills salamander (*Phaeognathus hubrichti*) populations.

Discussion and Recommendations

It has been well over thirty years since *P. hubrichti* was listed as a federally threatened species. Yet, there are no signs that there has been any significant recovery. The species was originally listed under concerns that habitat destruction in conjunction with a limited distribution, unique life history, and restricted habitat requirements could lead to the species becoming critically endangered (U.S. Fish and Wildlife Service, 1976). Yet, significant habitat destruction continues to occur across the range of the species. Since the listing the main tool for *P. hubrichti* conservation efforts has been the

employment of HCPs with large landowners. In theory this practice would eliminate a large amount of habitat destruction. However, the uncertainty associated with HCPs make them less than ideal for the long-term conservation of *P. hubrichti* (Godwin, 2008). Furthermore, HCPs are not required to insure that they contribute to the recovery of a listed species, but rather to minimize and mitigate habitat losses (Bonnie, 1999). Therefore, it is imperative that recovery efforts are centered on a number of key land acquisitions and supplemented with an incentive based recovery program such as safe harbor agreements or conservation banking (see Wilcove and Lee, 2004).

The recovery of *P. hubrichti* has recently received a major enhancement. In 2009 the Alabama Forever Wild Land Trust, along with the USFWS and The Nature Conservancy acquired 1 048 ha in four continuous parcels on the western edge of *P. hubrichti*'s range. This gives a total amount of 1 117 ha of protected land when combined with the Haines Island park operated by the Army Corps of Engineers. The actual amount of *P. hubrichti* habitat within these tracts is yet to be determined, but we can say for certain that it will be far less than 1 117 ha. While this acquisition is a major accomplishment, ultimately several more tracts throughout the range of *P. hubrichti* must be secured to ensure their long-term survival.

Recommendations for habitat acquisition

The acquisition of viable *P. hubrichti* habitat followed by proper habitat management is by far the most effective method for ensuring their long-term survival. However, the effectiveness of this approach must be based on reliable data in order to

maximize the conservation impact of limited funds. Dodd (1988) recommended 23 sites for this purpose based on his assessment of burrow abundance and status of the habitat (Fig. 7). He also made an attempt to select sites from across the range of the species in order to preserve genetic diversity. However, the knowledge of the species' genetic diversity at that time was limited to a small amount of variation found in allozymes (McKnight et al. 1991). This data indicated that there were two major populations, separated by the Sepulga River (McKnight et al. 1991). Recent data using microsatellites (Apodaca 2010) indicate that in fact there are 5 populations that should be considered as unique management units (Fig. 3). Based on these findings as well as the recent land purchases by state of Alabama Forever Wild Land Trust the we have reassessed Dodd's (1988) recommendations and updated them to reflect the current state of knowledge on the species. We will frame and discuss recommendations based on preserving the genetic diversity of the five distinct populations (Fig. 3) and with the goal of increasing gene flow between them. Additionally, since specific land tracts may not become available and unique land purchasing opportunities may arise we will tend to focus on general areas rather than specific tracts.

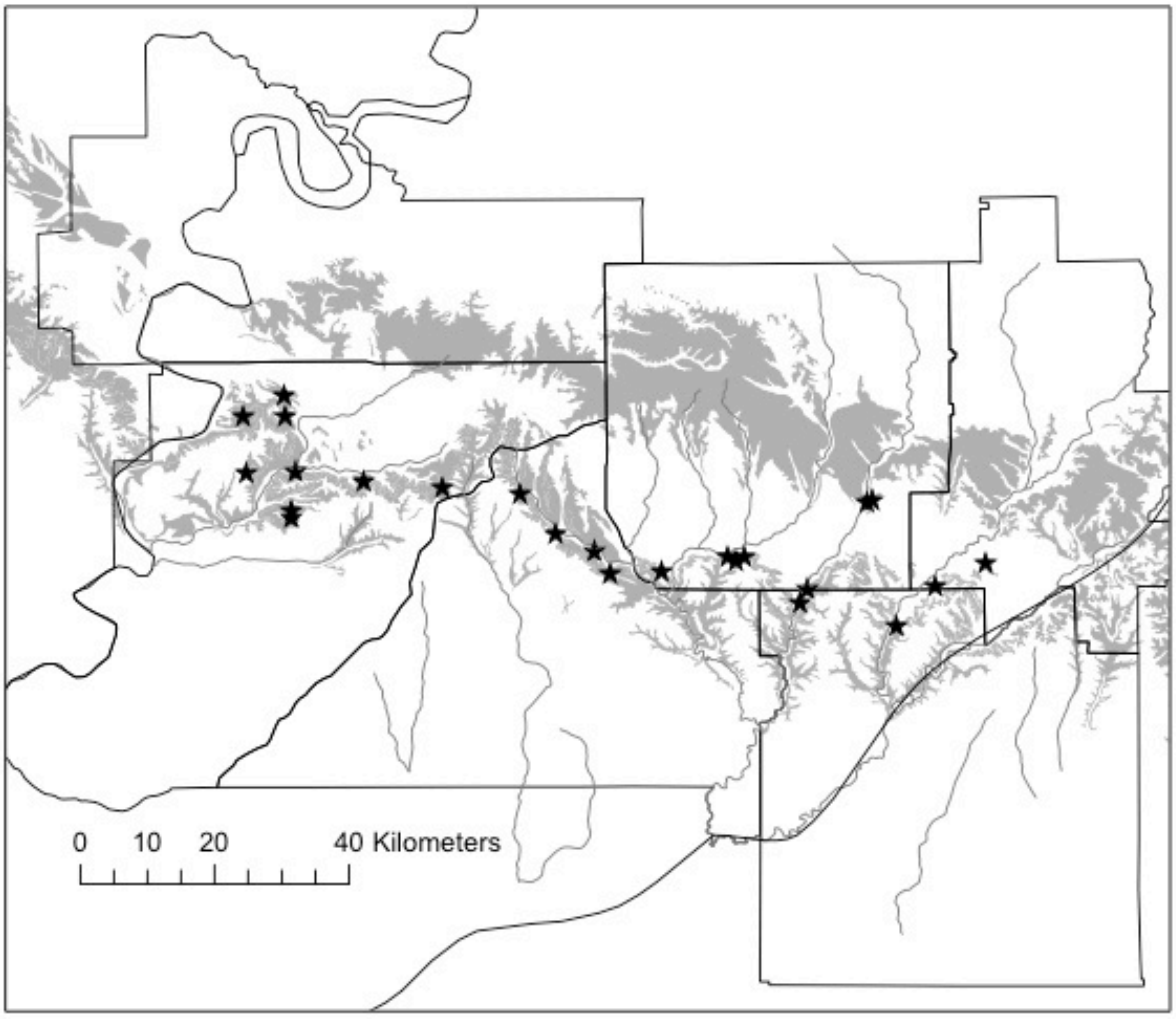

Fig. 7. Red Hills salamander (*Phaeognathus hubrichti*) localities suggested for acquisition by Dodd (1988).

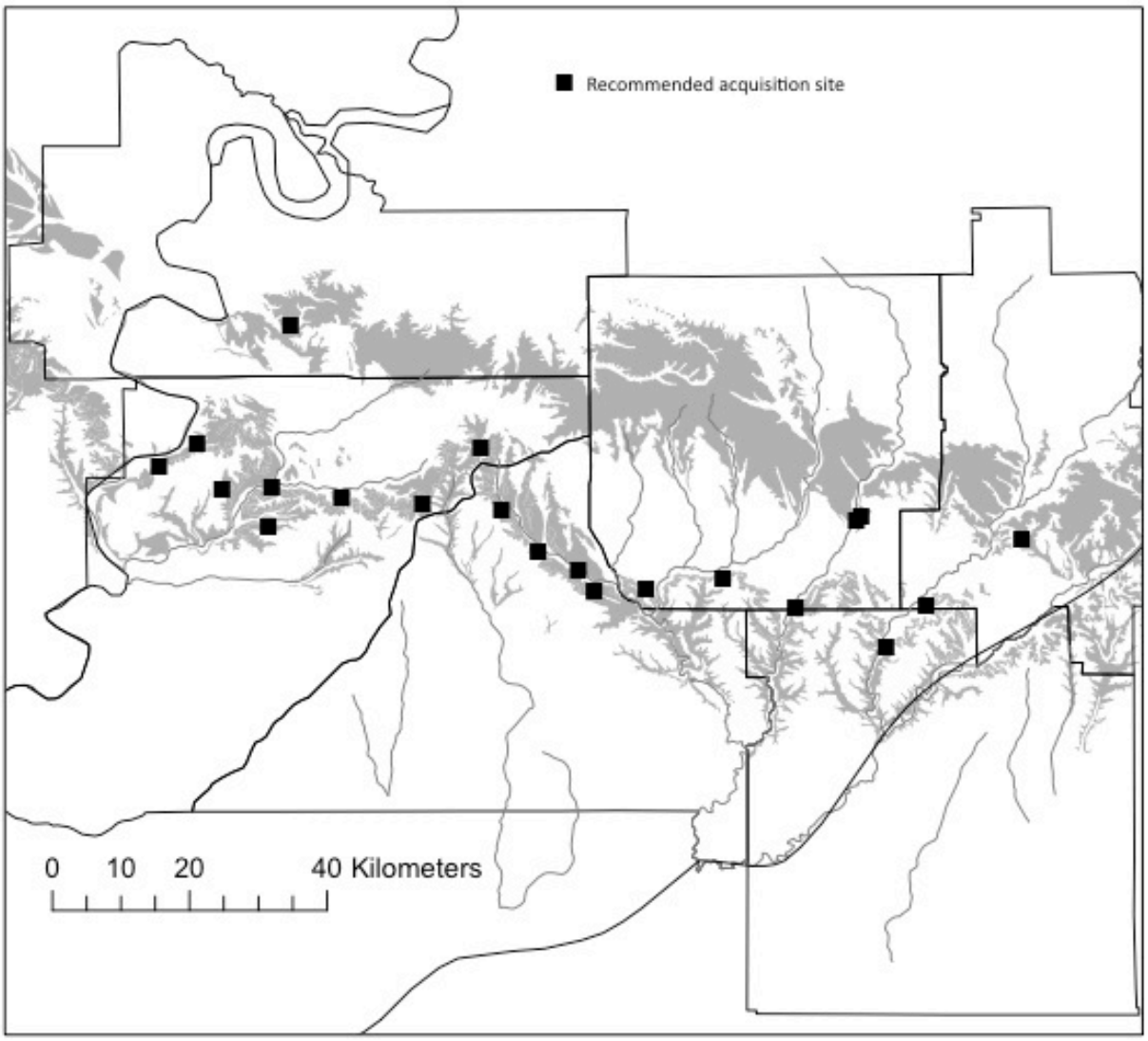

Fig. 8. Sites suggested for acquisition to promote the recovery of the Red Hills salamander (*Phaeognathus hubrichti*).

Population 1 is the smallest population in terms of geographic distribution and has approximately 69 ha in permanent habitat protection from the Haines Island Park. Therefore, we suggest a maximum of two more habitat acquisitions for this population. Dodd (1988) did not suggest any additional sites for this area, yet our model suggests that proper habitat conditions are readily available in the area (Fig. 4). Thus, we suggest one

site to the southwest of Haines Island (site 1) and one site to the northeast of Haines Island (site 2). Of these two sites, site 2 should be a higher priority, as it would help to facilitate gene flow between populations 1 and 2.

Population 2 includes a large amount of good habitat for *P. hubrichti* (Fig. 3). This area includes four of Dodd's (1988) recommended sites (Dodd sites: 4, 125, 39, and 41). Three of these sites (125, 39, and 41) are located very near the recently purchased Forever Wild Land tract. For that reason, we are removing those three sites from our recommendations. We suggest two sites (3 &4) to the south of the Forever Wild Land Tract. Site 3 is placed in an effort to enhance gene flow between populations 2 and 3, and if possible should be purchased in close proximity to Big Flat Creek (likely the major hindrance to migration between these two populations). Site 4 is Dodd's (1988) original site number 4. Population 2 also includes the recently discovered population in Wilcox County (Bailey and Miller, 2004). This area is in desperate need of surveys to discover the true nature of populations in this area. For the time being we suggest one site for Wilcox County (site 5). It is highly possible that many new populations will be discovered in this area, thus increasing the need for habitat protection.

Population 3 consists of a long narrow band of *P. hubrichti* localities, often dispersed among fragmented habitat (Fig.4). This area includes 7 of Dodd's (1988) recommended sites (44, 48, 96, 103, 104, 23, and 137). We agree with these recommendations and add an additional site (6) that may help connect eastern and western localities within the population. These recommended sites are crucial to preserving the genetic diversity of *P.*

hubrichti due to the fact that this area of the species' range is comprised of a very narrow band of habitat. Therefore, it would not take a significant amount of habitat loss in this area to completely sever the already minimal amount of gene flow between eastern (4 &5) and western populations (1 & 2) (see Apodaca 2010).

Population 4 is similar to population 3 in that it is very narrow. Though, our model suggests that there may be additional unknown localities along the Sepulga River (Fig. 3). This area contains six of Dodd's (1988) recommended sites (29, 138, 49, 52, 59, and 142). We do not recommend any additional sites for this area, though we do note that sites 52, 59, and 142 would most likely be included in a single land purchase. Therefore, the actual number of recommended sites for this population is four.

Population 5 is geographically large compared to the other populations. Though, suitable habitat is not as dense as in other areas of *P. hubrichti*'s range (Fig. 3). In fact, Mohr (1901) noted that the hills in this region become less prominent. Thereby reducing the amount of slope available as habitat. This area includes seven of Dodd's (1988) original recommendations (60, 63, 90, 91, 121, 78, and 84), though sites 90 and 91 could be considered one site. We recommend one additional site (7) for this area. We note that our model suggests that there is a great deal of suitable habitat in the area that may harbor unknown populations (Fig. 3).

Recommendations concerning surveys for unknown populations

In order to fully protect *P. hubrichti* we must have a strong understanding of their geographic distribution. Unknown populations may provide important sources of genetic variation or be fundamental to the connectivity between other populations. Thus, it is important that we identify and protect such populations. We have identified 14 sites that we feel have a strong possibility of containing unknown populations of *P. hubrichti*. We suggest that at the very minimum the sites we have ranked as highly probable to contain populations (Table 2) are surveyed as soon as possible.

Table 2. Estimated probabilities of the potential for identified sites to harbor unknown populations of the Red Hills salamander (*Phaeognathus hubrichti*). Estimates are based on: 1.) Amount of available habitat, 2.) Proximity to known populations, and 3.) is the area separated from other populations by a major barrier (i.e. Alabama or Conecuh Rivers, etc.).

Site Number	Probability Of Occurrence
1	High
2	High
3	High
4	High
5	High
6	High
7	High
8	High
9	High
10	Medium
11	Medium
12	Medium
13	Low
14	Low

Recommendations for habitat improvement

While the acquisition of viable *P. hubrichti* habitat is the most effective method for stabilizing the species it may not lead to a return to adequate levels of gene flow

between populations. Without adequate levels of gene flow populations are extremely vulnerable to the loss of genetic variability and extinction due to inbreeding and the susceptibility to demographic, environmental, and genetic stochasticity (Conner and Hartl, 2004; Frankham et al., 2007). In fact, each of the five *P. hubrichti* populations were found to have an extremely low effective population size (> 46), indicating that the populations are even more susceptible to the perils of small population size than previously believed (Apodaca 2010). Genetic data has revealed that recent levels of gene flow have been greatly reduced when compared to pre-timber harvest conditions (Apodaca 2010). It is likely that these reduction are a result of the loss of viable habitat between populations caused by timber practices such as the clear cutting of intervening habitat followed by the replanting of pine plantations. Therefore, a full recovery of the species will depend not only on preserving currently recognized habitat but also restoring historical conditions. Therefore, we recommend the initiation of a *P. hubrichti* safe harbor program. The goal of this program would be to convince major landowners to enter into safe harbor agreements with both state and federal agencies.

Safe harbor programs are most well known for their involvement in the recovery of the endangered Red-cockaded Woodpecker (*Picoides borealis*). There are over 35 safe harbor agreements in 16 states protecting a wide variety of species, including 3 mussel species within the state of Alabama (www.edf.org). Essentially a safe harbor agreement is a binding agreement where the landowners agree to protect a pre-established baseline population (similar to an HCP) and to enhance additional habitat for the species. For example, in the case of the Red-cockaded Woodpecker several large landowners

throughout the southeast have used prescribed fire, mid-story hardwood removal, drilling of artificial cavities, and other means to create additional habitat for the species (Bonnie, 1997). In return for their efforts, landowners are not responsible for additional individuals not counted under the baseline population, thereby removing uncertainty in forestry management (Zhang and Mehmood, 2002). One of the main goals of this program is to remove the fear of regulatory consequences that prevents some landowners from participating in habitat restoration (Wilcove and Lee, 2004). The major advantage of this program for *P. hubrichti* is that it would protect currently known populations (similar to an HCP) and additionally it would add habitat and reduce population fragmentation. Traditionally safe harbor programs do not include a financial reward to the owner. Though, landowners have often received financial aid from federal, state, or private sources to help offset some of the costs associated with habitat restoration (Wilcove and Lee, 2004). We suggest that safe harbor agreements for *P. hubricthi* would not be cost intensive, as most of the modifications could occur after a harvest and would not require land modification.

We recommend that safe harbor agreements for *P. hubrichti* implement the following recommendations:

- Any area that is currently managed for non-historical plant assemblages (i.e. loblolly or evergreen pine plantations, etc), has a slope greater than 35°, is within 1 km of known *P. hubrichti* habitat, and is found within the proper geologic formations (Tallahatta, Hatchitigbee, or Nanafalia) should be restored to a native assemblage of hardwood species (see

Mohr, 1901; Harper, 1943; Jordan, 1975; Diamond, 1987; Godwin, 2008) after the next scheduled harvest.

- Mechanical preparation of restored sites should be avoided.
- A buffer should be placed at the top of any slope containing *P. hubrichti*, preferably greater than 25 meters and comprised of a natural system (i.e. Long-leaf Pine).
- Chemical preparation of any kind should be avoided near any slope known to contain *P. hubrichti* or on any restored site.
- Timber harvest should be completely avoided on any slope containing *P. hubrichti* that has a slope greater than 18°.
- Harvest on any slope (< 18°) known to contain *P. hubrichti* should be limited to 35% of the bottom third of the slope. Plantation style pine assemblages should not be replanted after this harvest.
- We also suggest all of the recommendations of Dodd (1991) be followed.

Conclusions

We have outlined an extensive plan that would lead to a substantial recovery of *P. hubrichti*. This plan includes the purchase a total of 21 sites (Fig. 8) dedicated to preserving *P. hubricthi* and the other biodiversity of the region. We have based this plan on *P. hubrichti* habitat requirements, climatic suitability, available habitat, habitat fragmentation, gene flow patterns, genetic structure and genetic variability. We strongly believe that if followed this combination of habitat acquisitions and a series of safe

harbor agreements will allow for the future recovery and possibly delisting of *P. hubrichti.*

The Red Hills of Alabama are a unique and biologically diverse region of the state. This physiographic province supports at least 24 vertebrates of conservation concern (Table 3) and a unique assemblage of flora (Diamond, 1987), including a recently discovered azalea species that is endemic to the Red Hills (Zhou et al. 2008). Given the unique nature of the slope habitat occupied by *P. hubrichti* it is likely that there are other unidentified distinctive taxa endemic to the region. The conservation efforts put forth to conserve *P. hubrichti* will benefit this vast biodiversity, especially in the case of acquired and properly managed land where both ridge-tops and ravines can be returned to a natural state.

Table 3. Terrestrial vertebrates of the Red Hills physiographic province of high conservation concern. P1=endangered; P2=threatened.

Species	Common Name	Status
Phaeognathus hubrichti	**Red Hills salamander**	**P2**
Eumeces anthracinus	Coal skink	P2
E. inexpectatus	Southeastern five-lined skink	P2
Lampropeltis getula holbrooki	Speckled kingsnake	P2
Gopherus polyphemus	Gopher tortoise	P2
Picoides borealis	Red-cockaded woodpecker	P1
Hylocichla mustelina	Wood thrush	P2
Helmitheros vermivorus	Worm-eating warbler	P2
Limnothypis swainsonii	Swainson's warbler	P2
Oporonis formosus	Kentucky warbler	P2
Aimophila aestivalis	Bachman's sparrow	P2
Columbina passerina	Common ground dove	Watch List
Otus asio	Eastern screech owl	Watch List
Bubo virginianus	Great horned owl	Watch List
Caprimulgus carolinensis	Chuck-will's-widow	Watch List
Ceryle alcyon	Belted kingfisher	Watch List
Melanerpes erythrocephalus	Red-headed woodpecker	Watch List
Picoides pubescens	Downy woodpecker	Watch List
P. villosus	Hairy woodpecker	Watch List
Parula americana	Northern parula	Watch List
Dendroica discolor	Prairie warbler	Watch List
Protonotaria citrea	Prothonotary warbler	Watch List
Seiurus motacilla	Louisiana warbler	Watch List

Literature Cited

Apodaca JJ (2010) Prioritizing regions for the conservation of amphibians with special emphasis on the Red Hills salamander (Phaeognathus hubrichti) Dissertation, The University of Alabama

Bailey MA, Means DB (2004) Red Hills Salamander Phaeognathus hubrichti Highton. . In: Alabama Wildlife.Volume 3. Imperiled amphibians, reptiles, birds, and mammals. (eds. Mirarchi RE, Bailey MA, Haggerty TM, Best TL), pp. 34-36. The University of Alabama Press Tuscaloosa, AL.

Bailey MA, Miller DA (2006) Phaeognathus hubricthti. Herpetological Review 37, 357.

Bakkegard KA (2002) Activity patterns of Red Hills salamanders (Phaeognathus hubrichti) at their burrow entrances. Copeia, 851-856.

Balmford A, Green MJB, Murray MG (1996) Using higher-taxon richness as a surrogate for species richness .1. Regional tests. Proceedings of the Royal Society of London Series B-Biological Sciences 263, 1267-1274.

Bonnie R (1997) Safe harbor for the red-cockaded woodpecker. Journal of Forestry 95, 17-22.

Bonnie R (1999) Endangered species mitigation banking: promoting recovery through habitat conservation planning under the Endangered Species Act. Science of the Total Environment 240, 11-19.

Conner JK, Hartl DL (2004) A primer of ecological genetics Sinauer Associates Inc. , Sunderland, MA.

Diamond A (1987) A flora of the mesic ravines of the central Red Hills of Alabama Auburn University, Auburn Alabama

Dodd CK (1988) A re-examination of the status of the Red Hills salamander, Phaeognathus hubrichti, Alabama, USA, 1976-1988. Report to the U.S. Fish and Wildlife Service, Jackson, M.S., 74 pp. .

Dodd CK (1991) The Status of the Red Hills Salamander Phaeognathus-Hubrichti, Alabama, USA, 1976-1988. Biological Conservation 55, 57-75.

Elith J, Graham CH, Anderson RP, et al. (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography 29, 129-151.

Frankham R, Ballou JD, Briscoe DA (2007) Introduction to conservation genetics Cambridge University Press, Cambridge, UK.

Godwin J (2008) Red Hills salamander habitat delineation, breeding bird surveys, and habitat restoration recommendations on commercial timberlands Unpublished report prepared for The Alabama Department of Conservation and Natural Resources.

Gunzburger MS, Guyer C (1998) Longevity and abandonment of burrows used by the Red Hills salamander (Phaeognathus hubrichti). Journal of Herpetology 32, 620-623.

Harding EK, Crone EE, Elderd BD, et al. (2001) The scientific foundations of habitat conservation plans: a quantitative assessment. Conservation Biology 15, 488-500.

Harper RM (1920) Resources of southern Alabama: a statistical guide for

investors and settlers, with an exposition of some of the general principles

of economic geography. Geological Survey of Alabama, Special Report 11, 152 pgs. .

Highton R (1961) A new genus of Lungless salamander from the coastal plain of Alabama Copeia 1, 65-68.

Hunter MLJ, Gibbs J (2007) Fundamentals of Conservation Biology 3rd edn. Blackwell Publishing, Malden, MA.

Jenks GF (1967) The data model concept in statistical mapping. International Yearbook of Cartography 7, 186-190.

Jordan RJ, Mount RH (1975) The status of the Red Hills salamander, Phaeognathus hubrichti Highton. Journal of Herpetology, 211-215.

Klein BC (1989) Effects of Forest Fragmentation on Dung and Carrion Beetle Communities in Central Amazonia. Ecology 70, 1715-1725.

Kleiner K, Mackenzie M, Silvano A, et al. (2007) GAP land cover map of ecological systems for the state of Alabama (Provisional). Alabama Gap Analysis Program Acessed 8-2009 from www.auburn.edu/gap.

Kozak KH, Mendyk RW, Wiens JJ (2009) Can Parallel Diversification Occur in Sympatry? Repeated Patterns of Body-Size Evolution in Coexisting Clades of North American Salamanders. Evolution 63, 1769-1784.

Mcknight ML, Dodd CK, Spolsky CM (1991) Protein and Mitochondrial-DNA Variation in the Salamander Phaeognathus-Hubrichti. Herpetologica 47, 440-447.

Means DB (2003) Notes on the reproductive biology of the Alabama Red Hills salamander (Phaeognathus hubrichti). Contemporary Herpetology 2003, 1-5.

Mohr C (1901) Plant life of Alabama Contribution from the U.S. National Herbarium 4, 921 pgs. .

Mount RH (1975) The Reptiles & Amphibians of Alabama Alabama Agricultural Experiment Station, Auburn University Auburn, AL.

Olden JD, Lawler JJ, Poff NL (2008) Machine learning methods without tears: A primer for ecologists. Quarterly Review of Biology 83, 171-193.

Parker CA (1989) Soil biota and plants in the rehabilitation of degraded agricultural soils. In: Animals in primary succession. The role of fauna in reclaimed lands (ed. Majer J). Cambridge University Press, Cambridge, UK.

Pearson RG, Raxworthy CJ, Nakamura M, Peterson AT (2007) Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. Journal of Biogeography 34, 102-117.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecological Modelling 190, 231-259.

Raxworthy CJ, Martinez-Meyer E, Horning N, et al. (2003) Predicting distributions of known and unknown reptile species in Madagascar. Nature 426, 837-841.

Redford KH, Richter BD (1999) Conservation of biodiversity in a world of use. Conservation Biology 13, 1246-1256.

Rodrigues ASL, Gaston KJ (2002) Maximising phylogenetic diversity in the selection of networks of conservation areas. Biological Conservation 105, 103-111.

Schwaner TD, Mount RH (1970) Notes on the distribution, habits, and ecology of the salamander Phaeognathus hubrichti Highton. Copeia 1970, 571-573.

Scott JC (1972) The geology of Monroe County, Alabama. Alabama Geological Survey Map 101.

Service UFaW (1976) Determination that the Red Hills salamander is a threatened species. . federal Registry 41, 53032-53034.

Soule ME (1991) Conservation - Tactics for a Constant Crisis. Science 253, 744-750.

Vertessy RA (2001) Impacts of plantation forestry on catchment runoff. In: Plantations, Farm Forestry and Water: Workshop Proceedings Publication No. 01/20 (eds. Nambiar EKS, Brown AG). Rural Industries Research and Development Corporation.

Vieites DR, Min MS, Wake DB (2007) Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. Proc Natl Acad Sci U S A 104, 19903-19907.

Wilcove DS, Lee J (2004) Using economic and regulatory incentives to restore endangered species: Lessons learned from three new programs. Conservation Biology 18, 639-645.

Zhang DW, Mehmood SR (2002) Safe Harbor for the red-cockaded woodpecker: Private forest landowners share their views. Journal of Forestry 100, 24-29.

Zhou W, Gibbons T, Goetsch L, Hall B (2008) Rhododendron colemanii: A New species of deciduous Azalea (Rhododendron section Pentanthera; Ericaceae) from the coastal plain of Alabama and Georgia. Journal of the American Rhododendron Society 75, 72-78.